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Role of *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms in the response to chemotherapy and overall survival of advanced non-small cell lung cancer

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ABSTRACT. We evaluated the association between *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms and treatment outcomes of advanced non-small cell lung carcinoma. Between January 2010 and December 2012, a total of 244 patients with non-small cell lung carcinoma were recruited from Yiwu Central Hospital. The *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism and the results were statistically analyzed. Conditional regression analysis, showed that individuals carrying the null *GSTM1* were associated with an increased risk of response to chemotherapy when

compared to the present *GSTM1* (odds ratio = 1.88, 95% confidence interval (CI) = 1.01-3.47). Moreover, the GG genotype of *GSTP1* IIe105Val was associated with a better response to chemotherapy compared to the AA genotype (odds ratio = 2.77, 95%CI = 1.14-6.64). The null *GSTM1* genotype was associated with a lower risk of death from all causes when compared with the present *GSTM1* genotype (hazard ratio = 2.16, 95%CI = 1.10-4.38). Moreover, the GG genotype of *GSTP1* IIe105Val was correlated with a reduced risk of death from all causes compared with the AA genotype (hazard ratio = 2.94, 95%CI = 1.11-8.68). In conclusion, we found that the null *GSTM1* and the GG genotype of *GSTP1* IIe105Val were correlated with a good response to chemotherapy and improved overall survival of advanced non-small cell lung carcinoma patients.

Key words: Advanced non-small cell lung carcinoma; Polymorphism; *GSTM1*, *GSTP1*, and *GSTT1* IIe105Val

INTRODUCTION

Lung cancer is one of the most common malignant tumors worldwide and is the leading cause of cancer-related deaths (IARC, 2012). It is estimated that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide. More than 50% of lung cancer cases occur in less-developed regions (IARC, 2012). There are two types of lung cancer, including small cell lung cancer and non-small cell lung cancer (NSCLC) (Liu et al., 2013). The 5-year survival rate of NSCLC is estimated to be 15% and the median survival time is less than 10 months (NCCN, 2011). An increasing number of genomic studies have reported potential therapeutic targets in lung cancer, such as *XRCC1*, *XPF*, *XPG*, *CYP1B1*, and *OPN* genes (Hao et al., 2014; Liu et al., 2015; Shi et al., 2015; Vasile et al., 2015; Zou and Zhao, 2015).

Glutathione-*S*-transferases (GSTs) comprise a family of cytosolic enzymes and play important roles in the metabolism and detoxification of chemotherapeutic agents (Hayes and Pulford, 1995). The *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val genes play an important role in lowering the intracellular concentration of chemotherapeutic agents (Deng et al., 2015). Previous studies have shown the potential role of genetic variability of the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val genes in the treatment outcomes of NSCLC; however, the results are inconclusive (Tkácová et al., 2004; Booton et al., 2006; Ada et al., 2010; Piao et al., 2013; Yang and Xian, 2014). Therefore, we evaluated the association between *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms and treatment outcomes of advanced NSCLC.

MATERIAL AND METHODS

Patients

Between January 2010 and December 2012, a total of 265 patients with NSCLC were recruited from Yiwu Central Hospital. All patients were histopathologically confirmed by two pathologists. The exclusion criteria for patients with NSCLC were as follows: any

serious concomitant systemic disorder, unable to receive chemotherapy, concurrent chemo-radiotherapy, and brain metastasis. Among these, 244 NSCLC patients agreed to participate in this study (participation rate, 92.08%).

Cisplatin-based chemotherapy regimens were used to treat all advanced NSCLC patients. The response to chemotherapy was evaluated based on the Response Evaluation Criteria in Solid Tumors. Patients' responses to chemotherapy were divided into 2 groups: response and non-response. For long-term survival, overall survival (OS) was defined as the time from the beginning of study (January 2010) to the date of death from any cause or the end of follow-up. All patients were followed up until the end of December 2014, and patients were followed up by attending the clinics or by telephone every 4 weeks. All patients signed informed consent forms, and the protocol of our study was approved by Yiwu Central Hospital.

DNA extraction and genotyping

Prior to the beginning of chemotherapy, 2 mL peripheral blood was acquired from each patient for DNA extraction using the TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). The extracted DNA samples were stored at -80°C until use. The *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms were analyzed by a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay. The primers used for amplification were designed using Assay Designer 4.0 (Sequenom, Inc., San Diego, CA, USA). The PCR conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 45 s. To verify the results, 5% DNA samples were randomly selected for duplicate assays.

Statistical analysis

SPSS 21.0 package (SPSS Inc., Chicago, IL, USA) was used for the analyses. The associations between the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms and the response to chemotherapy were evaluated by logistic regression analysis. The results are reported as odds ratio (OR) and 95% confidence interval (95%CI). Multivariate Cox proportional hazard regression analysis and hazard ratio (HR) with the corresponding 95%CI were used to evaluate the effect of *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms on OS. The Kaplan-Meier method was used to compare OS among different genotypes. All statistical tests were two-sided, and $P < 0.05$ was considered to indicate significance.

