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Genetic variability of DNA repair mechanisms and glutathione-S-transferase genes influences treatment outcome in osteosarcoma

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ABSTRACT

Background: Osteosarcoma patients are commonly treated with cisplatin-based preoperative and postoperative chemotherapy. Cisplatin binds to DNA and forms both intrastrand and interstrand crosslinks, inhibiting DNA replication. Glutathione-S-transferases (GSTs) participate in cisplatin detoxification, while several independent DNA repair mechanisms repair cisplatin-induced lesions. The aim of our study was to investigate the influence of genetic variability of DNA repair mechanisms and GSTs on efficacy and toxicity of cisplatin-based chemotherapy in osteosarcoma patients.

Methods: A total of 66 osteosarcoma patients were genotyped for *ERCC1*, *ERCC2*, *NBN*, *RAD51*, *XRCC3*, and *GSTP1* polymorphisms, as well as *GSTM1* and *GSTT1* gene deletion. We determined the influence of polymorphisms on survival and treatment outcome using Cox regression and logistic regression.

Results: Carriers of at least one polymorphic *ERCC2* rs1799793 allele had longer event-free survival (EFS) ($P = 0.006$; hazard ratio (HR) = 0.28; 95% confidence interval (CI) = 0.11–0.70). Polymorphic *GSTP1* rs1138272 allele was associated with both shorter EFS and OS ($P = 0.005$; HR = 3.67; 95%CI = 1.47–9.16; and $P = 0.004$; HR = 3.52; 95%CI = 1.51–8.22, respectively). Compared to the reference *NBN* CAA haplotype, *NBN* CGA haplotype was associated with shorter EFS ($P = 0.001$; HR = 4.12; 95%CI = 1.77–9.56).

Conclusions: Our results suggest that DNA repair polymorphisms and GST polymorphisms could be used as predictive factors for cisplatin-based chemotherapy in osteosarcoma patients and could contribute to treatment personalization.

1. Introduction

Osteosarcoma is a rare cancer, however in children and adolescents it is the most common malignant bone tumor. It has a first peak of incidence in adolescents around 16 years, and a second peak in patients older than 60 years [1]. Different etiology might contribute to the bimodal incidence distribution. The first peak among adolescents overlaps with the time of rapid adolescent bone growth and may be associated with rapid bone proliferation. On the other hand, osteosarcoma among adults may be associated more with exposure to environmental factors and can also represent a secondary malignancy [1,2]. With the use of chemotherapy the survival has significantly improved compared to only surgical treatment [3], but despite advances in chemotherapy, survival rates have reached a plateau [4]. Most treatment protocols are based on multiagent preoperative and postoperative chemotherapy that include cisplatin in combination with doxorubicin, high-dose methotrexate, and/or ifosfamide [5]. Although the prognosis has improved, considerable interindividual differences in treatment outcome are observed between patients. Up to 50% of patients have poor clinical outcome [6] and around 30% of patients relapse locally or develop metastases [4]. Genetic variability of

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mechanisms involved in response to chemotherapeutic agents could influence both survival and treatment related toxicity, therefore identification of predictive markers could lead to improved drug selection and treatment outcomes.

Cisplatin is a platinum analog, frequently used in treatment of various cancer types. It binds to DNA and forms DNA adducts, both intrastrand and interstrand crosslinks (ICLs), and inhibits DNA replication [7]. DNA repair mechanisms are therefore important factors that may determine the response to cisplatin. Nucleotide excision repair (NER) is the key mechanism involved in repair of cisplatin-induced DNA damage, because intrastrand crosslinks present the majority of cisplatin-induced DNA damage. However, because ICLs affect both DNA strands, they are more cytotoxic, and their repair through different pathways including homologous recombination repair (HRR) can be crucial for genomic stability [8].

All DNA repair pathways are complex and involve many different enzymes. NER is mainly involved in the repair of various distorting helix-distorting lesions [9]. After the recognition of the damage, helicases and endonucleases enable removal of the damaged region and polymerases and ligases fill and close the gap. Two enzymes of this pathway most often associated with resistance to cisplatin are helicase XPD, encoded by the excision repair cross-complementation group 2 (*ERCC2*) gene, and excision repair cross-complementation group 1 (ERCC1), which is a part of an endonuclease complex.

More complex DNA damage requires different mechanisms such as HRR for successful repair. In HRR, nibrin (NBN) is part of the complex involved in recognition of DNA damage, while RAD51 recombinase (RAD51) catalyses homologous search and strand invasion with the help of other proteins, including X-ray complementing defective repair in Chinese hamster cells 3 (XRCC3).

