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ORIGINAL ARTICLE

Glutathione S-transferase P1 polymorphisms are associated with time to tumor progression in small cell lung cancer patients

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Summary

Purpose: *Many of commonly used chemotherapeutics in lung cancer treatment are metabolized by glutathione-S transferases (GSTs). The placental isoform of GST (GSTP1) is the most abundant isoform in the lung. Polymorphisms within the GSTP1 may result in alterations in enzyme activity and change sensitivity to platinum-based chemotherapy. We investigated whether the polymorphism within the exons 5 and 6 of GSTP1 gene may change response to therapy, time to tumor progression (TTP) and overall survival in small cell lung cancer (SCLC) patients.*

Methods: *Ninety-four histologically confirmed patients with SCLC were enrolled in this study during 1995-2006. GSTP1 Ile105Val polymorphism in exon 5 and GSTP1 Ala-114Val polymorphism in exon 6 were determined by using PCR-RFLP techniques. Associations between the GSTP1 polymorphisms and treatment response were evaluated using the chi-square test. Associations between the GSTP1 polymorphisms and TTP and overall survival were compared using Kaplan-Meier survival curves.*

Results: *We found no significant associations between exon 5 and exon 6 GSTP1 gene polymorphisms and response to therapy or overall survival. Patients carrying both variant exon 5 (Ile/Val or Val/Val) and variant exon 6 (Ala/Val) genotypes had significantly shorter TTP (5 vs. 8 months, p = 0.04). Moreover, patients with heterozygote exon 6 variant had presented with extensive-stage disease.*

Conclusion: *No individual effect of variant alleles was found in relation to chemotherapy response, median TTP and overall survival. The carriage of both types of variant alleles may predict worse outcome.*

Key words: Ala114Val, GSTP1 polymorphisms, Ile105Val, platinum based chemotherapy, small cell lung cancer, time to tumor progression

Introduction

Lung cancer is the leading cause of cancer-related mortality in many countries [1,2]. Approximately 15% of lung cancers are SCLC [3,4]. Cisplatin and etoposide combination has been the standard therapy since decades both for the limited and extensive stages [5,6].

Multiple genetic and environmental factors interact in the etiopathogenesis of various cancer subtypes. However, multiple protecting mechanisms consisting of detoxifying enzymes, antioxidants, and some barriers in the body, like skin, work against harmful substances, like different chemicals and overcome genetic susceptibility [7-9].

GSTs are a family of phase II metabolizing enzymes that catalyze the conjugation of reduced glutathione - via a sulfhydryl group - to electrophilic centers on a wide variety of substrates [10]. This conjugation results in detoxification of endogenous compounds such as peroxidized lipids, as well as breakdown of exogenous compounds such as cytotoxics, mutagens and carcinogens [11]. Among the various isoforms, GSTP1 is expressed more abundantly in alveoli, alveolar macrophages, and respiratory bronchioles. In addition to carcinogens, substrates for GSTP1 include a number of chemotherapeutic agents, among them cisplatin [12].

Rather than being present or absent, GSTP1 gene has alleles that encode enzymes with different activities.

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A polymorphism is a variation within a gene in which two or more alleles exist at a frequency of at least 1% in the general population. Genetic polymorphism has been identified in metabolic genes, and the biological consequence of such changes is an altered enzyme activity which may influence the ratio between activation and deactivation [13]. Activity of the GSTP1 is affected by substitution of isoleucine with valine (Ile105Val) in exon 5 and alanine with valine (Ala114Val) in exon 6 of this gene [14]. Variant alleles with decreased enzymatic activity were suggested to be accompanied with increased response to therapy and overall survival.

The aim of this study was to investigate whether the GSTP1 Ile105Val polymorphism in exon 5 and GSTP1 Ala114Val polymorphism in exon 6 can alter the chemotherapy response, median TTP or overall survival in SCLC patients.

