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Effect of variation of ABCB1 and GSTP1 on osteosarcoma survival after chemotherapy

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ABSTRACT. We conducted a comprehensive study to investigate the role of genes involved in metabolic and transport pathways in response to chemotherapy and clinical outcome of osteosarcoma patients. Genotyping of seven gene polymorphisms was performed on a 384-well plate format on the Sequenom MassARRAY platform in 162 patients with osteosarcoma. We studied the correlation of the seven gene polymorphisms with response to chemotherapy and clinical outcome of patients. Individuals with the *ABCB1 TT* genotype had a higher probability of responding poorly to chemotherapy, indicated by an odds ratio (OR) of 2.64 (95%CI = 1.04-6.83). Similarly, the genotype of *GSTP1 GG* was significantly associated with improved responses to chemotherapy, indicated by an OR of 3.33 (95%CI = 1.26-8.99). The *ABCB1 TT* and *GSTP1 GG* genotypes were significantly associated with a shorter overall survival (OS). Our study found that two gene polymorphisms in two transporter genes and one Phase II metabolism enzymes are associated with response to chemotherapy and

OS in osteosarcoma patients, suggesting the potential of the two gene polymorphisms as prognostic biomarkers for osteosarcoma.

Key words: ATP-binding cassette; Glutathione S-transferases; Osteosarcoma; Clinical outcome

INTRODUCTION

Osteosarcoma is the most common malignant sarcoma tumor and a leading cause of death from cancer in children and adolescents (Ottaviani and Jaffe, 2009). Standard treatment of osteosarcoma involves neoadjuvant therapy before surgical resection of the primary tumor, followed by chemotherapy after operation (Longhi et al., 2006). The main chemotherapy drugs for osteosarcoma include methotrexate, cisplatin, cyclophosphamide, vincristine, and doxorubicin. Despite this, about 30% of these osteosarcoma patients show recurrence or metastasis during a five-year period (Ottaviani and Jaffe, 2009).

Individualized chemotherapy according to biomarkers may improve the response to chemotherapy and clinical outcome of patients. Therefore, better understanding of the role of pharmacogenetics could help establish an individualized chemotherapy, where patients would benefit more from chemotherapy to prolong their life. Genes which influence the clinical response to chemotherapeutics could control drug absorption, distribution, metabolism, and excretion. Most of these metabolisms are influenced by cytochrome P450 enzymes (Redlich et al., 2008). Many chemotherapeutic agents are metabolized by glutathione S-transferases (GSTs), which catalyze the conjugation of glutathione to a wide variety of xenobiotics (Hayes and Pulford, 1995). ATP-binding cassette, ABC proteins, are one main types of transport superfamilies and responsible for the majority of drug transport (Zhou et al., 2008). However, the genetic polymorphisms of these drug metabolism and transport genes may influence interindividual variability in the plasma concentration of chemotherapeutic drugs.

Our previous pharmacogenetic studies have shown that the polymorphism of nucleotide excision DNA repair pathway correlates with response to chemotherapy and clinical outcome of osteosarcoma (Hao et al., 2012). The genetic variation may affect the global response to treatment or cause adverse drug events. Therefore, we conducted a comprehensive study to investigate the role of four genes involved in metabolic and transport pathways in response to chemotherapy and clinical outcome of osteosarcoma patients.

MATERIAL AND METHODS

Patients, treatments and clinical variables

A total of 162 consecutive patients diagnosed with osteosarcoma at Juye County People's Hospital and The Third People's Hospital of Heze from January 2008 to July 2009 were collected in our study. Clinical data recorded at study entry included age at diagnosis. Blood samples were provided by all patients, and written informed consent was obtained from patients or their relatives. Our study was approved by the ethics committee of Juye County People's Hospital and The Third People's Hospital of Heze.

Patients were treated preoperatively with intravenous 25-30 mg/m^2 doxorubicin for three courses and one day, 14 mg/m^2 methotrexate for four courses and one day, and intra-arte-

rial 35 mg/m^2 cisplatin for three courses and three days. However, the adjuvant chemotherapy after surgery included 10 mg/m^2 methotrexate for one day, and alternate cycles of 0.45 mg/m^2 cisplatin or actinomycin D and 1.5 mg/m^2 vincristine for one day. The adjuvant chemotherapy was used for at most 48 weeks. If patients showed non-hematology toxicity higher than grade three, or showed febrile neutropenia and thrombocytopenia with bleeding, the dose of the chemotherapy drug was reduced by 25%.

The treatment response was determined by the extent of tumor necrosis. Patients with less than 90% necrosis were classified as poor responders and those with 90% necrosis or more, as good responders (Bacci et al., 2003). Our primary end point was overall survival (OS), calculated as the time from diagnosis until death from any cause or last known date alive. All the patients were followed up to death or the end of study (December 2012).

