RESEARCH ARTICLE

Single Nucleotide Polymorphisms in the NER Pathway and Clinical Outcome of Patients with Bone Malignant Tumor

Xiao-Hui Sun*, Wen-Gen Hou, Hong-Xing Zhao, Yi-Lei Zhao, Chao Ma, Ying Liu

Abstract

The effects of polymorphisms in ERCC5, ERCC6, XPC, CCNH and MMS19L on osteosarcoma response to chemotherapy and the survival of the affected patients were assessed. Genotyping of ERCC5, ERCC6, XPC, CCNH and MMS19L was performed by PCR-RFLP assay. The median PFS was 12.8 months, and the median OS was 18.6 months. Individuals carrying homozygous genotypes of ERCC5 rs17655 and ERCC5 rs1047768 were more likely to have good response to treatment, while those carrying homozygous genotypes of MMS19L rs29001322 showed poor response. Osteosarcoma patients carrying TT genotype of ERCC5 rs1047768 showed a significantly longer PFS (16.8 months) and OS (21.4 months) than CC genotype, with HRs (95% CI) of 0.31 (0.10-0.93) and 0.32 (0.06-0.97), respectively. Conversely, those with the TT genotype of MMS19L rs29001322 demonstrated shorter PFS and OS, the HRs (95% CI) being 2.23 (1.08-4.15) and 4.62 (1.45-16.08), respectively. Our findings showed polymorphisms in ERCC5 rs1047768 and MMS19L rs29001322 to be associated with clinical outcome of osteosarcoma patients undergoing chemotherapy.

Keywords: Single Nucleotide polymorphisms - osteosarcoma - chemotherapy - clinical outcome

Asian Pacific J Cancer Prev, 14 (3), 2049-2052

Introduction

Osteosarcoma derives from primitive bone-forming mesenchymal cells, and is the most common bone malignant tumor found in children and adolescents. Osteosarcoma often occurs during rapid skeletal growth with more than half of them developing in the long bones (Hattinger et al., 2010). Chemotherapy with methotrexate, cisplatin and adriamycin followed by surgery and postoperative chemotherapy were the standard treatment of osteosarcoma. Although these advanced treatment, almost 30% of these patients relapse or occur metastasis (Chou and Gorlick, 2006). The clinical response to chemotherapeutics is influenced by multiple factors, including genetic and environmental factors.

Previous pharmacogenetic studies have shown that the gene polymorphisms are correlated with the drug metabolism and transport (Zhou et al., 2008). It is suggested that deficiencies in DNA repair capacity could have a role in cancer onset or progression, and could have a role in affecting the response to DNA damaging agents (Goode et al., 2002; Le et al., 2006; Martin and Hamilton, 2008). Four main different pathways of DNA repair processes participate into repairing various types of DNA damages. Particularly, the nucleotide excision repair (NER) pathway repairs bulky lesions, and has been associated with tumor progression and response to platinum-based chemotherapy (Reed, 1998; Stoehlmaier et al., 2004). Various studies have indicated the SNPs of NER genes are related with the response to chemotherapy in osteosarcoma (Caronia et al., 2009; Biason et al., 2012; Dogan et al., 2012). But the response to chemotherapy of osteosarcoma by SNPs in the NER pathway, including ERCC5, ERCC6, XPC, CCNH and MMS19L, has not been identified. Therefore, in our study, we aimed to assess the effect of polymorphisms in ERCC5, ERCC6, XPC, CCNH and MMS19L on the response to chemotherapy in osteosarcoma, and the survival of these patients.

Materials and Methods

Subjects, treatments and clinical variables

One hundred and eighty two consecutive patients diagnosed with osteosarcoma were enrolled between May 2006 and May 2009 in our hospitals. All the cases were histologically confirmed. All the patients were followed up every two months by telephone until death or the end of follow-up. All samples were obtained with written informed consent from patients or their relatives. The ethical approval of the study was gained from the Ethics Committee of the Xinxiang Medical University.