Methods

Subjects

Ninety-four histologically confirmed consecutive SCLC patients who had provided informed consent were enrolled in this study during 1995-2006 at the Oncology Institute of Istanbul University. All patients were evaluated and staged at the first visit by medical history, physical examination including Eastern Cooperative Oncology Group (ECOG) performance status (PS), complete blood count, serum biochemistry analysis, chest X-ray and abdominal ultrasonography. Cranial computed tomography (CT) or magnetic resonance imaging (MRI) scans, thoracic and upper abdomen CT scans or abdominal MRI scans, and bone scans were also performed as indicated. Patients were treated according to their stage and PS. Limited-stage disease was defined as disease confined to the chest and encompassable in a radiation field. The definition included bilateral supraclavicular nodal disease, but excluded pleural fluid identified by chest X-ray or thoracic CT. Disease outside the limited area was defined as extensive-stage disease. Limited-stage patients were treated with chemotherapy and involved field radiotherapy, either concomitantly or sequentially. Prophylactic cranial irradiation was given to patients achieving complete remission. Extensive-stage patients received systemic chemotherapy for 4-6 cycles and palliative radiotherapy when needed. Standard combination chemotherapy with cisplatin 75 mg/m^2, day 1 and etoposide 120 mg/m^2, days 1-3 for 4-6 cycles was administered to all patients. The doses were reduced in patients receiving concomitant radiotherapy (cisplatin 60 mg/m^2, etoposide 100 mg/m^2). Response to treatment was assessed after 2-4 cycles of chemotherapy according to WHO response criteria. Overall survival was determined as the time elapsed between the time of histologic diagnosis and the date of death or the last follow-up visit. TTP progression was determined from the date of histologic diagnosis to the date of disease progression.

Patients and disease characteristics including age, ECOG PS, family history of cancer, stage, LDH levels, response to therapy, gender and smoking packet / year, TTP and overall survival were obtained from the patient records.

All patients gave written informed consent and the study was approved by the local ethics committee of Istanbul Medical Faculty.

DNA isolation and genotyping

Genomic DNA was extracted from whole blood using the QIAamp Blood Kit (QIAGEN Inc, California, USA). Two GSTP1 polymorphisms in exon 5 (Ile105Val) and exon 6 (Ala114Val) were characterized by the PCR and restriction fragment length polymorphism (RFLP) (12). Primer sequences used were as follows: for exon 5 (forward) 5'-GTAGTTTGCCCAAGGTCAAG-3', (reverse) 5'-AGCCACCTGAGGGGTAAG-3' and exon 6 (forward)5'-GGGAGCAAGCAGAGGAGAAT-3', (reverse)5'-GGTTGTAGTCAGCGAAGGAG-3'. The PCR products for exon 5 (Ile105Val) were digested for 2 units of *Alw26I* (Fermentas Inc, Vilnius, Lithuania). Exon 6 (Ala114V) PCR products were digested for 2 h at 37^o C with 5 units of *AciI* (New England BioLabs Inc, Massachusetts, USA). The fragments were visualized on a 3% agarose gel stained with ethidium bromide.

Statistical analysis

Frequency tables and statistical comparisons were calculated with SPSS 16 (SPSS Inc., USA). The differences of the distributions of GSTP1 Ile105Val polymorphism in exon 5 and GSTP1 Ala114Val polymorphism in exon 6 were compared with chi-square test. The effect of GSTP1 Ile105Val polymorphism in exon 5 and GSTP1 Ala114Val polymorphism in exon 6 on survival were calculated with Kaplan-Meier survival plots. Log-rank test was used for the survival differences between the groups. A level of $p < 0.05$ was considered as statistically significant.

Results

The study population consisted of 13 female and 81

male patients. Patient ethnicity was not recorded because the vast majority of patients were Turkish descent. Genotyping for GSTP1 exon 5 and exon 6 were successful for 90 patients and 84 patients, respectively. The distribution of genotypes was not related to age, gender, smoking status, LDH, PS, family history or response to therapy.

There was a significant association between exon 6 genotype and stage at diagnosis: patients with heterozygote exon 6 variant had extensive-stage disease (p=0.03). Due to the small number of variant type exon 6 patients (n=8), the importance of this observation remains to be clarified. Because there were no homozygous patients with variant exon 6 allele and only 6 patients with mutant type exon 5, these groups were combined with heterozygous groups of each exons for all future analyses.

The blood sample and data collections at baseline were performed carefully, however during the follow up period, information about response to therapy (n=1), TTP (n=11) or overall survival (n=6) could not be completely recorded in all patients. Of 94 patients, 60 had shown response to treatment (complete response + partial response) and 33 no response (stable disease+progression). Median follow up of the study population was 13 months (range 1-170).