Genotyping

A sample of 5 mL venous blood was drawn from all patients, and was kept at -20°C. Genomic DNA was extracted using the TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Genotyping of ABCB1 1236C>T, GSTP1 313A>G, GSTT1, and GSTM1 was performed on a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA). Primers for polymerase chain reaction amplification and single base extension assays were designed using the Sequenom Assay Design 3.1 software (Sequenom®) according to manufacturer instructions. PCR was carried out in a reaction volume of 20 μL, containing 50 ng genomic DNA, 200 μM dNTP, 2.5 U Taq DNA polymerase (Promega Corporation, Madison, WI, USA), and 200 μM primers. PCR conditions were as follows: 94°C for 2 min, 35 cycles of 94°C for 30 s, an annealing temperature reduced to 64°C for 30 s and 72°C for 1 min. The PCR products were analyzed by 1.0% agarose gel electrophoresis. For quality control, genotyping was performed without knowledge of the case/control status of the subjects and 5% of the total number of case and control patients were selected at random and re-genotyped by different investigators; the reproducibility was 100%.

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences software 13.0 for Windows. Correlation between polymorphisms in ABCB1 1236C>T, GSTP1 313A>G, GSTT1, and GSTM1 and response to chemotherapy were assessed using odds ratios (OR) [95% confident interval (CI)] with logistic regression analysis by comparing genotype frequencies in good and poor responders. The homozygote for the most frequent allele was used as the reference group. The association between variants of ABCB1 1236C>T, GSTP1 313A>G, GSTT1, and GSTM1 genotypes and OS was assessed by Cox proportional hazards model with hazard ratios (HR) and their CI. OS curves were plotted using the Kaplan-Meier method. All P values were two-tailed, and a difference was considered to be statistically significant when $P < 0.05$.

RESULTS

The main clinical and pathological characteristics of 162 patients are presented in

Table 1. Sixty-three patients (38.9%) died during the follow-up period. The median age of patients was 15.7 years and ranged from 9.1 to 48.6 years, and 92 (56.7%) of the patients were males. At the time of diagnosis, 23 (14.3%) of the patients already presented with metastasis. The percentage of good responders to therapy was 54.2%, and poor responders made up 45.8%. The median follow-up time was 33.6 months.

Table 1. Clinical and pathological characteristics of the study.

	Patients (N = 162)	(%)
Age at diagnosis (years)		
Median (range)	15.7 (9.1-48.6)	
Gender		
Male	92	56.7
Female	70	43.3
Tumor location		
VFemur	82	50.6
Tibia/fibula	52	32.2
Arm	14	8.6
Central	14	8.6
Histological response		
Good	88	54.2
Poor	74	45.8
Metastasis at diagnosis		
No	139	85.7
Yes	23	14.3

Our results indicated a significant effect of ABCB1 1236C>T and GSTP1 313A>G polymorphisms on responses to chemotherapy ($P < 0.05$) (Table 2). Individuals with the ABCB1 TT genotype were more likely to have a poor response to chemotherapy, with an OR of 2.64 (95%CI = 1.04-6.83). Similarly, individuals with the GSTP1 GG genotype showed a significantly poorer response to chemotherapy (OR = 3.33; 95%CI = 1.26-8.99). However, we did not find any association of GSTT1 or GSTM1 with responses to chemotherapy.

Table 2. Correlation of four single nucleotide polymorphisms with tumor response.

Genotype		Patients	Tumor response				OR (95%CI)	P value
			Poor	%	Good	%		
ABCB1 1236C>T	CC	68	25	33.8	43	48.9	-	-
	CT	61	29	39.2	32	36.4	1.43 (0.67-3.06)	0.32
	TT	33	20	27.0	13	14.8	2.64 (1.04-6.83)	0.02
GSTP1 313A>G	AA	68	24	32.4	44	50.0	-	-
	AG	63	30	40.5	33	37.5	1.67 (0.78-3.57)	0.15
	GG	31	20	27.0	11	12.5	3.33 (1.26-8.99)	0.006
GSTT1	Present	72	32	43.5	40	45.4	-	-
	Null	90	42	56.5	48	54.6	1.09 (0.56-2.14)	0.78
GSTM1	Present	100	45	60.7	55	62.7	-	-
	Null	62	29	39.3	33	37.3	1.07 (0.54-2.13)	0.82

Results from the analysis of OS are presented in Table 3. We identified two genes that were associated with the OS of osteosarcoma patients. The ABCB1 T/T genotype was significantly associated with shorter OS (Figure 1) with HR (95%CI) of 3.17 (1.14-6.67). Moreover, GSTP1 GG genotype was significantly correlated with reduced OS (Figure 2), with HR (95%CI) of 3.86 (1.41-10.2).

