RESEARCH COMMUNICATION

Predictive Potential of Glutathione S-Transferase Polymorphisms for Prognosis of Osteosarcoma Patients on Chemotherapy

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Abstract

Objective: To evaluate the predictive value of glutathione S-transferase (GST) gene polymorphisms for the prognosis of osteosarcoma patients receiving chemotherapy. Methods: A total of 159 patients were included in our study between January 2005 and December 2007., with follow-up until January 2012. Genotyping was based upon the duplex polymerase-chain-reaction with the PCR-CTPP method. Results: At the time of diagnosis, 15.4% of the patients presented with metastasis, while 22.3% developed metastasis during follow-up. At the time of final analysis on January 2012, the median follow-up was 45.5 months. Patients with null GSTM1 and GSTT1 had a higher event free survival rate than non-null genotype, but no significant association was found between the two genotypes and prognosis of osteosarcoma. Individuals with GSTP1 Val/Val genotype tended to live shorter than with the IIe/IIe genotype, and we found a significantly higher risk of death from osteosarcoma (adjusted HR=2.35, 95% CI=1.13-4.85). Conclusion: The GSTP1 gene polymorphism may have an important role in the prognosis of osteosarcoma patients with chemotherapy. Further analyses with larger samples and more genes encoding metabolizing and DNA repair enzymes are warranted.

Keywords: GSTs - polymorphisms - osteosarcoma - predictive role

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Introduction

Osteosarcoma derives from primitive bone-forming mesenchymal cells and is the most common primary bone malignancy, and which is the most common malignant bone tumor 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 (ACS, 2007; 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). The administration of preoperative chemotherapy with multiple chemotherapeutic agents including cisplatin, doxorubicin, carboplatin and ifosfamide could improve the prognosis of osteosarcoma (Rosen et al., 1976). Nevertheless, multi-drug resistance and poor clinical outcome are the main problems of 50% osteosarcoma patients, and the prognosis of osteosarcoma depends on clinical-, genetic- and treatment- related factors (Sakkadech et al., 2011). Identified the prognostic genetic factors for the chemotherapeutic agents could improve targeted therapy for patients at different risk.

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. In humans, 4 major subfamilies of GSTs can be distinguished and are designated as GSTa, GSTμ, GSTu, and GSTp (Mannervik et al., 1992). Each of these subfamilies is composed of several members, some of which display genetic polymorphism. Within the GSTμ subfamily, the gene coding for GSTM1 exhibits a deletion polymorphism, which in case of homozygozity (GSTM1 null) leads to absence of phenotypic enzyme activity (Seidegard et al., 1988).4 A similar mechanism is described for GSTT1 within the GSTu subfamily (Pemble et al., 1994), whereas the gene coding for GSTP1, a member of the GSTp subfamily, displays polymorphisms within its coding region at codon 105 (Ile105Val) and codon 114 (Ala114Val) (Board et al., 1989; Ali-Osman et al., 1997; Lo et al., 1997; Harries et al., 1997; Watson et al., 1998). 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

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and resistance mechanisms (Wang et al., 2002).

GSTM1, GSTT1, and GSTP1 genotype status have been reported to associated with various malignancies such as smoking-induced lung cancer, baldder, breast, or gastrointestinal cancer (Seidegard et al., 1990; Strange et al., 1991; Nazar-Stewart et al., 1993; Bell et al., 1993; Ambrosone et al., 1995; Deakin et al., 1996; Kelsey et al., 1997; Helzlsouer et al., 1998). Our previous study showed the GSTM1 and GSTT1 are associated with the risk of osteosarcoma (Lu et al., 2011). The GSTM1, GSTT1, and GSTP1 gene polymorphism were reported to confer resistance to cytotoxic chemotherapeutic agents used to treat cancer. GST genotypes conferring lower enzyme activity may be of advantage for individuals undergoing chemotherapeutic treatment for neoplastic disease because reduced detoxification potentially enhances effectiveness of cytotoxic drugs. Anticancer drugs that have been shown to be substrates for GSTs are, for example, chlorambucil, melphalan, cyclophosphamide metabolites, and steroids (Yuan et al., 1991; Listowsky, 1993; Tew, 1994). Indirect evidence for a role of GSTs in modulating drug effects through deactivation of drug-generated hydroperoxides or other reactive oxygene species exists for adriamycin, mitomycin C, carboplatin, and cisplatin (Black et al., 1990; Nakagawa et al., 1990; Tew, 1994).

Despite the fact that GSTs gene polymorphism have been widely examined and related to the survival of several cancer, their role in osteosarcoma survival in Chinese population has not been established. Therefore, we conducted this prospective study in an Chinese population to detect the association between the GSTs gene polymorphisms and survival of osteosarcoma.

Materials and Methods

A perspective study was undertaken in our study. A total of 159 patients included in this study were newly diagnosed osteosarcoma between Jan. 2005 to Dec. 2007 in our hospitals. All the included cases in our study were histologically confirmed. All the cases received chemotherapy including cisplatin, doxorubicin, carboplatin and ifosfamide. All the patients were followed up until January 2012. All interviews and blood samples collection were conducted after obtaining signed informed consent from participants.

