RESEARCH ARTICLE

Prediction Value of XRCC 1 Gene Polymorphism on the Survival of Ovarian Cancer Treated by Adjuvant Chemotherapy

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Abstract

Objective: We conducted a prospective study to test the association between three amino acid substitution polymorphismic variants of DNA repair genes, XRCC1 (Arg194Trp), XRCC1(Arg280His) and XRCC1 (Arg399Gln), and clinical outcome of ovarian cancer patients undergoing adjuvant chemotherapy. Methods: 195 patients with primary advanced ovarian cancer and treated by adjuvant chemotherapy were included in our study. All were followed-up from Jan. 2007 to Jan. 2012. Genotyping of XRCC1 polymorphisms was conducted by TaqMan Gene Expression assays. Results: The XRCC1 194 Trp/Trp genotype conferred a significant risk of death from ovarian cancer when compared with Arg/Arg (HR=1.56, 95%CI=1.04-3.15). Similarly, those carrying the XRCC1 399 Gln/Gln genotype had a increased risk of death as compared to the XRCC1 399Arg/Arg genotype with an HR (95% CI) of 1.98 (1.09-3.93). Conclusion: This study is the first to provide evidence that XRCC1 gene polymorphisms would well be useful as surrogate markers of clinical outcome in ovarian cancer cases undergoing adjuvant chemotherapy.

Keywords: Ovarian cancer - XRCC1 - adjuvant chemotherapy - polymorphism

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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 the ovarian cancer patients are diagnosed at an advanced stage (Hogberg et al., 2001). Several regimens of combination chemotherapeutic therapy have been introduced to treat patients with advanced ovarian cancer (Hogberg et al., 2001), Currently, the initial surgery followed by adjuvant chemotherapy is a main methods for ovarian cancer patients (Williams et al., 1989; Harries et al., 2001; Piccart et al., 2001).

As we know, the chemotherapeutic drugs cancer damage DNA directly, through intercalation and also by lipid peroxidation and the formation of by-products, such as reactive oxygen species (La et al., 1997; Weijl e tal., 1997). The in vitro and in vivo studies have shown associations between alteration in DNA repair and cell cycle control genes and/or proteins and sensitivity to a broad range of drugs and patient survival (Allan et al., 2004; Zhou et al., 2004; Simon et al., 2005). The single nucleotide polymorphisms (SNPs) in gene involved in DNA repair and cell cycle control can affect repair efficiency, increase cancer risk and significantly alter patient responses to cancer treatments (Allan et al., 2004; Price et al., 2004; Zhou et al., 2004; Zhou et al., 2004). XRCC1 is a base excision repair and single strand break repair protein that may play an important role in resistance to variety of DNA damaging agents. The polymorphisms of three SNPs, resulting in amino acid substitutions, were detected at codons 194 (Arg-Trp), 280 (Arg-His) and 399 (Arg-Gln). Most studies report an increased risk of death from cancer among patients with 194Trp allele (Cui et al., 2012). The 399 polymorphism was reported to be associated with the prognosis of a number of cancers, although results have been inconsistent (Liang et al., 2010; Liu et al., 2011; Cui et al., 2012; Liao et al., 2012). Few studies have investigated the association between the XRCC1 280His allele and risk of death from cancers.

Therefore, in this present study, we conducted a prospective study to test the association between three amino acid substitution variants of DNA repair genes, XRCC1 (Arg194Trp), XRCC1(Arg280His) and XRCC1 (Arg399Gln) polymorphisms and the clinical outcome ovarian cancer patients with adjuvant chemotherapy.

Materials and Methods

Patients

Patients included in our study had primary advanced ovarian cancer and treated by adjuvant chemotherapy. A total of 210 eligible cases were included between Jan. 2005 to Jan 2007. Among them, 195 patients were interviewed

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Table 1. Characteristics of Included Cases in Our Studies

Variables	Cases N=195	%
Median age(range), years	46.5(25.6-72.3)	
<40	30	15.4
40-60	113	57.9
>60	52	26.7
Surgical stage		
I	16	8.2
II	21	10.8
III	139	71.3
IV	19	9.7
Optimal debulking operation as primary operation		
No	62	31.8
Yes	133	68.2
Histological subtype		
Serous adenocarcinoma	109	55.9
Endometrioid adenocarcinoma	23	11.8
Clear cell carcinoma	34	17.4
Mucinous adenocarcinoma	12	6.2
Other adenocarcinomas	17	8.7
Menopausal status		
Pre	63	32.3
Post	132	67.7

with a participation rate of 92.9%. After patients provided informed consent, every patient required to provide 5 ml bloods. All the patients were followed every 2 months until death or the end of follow-up. Survival time was calculated from the date of diagnosis to the date of death or the end of follow-up. All the 195 patients were followed-up from Jan. 2007 to Jan. 2012.

Adjuvant chemotherapy

The treatment program consisted of bleomycin 15 mg/day on days 1 to 3 as an intravenous (IV) infusion in 1000 ml normal saline for 24 h. Etoposide was given at a dose of 100 mg/m^2/day IV on day 1 to 3 for 2 h and cisplatin 20 mg/m^2/day IV on days 1 to 5 for 1 h. The cycles were repeated every 3 weeks.

Genotyping

Genotyping of 3 SNPs of XRCC1 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 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 previous studies (Butkiewicz et al., 2011). We used replicates for 10% samples for quality control.

