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Original Article

Association of GSTs gene polymorphisms with treatment outcome of advanced non-small cell lung cancer patients with cisplatin-based chemotherapy

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Abstract: We evaluated the association of GSTM1 null/present, GSTT1 null/present, and GSTP1 Ile105Val polymorphisms with the clinical response to chemotherapy and treatment outcome of NSCLC. Between October 2009 and October 2012, a total of 282 patients with advanced NSCLC were enrolled into our study, and they were followed up until October 2014. The genotypes of GSTM1, GSTT1, and GSTP1 Ile105Val were performed by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). By logistic regression analysis, our study found that the Val/Val genotype of GSTP1 Ile105Val was associated with more CR+PR response to chemotherapy when compared with the Ile/Ile genotype, and the OR (95% CI) was 2.18 (1.16-4.12). By multivariate Cox proportional hazards regression analysis, we found the Val/Val genotype of GSTP1 was correlated with lower risk of death in advanced NSCLC (HR, 0.48; 95% CI, 0.25-0.93). However, no association was found between GSTT1 and GSTM1 polymorphisms and response to chemotherapy and overall survival of advanced NSCLC. Moreover, the Ile/Val + Val/Val genotypes of GSTP1 were associated with lower risk of death in never smokers, and the adjusted HR (95% CI) was 0.34 (0.12-0.93). In conclusion, we found that the GSTP1 polymorphism was correlated with better response to chemotherapy and lower risk of death in advanced NSCLC patients.

Keywords: GSTM1, GSTT1, GSTP1, polymorphism, NSCLC, chemotherapy

Introduction

Worldwide, lung cancer is the most common cancer among men in terms of both incidence and mortality, and among women it has the third highest incidence and the second mortality rate [1]. In 2012, lung cancer is the number one cause of death among people with malignant tumors in China, and there are 652,842 new cases with lung cancer [1]. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Although both individually and in combination of treatment, such as surgery, radiotherapy and chemotherapy, have improved survival patients with of NSCLC in recent years, the long-term survival still has room to improve. TNM classification is the basis for prognostic management of NSCLC; however, it does not provide sufficient information about biological tumor progression [2]. There is still demanding for revealing biomarkers for patients' survival. Increasing evidences have shown that drug-metabolizing enzyme (DME) have a role in determining inter-individual variations in therapeutic response [3, 4].

The human glutathione S-transferases (GSTs), a superfamily of dimeric phase II metabolic enzymes, play an important role in the cellular defense system [5]. The GSTs enzymes detoxify chemotherapeutic drugs or their metabolites by catalyzing the reduction of these compounds through its conjugation with glutathione. It has been suggested that genetic polymorphisms in GSTs genes could reduce the effectiveness of detoxifying cytotoxins generated by chemotherapeutic agents in the treatment of NSCLC [3, 4]. The GSTs family consists of six dimeric isoenzymes: GSTs alpha (α), mu (μ), pi (π), theta (τ), zeta (ζ), and omega (ω). Allelic deletions in

GSTM1 and GSTT1 genotypes result in polymorphism, and complete deletion of both the alleles (null-type) is associated with reduced enzyme activity [6]. The rs1695 of GSTP1 results in an amino acid change at codon 105 (Ile/Val), which is correlated with lower substrate specific catalytic activity. Polymorphisms of GSTs could alter the metabolism of chemotherapeutic drugs and modify the effectiveness of therapy, as suggested by reports that GSTs polymorphisms predict differences in outcomes of treatment for NSCLC [7, 8]. Analysis of the GSTs polymorphisms could be helpful in optimizing chemotherapy treatment.

Only two studies reported the association between GSTs gene polymorphisms and treatment outcome of NSCLC, and no study reported their association in Chinese population [7, 8]. We conducted a study to evaluate the clinical response to chemotherapy and treatment outcome of advanced NSCLC in the presence of GSTM1 null/present, GSTT1 null/present, and GSTP1 Ile105Val polymorphisms.

