RESEARCH ARTICLE

Glutathione S-transferase P1 and DNA Polymorphisms with the Response to Chemotherapy and the Prognosis of Bone Tumor

Li-Min Yang[1], Xiu-Hua Li[1]*, Cui-Fen Bao[2]

Abstract

Osteosarcoma is the most common primary bone malignancy in children and adolescents, and its clinical outcome is poor. We evaluated the response of GSTP1, ERCC1 and ERCC2 to chemotherapy among osteosarcoma patients, and the role of these genes on the prognosis of osteosarcoma. 187 patients with osteosarcoma were administered with methotrexate, cisplatin/adriamycin, actinomycin D, cyclophosphamide, or vincristine treatment. GSTP1, ERCC1 and ERCC2 polymorphism was genotyped by PCR-RFLP assay. The results showed the average survival time of 187 patients were 38.4 months. 97 patients showed response to neoadjuvant chemotherapy. The GSTP1 Val and ERCC2 A/A genotypes had significantly higher rates of response to chemotherapy, with adjusted OR (95% CI) of 2.19 (1.15-6.21) and 2.88 (1.14-13.25). Individuals with ERCC2 A/A genotype were likely to have a lower risk of death from oseosarcoma, and the adjusted HR was 0.32 (0.13-0.95). Our study indicated test of GSTP1 and ERCC2 Lys751Gln polymorphisms might be a candidate pharmacogenomic factors to be explored in the future to identify the osteosarcoma patients who might benefit from chemotherapy.

Keywords: GSTP1 - DNA repaird gene - chemotherapy - polymorphisms - response

Asian Pacific J Cancer Prev, **13 (11)**, 5883-5886

Introduction

Osteosarcoma derives from primitive bone-forming mesenchymal cells and is the most common primary bone malignancy in children and adolescents. The risk of being diagnosed with cancer increases as an individual ages, and 77% of all cancers are diagnosed in persons aged 55 years and above. As a lifetime risk, the probability that an individual, over the course of a lifetime, will develop a cancer is slightly less than one in two for men and a little more than one in three for women (US Cancer Statistics Working Group, 2007; Ries et al., 2009). The etiology of OS still remains unknown, and the progression might be influenced by the genetic factors (Fuchs et al., 2001). Standard treatment of osteosarcoma is based on a combination of different drugs: neoadjuvant therapy with methotrexate, cisplatin, and adriamycin followed by surgery and post-operative chemotherapy (methotrexate, cisplatin, adriamycin, cyclophospharmide, and vinciristine). Nevertheless, multi-drug resistance and poor clinical outcome are the main problems of 50% osteosarcoma patients.

Clinical response to chemotherapeutics is a complex trait that is influenced by genetic and environmental factors. Anticancer therapies have a narrow therapeutic range so that a higher concentration in patient's body causes toxicity and a lower concentration reduces the efficacy of the drug. Interindividual differences in pharmacokinetics and pharmacodynamics determine the global response and toxicity profile of each drug. In this process, the genes involved are the ones that control drug absorption, distribution, metabolism, and excretion. Our previous study indicated ERCC1 and ERCC2 might have a role in the prognosis of bone tumor (Hao et al., 2012). Glutathione S-transferases (GSTs) are a family of cytosolic enzymes involved in the detoxification of various exogenous as well as endogenous reactive species (Ketterer, 1998; Hengstler et al., 1998). GSTs function as dimers by catalyzing the conjugation of mutagenic electrophilic substrates to glutathione. The coding region polymorphisms within GSTP1 have been suggested to confer different catalytic activities (Zimniak et al., 2002). The effects of these polymorphisms on drug metabolism, including chemotherapeutic agents, make these genes candidates for investigation of toxicity and resistance mechanisms (Wang et al., 2002).

