RESEARCH COMMUNICATION

Predictive Value of XRCC1 and XRCC3 Gene Polymorphisms for Risk of Ovarian Cancer Death After Chemotherapy

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Abstract

Objective: To investigate any association between XRCC1 and XRCC3 polymorphisms and outcome of platinum-based chemotherapy in ovarian cancer patients. Methods: With a prospective study design was cases were consecutively collected from January 2005 to January 2007. All 310 included patients were followed-up until the end of January 2010. Genotyping of XRCC1 and XRCC3 polymorphisms was conducted by TaqMan Gene Expression assays. Results: A total of 191 patients died during follow-up. Our study showed a lower survival rate in XRCC1 399 Arg/Arg genotype than Gln/ Gln, with a significant increased risk of death (HR=1.69, 95%CI=1.07-2.78). Similarly, those carrying XRCC3 Thr/ Thr genotype had a increased risk as compare to the Met/Met genotype, with a HR (95% CI) of 1.90 (1.12-3.41). There was no significant association between XRCC1 Arg194Trp and XRCC1Arg280His gene polymorphisms and ovarian cancer death. Conclusion: Our study demonstrates that polymorphisms in DNA repair genes have roles in the susceptibility and survival of ovarian cancer patients.

Keywords: Ovarian cancer - XRCC1 - XRCC3 - polymorphism - Chinese population

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Introduction

Ovarian cancer is the leading cause of death from gynaecologic malignancy. The vast majority of malignant ovarian cancers are of epithelial origin and can be classified into four major subtypes: serous, mucinous, endometrioid, and clear cell. More than 50% of ovarian cancer patients are diagnosed at an advanced stage (Hogberg et al., 2001). Though several active chemotherapeutic agents are available, the platinum-based doublet chemotherapy remains the standard initial treatment for ovarian cancer patients and their overall survival is till dismal. However, despite this initial success, approximately 50% of patients will develop recurrent disease within 3 years of diagnosis(Hogberg et al., 2001). Paradoxically, although most patients initially respond to platinum chemotherapy, the majority eventually die from chemotherapy-resistant disease (Harries et al., 2001; Piccart et al., 2001). The identification of molecular agents that effectively target the mechanisms of chemotherapy resistance could represent a significant advancement in our ability to treat these often fatal malignancies (Giaccone et al., 2000).

DNA bears indispensable inheritance information in human beings and its damage is critical to carcinogenesis (Hoeijmakers, 2001). At least four major pathways of DNA repair have been described that operate on specific types of damaged DNA, including base excision repair (BER), mismatch repair, nucleotide excision repair, and double-strand break repair. BER operates on small lesions including oxidized or reduced bases, fragmented or nonbulky adducts, or those produced by methylating agents (Goode et al., 2002). Of the multiple proteins involved in the BER pathway, X-ray repair cross-complementing group 1 (XRCC1) and group 3 are two important ones.

The DNA repair gene XRCC1 gene codes for a protein involved in the repair of single-strand breaks (SSB) and in base excision repair (BER) of damaged bases caused by endogenous and exogenous oxidants. Three polymorphisms occurring at conserved sequences in the XRCC1 gene were reported by Shen et al. (1998). These coding polymorphisms, resulting in amino acid substitutions, were detected at codons 194 (Arg-Trp), 280 (Arg-His) and 399 (Arg-Gln). Most studies report a reduced risk of cancer associated with the 194Trp allele (Goode et al., 2002). The 399 polymorphism have been associated with a number of cancers, although results have been inconsistent (Abdel-Rahman et al., 2000; Goode et al., 2002; Mort et al., 2003; Nexo et al., 2003; Vogel et al., 2004; Hong et al., 2005; Yeh et al., 2005). Few studies have investigated the association between the XRCC1 280His allele and risk of cancer. No association has been observed with colorectal cancer (Hong et al., 2005), but an increased risk has been reported with lung cancer (Ratnasinghe et al., 2001) and breast cancer (Moullan et al., 2003).