Material and methods

Study subjects

Between October 2009 and October 2012, total of 318 patients who were histopathologically confirmed, without previous chemotherapy or radiotherapy, and underwent lung cancer surgery were enrolled from the Sichuan Cancer Hospital. All the NSCLC patients were inoperable stage IIIA, IIIB and IV NSCLC. The diagnosis of NSCLC were confirmed by bronchoscopic or needle aspiration biopsies. Patients who had any serious concomitant systemic disorder unable to receive chemotherapy, concurrent chemo-radiotherapy, brain metastasis with symptoms which may affect the safety of patients or the evaluation of results were excluded from our study. Finally, 282 patients were included into our study, and the participation rate was 88.68%.

Patients with NSCLC were investigated by trained nurses to collect the demographic and clinical characteristics, such as sex, age, tobacco smoking, alcohol drinking, and family history of cancer, histological classification, TNM stage, and response to chemotherapy. The TNM stage was defined according to the 7th edition of the TNM staging system of the International Union Against Cancer (UICC)/ American Joint Committee on Cancer (AJCC).

All participants received cisplatin-based combination chemotherapy regimen. The chemotherapy was repeated at three-weekly intervals for up to six cycles unless unacceptable toxicity, disease progression or patients' refusal to continue treatment. The patients were required to complete two cycles of chemotherapy at least to evaluate treatment response according to RECIST 1.1. Patients who showed complete response (CR) or partial remission (PR) were considered as good responder, and patients who presented stable disease (SD) and progressive disease (PD) were regarded as poor responder. For long-term survival, overall survival (OS) was defined as the period between the date of chemotherapy and the data of death from any cause. All the patients with advanced NSCLC were followed up until the date of October 2014. All patients with advanced NSCLC signed written informed consents before enrolling into our study. The protocol of this study was approved by the ethics committee of our hospital.

DNA extraction and genotyping

5 ml peripheral blood sample was drawn from each patient with advanced NSCLC, and the peripheral blood samples were kept in -20°C until use. Genomic DNA was isolated from peripheral blood lymphocytes using Qiagen blood mini kit (Qiagen, Germany) by the manufacturer's protocol. The genotypes of GSTM1, GSTT1, and GSTP1 Ile105Val were performed by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primer sequences of GSTM1, GSTT1, and GSTP1 Ile105Val were designed using Sequenom Assay Design 3.1 software. The forward and reverse primers for GSTT1 were 5'-GAACTTGAACCGCTAAGC-3' and 5'-GTTCCTCGGTATACGGTGG-3', respectively. The forward and reverse primers for GSTM1 were 5'-CCTTACTGGCTTCTACATCTC-3' and 5'-TCACCGGATCAGCCGAGCA-3', respectively. The forward and reverse primers for GSTP1 Ile105Val were 5'-ACCAGGGCTCTATGGCCAA-3' and 5'-TGACCCGAGAAGAACGGGT-3', respectively. The reaction conditions were as follows: 94°C for 4 min; 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec with 35 cycles; 72°C for 7 min. The PCR

Table 1. Characteristics of patients with advanced NSCLC

Variables	Patients	%
Age, years		
< 55	117	41.49
≥ 55	165	58.51
Sex		
Female	101	35.82
Male	181	64.18
Tobacco smoking		
Never	89	31.56
Ever	193	68.44
Alcohol drinking		
Never	199	70.57
Ever	83	29.43
Family history of cancer		
No	253	89.72
Yes	29	10.28
Histological types		
Squamous Carcinoma	120	42.55
Adenocarcinoma	162	57.45
TNM stage		
IIIA and IIIB	204	72.34
IV	78	27.66
Response to chemotherpy		
CR+PR response	123	43.62
SD+PD response	159	56.38

products were analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Statistical analysis