Pharmacogenetic studies have shown that germline polymorphisms in genes related to drug metabolism and transport can have a major effect on the pharmacokinetics and pharmacodynamics of these drugs (Zhou et al., 2008). We previously performed a study of the nucleotide excision DNA repair pathway in relation to response to cisplatin and observed an association between osteosarcoma outcome and a polymorphism in ERCC1 and ERCC2 (Hao

*[1]Department of Orthopaedics, The First Affiliated Hospital of Liaoning Medical College, [2]Central Laboratory of Liaoning Medical University, Jinzhou, China *For correspondence: qiangwangw_2001@163.com*

Table 1. The Primer Sequences of GSTM1, GSTT1, GSTP1, ERCC1 and ERCC2 Polymorphisms

Gene	Forward primer	Reverse primer
GSTM1	5'- GAACTCCCTGAA AAGCTA AAG C -3'	5'- CTTGGGCTCAAATATACGGTGG -3'
GSTT1	5'- CTTCCTTACTGGTCCTCACATCTC -3'	5'- TCACCGGATCAT GGCCAGCA -3'
GSTP1 I105V	5'-ACCCCAGGGCTCTATGGGAA-3'	5'-TGAGGGCACAAGAAGCCCCT-3'
ERCC1 C118T	5'-GCCCCGTCCCAGGTA-3'	5'-AGCCCCAAGACCCTTTCACT-3'
ERCC2 Lys751Gln	5'-TTGTGCTTTCTCTGTGTCCA-3'	5'-TCCTCCAGCCTTTTCTGATA-3'

et al., 2012). In this study we studies a comprehensive set of three SNPs and identified the prognostic genetic factors for the chemotherapeutic agents, and their association with drug response.

Materials and Methods

187 patients diagnosed with osteosarcoma were collected at department of traumatic orthopedics of Zhengzhou hospital, 91 Central Hospital of PLA, Children Hospital of Zhengzhou and the Second Affiliated Hospital of Inner Mongolia Medical University between January 2005 and January 2007. All the included cases in our study were histologically confirmed. All interviews and blood samples collection were conducted after obtaining signed informed consent from participants. Patients were treated with neoadjuvant chemotherapy based on doxorubicin, methotrexate, cisplatin and ifosfamide before and after surgery.

Patients were subsequently grouped as responders (complete+partial response) or nonresponders (stable+progressive disease). Cases with secondary or recurrent tumors were excluded. All patients were followed up till January 2012.

DNA extraction and quantification

The DNA samples were obtained from peripheral blood lymphocytes by using the Qiagen Blood Kit (Qiagen, Chastworth, CA). Genotyping for GSTP1, ERCC1 and ERCC2 polymorphisms by PCR-RFLP assay was done following a modified method of Ateş et al (Ateş et al., 2005). The primer sequences of GSTP1, ERCC1 and ERCC2 polymorphisms were showed in Table 1. Polymerase chain reaction was started at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 61°C for 30 s, and 72°C for 30 s, and a final elongation step at 72°C for 7 min. We also performed the genotyping of internal positive control samples, use of no template controls, and use of replicates for 10% samples for quality control. These results of the quality control analysis confirmed 100% concordance.

Statistical analysis

The SPSS software package version 16.0 (SPSS Inc. Chicago, USA) was used for statistical analysis. Association between the various parameters was assessed by using Chi-squared test or Fisher's exact test. The association of polymorphisms of genotypes with response to chemotherapy was calculated by odds ratios (OR) with a corresponding 95% confidence interval (CI). The overall survival curves were plotted using the Kaplan-Meier product limit method, and the statistical differences in survival among subgroups were compared by log-rank test. The correlations between different genotypes of GSTP1, ERCC1 and ERCC2 and response were analyzed by using multiple logistic analyses. The independent prognostic values of different polymorphism including GSTP1, ERCC1 and ERCC2 their association with survival of bone cancer were analyzed by Cox Hazard regression model. Cox hazard ratio and its 95% confidence interval (CI) were analyzed accordingly. P-value less than 0.05 was considered statistically significant.

Table 2. Clinical Characteristics of Osteosarcoma Patients with Adjuvant Chemotherapy (N=187)

		Patients No=187	%
Sex	Female	81	43.32
	Male	106	56.68
Age	<10	10	5.35
	10~14	48	25.67
	15-30	77	41.18
	>30	52	27.81
Subtype	Osteoblastic	102	55.08
	Chondroblastic	34	18.18
	Other	50	26.74
Tumor size	<50	77	41.18
	≥50	110	58.82
Anatomic location	Tibia/femur	119	63.64
	Elsewhere	68	36.36
Metastasis	No	97	51.87
	At diagnosis	38	20.32
	At follow-up	52	27.81
Surgical stage	IIA	77	41.18
	IIB-III	110	58.82

Results

The clinical features of 187 osteosarcoma patients were showed in Table 2. The median age at diagnosis is 17.7±9.6 years (range: 7-39 years old). Most of the patients were less than 30 years, and more than 50% of the patients were at the range of 10-30 years old. The diagnosis was established with histological examination in all cases. Among 187 patients, most of them were males (56.68%). 55.08% of the osteosarcoma were osteoblastic, and 18.18% of them were chondroblastic. The remainders were regarded as other type. When the cases included in our study, 20.32% of the patients were metastasis, whereas 27.81% of them had developed metastasis since January 2012.