Overall median survival was 13 months (range 1-122) and median TTP 7 months (range 1-48). The Kaplan-Meier survival functions for overall survival and TTP according to GSTP1 genotypes, LDH, age, smoking, PS, family history and response to therapy are presented in Tables 1 and 2. Overall survival between different genotypes was similar (p >0.05). Figure 1 shows

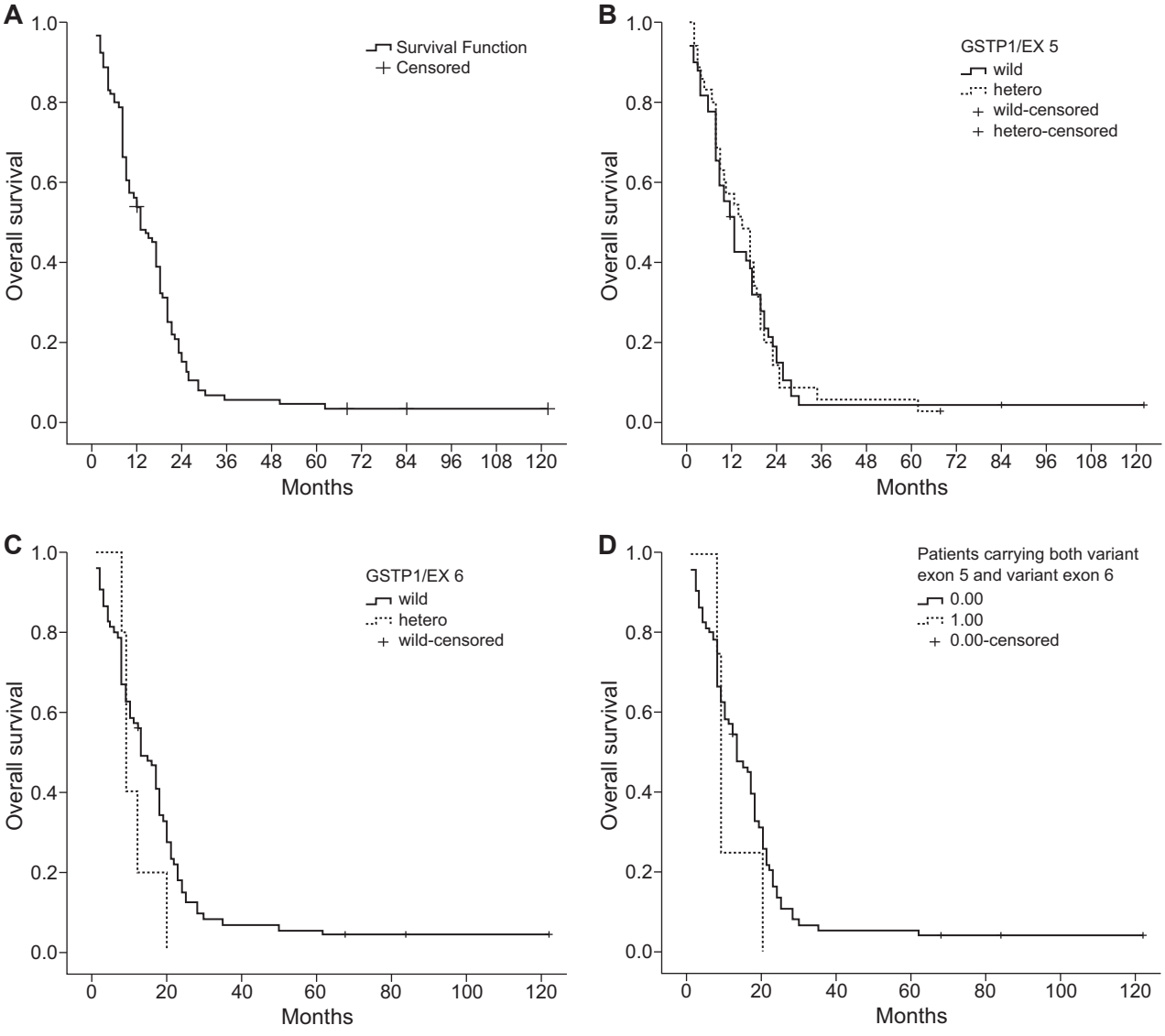

Figure 1. Survival of patients. **A:** Overall survival of all patients; **B:** Overall survival of patients carrying wild type GSTP1 exon 5 and variant type GSTP1 exon 5; **C:** Overall survival of patients carrying wild type GSTP1 exon 6 and variant type GSTP1 exon 6; **D:** Overall survival of patients carrying both variant type GSTP1 exon 5 and variant type GSTP1 exon 6, and others.

overall survival of all patients, each exons and patients carrying both variant alleles. Only 3 of 94 patients were alive during the follow up period and both exons 5 and 6 were wild in these patients.

