JBUON 2018; 23(4): 1130-1135
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Association between GPX1 and SOD2 genetic polymorphisms and overall survival in patients with metastatic urothelial bladder cancer: a single-center study in Serbia

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Summary

Purpose: *Urothelial bladder cancer (UBC) is the most common malignancy of urinary tract in the developed world. In metastatic UBC, systemic chemotherapy still remains the mainstay of initial treatment. Inter-individual differences in treatment outcome partially may be the consequence of genetic variations in enzymes that modulate oxidative stress. Therefore, we aimed to determine the potential prognostic role of single nucleotide polymorphism (SNP) of the two antioxidant enzymes glutathione peroxidase 1 (GPX1) and superoxide dismutase 2 (SOD2) in metastatic UBC patients treated with cisplatin-based chemotherapy.*

Methods: *This prospective single-center hospital-based case-control study included 33 patients with metastatic UBC treated with cisplatin-based chemotherapy and 227 healthy controls. GPX1 SNP (rs1050450) was assessed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and SOD2 SNP (rs4880) was determined by quantitative PCR (q-PCR). Overall survival (OS) was evaluated using Kaplan–Meier survival analysis during 2-year follow up period, with the log-rank test for prognostic significance.*

Results: *No significant difference was observed in the distributions of GPX1 and SOD2 gene variants between patients and controls ($p>0.05$). Regarding GPX1 polymorphism, no impact of GPX1 polymorphism on OS could be demonstrated ($p>0.05$). Finally, Kaplan-Meier survival analysis showed no association between SOD2 polymorphism and OS ($p>0.05$).*

Conclusions: *No association was found between polymorphism of GPX1 and SOD2 and OS in patients with metastatic urothelial bladder cancer treated with cisplatin-based chemotherapy.*

Key words: *chemotherapy, genetic polymorphisms, GPX1, metastatic urothelial bladder cancer, overall survival, SOD2*

Introduction

Urothelial bladder cancer (UBC) is the most common malignancy of urinary tract in the developed world and the 11th most common cancer diagnosed worldwide, with prominent gender predilection for males, both in incidence and mortality rates [1]. At the time of diagnosis, metastatic disease is found in about 4% of the patients [2]. Furthermore, around half of the patients who progress after cystectomy develop distant metastases and/or local recurrence [3]. In metastatic disease, systemic chemotherapy still remains the mainstay of initial treatment. Cisplatin-based chemotherapy has been introduced in late 1980s. For cisplatin eligible patients, MVAC (methotrexate, vinblastine, doxo-

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Received: 29/11/2017; Accepted: 04/01/2018

rubicin, and cisplatin) and GC (gemcitabine and cisplatin) are two multi-agent combinations commonly used in the first-line setting. Both combinations are comparable in terms of efficacy, but the lower toxicity of GC makes it a preferred regimen [4]. Even though UBC is a chemo-sensitive tumor, only 40-50% of the cases will respond to cisplatin-based chemotherapy with a median OS around 14 months [5]. Development of drug resistance is a major reason why cisplatin-based chemotherapy has limited efficacy in metastatic UBC. The cytotoxic effect of cisplatin is believed to be primarily the result of formation of nuclear DNA adducts that, if not removed, cause unrepaired damage and cell death [6]. Moreover, both *in vitro* and *in vivo* studies have shown that cisplatin increases oxidative stress which may lead to cisplatin-induced toxicity [7]. However, the contribution of cisplatin-induced oxidative stress in cytotoxicity in normal and cancer cells is still poorly understood. At higher levels, free radicals are important intracellular carcinogens associated with DNA damage and disease progression. However, high levels of free radicals are also necessary to facilitate the cytotoxic effect of chemotherapy or radiation [8]. It is well known that there are inter-individual differences in drug response which partially may be the consequence of genetic variations in enzymes that modulate oxidative stress. Studies focusing on the role of drug-induced oxidative stress and treatment efficacy have reported contradictory results [9,10].

Enzymes involved in the defense against oxidative stress are polymorphic, such as glutathione peroxidase (*GPX*). *GPX* is a family of selenium-dependent enzymes catalyzing the reduction of hydrogen peroxide to water [11]. *GPX1* is the most abundant and ubiquitous isoform. One of the most extensively studied polymorphisms represents modified *C > T* (GPX1C593T, dbSNP ID rs1050450), which leads to a change from proline to leucine at amino acid 200 (*Pro200Leu*) [11]. The *GPX1* C593T variant is supposed to result in lower enzyme activity and mRNA expression in the presence of *Leu*-allele compared with the *Pro*-allele [12]. This polymorphism has been linked to breast [13], lung [14] and bladder cancer [15], as well as coronary heart disease [16] and diabetic peripheral neuropathy [17].