Among 187 patients, 97 patients showed response to neoadjuvant chemotherapy. Among 97 responders, 17.53% took GSTP1 Val/Val genotype, 12.37% took ERCC1 T/T genotype and 9.28% took ERCC2 A/A genotype (Table 3). GSTP1 Val/Val genotype had significantly higher rates of response to chemotherapy when compared to GSTP1 Ile/ Ile genotype, and the adjusted OR (95% CI)

Table 3. Distribution of Polymorphisms in GSTM1, GSTT1, GSTP1, ERCC1 and ERCC2 Genes and Response to Neoadjuvant Chemotherapy

Genotype		Responders, N=97	%	Nonresponders, N=90	%	Odds ratio (95% CI)[1]	Odds ratio (95% CI)[2]
GSTM1	Non-null	48	49.48	47	52.22	1.0 (Ref.)	-
	Null	49	50.52	43	47.78	1.12(0.60-2.06)	1.23(0.67-2.98)
GSTT1	Non-null	35	36.08	34	37.78	1.0 (Ref.)	-
	Null	62	63.92	56	62.22	1.07(0.57-2.03)	1.43(0.61-2.51)
GSTP1 I105V	IIe/IIe	54	55.67	56	62.22	1.0 (Ref.)	-
	IIe/Val	26	26.8	23	25.56	1.17(0.57-2.43)	1.32(0.68-2.76)
	Val/Val	17	17.53	11	12.22	1.60 (0.84-4.15)	2.19(1.15-6.21)
ERCC1 C118T	C/C	47	48.45	46	51.11	1.0 (Ref.)	-
	C/T	38	39.18	35	38.89	1.06(0.55-2.05)	1.34(0.64-2.45)
	T/T	12	12.37	9	10	1.30(0.45-3.86)	1.65(0.65-4.13)
ERCC2 Lys751Gln	G/G	54	55.67	55	61.11	1.0 (Ref.)	-
	G/A	35	36.08	32	35.56	1.11(0.57-2.14)	1.34(0.75-2.78)
	A/A	9	9.28	4	4.44	2.29 (0.61-10.83)	2.88(1.14-13.25)

[1]Non-adjusted; [2]Adjusted for sex, age, subtype, location, metastasis, tumor size and anatomic location

Table 4. Associations of GSTM1, GSTT1, GSTP1, ERCC1 and ERCC2 Gene Polymorphisms and Survival of Osteosarcoma

Genotypes		Cases N=187	%	Patient deaths N=79	%	Median overall survival (months)	HR (95% CI)[1]	P
GSTM1	Non-null	95	50.8	42	53.26	37.63	1.0 (Ref.)	-
	Null	92	49.2	37	46.74	39.25	0.91(0.51-1.77)	0.74
GSTT1	Non-null	69	36.9	32	40.45	37.44	1.0 (Ref.)	-
	Null	118	63.1	47	59.55	39.52	0.92(0.57-1.76)	0.75
GSTP1 I105V	IIe/IIe	110	58.82	51	64.29	35.31	1.0 (Ref.)	-
	IIe/Val	49	26.2	19	24.5	37.21	0.85 (0.51-1.42)	0.54
	Val/Val	28	14.97	9	11.21	40.84	0.53 (0.24-1.16)	0.32
ERCC1 C118T	C/C	93	49.73	42	53.79	36.26	1.0 (Ref.)	-
	C/T	73	39.04	30	37.65	37.55	0.81(0.40-1.33)	0.7
	T/T	21	11.23	7	8.56	39.39	0.74 (0.28-1.94)	0.61
ERCC2 Lys751Gln	G/G	107	58.29	50	63.05	34.9	1.0 (Ref.)	-
	G/A	67	35.83	26	33.5	38.22	0.73(0.40-1.12)	0.35
	A/A	13	6.95	3	3.45	41.53	0.32 (0.13-0.95)	<0.05

[1]Adjusted for sex, age, subtype, location, metastasis, tumor size and anatomic location