Genotyping

Genomic DNA was extracted from whole-blood samples using the Qiagen Blood Kit (Qiagen, Chastworth, CA). Genotyping was conducted using TaqMan assays (Applied Biosystems, Foster City, CA). Primer and probe sets were designed and manufactured using Applied Biosystems 'Assay-by-Design' custom service (Applera, Austria). General TaqMan reaction conditions were as described previously (Salinas-Souza et al., 2010). 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.

Statistical analysis

Follow-up began on the first day of participating. The overall survival was the time from study entry until death regardless of cause. All statistical tests are two sided. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software 13.0 for windows. The main statistical methods used are Kaplan-Meier method, and the Cox Hazard regression model. Two of the censored times in the Kaplan-Meier plots presented are caused by patients being disease recurrence, development of lung or bone metastases, death from any cause or lost to follow-up. The main outcome variable analyzed is the presence of polymorphisms of GSTs in the prognosis of osteosarcoma. The active genotype of GSTs was taken as reference group. Therefore, in the Cox regression model, we divided patients into different groups according to a specific gene polymorphism. Similarly, in the Kaplan-Meier analysis, gene-by-gene comparisons can be made.

Table 1. Clinical Characteristics of Osteosarcoma Patients (N=159)

		No	Patients %
Mean age		14.7±9.6	
Sex	Female	68	42.7
	Male	91	57.3
Subtype	Osteoblastic	91	57.3
	Chondroblastic	34	21.5
	Other	34	21.2
Location	Femur	76	47.7
	Tibia	58	36.4
	Arm	11	7.1
	Central	14	8.8
Necrosis	Good	87	54.7
	Poor	72	45.3
Metastasis	No	99	62.3
	At diagnosis	24	15.4
	At follow-up	35	22.3
Status	Alive	104	65.4
	Dead	55	34.6

Table 2. Associations Between Polymorphisms in GSTs Genes and the Risk of Osteosarcoma

Genotype	Cases, N (%)	Events, N (%)	Event-free survival rate (%)	Survival, HR (95%) Unadjusted	Adjusted[1]
GSTM1					
Non-null	67(42.3)	39(51.4)	75.8	-	-
Null	92(57.7)	36(48.6)	77.1	0.68(0.37-1.21)	0.62(0.30-1.14)
GSTT1					
Non-null	54(34.1)	31(41.3)	80.5	-	-
Null	105(65.9)	44(58.7)	72.3	0.73(0.41-1.35)	0.70(0.39-1.33)
GSTP1					
IIe/IIe	93(58.7)	33(52.4)	75.3	-	-
IIe/Val	40(25.2)	23(26.5)	87.5	1.62 (0.80-3.25)	1.93(0.98-3.45)
Val/Val	26(16.1)	19(21.1)	90	2.06(0.94-4.45)	2.35(1.13-4.85)

[1]Adjusted for sex, age, subtype, location and necrosis

Results

The clinical features of 159 osteosarcoma patients are summarized in Table 1. The median age at diagnosis is 14.7±9.6 years (range 7 to 39 years). About 57.3% of the patients are males. Most of the osteosarcoma are osteoblastic, however, 21.5% of them are chondroblastic. At the time of diagnosis, 15.4% of the patients presented metastasis, while 22.3% developed metastasis during follow-up. At the time of final analysis on January 2012,

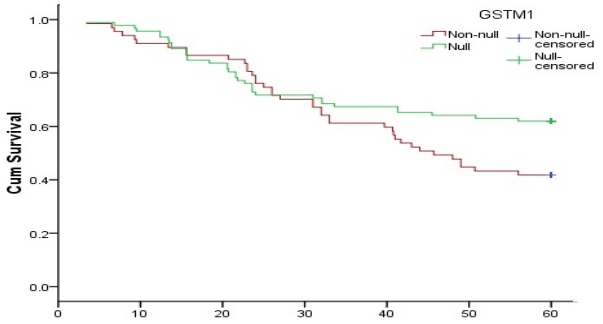

Figure 1. Kaplan-Meier Curves for Event-free Survival of Osteosarcoma Patients Treated with Chemotherapy. Analysis for GSTM1 Gene Polymorphism

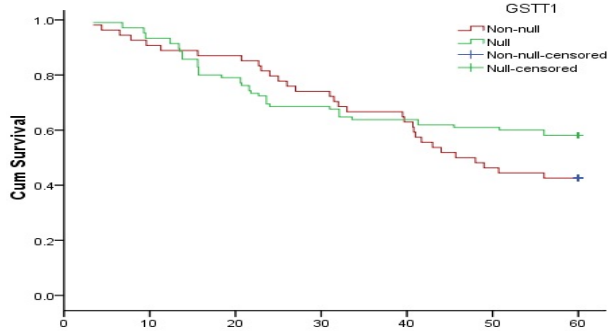

Figure 2. Kaplan-Meier Curves for Event-free Survival of Osteosarcoma Patients Treated with Chemotherapy. Analysis for GSTT1 Gene Polymorphism