Patients were treated preoperatively with 25 mg/m² adriamycin intravenous on days one for three days, or 14 g/m² methotrexate plus 35 mg/m² cisplatin on day one for three days. After surgery, the adjuvant chemotherapy included methotrexate 10 g/m² on day one, 25 mg/m²...
cisplatin or adriamycin, 0.45 mg/m$^2$ one day one for three days, 500 mg/m$^2$ cyclophosphamide on day one for three days, and 1.5 mg/m$^2$ vincristine on day one for one day. The response to chemotherapy was evaluated in accordance with the response evaluation criteria from European Organization for Research and Treatment of Cancer. The response was assessed after four weeks of treatment. The response to chemotherapy was divided into good responders and poor responders. The good responders were defined as complete response or partial response, and the poor responders were defined as stable disease or progressive disease. Overall survival (OS) was calculated from the date of entry to the date of death or last clinical follow-up.

DNA extraction and quantification

Genomic DNA was extracted from peripheral blood lymphocytes using the Qiagen Blood Kit (Qiagen, Germany) according to the manufacturer’s instructions. The DNA extraction was determined by Agarose gel electrophoresis method. When a stripe was shown, the DNA was successfully extracted. Genotyping of ERCC5, ERCC6, XPC, CCNH and MMS19L was performed by PCR-RFLP assay. The forward and reverse primers for polymerase chain reaction (PCR) amplification and single base extension (SBE) assays were determined by Primer 5.0 software. Polymerase chain reaction (PCR) conditions were used as follows: an initial melting step of 5 min at 94°C; 35 cycles of denaturation for 30 s at 94°C; annealing for 30 s at 64°C; extension for 60 s at 72°C, followed by a 5 min final extension at 72°C.

Table 1. Clinical Characteristics of Subjects

| Patients | No | % |
|----------|----|---|
| Age, yr (Mean±SD) | 15.3±8.2 | |
| Sex | Female | 72 | 39.6 |
| | Male | 110 | 60.4 |
| Subtype | Osteoblastic | 104 | 57.1 |
| | Chondroblastic | 47 | 25.6 |
| | Not specified | 31 | 17.3 |
| Location | Femur | 94 | 51.4 |
| | Tibia | 61 | 33.7 |
| | Arm | 15 | 8.4 |
| | Central | 12 | 6.5 |
| Response to treatment | Good | 94 | 51.6 |
| | Poor | 88 | 48.4 |
| Metastasis | No | 97 | 53.2 |
| | At diagnosis | 48 | 26.1 |
| | At follow-up | 38 | 20.7 |
| Status at the end of follow-up | Alive | 109 | 59.9 |
| | Dead | 73 | 40.1 |

Table 2. Allele Frequencies of Gene Polymorphisms in 182 Patients Treated with Chemotherapy

| Gene | SNP | Alleles | MAF | Wide-zygous | Hetero-zygous | Homo-zygous |
|------|-----|---------|-----|-------------|--------------|-------------|
| ERCC5 | rs1047768 | C/T | 0.4881 | 131 | 35 | 16 |
| ERCC6 | rs2228526 | A/G | 0.1566 | 80 | 76 | 27 |
| XPC | rs2228001 | A/C | 0.3439 | 136 | 28 | 18 |
| CCNH | rs2266690 | C/T | 0.1378 | 131 | 34 | 17 |
| 17MMS19L | rs29001322 | C/T | 0.3054 | 80 | 76 | 26 |

Table 3. Response to Platinum-based Chemotherapy According to Genotypes

| Genotype | Cases | $\chi^2$ | P value |
|----------|-------|---------|---------|
| ERCC5 rs1047768 | | | |
| CC | 60 | 63.5 | 72 | 81.3 |
| CT | 21 | 22.1 | 14 | 16.3 |
| TT | 14 | 14.4 | 2 | 2.4 |
| ERCC6 rs2228526 | | | |
| AA | 38 | 40.4 | 42 | 47.2 |
| AG | 40 | 42.2 | 36 | 40.8 |
| GG | 16 | 17.4 | 11 | 12 |
| XPC rs2228001 | | | |
| AA | 68 | 72.5 | 67 | 76.6 |
| AC | 15 | 16.2 | 13 | 14.5 |
| CC | 11 | 11.3 | 8 | 8.8 |
| CCNH rs2266690 | | | |
| CC | 70 | 74.5 | 61 | 69.7 |
| CT | 16 | 17.2 | 18 | 20.1 |
| TT | 8 | 8.3 | 9 | 10.2 |
| MMS19L rs29001322 | | | |
| CC | 49 | 52.2 | 31 | 35.4 |
| CT | 36 | 38.4 | 39 | 44.8 |
| TT | 9 | 9.4 | 17 | 19.7 |

