RESEARCH ARTICLE

Effect of Variation of ABCB1 and ABCC3 Genotypes on the Survival of Bone Tumor Cases after Chemotherapy

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Abstract

We conducted a comprehensive study to investigate the role of genes involved in transport pathways in response to chemotherapy and clinical outcome of osteosarcoma cases. Genotyping of six SNPs was performed in a 384-well plate format on the Sequenom MassARRAY platform for 208 osteosarcoma patients to reveal any correlations of the six SNPs with response to chemotherapy and clinical outcome. Individuals with the ABCB1 rs1128503 TT and ABCC3 rs4148416 TT genotypes had a higher probability of responding poorly to chemotherapy, indicated by odds ratios (ORs) of 2.46 (95%CI, 1.21-5.74) and 3.78 (95% CI, 1.20-13.85), respectively. Moreover, the ABCB1 rs1128503 TT and ABCC3 rs4148416 TT genotypes were significantly associated with shorter disease-free survival (DFS) and overall survival (OS). Our study found the two SNPs in two transporter genes and one phase II metabolism enzyme to be associated with response to chemotherapy and overall survival in osteosarcoma patients, suggesting potential prognostic biomarker applications of the two SNPs.

Keywords: APT-binding cassette - osteosarcoma - clinical outcome

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Introduction

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

Individualized chemotherapy by biomarkers may improve the response to chemotherapy and clinical outcome of patients. Therefore, better understanding the role of pharmacogenetics could help establishing an individualized chemotherapy and patients benefit more from chemotherapy to prolong their life. Genes which influence the clinical response to chemotherapeutics could control drug absorption, distribution, metabolism and excretion. APT-binding cassette, ABC proteins, are one main type of transport suferfamilies and responsible for majority of drug transport (Zhou et al., 2008). However, the genetic polymorphisms of these drugs metabolized and transport genes may influence the interindividual variability in the plasma and concentration of chemotherapeutic drugs.

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

Materials and Methods

Patients, treatments and clinical variables

208 consecutive patients diagnosed with osteosarcoma were collected at Department of Pediatric Orthopedics of Shanghai Children's Medical Center of Shanghai Jiaotong University between May 2008 and November 2009. Clinical data recorded at study entry included age at diagnosis. All the blood samples were provided by all patients, and written informed consents were gained from patients or their relatives. Our study was approved by the ethics committee of Shanghai Children's Medical Center of Shanghai Jiaotong University.

Patients were treated preoperatively with intravenous 25-30 mg/m^2 adriamycin for three courses and one day, 14 mg/m^2 methotrexate for four courses and one day, and intra-arterial 35 mg/m^2 cisplatin for three courses and three days. However, the adjuvant chemotherapy after surgery

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Table 1. Clinical and Pathological Characteristics of Included Study

Age at diagnosis, y	Patients, N	%
Total number of patients	208	
Median (range)	16.2 (9.4-47.5)	
Sex		
Male	120	57.7
Female	88	42.3
Tumor location		
Femur	109	52.4
Tibia/fibula	65	31.2
Arm	15	7.3
Central	19	9.1
Histological response		
Good	112	53.8
Poor	96	46.2
Metastasis at diagnosis		
No	180	86.5
Yes	28	13.5
Relapse		
No	153	73.6
Yes	55	26.4

Table 2. Correlation of Six SNPs Polymorphisms with Tumor Response

Genotype		Patients	Poor tumor response	Good tumor response	OR(95% CI)	*P* value
ABCB1	CC	84	32	52	-	-
rs1128503	CT	80	38	42	1.51(0.65-2.97)	0.22
	TT	44	26	18	2.46(1.21-5.74)	0.02
ABCB1	CC	96	43	53	-	-
rs1045642	CT	82	38	44	1.12(0.62-2.14)	0.82
	TT	30	15	15	1.30(0.51-3.14)	0.62
ABCG2	CC	150	68	82	-	-
rs2231142	CA	39	19	20	1.17(0.57-2.56)	0.71
	AA	19	9	10	1.13(0.41-3.27)	0.86
ABCC1	AA	134	60	74	-	-
rs246240	AG	42	20	22	1.16(0.54-2.41)	0.72
	GG	32	16	16	1.27(0.54-2.89)	0.56
ABCC2	CC	140	64	76	-	-
rs717620	CT	38	18	20	1.09(0.51-2.42)	0.83
	TT	30	14	16	1.05(0.45-2.57)	0.92
ABCC3	CC	163	67	95	-	-
rs4148416	CT	28	16	12	1.92(0.81-4.72)	0.12
	TT	17	13	5	3.78(1.20-13.85)	0.01

included 10 g/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 presented non-hematology toxicity higher than grade three, or showed febrile neutropenia and thrombocytopenia with bleeding, the dosage of chemotherapy drug would be 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. Disease-free survival (DFS) following treatment was calculated from the initiation of therapy to first recorded date of progression, death, or last follow-up evaluation. All the patients were followed up to death or the end of study (May 2012).