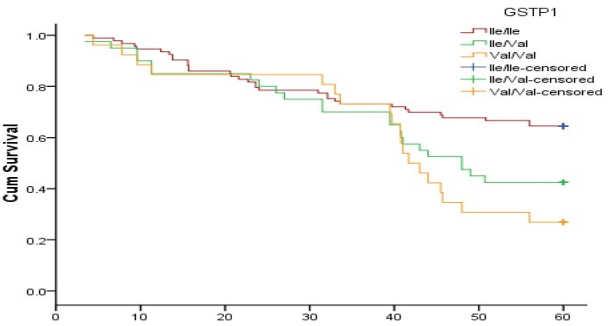

Figure 3. Kaplan-Meier Curves for Event-free Survival of Osteosarcoma Patients Treated with Chemotherapy. Analysis for GSTP1 Gene Polymorphism

the median follow-up was 45.5 months.

The relationship of GSTM1, GSTT1 and GSTP1 gene polymorphisms with prognosis of osteosarcoma was showed in Table 2. Polymorphisms in null GSTM1 and GSTT1 had a higher event free survival rate than non-null genotype (Figure 1 and Figure 2), whereas no significant association was found between the two genotypes and prognosis of osteosarcoma. The adjusted HRs (95% CI) of null GSTM1 and GSTT1 for the survival of osteosarcoma were 0.62 (0.30-1.14) and 0.70 (0.39-1.33), respectively. Individuals with GSTP1 Val/Val genotype tended to live shorter than IIe/IIe genotype (Figure 3), and we found a significantly higher risk of death from osteosarcoma (adjusted HR=2.35, 95% CI=1.13-4.85).

Discussion

In the present study, we attempted to identify predictive genetic polymorphism for survival to chemotherapy in patients with osteosarcoma. Our study found the GSTP1 Ile105Val polymorphism may influence cisplatin efficacy in patients with osteosarcoma. The patients with a homozygous IIeI/IIe genotype had a significantly longer survival than patients with one or two Val alleles. Our findings, together with existing data on the prevalent expression of GSTP1 in cancer cells, are in good agreement with the results of an in vitro experiment in which the human 105 Val variant of the GSTP1 enzyme was significantly more active against cisplatin than was the enzyme containing the IIe residue (Ishimoto et al., 2002; McIlwain et al., 2006). However, our results are not universally consistent with the epidemiology and clinical studies. Several studies have confirmed there was association between GSTP1 105 IIe allele and platinum-containing chemotherapy (Howells et al., 2004; Lee et al., 2005). However, some other studies have reported opposite associations or no relationship between the GSTP1 IIe105 Val polymorphism and survival (Stoehlmacher et al., 2002; Beeghly et al., 2006; Marsh et al., 2007; Nagle et al., 2007). These contradictions may be partly attributable to differences in the chemical structures and reaction kinetics of chemotherapy drugs. Furthermore, different studies have used different agents together with the platinum drugs. From our study, we found 93 patients with the GSTP1 105 IIe / IIe genotype tended to live longer than IIe/Val and Val/Val.

The results of our study here are in strong agreement with the current understanding of GSTP1 involvement in cisplatin detoxification. Our findings support the hypothesis that increased cisplatin sensitivity may in part be due to impaired GSTP1 zenzyme function. Previous studies found the homozygosity GSTP1 105 Val was even more protective against cisplatin-related toxicity in testicular and ovarian cancer (Choueiri et al., 2007; Oldenburg et al., 2007). In our study, we did not observe such association, we found the patients with Val/Val genotype had a higher risk of death from osteosarcoma (HR=2.35, 95% CI=1.13-4.85). The difference results might be due to variation of ethnicity, patients characteristics or by chance.

We further analyze the relationship of GSTT1 and GSTM1 deletion gene with the survival of patients with osteosarcoma. We found the null GSTT1 and GSTM1 are associated with a better survival. However, we did not find a significant differences in the five years survival rate of patients with homozygous null GSTT1 and GSTM1 and those with functional variants. Regarding the GSTM1 and GSTM1null genotypes, our previous study showed the two enzymes had a important role in the risk of osteosarcoma in Chinese population (Lu et al., 2011), and previous study conducted in Brazil showed the two enzymes had association with the clinical outcome of osteosarcoma. However, we were not able to find a significant difference in our study, as we had a limited number of patients to draw further conclusions.

Our study is limited by the relatively small sample size. In addition, meanwhile, more polymorphic genes involved in chemotherapy drugs have been identified, including XRCC1, XRCC3 and ERCC1, MTHFR, that may also impact the effect of these drugs. Therefore,

in order to confirm our findings from this study, we are currently analyzing a panel of 22 genes of metabolizing and DNA repair enzymes in a perspective study involving more than 200 patients.

In conclusion, this study showed the GSTP1 gene polymorphism may have an important role in the prognosis of osteosarcoma patients with chemotherapy, and no association of GSTM1 and GSTT1 with osteosarcoma was found. To our knowledge, this is the first report demonstrating a predictive value of GSTs genotypes in osteosarcoma patients with chemotherapy.

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