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 method used is the Cox Hazard regression model. Survival distributions were estimated by using the Kaplan-Meier method and assessed using the log-rank test. The association between genotype and survival was estimated by hazard ratios (HR) their confidence intervals (CI) from multivariate Cox proportional hazards model. The adjusted hazard ratios (HR) with 95% CI was used to assess the association between ERCC5, ERCC6, XPC, CCNH and MMS19L polymorphisms and OS of osteosarcoma. P value less than 0.05 was considered to be significant. All tests were two-sided and analyzed by SPSS 11.0 software.

Results

The clinical characteristics of 182 osteosarcoma patients were shown in Table 1. The median age of the patients at the diagnosis was 15.3±8.2 years (range 8.5 to 31.6 years). At the time of recruitment, only 33% of the patients were older than 18 years, and 60.5% of them were males. More than 50% of them patients were osteosarcoma, whereas the remainders were osteoblastic and chondroblastic osteosarcoma. Most of the location of cancer was in Femur (51.4%), and followed by tibia (33.7%) and arm (8.4%). Among 182 patients, 51.6% of them showed good response to treatment, and 46.8% of the patients showed metastasis at diagnosis or follow-up. At the end of follow-up, 73 patients died and 109 patients were alive. At the time of final analysis on May 2012, the median follow-up was 41.5 months.
The genotypic frequencies of the five SNPs were showed in Table 2. All the SNPs were common SNPs with minor allele frequencies between 0.13 and 0.48. 94 patients were classified as good responders. The results of tumor response to treatment by genotype were showed in Table 3. We found significant difference in the response to treatment in the polymorphisms in ERCC5 rs1047768 and MMS19L rs29001322 (P<0.05). Individuals carrying homozygous genotypes of ERCC5 rs1047768 were more like to have good response to treatment, while those carrying homozygous genotypes of MMS19L rs29001322 had significantly poor response to treatment (Table 3). No evidence of association was found for the other polymorphisms in the NER pathway.

In our study, the median PFS was 12.8 months, and the median OS was 18.6 months. Patients carrying TT genotype of ERCC5 rs1047768 showed a significantly different median OS compared to CC genotype. We found a significantly decreased risk of death from osteosarcoma among patients carrying TT genotype of ERCC5 rs1047768, and HR (95% CI) was 0.31(0.10-0.93) when compared to CC genotype. We found a significantly decreased risk of death from osteosarcoma when patients carrying TT genotype of ERCC6 rs2228526 were compared to AA genotype. The HRs (95% CI) were 0.31(0.10-0.93) when compared to AA genotype.