Table 3. Cox regression analysis of four gene polymorphisms with overall survival (OS).

Genotype		Patients	Events		OS	
					HR (95%CI)	P value
ABCB1 1236C>T	CC	68	23	36.5	-	-
	CT	61	24	38.3	1.27 (0.63-2.77)	0.42
	TT	33	16	25.2	3.17 (1.14-6.67)	0.01
GSTP1 313A>G	AA	68	19	30.2	-	-
	AG	63	26	41.1	1.84 (0.85-4.16)	0.12
	GG	31	18	28.7	3.86 (1.41-10.2)	0.004
GSTT1	Present	72	27	42.8	-	-
	Null	90	36	57.2	1.26 (0.63-2.42)	0.66
GSTM1	Present	100	37	59.1	-	-
	Null	62	26	40.9	1.45 (0.75-2.71)	0.40

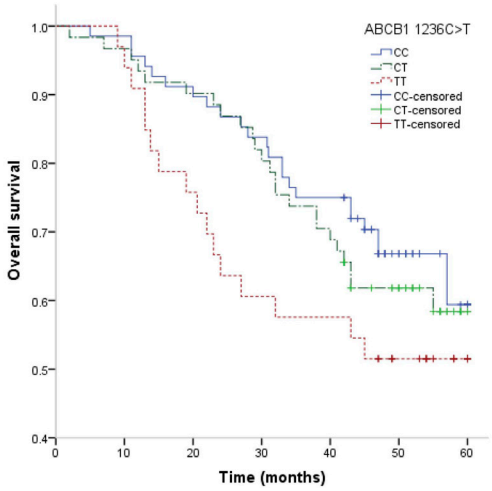

Figure 1. Kaplan-Meier survival curves for osteosarcoma patients for ABCB1 1236C>T.

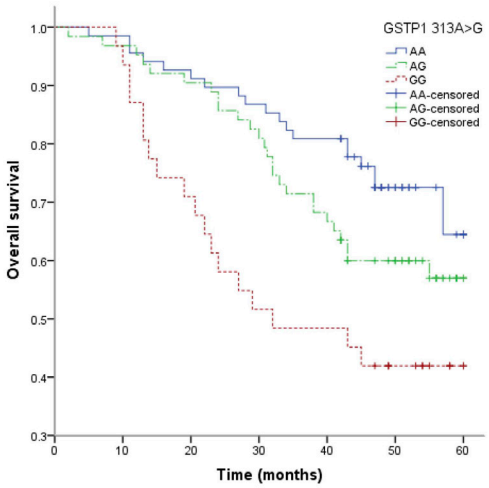

Figure 2. Kaplan-Meier survival curves for osteosarcoma patients for GSTP1 313A>G.

DISCUSSION

This study assessed the most comprehensive pharmacogenetic SNPs of GSTs and ATP-binding cassette, which involved in cisplatin, doxorubicin, methotrexate, vincristine, and cyclophosphamide pathways in osteosarcoma patients receiving chemotherapy. Our study suggested that polymorphisms of ABCB1 1236C>T and GSTP1 313A>G had a higher probability of negative response to chemotherapy when compared with wild-type genotype, and these three gene polymorphisms were associated with a reduced OS of patients.

ABCB1 (P-glycoprotein, multidrug resistance 1) is a transmembrane protein that acts as an energy-dependent drug efflux pump for chemotherapeutic drugs, including platinum-based chemotherapy drugs (Clarke et al., 2005). Polymorphisms of ABCB1 may increase the efflux of chemotherapeutic agents from tumor cells or increase their elimination from the body, reduce the plasma concentrations, and thus influence their therapeutic efficacy. Previous studies have indicated that polymorphisms of ABCB1 are associated with response to chemotherapy in various cancers, such as breast cancer and ovarian cancer (Caronia et al., 2011; Ehrlichova et al., 2013; Lévy et al., 2013). A study conducted in the Czech Republic reported that a low expression of ABCB1 was found in ovarian cancer tissues when compared with normal tissues, and that ABCB1 could be used as a surrogate marker of ovarian cancer progression and associated with therapy outcome (Ehrlichova et al., 2013). Another study in 101 breast cancer patients indicated that ABCB1 C3435T polymorphism are involved in the response to neoadjuvant chemotherapy, and that the variant of this gene could be used to optimize individualized therapy (Lévy et al., 2013).