RESULTS

The lifestyle and clinical characteristics of the included subjects are shown in Table 1. The mean age of the included NSCLC patients was 57.50 ± 11.25 years. There were 84 (34.43%) females and 160 (65.57%) males in the present study. A total of 108 (44.26%) of the included patients with advanced NSCLC had a habit of tobacco smoking, 75 (30.74%) had a habit of alcohol drinking, 99 (40.57%) were at TNM stage IIIA or IIIB, 145 (59.43%) were in TNM stage IV, 141 (57.79%) were adenocarcinoma, and 83 (34.02%) were squamous carcinoma. There were 179 (73.36%) patients who showed a non-response to chemotherapy and 65 (26.64%) patients who responded to chemotherapy.

Table 1. Characteristics of included patients with advanced NSCLC.

Characteristics	Patients	%
Age (years)		
≤60	137	56.15
>60	107	43.85
Gender		
Female	84	34.43
Male	160	65.57
Tobacco smoking		
No	136	55.74
Yes	108	44.26
Alcohol drinking		
No	169	69.26
Yes	75	30.74
TNM stage		
IIIA or IIIB	99	40.57
IV	145	59.43
Histology		
Adenocarcinoma	141	57.79
Squamous carcinoma	83	34.02
Other	20	8.20
Response to chemotherapy		
Non-response	179	73.36
Response	65	26.64

According to the results of the chi-squire test, there were significant differences in the genotype distribution of *GSTM1* between the response and non-response to chemotherapy in advanced NSCLC ($\chi^2 = 4.64$, P = 0.03; Table 2). Conditional regression analysis showed that individuals carrying the null *GSTM1* were associated with a better response to chemotherapy compared to the present *GSTM1* (OR = 1.88, 95%CI = 1.01-3.47). Moreover, the GG genotype of *GSTP1* IIe105Val was associated with a better response to chemotherapy compared to the AA genotype (OR = 2.77, 95%CI = 1.14-6.64).

Table 2. Association between response to chemotherapy and *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms in advanced NSCLC patients.

Genes	Response	%	Non-response	%	χ^2 test	P value	OR (95%CI)[1]	P value
GSTM1								
Present	33	50.77	118	65.92			1.0 (Ref.)	-
Null	32	49.23	61	34.08	4.64	0.03	1.88 (1.01-3.47)	0.03
GSTT1								
Present	28	43.08	83	46.37			1.0 (Ref.)	-
Null	37	56.92	96	53.63	0.21	0.65	1.14 (0.62-2.11)	0.65
GSTP1 IIe105Val								
AA	21	32.31	80	44.69			1.0 (Ref.)	-
AG	28	43.08	77	43.02			1.38 (0.69-2.80)	0.32
GG	16	24.62	22	12.29	6.42	0.04	2.77 (1.14-6.64)	0.01

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

A total of 187 patients died during the follow-up period by the end of December 2014, and the 5-year survival rate was 23.36%. The mean overall survival time was 23.77 ± 1.20 months. We found that patients with null *GSTM1* (25.36 ± 1.72 months) had significantly longer survival times compared with *GSTM1* (21.98 ± 1.20 months) (P for log-rank test = 0.03; Figure 1 and Table 3). Moreover, patients with the GG genotype of *GSTP1* IIe105Val (26.64 ± 2.20 months) were associated with a longer overall survival time compared to the AA

genotype (19.55 ± 2.15 months) (P for log-rank test = 0.01; Figure 2 and Table 3). According to the Cox proportional hazard model, the null *GSTM1* genotype was associated with a lower risk of death from all causes compared with the present *GSTM1* genotype (HR = 2.16, 95%CI = 1.10-4.38). Moreover, the GG genotype of *GSTP1* IIe105Val was correlated with a reduced risk of death from all causes compared with the AA genotype (HR = 2.94, 95%CI = 1.11-8.68).

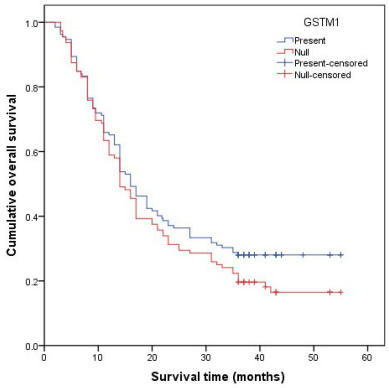

Figure 1. Kaplan-Meier survival curves for overall survival of advanced NSCLC by *GSTM1*.

Table 3. Association between survival of advanced NSCLC patients and *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms.