Continuous variables were shown as the mean ± standard deviation (SD) and categorical variables were expressed as number (N) and percentage (%). Homozygote for the most frequent genotype was regarded as the reference group. The associations of GSTM1 null/present, GSTT1 null/present, and GSTP1 Ile105Val polymorphisms with the response to chemotherapy in advanced NSCLC were analyzed by logistic regression analysis, and the results were expressed by odds ratio (ORs) and 95% confidence interval (CI). The prognostic values of GSTM1 null/present, GSTT1 null/present, and GSTP1 Ile105Val polymorphisms for the OS were estimated using the multivariate Cox proportional hazards regression analysis and the results were illustrated using the hazard ratio (HR) and 95% CI. Survival distributions were estimated using the Kaplan-Meier method. Baseline characteristics, such as age, sex, smoking history, histological types and TNM stage at entry, were adjusted in order to avoid potential confounding effects. *P* values < 0.05 with two-sided were considered statistical differences. Statistical analyses were conducted with SAS software (version 9.1.3; SAS Institute, Cary, NC, USA).

Results

The median ages of included patients with advanced NSCLC were 59.15±10.50 years. There were 101 (35.82%) females and 181 (64.18%) males in our study. Of 282 patients with NSCLC, 193 (68.44%) patients were ever smokers, 83 (29.43%) were drinkers, 29 (10.28%) showed family history of cancer in the first relatives, 120 (42.55%) were squamous cell carcinoma, 162 (57.45%) were adenocarcinoma, 204 (72.34%) were at IIIA and IIIB TNM stage, 78 (27.66%) were at IV TNM stage, and 123 (43.62%) showed CR+PR response to chemotherapy in advanced NSCLC (**Table 1**).

Association between the GSTM1 null/present, GSTT1 null/present, and GSTP1 Ile105Val polymorphisms and response to chemotherapy in advanced NSCLC was shown in **Table 2**. Of 282 patients with advanced NSCLC, the good response rate was 43.62%. By logistic regression analysis, our study found that the Val/Val genotype of GSTP1 Ile105Val was associated with more CR+PR response to chemotherapy when compared with the Ile/Ile genotype, and the OR (95% CI) was 2.18 (1.16-4.12). However, the GSTT1 and GSTM1 polymorphisms were not correlated with response to chemotherapy in advanced NSCLC.

By the end of follow-up, we found that the Val/Val genotype of GSTP1 was associated with longer overall survival time of advanced NSCLC when compared with the Ile/Ile genotype (17.45 months vs. 15.30 months) (**Figure 1**). By multivariate Cox proportional hazards regression analysis, we found the Val/Val genotype of GSTP1 was correlated with lower risk of death in advanced NSCLC (HR, 0.48; 95% CI, 0.25-0.93) (**Table 3**). However, the GSTT1 and

Table 2. Association between GSTM1 null/present, GSTT1 null/present, and GSTP1 Ile105Val polymorphisms and response to chemotherapy in advanced NSCLC

SNPs	Patients	%	Good response	%	Poor response	%	OR (95% CI)[1]	*P* value
GSTM1								
Present	168	59.57	68	55.28	100	62.89	1.0 (Ref.)	-
Null	114	40.43	55	44.72	59	37.11	1.37 (0.82-2.28)	0.2
GSTT1								
Present	161	57.09	69	56.10	92	57.86	1.0 (Ref.)	-
Null	121	42.91	54	43.90	67	42.14	1.07 (0.65-1.78)	0.77
GSTP1								
Ile/Ile	120	42.55	42	34.15	78	49.06	1.0 (Ref.)	-
Ile/Val	89	31.56	41	33.33	48	30.19	1.59 (0.87-2.89)	0.11
Val/Val	74	26.24	40	32.52	34	21.38	2.18 (1.16-4.12)	0.009

[1]Adjusted for age, sex, tobacco smoking, alcohol drinking, family history of cancer in the first relatives and histological types.