SNP genotyping

5 ml venous blood was drawn from all patients, and was kept in -20°C. Genomic DNA was extracted using the TIANamp blood DNA kit (Tiangen Biotech, Beijing, China) with centrifuging for 3 min at 13.400 x g (12.000 rpm). Genotyping of ABCB1 rs1128503, ABCB1 rs1045642, ABCG2 rs2231142, ABCC1 rs246240, ABCC2 rs717620 and ABCC3 rs4148416 was performed in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers for polymerase chain reaction amplification and single base extension assays were designed using Sequenom Assay Design 3.1 software (Sequenom®) according to the manufacturer's instructions. PCR was carried out in a reaction volume of 20 μl, containing 50 ng of genomic DNA, 200 μM dNTP, 2.5 units of Taq DNA polymerase (Promega Corporation, Madison, WI, USA) and 200 μM of primers. The conditions of the PCR were as follows: 94˚C for 2 min, 35 cycles of 94˚C for 30 sec, an annealing temperature reduced to 64˚C for 30 sec and 72˚C for 1 min. The PCR products were analyzed using electrophoresis on 1.0% agarose gel. 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 was 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 (SPSS) software 13.0 for windows. Correlation between polymorphisms in ABCB1 rs1128503, ABCB1 rs1045642, ABCG2 rs2231142, ABCC1 rs246240, ABCC2 rs717620 and ABCC3 rs4148416 and response to chemotherapy were assessed using odds ratios (95% confident interval) with logistic regression analysis by comparing genotype frequencies in good and poor responders. Homozygote for the most frequent allele was used as the reference group. The association between variants of ABCB1 rs1128503, ABCB1 rs1045642, ABCG2 rs2231142, ABCC1 rs246240, ABCC2 rs717620 and ABCC3 rs4148416 genotypes and DFS and OS was assessed by Cox proportional hazards model with hazard ratios (HR) and their confidence intervals (CI). OS curves were plotted using the Kaplan-Meier method. The All P values were two-tailed, and difference was considered statistically significant when a value of $P<0.05$.

Results

The main clinical and pathological characteristics of 208 patients are presented in Table 1. 95 patients (45.7%) died during the follow-up period. The median age of patients was 16.2 years and ranged from 9.4 to 47.5 years, and 120 (57.7%) of the patients were males. At the time of diagnosis, 28 (13.5%) of the patients already presented metastasis, while 59(28.4%) patients developed metastasis

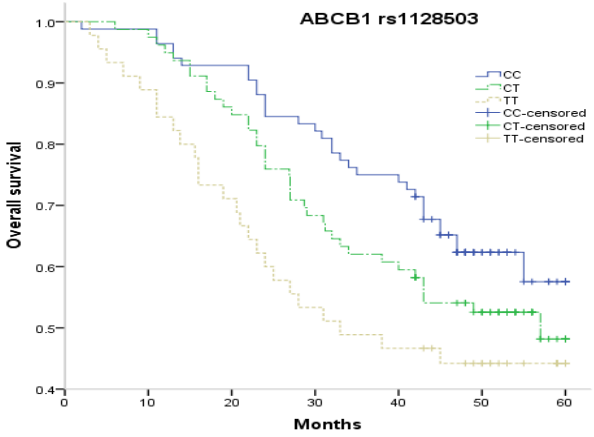

Figure 1. Kaplan-Meier Survival Curves for Osteosarcoma Patients for ABCB1 rs1128503

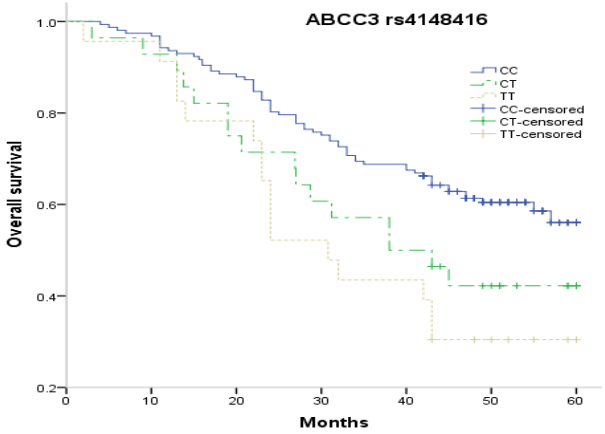

Figure 2. Kaplan-Meier Survival Curves for Osteosarcoma Patients for ABCC3 rs4148416