The XRCC3 gene codes for a protein involved in homologous recombinational repair (HRR) of double-strand DNA and is required for genomic stability (Cui

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et al., 1999; Griffin et al., 2000). The XRCC3 gene has a sequence variation in exon 7 (C18067T), which results in an amino acid substitution at codon 241 (Thr241Met) that may affect the enzyme's function and/or its interaction with other proteins involved in DNA damage and repair (Matullo et al., 2001). Molecular epidemiological studies have linked this XRCC3 polymorphism to increased risk of breast cancer (Kuschel et al., 2002), lung cancer (Jacobsen et al., 2004), skin cancer (Winsey et al., 2000) and colorectal cancer (Mort et al., 2003). The results have been inconsistent (Jacobsen et al., 2003).

In the present study, we conducted a prospective study to test the association between four amino acid substitution variants of DNA repair genes, XRCC1 (Arg194Trp), XRCC1(Arg280His), XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) polymorphisms and the clinical outcome ovarian cancer patients with platinum-based chemotherapy.

Materials and Methods

Patients

Patients were histological diagnosed as ovarian cancer during January 2005 to January 2007 were collected in our study. Among a total of 321 eligible cases, 310 were interviewed with a participation rate of 96.6%. After patients provided informed consent, every patient required to provide 5ml blood. Patients were treated with surgical cyoreduction followed by platinum-based chemotherapy regimen and had corresponding clinical and follow-up information. All the patients were followed every 2 months until death. Overall survival was the end point in the present study. Survival time was calculated from the date of diagnosis to the date of last follow-up from any causes. All the 310 patients were followed-up from January 2007 to January 2010.

The Genomic DNA was isolated from blood samples according to standard procedures with minor modifications (Miller et al., 1988). In brief, whole blood samples was mixed with a threefold volume of lysis buffer and incubated at 4 °C for at least 30 min. The lysate was then centrifuged, and pellet of intact leukocytes was resuspended in 10 ml SE buffer (75 mM NaCl, 24 mM EDTA pH 8.0) 500 µl SDS (20%) and 50 µl Proteinase K (20 mg/ml) and incubated overnight at 40 °C. After digestion, 3.5 ml 6 M NaCl was added to the lysate and the mixture was shaken vigorously and then centrifuged to pellet the cellular proteins. DNA in the supernatant was then precipitated with 2 volumes of absolute ethanol, washed in 70% ethanol and resuspended in TE buffer.

Genotype analysis of four single nucleotide polymorphisms (SNPs) of the DNA repair gene XRCC1 and XRCC3 was carried out by using the TaqMan allelic discrimination assay on a Sequence Detection System ABI 700 (Applied Biosystems). The XRCC1 polymorphisms (XRCC1 Arg194Trp, XRCC1 Arg280His and XRCC1 Arg399Gln as well as XRCC3 Thr241Met were determined in a 12 µl reactions containing 1× MasterMix, 200 nM of each probe, 900 nM primers, and 50-100 ng of genomic DNA. Cycling conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 s and 60 °C for 1 min. Primers and probes are described in Table 1.We used replicates for 10% samples for quality control.

Statistical analysis

SPSS Version 13.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistic calculations. The outcome for the study was overall survival, which was estimated using the Kaplan-Meir method. A univeariate Cox's regression analysis was used to assess the association between XRCC1 and XRCC3 gene polymorphism and survival of ovarian cancer. The primary death of ovarian cancer was defined as the failure event and the time of survival was the time between diagnosis and death. If a patient died of other causes rather than ovarian cancer, she was censored at the date of death. All survived patients were censored at the date of last follow-up.The relative risk [hazard ratio(HR)] and 95% CI were calculated from the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). All statistical tests were two sided, and differences were taken as significant when the P value was less than 0.05.

Results

All the 310 patients were followed-up till January 2010. The median follow-up time was 30.1 months, and 191 patients died during the four years follow-up. The clinical characteristics of cases showed in Table 1. The average age of included cases was 47.1±4.6 years old. 67.4% of the patients were at the stage of III and IV, and 61.5% of the patients were serous adenocarcinoma.