Table 4. Univariate Analysis of Gene Polymorphisms in Relation to PFS and OS

| Gene       | Case | event | Median (month) | Log-rank | HR(95%CI) | event | Median (month) | Log-rank | HR(95%CI) |
|------------|------|-------|----------------|----------|-----------|-------|----------------|----------|-----------|
| ERCC5 rs1047768 |      |       |                |          |           |       |                |          |           |
| CC         | 131  | 75    | 11.2           | 1.0      | 58        | 14.7  | 1              |          |           |
| CT         | 35   | 14    | 15             | 0.70(0.33-1.44) | 11 | 19.4  | 0.51(0.20-1.22) | 4 | 21.4  | 0.32(0.06-0.97) |
| TT         | 16   | 4     | 16.8           | <0.05    | 0.31(0.10-0.93) | 4     | 21.4           | <0.05    | 0.32(0.06-0.97) |
| ERCC6 rs2228526 |      |       |                |          |           |       |                |          |           |
| AA         | 80   | 43    | 13.1           | 1        | 34        | 18.3  | 1              |          |           |
| AG         | 76   | 36    | 13.6           | 0.89(0.49-1.57) | 28 | 18.7  | 0.76(0.37-1.51) |          |           |
| GG         | 27   | 14    | 13.4           | 0.51     | 11        | 19.1  | 0.13           |          | 0.92(0.33-2.57) |
| XPC rs2228001 |      |       |                |          |           |       |                |          |           |
| AA         | 136  | 71    | 12.2           | 1        | 56        | 18.2  | 1              |          |           |
| AC         | 28   | 14    | 13.4           | 0.96(0.44-2.02) | 11 | 19     | 0.91(0.34-2.36) |          |           |
| CC         | 18   | 9     | 13.6           | 0.29     | 6         | 19.2  | 0.29           |          | 0.77(0.21-2.61) |
| CCNH rs2266690 |      |       |                |          |           |       |                |          |           |
| CC         | 131  | 64    | 14.1           | 1        | 51        | 20.3  | 1              |          |           |
| CT         | 34   | 19    | 12.8           | 1.14(0.57-2.25) | 15 | 18.4  | 1.31(0.54-3.17) |          |           |
| TT         | 17   | 10    | 12.6           | 0.73     | 7         | 19.2  | 0.31           |          | 1.32(0.37-4.69) |
| MMS19L rs29001322 |      |       |                |          |           |       |                |          |           |
| CC         | 80   | 32    | 16.5           | 1        | 26        | 22.5  | 1              |          |           |
| CT         | 76   | 41    | 11.4           | 1.34(0.74-2.45) | 32 | 16.8  | 1.69(0.81-3.51) |          |           |
| TT         | 26   | 20    | 10.9           | <0.05    | 2.23(1.08-4.15) | 15 | 17.1           | <0.05    | 4.62(1.45-16.08) |

* Adjusted for potential risk factors, including sex, age, location of tumor and metastasis

**Discussion**

Osteosarcoma is a rare disease, and accounts for eighth most common cancer in children. Customized chemotherapy according to reliable molecular prognostic and predictive markers could play an important role in the treatment of patients with cancer. Many preclinical and clinical studies have extensively investigated the association between expression levels of DNA repaired gene and chemotherapy response in osteosarcoma patients. In our study, we found SNPs of ERCC5 and MMS19L could predict the response to chemotherapy and clinical outcome of osteosarcoma patients receiving chemotherapy by multivariate analysis.

Our findings have important prognostic and therapeutic implications. Tumors with dysfunctional ERCC5 expression would be predicted to demonstrate sensitivity to cisplatin. ERCC5 (ERCC5) is a structure-specific endonuclease, which participates in two incision steps that are critical to the DNA repair process. ERCC5 cleaves the damaged DNA. The 5' incision mediated by the ERCC1 and ERCC4 heterodimer, and stabilizes the DNA repair complex to the damaged DNA. ERCC5 levels are associated with cytotoxicity to cisplatin and iofulven, and potentially to be an important therapeutic target (Kopp et al., 2004).

Recently studies have indicated that ERCC5 is involved in the efficacy of cisplatin neoadjuvant chemotherapy in various cancers (Ott et al., 2011; Liu et al., 2012; Massuti et al., 2012). The low efficiency genotypes involved in DNA repair and replication may contribute to the difference in susceptibility of cancer. Only one previous study has indicated high expression of ERCC5 identifies a highly sensitive population of sarcomas with significantly improved treatment outcome (Schöffski et al., 2011). Our study also has showed variation of ERCC5 is correlated with susceptibility of osteosarcoma.
with good response to cisplatin in osteosarcoma. MMS19 splice variants have specific distinct functional domains, and this gene exerts its function in repairing and transcripting. Specific MMS19 domains a specific role in NER pathway and transcription and contributes to regulating the switch between transcription and NER (Hatfield et al., 2006). Previous two studies reported that the association between MMS19L and risk of cancer or its prognosis (McWilliams et al., 2009; Zhang et al., 2012). Our study has showed polymorphism in MMS19 is associated with good response of cisplatin chemotherapy in osteosarcoma, and our study provides evidence for further study to clarify their association.

There were some limitations in our study. Firstly, the sample size is relatively small in our study, which would decrease the statistical power of our study and thus lower the power to find the differences between groups. Secondly, lots of factors involved in cellular response to chemotherapy. However, we only analyzed nine SNPs of DNA repair genes, and some other factors might have interaction with these nine SNPs. Thus some bias may be existed. Therefore, in order to confirm our findings, we are currently analyzing a panel of 30 genes of metabolizing and DNA repair enzymes in a perspective study involving more than 200 patients.