Genes	Response	%	Non-response	%	χ^2 test	P value	OR (95%CI)[1]	P value
GSTM1								
Present	33	50.77	118	65.92			1.0 (Ref.)	-
Null	32	49.23	61	34.08	4.64	0.03	1.88 (1.01-3.47)	0.03
GSTT1								
Present	28	43.08	83	46.37			1.0 (Ref.)	-
Null	37	56.92	96	53.63	0.21	0.65	1.14 (0.62-2.11)	0.65
GSTP1 IIe105Val								
AA	21	32.31	80	44.69			1.0 (Ref.)	-
AG	28	43.08	77	43.02			1.38 (0.69-2.80)	0.32
GG	16	24.62	22	12.29	6.42	0.04	2.77 (1.14-6.64)	0.01

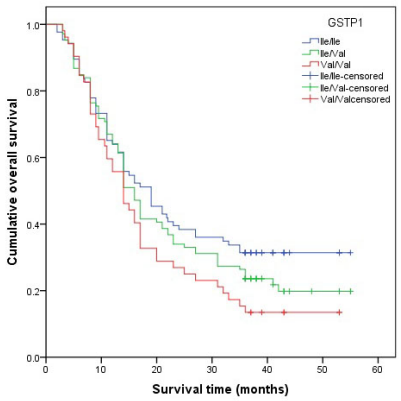

Figure 2. Kaplan-Meier survival curves for overall survival of advanced NSCLC by *GSTP1* IIe105Val.

DISCUSSION

In this present study, we conducted a case-control study to evaluate the role of *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms in the treatment outcomes of advanced NSCLC patients treated with cisplatin-based chemotherapy in a Chinese population. The results of our study indicated that the null *GSTM1* and the GG genotype of *GSTP1* IIe105Val was correlated with a good response to chemotherapy and improved the OS of advanced NSCLC.

Many previous epidemiological studies have reported that GSTs may affect the efficacy of chemotherapy on cancer (Djukic et al., 2013; Duggan et al., 2013; Oliveira et al., 2014; Kap et al., 2014; Goričar et al., 2015). Djukic et al. (2013) reported that the *GSTT1* active genotype was associated with the prognosis of patients with muscle invasive bladder cancer in a Serbian population. Duggan et al. (2013) conducted a study in an American population with breast cancer and found that the *GSTP1* IIe105Val gene polymorphism increased the risk of all-cause mortality in breast cancer survivors. Another study conducted in a Brazilian population reported that the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms contribute to better OS and disease-free survival in breast cancer patients (Oliveira et al., 2014). Kap et al. (2014) examined 755 colorectal cancer patients with stage II-IV diseases in Germany, and they suggested that *GSTM1* influenced the survival of colorectal cancer patients. Goričar et al. (2015) reported that the *GSTP1* IIe105Val gene polymorphisms were associated with the survival and treatment outcomes of patients with osteosarcoma. The above results suggest that GST superfamily enzymes are associated with the treatment outcome of chemotherapy in cancer patients.

Several previous studies have reported associations between the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val gene polymorphisms and patient outcomes, but the results are inconclusive (Lu et al., 2006; Booton et al., 2006; Kalikaki et al., 2009; Ada et al., 2010; Yang and Xian, 2014; Liu et al., 2015).

Booton et al. (2006) reported that the *GSTP1* IIe105Val polymorphism was correlated with the response and survival of NSCLC patients. Liu et al. (2015) conducted a study in a Chinese population and found that the *GSTP1* IIe105Val polymorphism may affect the clinical outcome of patients with advanced NSCLC. Li et al. (2008) reported that the null *GSTM1* was associated with a better response to chemotherapy than the non-null *GSTM1* type in NSCLC patients who received platinum chemotherapy. However, some studies reported inconsistent results with ours. Lu et al. (2006) conducted a study in a Chinese population and Ada et al. (2010) performed a study in a Turkish population, and they found that the GSTP1 Ala114Val polymorphism but not *GSTP1* IIe105Val was associated with improved OS among patients with advanced NSCLC. Kalikaki et al. (2009) reported no significant association between the *GSTP1* IIe105Val, *GSTT1*, and *GSTM1* gene polymorphisms and the OS of advanced NSCLC patients. Yang and Xian (2014) performed a meta-analysis of 9 studies, and they found that the *GSTM1* and *GSTP1* IIe105Val gene polymorphisms may influence the treatment response of platinum-based chemotherapy in East-Asian populations. In our study, we found that the *GSTM1* and *GSTP1* IIe105Val gene polymorphisms were correlated with the response to chemotherapy and OS of advanced NSCLC patients.

In conclusion, we found that the null *GSTM1* and the GG genotype of *GSTP1* IIe105Val was correlated with a good response to chemotherapy and improved the OS of advanced NSCLC patients.

Conflicts of interest

The authors declare no conflict of interest.

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