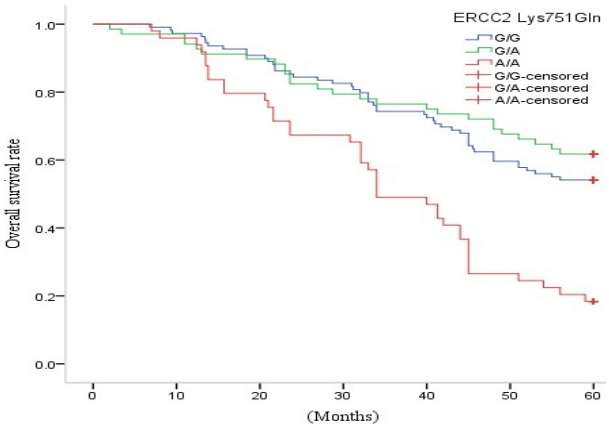

Figure 1. Kaplan-Meier Curves for Relationship Between ERCC2 Lys751Gln and Overall Survival of Osteosarcoma Patients with Neoadjuvant Chemotherapy

of 2.19(1.15-6.21). ERCC2 A/A genotype also presented a higher rate of response to chemotherapy (OR=2.88, 95% CI=1.14-13.25). However, we did not find a significant risk of response to neoadjuvant chemotherapy with other genotypes among osteosarcoma patients.

The average survival time of 187 patients were 38.4 months. The association between GSTP1, ERCC1 and ERCC2 gene polymorphisms with prognosis of osteosarcoma with neoadjuvant chemotherapy was showed in table 4. Polymorphisms in GSTP1 Val/Val and ERCC1 T/T had a higher survival rate than non-null genotype, whereas no significant association was found between these two genotypes and prognosis of osteosarcoma with neoadjuvant chemotherapy(For GSTP1 Val/Val, HR=0.53, 95%CI: 0.24-1.16; for ERCC1 T/T, HR=0.74, 95%CI: 0.28-1.94). Individuals with ERCC2 A/A genotype were likely to have a lower risk of death from oseosarcoma, and they were tended to live longer than ERCC2 G/G genotype (Figure 1). A significantly adjusted Hazard ratio was found between the ERCC2 A/A and prognosis of osteosarcoma (adjusted HR=0.32, 95% CI=0.13-0.95).

Discussion

In the present study, we provide evidence that glutathione S-transferases and DNA repaired gene are involved in response to adjuvant chemotherapy in Chinese osteosarcoma patients. Our results showed a significant relationship with response to chemotherapy among individuals with GSTP1 Val/Val and ERCC2 A/A genotypes. Results of various subsequent studies have shown that consistent of nature of this relationship (Riddick et al., 2005). A similar association is found in

previous studies (Pasello et al., 2008; Pasello et al., 2011; Windsor et al., 2012; Zhang et al., 2012). Zhang et al., in 2012, studies 159 patients with osteosarcoma treated with neoadjuvant chemotherapy and did not find any association of increased risk of death from osteosarcoma among patients with GSTP1 Val/Val genotype (Zhang et al., 2012). However, another two studies conducted in Italy reported GSTP1 Val/Val was associated with the outcome of osteosarcoma, and GSTP1 could be considered a promising new therapeutic possibility for osteosarcoma patients (Pasello et al., 2008; Pasello et al., 2011). The inconsistencey of these results may be explained by differences in different ethnicities, source of control, sample size and etc. Further confirmation of these results is needed in future studies.

Numbers of studies have suggested an important role for drug-metabolizing enzymes in determining interindividual variations in therapeutic response to chemotherapy among cancer patients. GSTs make up a family of multifunctional enzymes that detoxify a variety of eletrophilic compounds. The explanation of polymorphisms in GSTP1 gene on the efficacy of detoxifying cytotoxins generated by chemotherapeutics might be impairment of the GSTP1 capacity caused by the A→G substitution, patients with the Val variant allele may be less capable of detoxifying oxaliplatin compared to patients with wide-type allele. Our study indicated GSTP1 Val/Val has forable overall survival, which is agreement with previous reports in various cancers, including breast, colorectal (Stoehlmacher et al., 2002; Kweekel et al., 2008). ERCC1 and ERCC2 are potentially relevant to cancer because of their involvement in the process of nucleotide expcision repair (NER) (Goode et al., 2002), and the NER acts a role in repairmen of DNA damaged by environment and UV damage, and the polymorphisms in the two DNA repared genes may change the function of NER in repairing DNA damage by chemotherapy. Our study indicated ERCC2 A/A genotype also presented a higher rate of response to chemotherapy, and ERCC2 A/A was significantly associated with higher risk of death from osteosarcoma. Previous studies showed ERCC2 was associated with response to chemotherapy among osteosarcoma (Caronia et al., 2009), and survival of this cancer (Hao et al., 2012). Our results here are in line with these previous studies.