The frequencies of XRCC1 Arg194Trp, Arg280His,

Table 1. Characteristics of Included Cases in Our Studies

Variables	Cases N=310	%
Age (mean±SD, years)	47.1±4.6	
Disease stage		
I	23	7.5
II	36	11.7
III	224	72.1
IV	27	8.7
Histological subtype		
Serous adenocarcinoma	173	55.7
Endometrioid adenocarcinoma	40	12.6
Clear cell carcinoma	51	16.6
Mucinous adenocarcinoma	17	5.6
Other adenocarcinomas	29	9.2

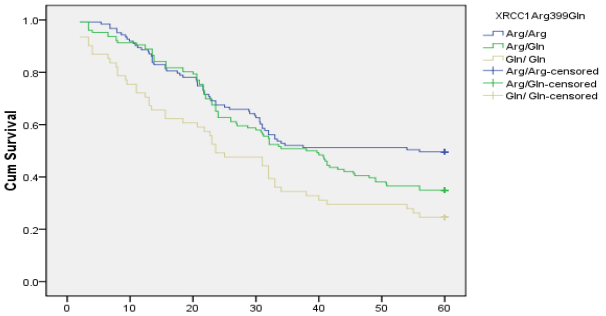

Figure 1. Kaplan-Meier Estimates of Overall Survival with XRCC1 Arg194Trp Polymorphism

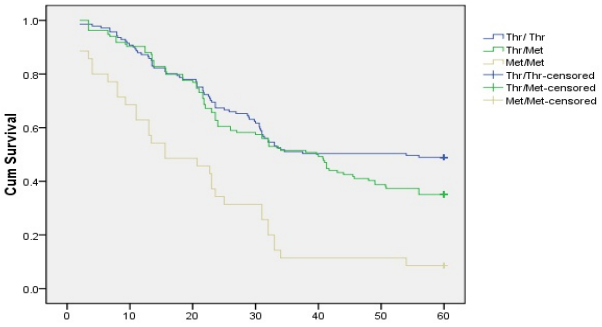

Figure 2. Kaplan-Meier Estimates of Overall Survival with XRCC1Arg280His Polymorphism

Table 2. Associations Between XRCC1 and XRCC3 Thr241Met Gene Polymorphisms and Ovarian Cancer Risk

Genotype	No. of patients (%) N=335	Death of patients N=229	HR(95% CI)[1]	P
XRCC1 Arg194Trp				
Arg/Arg	218(70.4)	131(68.7)	1.0(Ref.)	-
Arg/Trp	65(20.9)	41(21.7)	1.04(0.65-1.67)	0.84
Trp/Trp	27(8.7)	18(9.6)	1.11(0.55-2.18)	0.76
XRCC1Arg280His				
Arg/Arg	259(83.5)	156(81.9)	1.0(Ref.)	-
Arg/His	41(13.3)	27(10.7)	1.09(0.62-1.90)	0.74
His/ His	10(3.2)	7(2.7)	1.16(0.37-3.46)	0.77
XRCC1Arg399Gln				
Arg/Arg	123(39.7)	62(32.2)	1.0(Ref.)	-
Arg/Gln	126(40.8)	82(42.7)	1.33(0.87-2.13)	0.21
Gln/ Gln	60(19.5)	48(25.1)	1.69(1.07-2.78)	<0.05
XRCC3 Thr241Met				
Thr/Thr	140(45.1)	72(37.6)	1.0(Ref.)	-
Thr/Met	135(43.7)	87(45.7)	1.34(0.93-1.97)	0.25
Met/Met	35(11.2)	32(16.7)	1.90(1.12-3.41)	<0.05