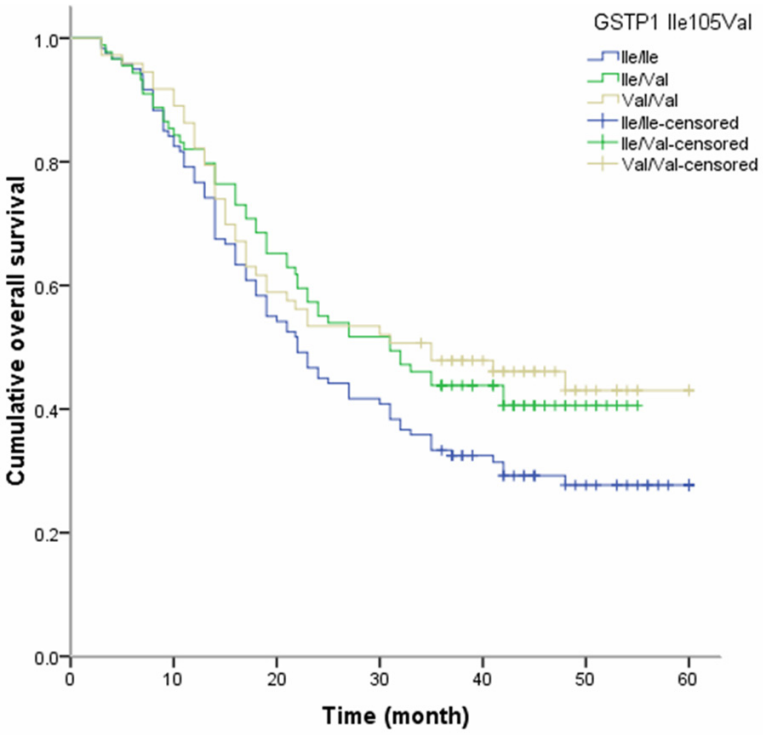

Figure 1. Kaplan-Meier survival curves by GSTP1 Ile105Val polymorphisms in patients with advanced NSCLC.

GSTM1 polymorphisms did not contribute to the overall survival of advanced NSCLC.

By stratification analysis, we found that the Ile/Val + Val/Val genotype of GSTP1 was associated with lower risk of death in never smokers, and the adjusted HR (95% CI) was 0.34 (0.12-0.93) (**Table 4**). However, no correlation was found between the GSTP1 polymorphism and alcohol drinking, histological types and TNM stage in the overall survival of advanced NSCLC.

Discussion

It is well known that genetic factors may contribute to the individualized response to chemotherapy. The GST super-family of enzymes belongs to the phase II group of enzymes, which are responsible for the metabolism of a wide range of xenobiotics and drugs including a variety of cytotoxic cancer chemotherapeutic agents [9]. Since the first evidence that glutathione S-transferases may play an important role in chemotherapy efficacy [10], the results of various subsequent studies have shown the inconsistent nature of this relationship. Although many clinicopathologic characteristics, such as patient age and clinical disease stage, have been identified as being influential factors in predicting the efficacy of chemotherapy, and accumulated evidences have reported that drug-metabolizing enzyme plays a critical role in determining inter-individual variations in therapeutic response [11, 12]. In the present study, we examine the association of GSTM1, GSTT1, and GSTP1 Ile105Val polymorphisms with the treatment response to chemotherapy and treatment outcome of advanced

Table 3. Association between GSTM1 null/present, GSTT1 null/present, and GSTP1 Ile105Val polymorphisms and overall survival of NSCLC

Genotypes	Patients	%	Death	%	Alive	%	HR (95% CI)[1]	*P* value
GSTM1								
Present	168	59.57	102	57.63	66	62.86	1.0 (Ref.)	-
Null	114	40.43	75	42.37	39	37.14	1.24 (0.74-2.11)	0.39
GSTT1								
Present	161	57.09	105	59.32	56	53.33	1.0 (Ref.)	-
Null	121	42.91	72	40.68	49	46.67	0.78 (0.47-1.31)	0.33
GSTP1								
Ile/Ile	120	42.55	85	48.02	35	33.33	1.0 (Ref.)	-
Ile/Val	89	31.56	52	29.38	37	35.24	0.58 (0.31-1.07)	0.06
Val/Val	74	26.24	40	22.60	34	32.38	0.48 (0.25-0.93)	0.02

[1]Adjusted for age, sex, tobacco smoking, alcohol drinking, family history of cancer in the first relatives and histological types.