Table 3. Cox Regression Analysis of Polymorphisms with DFS and OS

Genotype		Patients	DFS		OS	
			HR(95% CI)	*P* value	HR(95% CI)	*P* value
ABCB1	CC	84	-	-	-	-
rs1128503	CT	80	1.86(0.82-4.07)	0.17	1.33(0.50-2.74)	0.51
	TT	44	3.74(1.63-7.40)	0.003	2.25(1.15-4.80)	0.03
ABCC3	CC	163	-	-	-	-
rs4148416	CT	28	2.05(0.83-4.92)	0.08	1.75(0.76-3.20)	0.22
	TT	17	4.32(1.75-15.65)	0.006	3.46(1.15-10.43)	0.02

during follow-up. The percentage of good responders to therapy was 53.8% (112 patients), and poor responders were 46.2% (96 patients). The median follow-up time was 38.5 months (range 4 to 60 months).

Our analysis detected a significant effect of ABCB1 rs1128503 and ABCC3 rs4148416 polymorphisms on responses to chemotherapy in Table 2 ($P < 0.05$): Individuals with the ABCB1 rs1128503 TT genotype were more likely to have a poor response to chemotherapy, with an OR of 2.46 (95%CI, 1.21-5.74). Similarly, individuals with the ABCC3 rs4148416 TT genotype showed a significantly poorer response to chemotherapy (OR, 3.78; 95%CI, 1.20-13.85). However, we did not find any association of ABCB1 rs1045642, ABCG2 rs2231142, ABCC1 rs246240 and ABCC2 rs717620 with responses to chemotherapy.

Results from the analysis of DFS and OS are presented in Table 3. We identified two genes that were associated with the DFS and OS of osteosarcoma. The ABCB1 rs1128503 T/T genotype was significantly associated with shorter DFS and OS (Figure 1) with hazard ratios (HRs) (95% CI) of 3.74(1.63-7.40) and 2.25(1.15-4.80), respectively. The ABCC3 rs4148416 TT genotype was more likely to decrease the DFS and OS among NSCLC patients receiving platinum-based chemotherapy (Figure 2) with HRs of 4.32 (95%CI, 1.75-15.65) and 3.46 (95%CI, 1.15-10.43), respectively.

Discussion

This study assessed the most comprehensive pharmacogenetic SNPs of APT-binding cassette which involved in platinum, adriamycin, methotrexate, vincristine and cyclophosphamide pathways in the osteosarcoma patients receiving chemotherapy. Our study suggested that polymorphisms of ABCB1 rs1128503 and ABCC3 rs4148416 had a higher probability to negative respond to chemotherapy when compared with wild-type genotype, and these two genes' polymorphisms have been associated with a reduced overall survival of patients.

ABCB1 (P-glycoprotein, multidrug resistance 1) is a transmembrane protein, which acts as an energy-dependent drug efflux pump for chemotherapeutic drugs, including platinum-based chemotherapy drugs (Clarke et al., 2005). ABCB1 eliminates the parent drug through hepatobiliary and intestinal secretion, and previous experimental studies have suggested that ABCB1 has a role in the proliferation and survival of epithelial cells and malignant cells during tumorigenesis, such as non-small cell lung cancer, intestinal cancer and gastric cancer (Tahara et al., 2007). More than 50 SNPs have been reported in ABCB1 gene, and some variants affect the expression and function of P-glycoprotein (P-gp) (Fromm, 2002; Salama et al., 2006). 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.

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

ABCC3 is a member of the multidrug resistance protein family, and showed expression in liver, gallbladder,

kidney and etc. (Borst et al., 2000; Rost et al., 2001). Previous experimental study has indicated that ABCC3 polymorphisms have function of transporting anticancer drugs, such as methotrexate (Zeng et al., 2000; Zelcer et al., 2001). Several chemotherapy drugs are suggested to be the substrates of ABCC3, such as vincristine, doxorubicin and cisplatin (Zeng et al., 2000; Zelcer et al., 2001). Previous studies have reported that expression of ABCC3 mRNA has been associated with drug resistance in various cancers (Partanen et al., 2012; Cheng et al., 2013; Tran, 2013). However, to the best of our knowledge, few studies reported the association between the genetic ABCC3 variants and clinical outcome of osteosarcoma patients. Only one study conducted in Spain has reported that ABCC3 rs4148616 variants were associated with an eight-fold risk of shorter survival time (Caronia et al., 2011). In our study, we also reported a significant association between polymorphisms in ABCC3 rs4148416 and prognosis of osteosarcoma patients.

In conclusion, our study found that two SNPs in two transporters genes are associated with response to chemotherapy and overall survival in osteosarcoma patients. Our study suggests that the two SNPs may be a potential prognostic biomarker for osteosarcoma, which could help in the design of individualized therapy.

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