In our study, we found that polymorphism of ABCB1 was associated with poor response to chemotherapy and shorter survival time. Our study is in line with previous studies on osteosarcoma (Caronia et al., 2011). A recent study conducted in China reported that three SNPs of ABCB1 (rs4148737, rs128503 and rs10276036) were significantly correlated with tumor response and OS (Caronia et al., 2011). Another study conducted in China reported ABCB1 examined GSTP1 and response to chemotherapy among osteosarcoma and soft tissue sarcoma patients, and found that its polymorphism was related to poor prognosis (Wei et al., 2006). However, the results are inconsistent. A study conducted in the UK indicated that ABCB1 polymorphism was not associated with response to chemotherapy but with toxicity (Windsor et al., 2012). The inconsistency of these results may be caused by different ethnicity, sample size and change.

Our study reported an effective role of GSTP1 in clinical outcome of osteosarcoma patients, which was in line with previous studies (Pasello et al., 2008; Yang et al., 2012; Zhang et al., 2012). Our study suggests that genetic variation of ABCB1 and GSTP1 could play a critical role in the effectiveness of treatment and overall cancer survival.

In conclusion, our study found that ABCB1 1236C>T and GSTP1 313A>G polymorphisms in two transporter genes and one phase II metabolism enzymes are associated with response to chemotherapy and OS in osteosarcoma patients. Our study suggests that ABCB1 1236C>T and GSTP1 313A>G may be potential prognostic biomarkers for osteosarcoma, which could help in the design of individualized therapy.

REFERENCES

Bacci G, Bertoni F, Longhi A, Ferrari S, et al. (2003). Neoadjuvant chemotherapy for high-grade central osteosarcoma of the extremity. Histologic response to preoperative chemotherapy correlates with histologic subtype of the tumor. *Cancer* 97: 3068-3075.

Caronia D, Patiño-Garcia A, Peréz-Martínez A, Pita G, et al. (2011). Effect of ABCB1 and ABCC3 polymorphisms on osteosarcoma survival after chemotherapy: a pharmacogenetic study. *PLoS One* 6: e26091.

Clarke R, Leonessa F and Trock B (2005). Multidrug resistance/P-glycoprotein and breast cancer: review and meta-analysis. *Semin. Oncol.* 32: S9-15.

Ehrlichova M, Mohelnikova-Duchonova B, Hrdy J, Brynychova V, et al. (2013). The association of taxane resistance genes with the clinical course of ovarian carcinoma. *Genomics* 102: 96-101.

Hao T, Feng W, Zhang J, Sun YJ, et al. (2012). Association of four ERCC1 and ERCC2 SNPs with survival of bone tumour patients. *Asian Pac. J. Cancer Prev.* 13: 3821-3824.

Hayes JD and Pulford DJ (1995). The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* 30: 445-600.

Lévy P, Gligorov J, Antoine M, Rezai K, et al. (2013). Influence of ABCB1 polymorphisms and docetaxel pharmacokinetics on pathological response to neoadjuvant chemotherapy in breast cancer patients. *Breast Cancer Res. Treat.* 139: 421-428.

Longhi A, Errani C, De Paolis M, Mercuri M, et al. (2006). Primary bone osteosarcoma in the pediatric age: state of the art. *Cancer Treat. Rev.* 32: 423-436.

Ottaviani G and Jaffe N (2009). The epidemiology of osteosarcoma. *Cancer Treat. Res.* 152: 3-13.

Pasello M, Michelacci F, Scionti I, Hattinger CM, et al. (2008). Overcoming glutathione S-transferase P1-related cisplatin resistance in osteosarcoma. *Cancer Res.* 68: 6661-6668.

Redlich G, Zanger UM, Riedmaier S, Bache N, et al. (2008). Distinction between human cytochrome P450 (CYP) isoforms and identification of new phosphorylation sites by mass spectrometry. *J. Proteome Res.* 7: 4678-4688.

Wei L, Song XR, Wang XW, Li M, et al. (2006). Expression of MDR1 and GST-pi in osteosarcoma and soft tissue sarcoma and their correlation with chemotherapy resistance. *Zhonghua Zhong. Liu Za Zhi.* 28: 445-448.

Windsor RE, Strauss SJ, Kallis C, Wood NE, et al. (2012). Germline genetic polymorphisms may influence chemotherapy response and disease outcome in osteosarcoma: a pilot study. *Cancer* 118: 1856-1867.

Yang LM, Li XH and Bao CF (2012). Glutathione S-transferase P1 and DNA polymorphisms influence response to chemotherapy and prognosis of bone tumors. *Asian Pac. J. Cancer Prev.* 13: 5883-5886.

Zhang SL, Mao NF, Sun JY, Shi ZC, et al. (2012). Predictive potential of glutathione S-transferase polymorphisms for prognosis of osteosarcoma patients on chemotherapy. *Asian Pac. J. Cancer Prev.* 13: 2705-2709.

Zhou SF, Di YM, Chan E, Du YM, et al. (2008). Clinical pharmacogenetics and potential application in personalized medicine. *Curr. Drug Metab.* 9: 738-784.