RESEARCH ARTICLE

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

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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 osteosarcoma, 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

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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/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 osteosarcoma, 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.
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.

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)
Discussion

In the present study, we provide evidence that glutathione S-transferases and DNA repaired gene polymorphisms were associated with the response to adjuvant chemotherapy in Chinese osteosarcoma patients. Our results showed a significant relationship between the GSTM1 and ERCC2 genes and overall survival of osteosarcoma patients with neoadjuvant chemotherapy. The GSTM1 null genotype was associated with a significantly lower risk of death compared to the non-null genotype (HR=0.32, 95% CI: 0.13-0.95). Similarly, the ERCC2 A/A genotype was associated with a higher risk of death compared to the non-null genotype (HR=2.19, 95% CI: 1.15-6.21). These findings suggest that genetic polymorphisms can influence the prognosis of osteosarcoma patients and may be useful in predicting treatment outcomes.

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

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) | Odds ratio (95% CI) |
|---------------|------------------|----|---------------------|----|--------------------|--------------------|
| 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 1105V   | Ile/Ile 54       | 55.67 | 56                   | 62.22 | 1.0 (Ref.)         | -                  |
|               | Ile/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(0.84-3.45)    | 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                   | 38.29 | 1.06(0.55-2.05)    | 1.34(0.64-2.45)    |
|               | T/T 12           | 12.37 | 9                    | 10.52 | 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)   |

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

| Genotype      | Cases N=187 | %  | Patient deaths N=79 | %  | Median overall survival (months) | HR (95% CI) | P       |
|---------------|-------------|----|---------------------|----|---------------------------------|-------------|---------|
| GSTM1         | Non-null    | 50.58 | 42                   | 53.26 | 37.63                          | 1.0 (Ref.) | -       |
|               | Null        | 49.2  | 37                   | 46.74 | 39.25                          | 0.91(0.51-1.77) | 0.74   |
| GSTT1         | Non-null    | 36.9  | 32                   | 40.45 | 37.44                          | 1.0 (Ref.) | -       |
|               | Null        | 63.1  | 47                   | 59.55 | 39.52                          | 0.92(0.57-1.76) | 0.75   |
| GSTP1 1105V   | Ile/Ile     | 58.82 | 51                   | 64.29 | 35.31                          | 1.0 (Ref.) | -       |
|               | Ile/Val     | 26.2  | 19                   | 24.5  | 37.21                          | 0.85(0.51-1.42) | 0.54   |
|               | Val/Val     | 14.97 | 9                    | 11.21 | 40.84                          | 0.53(0.24-1.16) | 0.32   |
| ERCC1 C118T   | C/C         | 49.73 | 42                   | 53.79 | 36.26                          | 1.0 (Ref.) | -       |
|               | C/T         | 39.04 | 30                   | 37.65 | 37.55                          | 0.81(0.40-1.33) | 0.7    |
|               | T/T         | 11.23 | 7                    | 8.56  | 39.39                          | 0.74(0.28-1.94) | 0.61   |
| ERCC2 Lys751Gln | G/G         | 58.29 | 50                   | 63.05 | 34.9                           | 1.0 (Ref.) | -       |
|               | G/A         | 35.83 | 26                   | 33.5  | 38.22                          | 0.73(0.40-1.12) | 0.35   |
|               | A/A         | 6.85  | 3                    | 3.45  | 41.53                          | 0.32(0.13-0.95) | <0.05  |

1Non-adjusted; 2Adjusted for sex, age, subtype, location, metastasis, tumor size and anatomic location
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 inconsistency 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 excision 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 repaired 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.

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