Although the sample size was small, median TTP was significantly shorter in patients carrying both exon 5 and 6 variant alleles compared with the others (p=0.04) (Table 1, Figure 2). Overall survival tended to be shorter in the same group (10 vs. 21 months; Table 2), but did not reach statistical significance (p>0.05). Patients carrying wild type of exon 6 tended to have longer median TTP (p=0.09).

Table 1. Univariate survival analysis of potential covariates (median time to tumor progression)

Covariates	*No. of patients*	*Median TTP (mos)*	*p-value*
LDH (U)			0.005
≤450	49	8	
>450	31	5	
Age (years)			0.55
>50	76	7	
≤49	10	9	
Smoking (pack-years)			0.55
>45	45	7	
≤44	35	8	
Response to therapy			<0.001
Responders (CR+PR)	52	10	
Nonresponders (SD+PD)	30	4	
Stage			<0.001
Limited	46	10	
Extensive	38	4	
ECOG performance status			0.07
0-1	68	8	
2-4	15	3	
Genotype			
homozygous wild type (ile/ile)	47	8	0.15
heterozygous (ile/val) + mutant (val/val)	36	7	
homozygous wild type (Ala/ala)	72	8	0.09
heterozygous (Ala/val)	7	5	
any heterozygous			0.71
positive	36	16	
others	44	24	
both homozygous			0.65
wild	38	8	
others	35	7	
both heterozygous and/or mutant	6	5	0.04
others	72	8	

CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, TTP: time to progression, ECOG: Eastern Cooperative Oncology Group, mos: months

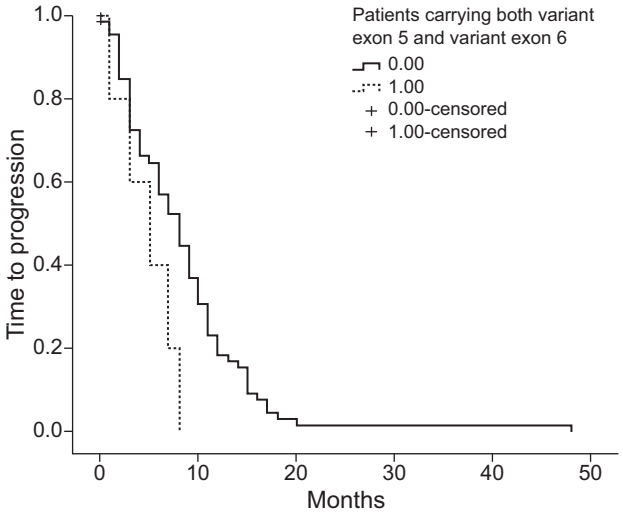

Figure 2. Median time to tumor progression is shorter for patients carrying both exon 5 and exon 6 variant alleles.

Table 2. Univariate median survival analysis of potential covariates

Covariates	*No. of patients*	*Median survival (mos)*	*p-value*
LDH (U)			0.001
≤450	50	16	
>450	32	8	
Age (years)			<0.001
>50	77	13	
≤49	11	23	
Smoking (pack - years)			0.13
>45	45	12	
≤44	37	17	
Response to therapy			<0.001
Responders (CR+PR)	58	26	
Nonresponders (SD+PD)	31	10	
Stage			<0.001
Limited	46	18	
Extensive	40	8	
ECOG performance status			0.002
0-1	67	14	
2-4	18	8	
Genotype			
homozygous wild type (ile/ile)	50	13	0.68
heterozygous (ile/val) + mutant (val/val)	35	15	
Homozygous wild type (Ala/Ala)	76	13	0.27
Heterozygous (Ala/Val)	5	9	
Any heterozygous positive	35	8	0.65
others	38	8	
Both homozygous wild	44	13	0.78
others	34	15	
Both heterozygous and/or mutant	6	10	0.19
others	76	21	

For abbreviations see footnote of Table 1

Discussion

The combination of cisplatin and etoposide is the standard chemotherapy regimen for patients with SCLC [6]. Epipodophylotoxins interact by poisoning Top2 isozymes, they are not DNA intercalators, whereas platinum compounds induce their cell killing effects through the development of covalent bifunctional DNA adducts with cellular DNA.