In conclusion, some SNPs in the NER pathways are correlated with response to chemotherapy and prognosis of osteosarcoma, especially for SNPs of ERCC5 and MMS19. Our findings would provide important evidence for prognostic and therapeutic implications in osteosarcoma.

Acknowledgements

This research is supported by Education Department of Henan Province (12B310020).

References

Biasón P, Hattinger CM, Innocenti F, et al (2012). Nucleotide excision repair gene variants and association with survival in osteosarcoma patients treated with neoadjuvant chemotherapy. *Pharmacogenomics J*, **12**, 476-83.

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.

Chou AJ, Gorlick R (2006) Chemotherapy resistance in osteosarcoma: current challenges and future directions. *Expert Rev Anticancer Ther*, **6**, 1075-85.

Dogan M, Karabulut HG, Tukun A, et al (2012). Relationship between antimetabolite toxicity and pharmacogenetics in Turkish cancer patients. *Asian Pac J Cancer Prev*, **13**, 1553-6.

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

Le MW, Longy M, Bonaiti-Pellie C, et al (2006). Genetic polymorphisms of the XPG and XPD nucleotide excision repair genes in sarcoma patients. *Int J Cancer*, **119**, 1732-5.

Hatfield MD, Reis AM, Obeso D, et al (2006). Identification of MMS19 domains with distinct functions in NER and transcription. *DNA Repair (Amst)*, **5**, 914-24.

Hattinger CM, Pasello M, Ferrari S, et al (2010). Emerging drugs for high-grade osteosarcoma. *Expert Opin Emerg Drugs*, **15**, 615-34.

Koeppl F, Poiridessous V, Lazár V, et al (2004). Irofulven cytotoxicity depends on transcription-coupled nucleotide excision repair and is correlated with XPG expression in solid tumor cells. *Clin Cancer Res*, **10**, 5604-13.

Liu D, Wu HZ, Zhang YN, et al (2012). DNA repair genes XP, XPG polymorphisms: relation to the risk of colorectal carcinoma and therapeutic outcome with Oxalplatin-based adjuvant chemotherapy. *Mol Carcinog*, **51**, E83-93.

Martin LP, Hamilton TC, Schilder RJ (2008). Platinum resistance: the role of DNA repair pathways. *Clin Cancer Res*, **14**, 1291-5.

Massutti B, Cobo M, Camps C, et al (2012). Trabectedin in patients with advanced non-small-cell lung cancer (NSCLC) with XPG and/or ERCC1 overexpression and BRCA1 underexpression and pretreated with platinum. *Lung Cancer*, **76**, 354-61.

McWilliams RR, Bamlet WR, de André M, et al (2009). Nucleotide excision repair pathway polymorphisms and pancreatic cancer risk: evidence for role of MMS19L. *Cancer Epidemiol Biomarkers Prev*, **18**, 1295-302.

Ott K, Rachakonda PS, Panzram B, et al (2011). DNA repair gene and MTHFR gene polymorphisms as prognostic markers in locally advanced adenocarcinoma of the esophagus or stomach treated with cisplatin and 5-fluorouracil-based neoadjuvant chemotherapy. *Ann Surg Oncol*, **18**, 2688-98.

Reed E (1998). Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. *Cancer Treat Rev*, **24**, 331-44.

Schöffski P, Taron M, Jimeno J, et al (2011). Predictive impact of DNA repair functionality on clinical outcome of advanced sarcoma patients treated with trabectedin: a retrospective multicentric study. *Eur J Cancer*, **47**, 1006-12.

Stoehlmacher J, Park DJ, Zhang W, et al (2004). A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. *Br J Cancer*, **91**, 344-54.

Zhang L, Gao G, Li X, et al (2012). Association between single nucleotide polymorphisms (SNPs) and toxicity of advanced non-small-cell lung cancer patients treated with chemotherapy. *PLoS One*, **7**, e48350.

Zhou SF, Di YM, Chan E, et al (2008) Clinical pharmacogenetics and potential application in personalized medicine. *Curr Drug Metab*, **9**, 738-84.