Table 4. Association between GSTP1 null/present and overall survival of NSCLC stratified by clinical characteristics

Characteristics	Ile/Ile		Ile/Val + Val/Val		HR (95% CI)[1]	*P* value
	Death	Alive	Death	Alive		
Tobacco smoking						
Never	39	12	20	18	0.34 (0.12-0.93)	0.02
Ever	46	23	72	52	0.69 (0.36-1.33)	0.24
Alcohol drinking						
Never	58	21	69	51	0.49 (0.25-0.94)	0.02
Ever	27	14	23	19	0.63 (0.23-1.67)	0.30
Histological types						
Squamous Carcinoma	32	15	41	32	0.55 (0.24-1.23)	0.11
Adenocarcinoma	53	20	51	38	0.49 (0.28-1.42)	0.17
TNM stage						
IIIA and IIIB	68	23	72	41	0.55 (0.32-1.07)	0.08
IV	17	12	20	29	0.43 (0.16-1.17)	0.07

[1]Adjusted for age and sex.

NSCLC. We found that the Val/Val genotype of GSTP1 was associated with better response to chemotherapy and longer overall survival time when compared with the Ile/Ile genotype.

GSTP1 is a kind of phase II detoxification enzymes, which is involved in the detoxification of cisplatin [13]. Over expression of GSTP1 in NSCLC patient could decrease the sensitivity to platinum agents in vitro [3]. The polymorphism of GSTP1 Ile105Val changes the thermal stability and conjugation capacity of GSTP1, which alters the ability of GSTP1 to detoxify chemotherapeutic agents and modulates drug responses. Therefore, enzyme activity with the GSTP1 Ile105Val is more effective on cisplatin than that with wild-type in vitro [14]. However, it is a controversy that GSTP1 Ile105Val polymorphism would enhance clinical response of cisplatin-based chemotherapy [15-21]. Oliveira et al. conducted a study in a Chinese population, and they found that GSTP1 rs1695 polymorphisms were associated with poor response to chemotherapy and shorter overall survival of breast cancer [20]. Goričar et al. suggested that GSTP1 polymorphism was associated with shorter overall survival of osteosarcoma [21]. Han et al. have reported that GSTP1 polymorphism was collected with a longer median survival time when compared with the wide-type

genotype [17]. These inconsistency results might be due to differences in ethnicities, conditions of patients, sample size, and by chance.

Only two previous studies reported the association of GSTs gene polymorphisms with the efficacy of chemotherapy treatment and overall survival of NSCL [7, 8]. Ada et al. conducted a study in a Greek population, and they found that variants of GSTP1 were associated an improved survival in the patients with advanced NSCLC [7]. Viachogeorgos et al. conducted a study with 39 patients with NSCLC, and they found that GSTP1 expression was associated inversely with response to chemotherapy and the survival of patients with advanced NSCLC [8]. In our study, we found that the null genotype of GSTP1 were correlated with better response to chemotherapy and lower risk of death in advanced NSCLC patients, which were in line with previous results. Moreover, we found that the GSTP1 polymorphism has interaction with tobacco smoking in the treatment efficacy of advanced NSCLC. Previous studies have reported an interaction between GSTP1 polymorphism and smoking in the risk of several diseases [22, 23], which suggests that GSTP1 polymorphism could have interaction with tobacco smoking in the treatment outcome of chemotherapy in NSCLC.

There were two limitations in our study. First, there may be selection bias may overestimate the true size of effect or lead to spurious findings. Second, the sample size of patients with advanced NSCLC is relatively small, which may limit the statistical power to find difference between groups. Therefore, the actual biological mechanism of effects of GSTs polymorphisms on the treatment outcome of advanced NSCLC required further large-scale studies in different ethnic groups and functional experiments to replicate our findings.

In conclusion, we found that the GSTP1 polymorphism was correlated with better response to chemotherapy and lower risk of death in advanced NSCLC patients. Future studies with larger sample size are greatly needed to confirm the role of GSTs polymorphisms in the clinical outcome of NSCLC.

Disclosure of conflict of interest

None.

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