Altered localization of Top2α decreases cellular expression of Top2α and impairs phosphorylation of Top2, which results in resistance to etoposide [15], whereas on the subcellular level there are more than 10 putative mechanisms through which cells may become sensitive or resistant to platinum compounds [16]. Cytosolic inactivation of the drug, which occurs at high levels of resistance, is mediated through the glutathione detoxification pathways and metallothioneins [17-19]. GSTP1 Ile105Val and GSTP1 Ala 114Val are two SNPs in the GSTP1 gene. They lead to an amino acid substitution in the enzyme's electrophile-binding site and are known to change the affinity and activity of GSTP1 for electrophilic substances [14]. Better response to therapy or increased survival are expected in patients carrying these variant alleles [20]. However, our patients with variant allele did not show improved response to therapy or overall survival ($p > 0.05$). Some recent studies which included non small cell lung cancer patients treated with platin-containing combination chemotherapy could not demonstrate any relationship between variant alleles and response to therapy, median TTP and overall survival [21]. Moreover, our patients carrying both types of variant alleles had shorter median TTP (5 vs. 8 months) and 3 patients who are still alive carried wild types of both exons.

The results of studies which show decreased enzymatic activity of GSTP1 in different types of carcinoma cells are conflicting [21,22]. It is expected that absence or decreased GSTP1 activity may lead to increased effectiveness of some chemotherapeutics and to decrease the defensive oxidative mechanisms of host cells and genome. For example, human prostate cancer cells devoid of GSTP1 are especially vulnerable to genome damage mediated by N-OH-PhIP, the charred meat carcinogen that causes prostate cancer when fed to rats, and by exposure to oxidant stress [23]. Furthermore, Martens et al. reported that GSTP1 occurs in normal cervical epithelium and in all stages of premalignant cervix, whereas the nucleus of the majority of cervical carcinoma cells stain weakly [24]. They suggested that absence or decreased GSTP1 activity in carcinoma cells may indicate that xenobiotic compounds are not catabolized and may therefore exert their mutagenic activity, resulting in tumor progression. In our study, patients carrying both exons 5 and 6 variants could be supposed to have decreased enzymatic activity which resulted in shorter TTP.

We could not demonstrate any gender or age difference between allele types because most of our patients were male and older than 50 years. Previous studies showed increased risk of lung carcinoma associated with the exon 6 variant allele that was especially evident in males and younger individuals [25], while our patients with variant exon 6 allele presented with extensive-stage disease and patients with wild type exon 6 had longer TTP. The variant type exon 6 may predict a worse prognosis. Although a very small percentage of our study group (9%) had the exon 6 variant allele, 75% of them had also the exon 5 variant allele.

To our knowledge, this is the first study in which GSTP exons 5 and 6 polymorphism was investigated in SCLC patients and evaluated response to therapy, TTP and overall survival in variant and wild type carriers. We found no difference in overall survival, TTP and response to therapy between the wild types and variant types of alleles. Since many GST genes regulate the production of the enzyme, a single abnormality - such as polymorphism of GSTP1 - may not be adequate to reduce the level of GST activity level, as the GST enzyme superfamily consists of many enzymes and several others are responsible for plasma GST activity.

Conclusion

Our results show that carrying both types of variant alleles may predict worse prognosis with shorter TTP. They may also indicate inadequate enzyme activity to detoxify ongoing carcinogenic assaults. Larger sample sized studies which could measure the activity of wild and variant types of alleles should be performed to confirm these results and to clarify the prognostic and predictive values of these findings. Because most of the SCLC patients respond well to chemotherapy but have short survival, it is hard to find out any prognostic or predictive value of any type of genetic polymorphism in SCLC. In the future, with better therapies, the importance of these polymorphisms could be established.

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