Another important antioxidant enzyme is superoxide dismutase (*SOD*): it is involved in the dismutation of superoxide into oxygen and hydrogen peroxide. Among three existing *SOD* isoforms, *SOD2* was found to modify cancer susceptibility [18]. The *SOD2* gene (6q25) has several SNPs, among which rs4880 (*T/C*) is the most well-studied. The SNP in the *SOD2* gene causes an amino acid substitution of valine (*Val*) with alanine (*Ala*) (*Val16Ala*). Individuals with the *CC* (*Ala16Ala*) genotype were found to have a higher *SOD2* activity compared to those with the *CT (Val 16Ala)* or *TT (Val16Val)* genotype [19].

The aim of this study was to explore the prognostic value of genetic polymorphisms of *GPX1* and *SOD2* in a population with metastatic UBC treated with cisplatin-based chemotherapy.

Methods

Study subjects

This study was designed as a prospective single-center hospital-based case-control study. The studied population included 33 patients with metastatic UBC in whom cisplatin-based chemotherapy was planned. Data were collected from October 1st 2014 to November 1st 2015. All patients were enrolled from the Clinic of Urology, Clinical Centre of Serbia, Belgrade. The control group included 227 subjects with nephrolithiasis admitted to the same hospital during the same period of time. None of the participants from the control group had any history of malignant disease.

Informed consent was obtained from all recruited subjects. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade (No. 29/VI-7), and the research was carried out in compliance with the Declaration of Helsinki.

Histopathological confirmation of UBC was obtained in all cases after transurethral resection or cystectomy.

Prior to starting chemotherapy, metastatic disease and/or local recurrence were confirmed in all patients with chest and abdomino-pelvic CT scan or MRI. Significant medical conditions such as cardiac, renal and liver impairment were documented, ECOG performance status was determined and laboratory analyses performed. Chemotherapy regimens included: GC - gemcitabine 1000 mg/m^2 (on days 1, 8 and 15) plus cisplatin 70 mg/m^2 (on days 1 or 2) every 28 days for a maximum of 6 cycles. MVAC - methotrexate 30 mg/m^2 (on days 1, 15, and 22) plus vinblastine 3 mg/m^2 (on days 2, 15, and 22) plus doxorubicin 30 mg/m^2 (on day 2) plus cisplatin 70 mg/m^2 (on day 2) every 28 days for a maximum of 4 cycles. OS was calculated from beginning of systemic treatment until death or last follow-up. Last follow up was November 1st, 2016.

DNA extraction

Genomic DNA was extracted from citrate-anticoagulated peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen, Inc., Chatsworth, CA, USA) in accordance with the manufacturer's instructions.

Analysis of the GPX1 and SOD2 genotypes

GPX1 (rs1050450) gene polymorphism was assessed by PCR-RFLP. Primers used for DNA amplification were: (forward) 5'-GCC GCC GCT TCC AGA CCA T-3', and (reverse) 5'-CCC CCC GAG ACA GCA GCA CT-3'. Amplified PCR fragments were digested with Apa1 enzyme

at 30°C overnight in order to determine *C>T* SNP. The restriction fragments (128bp for allele *T*, and 67bp + 61bp for allele *C*) were visualized after electrophoresis in 4% agarose gel stained by ethidium bromide. For this purpose ChemiDoc imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used.

SOD2 (rs4880) gene polymorphism was determined by q-PCR) using Applied Biosystems TaqMan® (Foster City, CA, USA). Drug Metabolism Genotyping assay (ID: C_8709053_10) was used according to the manufacturer's protocol. Allelic discrimination was performed on Mastercycler® ep realplex (Eppendorf, Germany).

Statistics

Statistical analyses were performed using IBM SPSS version 21.0 (IBM, Armonk, NY, USA). Chi-square test was used to analyze the frequency differences between two groups. The difference in the terms of age was assessed by Student t-test. Kaplan-Meier analysis was used to estimate the OS rates, with the log-rank test for prognostic significance. A p value less than 0.05 was considered statistically significant.