Single nucleotide polymorphisms (SNPs) of NER genes have already been associated with response to cisplatin-based treatment in different cancer types [10,11], including osteosarcoma, but all results are not concordant [12–15]. On the other hand, only a few studies have investigated the influence of HRR SNPs on cisplatin response [16–18], but not in osteosarcoma. In our previous studies we have shown that *XRCC3* and *NBN* SNPs modify DNA repair capacity and osteosarcoma risk [19,20].

Detoxification of cisplatin with glutathione-S-transferases (GSTs) may be also important for its efficacy. GST mu 1 (GSTM1), GST theta 1 (GSTT1), and GST pi 1 (GSTP1) are all responsible for lowering the intracellular concentration of cisplatin [7]. Several polymorphisms such as *GSTM1* or *GSTT1* gene deletion or non-synonymous *GSTP1* SNPs affect the expression or activity of GST enzymes. Previous studies have already shown the potential role of genetic variability of GSTs in osteosarcoma treatment; however the results are inconclusive [6,13,21,22].

The aim of the present study was to evaluate the influence of genetic variability in DNA repair pathways and *GST*s on the outcome of cisplatin-based treatment in osteosarcoma patients.

2. Patients and methods

2.1. Patients

Our retrospective study included Slovenian osteosarcoma patients diagnosed between 1990 and 2008 with sufficient formalin fixed, paraffin embedded (FFPE) material for DNA extraction and available medical records. All patients were treated with cisplatin-based chemotherapy at the Department of Hematology and Oncology, University Children's Hospital, Ljubljana, Slovenia or at the Institute of Oncology, Ljubljana, Slovenia. The study was approved by the Slovenian Ethics Committee for Research in Medicine and was carried out according to the Declaration of Helsinki.

2.2. Assessment of treatment outcome

Clinical and treatment data were obtained from the medical records. Histologically determined percentage of necrosis was used to assess the response to chemotherapy. To classify patients as good responders, more than 90% of necrosis had to be observed. Adverse events were evaluated according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm, accessed on 15.01.2014). The levels of neutropenia, leukopenia, anemia, or thrombocytopenia were used to determine hematological toxicity. Gastrointestinal (GI) toxicity was characterized as dyspepsia, diarrhea, constipation, nausea or vomiting, while elevated creatinine levels indicated renal toxicity. Event-free survival (EFS) and overall survival (OS) were defined as time from the beginning of treatment to an event or death, respectively. Event was defined as disease recurrence, development of metastases or death. Patients without an event or death at the time of the analysis were censored at the date of the last follow-up.

2.3. DNA extraction and genotyping

Extraction of genomic DNA from FFPE samples was performed using QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions as previously described [20]. Genotypes of *ERCC1* rs11615 (c.354T>C; p.Asn118=) and rs3212986 (c.*197G>T), *ERCC2* rs1799793 (c.934G>A; p.Asn312Asp) and rs13181 (c.2251A>C; p.Lys751Gln), *NBN* rs1805794 (c.553G>C; p.Glu185Gln), rs709816 (c.1197A>G; p.Asp399=) and rs1063054 (c.*1209A>C), *RAD51* rs1801320 (c.-98G>C), rs1801321 (c.-61G>T) and rs12593359 (c.*502T>G), and *XRCC3* rs1799794 (c.-316A>G) and rs861539 (c.722C>T; p.Thr241Met) were determined using Kaspar assay according to the manufacturer's instructions (KBiosciences, Herts, UK). Genotyping of *GSTP1* rs1695 (c.341C>T; p.Ile105Val) and rs1138272 (c.313A>G; p.Ala114Val) was carried out using TaqMan SNP Genotyping assays according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). *GSTM1* and *GSTT1* gene deletions were detected using multiplex PCR simultaneously amplifying *GSTM1*, *GSTT1*, and *BGLO* genes as described previously [23]. With this approach, we could identify homozygous *GSTM1* or *GSTT1* gene deletion, but we were not able to distinguish between carriers of one or two copies of each gene. Genotyping was repeated in 20% samples to check for genotyping accuracy.

2.4. Statistical analyses

Frequencies were used to describe the distribution of categorical variables and median and interquartile ranges were used for continuous variables. Standard chi-square test was used to assess deviation from Hardy–Weinberg equilibrium (HWE). Cox proportional hazards model was used in survival analysis to calculate hazard ratio (HR) and the 95% confidence interval (CI). Logistic regression was used to assess the influence of genetic polymorphisms or clinical variables with binary treatment outcomes and odds ratios (ORs) and their 95% CIs were determined. A dominant genetic model was used in all statistical analyses. All statistical analyses were carried out by IBM SPSS Statistics, version 19.0 (IBM Corporation, Armonk, NY, USA). Haplotype analysis was performed using Thesias software [24] as previously described [25]. To account for multiple comparisons, Benjamini–Hochberg false discovery rate (FDR) was used [26]. P values less than 0.010 were considered statistically significant after correction.