[1]Adjusted for age, disease stage, and histological subtype

Arg399Gln and XRCC3 Thr241Met gene polymorphisms in ovarian cancer cases were showed in Table 2. XRCC1 194 Arg/Arg, Arg/Trp and Trp/Trp had 70.4%, 20.9% and 8.7% in ovarian cancer cases, and had 68.7%, 21.7% and 9.6% in death patients. XRCC1 280 Arg/Arg, Arg/His and His/ His showed 83.5%, 13.3% and 3.2% in cases, and 81.9%, 10.7% and 2.7% in died patients. XRCC1 399 Arg/Arg, Arg/Gln and Gln/ Gln presented 39.7%, 40.8% and 19.5% in cases, and 32.2%, 42.7% and 25.1% in died cases. For XRCC3 Thr241Met, Thr/Thr, Thr/Met and Met/Met showed 45.1%, 43.7% and 11.2% in cases, and 37.6%, 45.7% and 16.7% in died cases. Our study showed a lower survival rate in XRCC1 399 Arg/Arg genotype than Gln/Gln, and with a significant increased death risk (HR=1.69, 95%CI=1.07-2.78) (Figure 1). Similarly, those carrying XRCC3 Thr/ Thr genotype had a increased risk of death than Met/Met genotype, HR (95% CI) of 1.90 (1.12-3.41) (Figure 2). No significant association between XRCC1 Arg194Trp and XRCC1Arg280His gene polymorphism and ovarian cancer death risk.

Discussion

This hospital-based prospective studies shown an association between SNP involved in DNA repair genes and ovarian cancer. Previous study showed increased risk of death from cancer after chemotherapy, including gastric, cervical, colorectal, breast cancer and lung cancer (Metzger et al., 1998; Britten et al., 2000; Shirota et al., 2001; Lord et al., 2002; Rosell et al., 2002; Ren et al., 2010; Krivak et al., 2011). However, the evidence of DNA repair gene on ovarian cancer in lacking. Only several experimental studies reported the association between XRCC1 gene polymorphism and ovarian cancer risk, and the results are conflicting (Jakubowska et al., 2010; Kudo et al., 2012; Siddiqui-Jain et al., 2012;). A study reported inactivation of XRCC1 could influence the effect of platinum-based chemotherapeutics in the clinical setting. However, another did not find a significant association between them.

The XRCC1 gene encodes the XRCC1 protein, which complexes with three other DNA repair enzymes involved in the BER pathways, including DNA ligase III, DNA polymerase and poly (ADP-ribose) polymerase PARP (Stern et al., 2001). The XRCC1 codon 194 and codon 280 polymorphisms are located in the linker region that separates the PARP interacting domain (Kubota et al., 1996). The codon 399 polymorphism resides on the COOH-terminal side of the PARP interacting domain, within the BRCT1 domain, that are thought to mediate several protein-protein interactions (Masson et al., 1998). Amino acid substitutions in the BRCT domain and in the DNA polymerase β interacting domain in hamster is reported to disrupt the functionality of XRCC1 (Shen et al., 1998). The mutations of XRCC1 polymorphisms may increase the risk of cancers by impairing the interaction of XRCC1 with other enzymatic proteins and consequently altering DNA repair activity (Basso et al., 2007; Tudek, 2007), and resulted in carcinogenesis development. Our study found significant an odds ratio of codon 399 Gln allele for ovarian cancer, which strongly implicates that these polymorphisms may alter the normal protein function by encoding for a twisted protein (Tuimala et al., 2002), resulting in altered affinity to its interactive proteins suggesting an association with a deficiency in DNA repair capacity. This finding indicated the XRCC1 gene variants in the impairment of DNA repair mechanism and their consequent biological effect could induce the carcinogenesis.

XRCC3 is one of the Rad51-related proteins and functions through complex interactions with other relevant proteins to repair double-strand breaks and to maintain genome integrity in multiple phases of a homologous recombination (Brenneman et al., 2000). Although polymorphisms of this gene may results in reduced DNA repair capacity, the evidence of direct functional research is limited, and the results of epidemiologic studies in terms of the associations with cancer susceptibility have proved inconsistent (Matullo et al., 2001; Savas et al., 2004; Matullo et al., 2005; Zhang et al., 2005; Ryk et al., 2006; Lee et al., 2007). Our ddata supported this hypothesis, and we found this gene polymorphism would be interact with drinking and AFB1-exposure levels in the process of AFB1-induced ovarian cancer.

Overall, our study demonstrates that polymorphisms in DNA repair genes have a role in the susceptibility and

survival of ovarian cancer patients. The limitation of our study was a lower number of patients with survival data. In future, studies with a higher sample size are warranted.

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