Results

Characteristics of patients with metastatic UBC and controls are presented in Table 1. Thirty-three patients (mean age 65.45 ± 9.07; 18 male and 15 female) with histologically confirmed UBC received chemotherapy. The control group consisted of 227 individuals with nephrolithiasis (mean age 63.42 ± 7.97; 154 male, 73 female) randomly selected from the same hospital within the same time period. As shown in Table 1, no significant difference in terms of age and gender was found between cancer patients and respective controls. Sixty-one percent and 49% were smokers in the metastatic UBC and control group, respectively. The distributions of *GPX1* (rs1050450) and *SOD2* (rs4880) genotypes in patients with metastatic UBC and controls are presented in Table 2. The frequencies of *GPX1* genotypes in patients were as follows: 49% *Pro200Pro*, 39% *Pro200Leu* and 12% *Leu200Leu*. However, the control group with the following *GPX1* genotype distribution, 39% *Pro200Pro*, 51% *Pro200Leu* and 10% *Leu200Leu*, didn't differ significantly from patients with metastatic UBC. In addition, no significant differences were observed in terms of *SOD2* genotype distribution. Frequencies of *SOD2* genotypes in patients were: 21% *Ala16Ala*, 52% *Val16Ala* and 27% *Val16Val*, while in controls: 22% *Ala16Ala*, 47% *Val16Ala* and 31% *Val16Val*.

In order to estimate the impact of *GPX1* and *SOD2* genotypes on OS, 33 patients with metastatic UBC were followed for two years. Regarding *GPX1* polymorphism, patients were dichotomized on those homozygous for referent allele *GPX1 Pro200Pro* (16 patients) and those who carried at least one variant allele *GPX1 Pro200Leu* and *GPX1 Leu200Leu* (17 patients). Kaplan-Meier survival analysis showed no impact of *GPX1* polymorphism

Table 1. Selected characteristics of patients with metastatic UBC and controls

Characteristics	*Patients, n (%)*	*Controls, n (%)*	*p value*
Age, years (mean ± SD)	65.45 ± 9.07	63.42± 7.97	0.75
Sex			
Male	18 (54)	154 (68)	
Female	15 (46)	73 (32)	0.131
Smoking			
No	13 (39)	112 (51)	
Yes	20 (61)	108 (49)	0.217

Table 2. *GPX1* and *SOD2* genotypes distribution

Genotype	*Patients, n (%)*	*Controls, n (%)*	*p value*
GPX1 rs1050450			
Pro200Pro	16 (49)	67 (39)	
Pro200Leu	13 (39)	89 (51)	
Leu200Leu	4 (12)	18 (10)	0.461
SOD2 rs4880			
Ala16Ala	7 (21)	47 (22)	
Val16Ala	17 (52)	99 (47)	
Val16Val	9 (27)	66 (31)	0.865

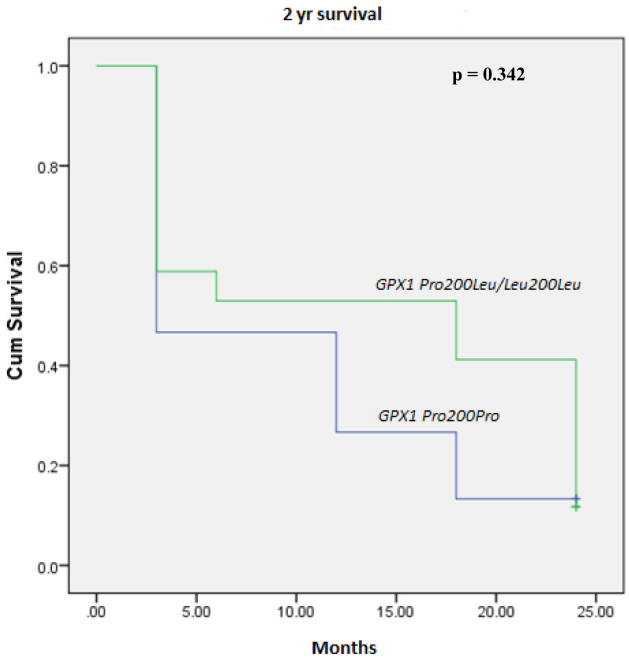

Figure 1. Kaplan–Meier 2-year survival according to *GPX1* polymorphism for mortality of metastatic urothelial bladder cancer patients on chemotherapy.