We found polymorphisms in GSTP1 and ERCC2 could modify the chemotherapy of patients with osteosarcoma, and they also have an important role in their prognosis. However, there was no significant association of ERCC1 C118T with osteosarcoma. We conclude that the test of GSTP1 and ERCC2 Lys751Gln polymorphisms might be a candidate pharmacogenomic factors to be explored in the future to identify the osteosarcoma patients who might benefit from chemotherapy.

Acknowledgements

This research is supported by the Liaoning Education Fund (L2010338).

References

Caronia D, Patiño-García A, Milne RL, et al (2009). Common variations in ERCC2 are associated with response to cisplatin chemotherapy and clinical outcome in osteosarcoma patients. *Pharmacogenomics J*, **9**, 347-53.

Fuchs B, Zhang K, Schabel A, et al (2001). Identification of twenty-two candidate markers for human osteogenic sarcoma. *Gene*, **278**, 245-52.

Goode EL, Ulrich CM, Potter JD (2002). Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol. *Biomarkers Prev*, **11**, 1513-30.

Hao T, Feng W, Zhang J, Sun YJ, Wang G (2012). Association of Four ERCC1 and ERCC2 SNPs with Survival of Bone Tumour Patients. *Asian Pac J Cancer Prev*, **13**, 3821-4.

Hengstler JG, Arand M, Herrero ME, et al (1998). Polymorphisms of N-acetyltransferases, glutathione S-transferases, microsomal epoxide hydrolase, and sulfotransferases: influence on cancer susceptibility. *Recent Results Cancer Res*, **154**, 47-85.

Ketterer B (1988). The protective role of glutathione transferases in mutagenesis and carcinogenesis. *Mutat Res*, **202**, 343-61.

Kweekel DM, Gelderblom H, Antonini NF, et al (2009). Glutathione-S-transferase pi (GSTP1) codon 105 polymorphism is not associated with oxaliplatin efficacy or toxicity in advanced colorectal cancer patients. *Eur J Cancer*, **45**, 572-8.

Mirabello L, Troisi RJ, Savage SA (2009). International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. *Int J Cancer*, **125**, 229-234.

Pasello M, Manara MC, Michelacci F, et al (2011). Targeting glutathione-S transferase enzymes in musculoskeletal sarcomas: a promising therapeutic strategy. *Anal Cell Pathol (Amst)*, **34**, 131-45.

Pasello M, Michelacci F, Scionti I, et al (2008). Overcoming glutathione S-transferase P1-related cisplatin resistance in osteosarcoma. *Cancer Res*, **68**, 6661-8.

Riddick DS, Lee C, Ramji S, et al (2005). Cancer chemotherapy and drug metabolism. *Drug Metab Dispos*, **33**, 1083-96.

Stoehlmacher J, Park DJ, Zhang W, et al (2002). Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. *J Natl Cancer Inst*, **94**, 936-42.

U.S. Cancer Statistics Working Group (2009). United States Cancer Statistics: 2004 Incidence and Mortality. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Cancer Institute. Available at: http://www.cdc. gov/cancer/npcr/npcrpdfs/US_Cancer_Statistics_2004_Incidence_and_Mortality.pdf. Oct. 2011.

Windsor RE, Strauss SJ, Kallis C, et al (2012). Germline genetic polymorphisms may influence chemotherapy response and disease outcome in osteosarcoma: a pilot study. *Cancer*, **118**, 1856-67.

Wang X, Zuckerman B, Pearson C, et al (2002). Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *JAMA*, **287**, 195-202.

Zhang SL, Mao NF, Sun JY, Shi ZC, Wang B, Sun YJ (2012). Predictive potential of glutathione S-transferase polymorphisms for prognosis of osteosarcoma patients on chemotherapy. *Asian Pac J Cancer Prev*, **13**, 2705-9.

Zimniak P, Nanduri B, Pikula S, et al (1994). Naturally occurring human glutathione S-transferase GSTP-1 isoforms with isoleucin and valine in position 104 differ in enzymic properties. *Eur J Biochem*, **224**, 893-9.