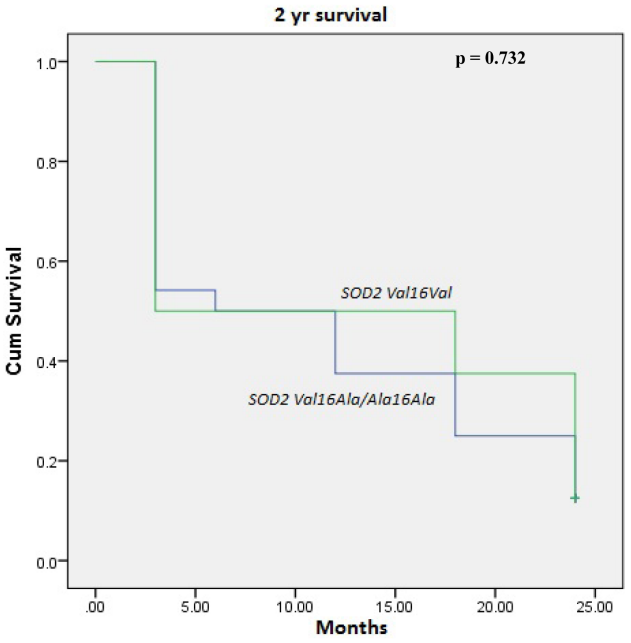

Figure 2. Kaplan–Meier 2-year survival according to *SOD2* polymorphism for mortality of metastatic urothelial bladder cancer patients on chemotherapy.

on OS (Figure 1). Additionally, the same analysis was performed in order to estimate the association between *SOD2* genotype and OS. Regarding *SOD2* polymorphism, patients were divided as follows: *SOD2 Ala16Ala* (7 patients), *SOD2 Val16Ala* and *SOD2 Val16Val* (26 patients). The assessed genotypes showed no effect on OS (Figure 2).

Discussion

To the best of our knowledge, this is the first report on the prognostic value of two SNPs of antioxidant enzymes *GPX1* (rs1050450) and *SOD2* (rs4880) in patients with metastatic UBC treated with cisplatin-based chemotherapy. In this single center hospital-based case-control study, we found no association between *GPX1* and *SOD2* genetic polymorphisms and OS. Findings from both *in vitro* and *in vivo* studies [20] have shown that oxidative stress is higher in cancer cells than in normal cells [20]. On one hand, moderate levels of reactive oxygen species (ROS) were shown to promote carcinogenesis and tumor growth, while high ROS levels - induced by chemotherapy - caused cancer cell death and tumor suppression [21]. Chemotherapeutic drugs used in UBC protocols (GC and MVAC) also were shown to produce high levels of ROS, leading to impaired tumor cell growth. In addition, a higher anti-oxidative status of cancer was found to be associated with drug resistance [22]. Functional polymorphisms in genes coding for antioxidant enzymes might be one of the factors involved in drug sensitivity. Indeed, the association between *MnSOD* and chemosensitivity of various cancer cell lines has been shown previously [23].

The lack of association between *GPX1* and *SOD2* genetic polymorphisms and OS in our patients is consistent with findings from the Danish Clinical Registry-Based Case-Control Study conducted in breast cancer patients. No association was found for the *SOD2* genotype and disease recurrence after adjuvant cyclophosphamide-based chemotherapy [24]. Similarly, another study which analyzed 9 oxidative stress-related SNPs showed no prognostic value of *SOD2* polymorphism and progression free survival (PFS) or OS in patients with metastatic gastric cancer who had received chemotherapy [25]. In contrast, Yao et al. reported an association of the *Ala*-allele in *SOD2* polymorphism, which led to a higher *MnSOD* antioxidant activity, with less treatment-related toxicity and shorter PFS after adjuvant chemotherapy in patients with breast cancer [26].

Currently, there is limited data on the prognostic value of *GPX1* and response to chemotherapy. Research conducted in patients who were treated with chemo-radiotherapy for head and neck squamous cell carcinoma revealed no significant influence of *GPX1* expression on treatment response or survival [27]. Conflicting results were reported from studies that focused on the potential role of various genetic polymorphisms of glutathione-

related enzymes in cisplatin-based chemotherapy response in NSCLC, ovarian, colorectal, endometrial and urothelial bladder cancer patients [28-32].

The limitation of our study includes first the small number of patients. This may be explained by the single-center recruitment. Second, only two genetic polymorphisms were genotyped. Third, all patients received multidrug chemotherapy regimens, thus it is difficult to estimate the role of *SOD2* and *GPX1* polymorphisms on treatment outcome for each individual drug. Since both the number of causal SNPs and the complex interactions between agents are still unknown, the single gene based approach in genetic studies may have limited value.

Conclusions

In conclusion, we found no association between a functional polymorphism of *GPX1* and *SOD2* and OS in patients with metastatic UBC treated with cisplatin-based chemotherapy. This is similar to the most of the findings published so far on this topic. Extensive research of numerous SNPs of genes encoding different antioxidant related enzymes in addition to *GPX1* and *SOD2* in large cohorts of patients is warranted.

Conflict of interests

The authors declare no conflict of interests.

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