Statistical analysis

Statistical analysis was performed by using the STATA statistical package (version 10.0, STATA, College Station, TX). The descriptive data for the major characteristics of study groups are expressed as mean and percent. Pearson's 2×2 χ^2-test (gender) and independent sample t-test(mean age) were used for analysis the differences of several qualitative and quantitative data. The primary death of ovarian cancer was defined as the failure event and the time of survival was the time between diagnosis and death. The cause of death was defined by specialists based on the clinical documents and reports by patient's family members. If patient died or 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 outcome for the study was overall survival, which was estimated using the Kaplan-Meir method (Figure 1 and Figure 2). In the multivariate analysis, a logistic regression model was applied to identify independent predictors associated with response to chemotherapy, and Cox's proportional hazard regression model was taken for the survival analysis. The analysis was adjusted for age, surgical stage, menopausal status and histological subtype. The hazard ratio (HR) and 95% confidence interval (CI) were also estimated. A cut-off P value of 0.05 was adopted for all the statistical analysis. Statistical significance was defined as a 2-sided P-value of less than 0.05.

Table 2. Associations Between XRCC1 Gene Polymorphisms and Survival of Ovarian Cancer Treated by Adjuvant Chemotherapy

Genotype	Patients N=195	%	Deaths N=114	%	Survival time (months)	HR(95% CI)[1]	P
XRCC1 Arg194Trp							
Arg/Arg	130	66.7	69	60.4	34.7	-	-
Arg/Trp	42	21.4	27	23.5	32.5	1.23(0.72-2.41)	0.51
Trp/Trp	23	11.9	18	16.1	26.2	1.56(1.04-3.15)	<0.05
XRCC1Arg280His							
Arg/Arg	141	72.4	79	68.9	35.2	-	-
Arg/His	33	16.7	20	17.2	30.6	1.13(0.61-2.43)	0.39
His/ His	21	10.9	16	13.9	39.1	1.46(0.72-3.01)	0.37
XRCC1Arg399Gln							
Arg/Arg	86	44.3	41	35.7	36.1	-	-
Arg/Gln	83	42.4	50	44.1	33.0	1.27(0.74-2.28)	0.36
Gln/ Gln	26	13.3	23	20.2	25.5	1.98(1.09-3.93)	<0.05

[1]Adjusted for age, surgical stage, menopausal status and histological subtype

Results

All the 195 patients were followed-up until Jan. 2012. The median follow-up time was 30.1 months, and 114 patients died during the five years follow-up. The clinical characteristics of cases showed in Table 1. The median age of included cases was 46.5(25.6-72.3) years, and about 58% of the patients were at the range of 40-60 years old. More than 50% of patients received optimal debulking operation as primary operation. 158 patients (81%) were at the stage of III and IV, and 109 patients (55.9%) were serous adenocarcinoma. 132 patients (67.7) were post menopausal status when diagnosis. The higher stage of ovarian cancer was likely to increase the risk of death from ovarian cancer [HR(95% CI)=2.21(1.23-4.57) for stage III and HR(95% CI)=2.68(0.53-6.13) for stage IV, data not shown].

The frequencies of XRCC1 Arg194Trp, Arg280His and Arg399Gln genotypes in ovarian cancer cases were showed in Table 2. Our study showed XRCC1 194 Trp/Trp genotype had a significant risk of death from ovarian cancer when compared with individuals with Arg/Arg, and with a significant increased death risk (HR=1.56, 95%CI=1.04-3.15) (Figure 1). Similarly, those carrying

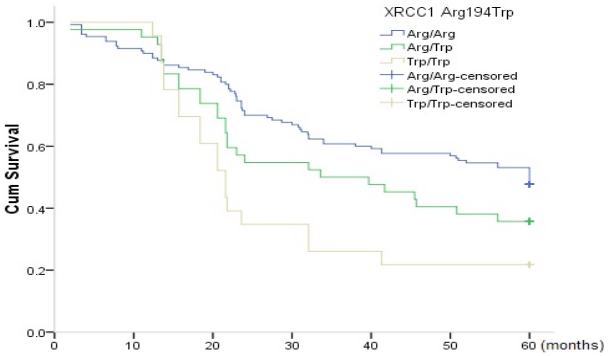

Figure 1. Kaplan-Meier Estimates of Overall Survival with XRCC1Arg194Trp Polymorphism

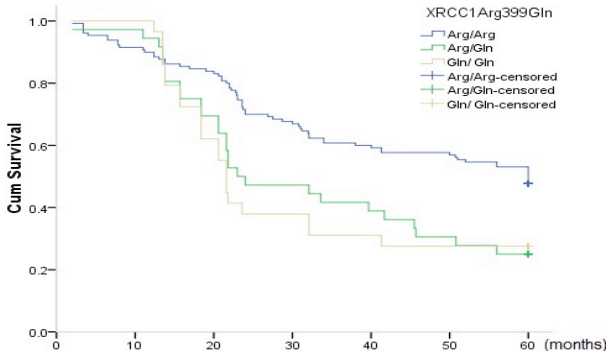

Figure 2. Kaplan-Meier Estimates of Overall Survival with XRCC1Arg399Gln Polymorphism

XRCC1 399 Gln/ Gln genotype had a increased risk of death than XRCC1 399Arg/Arg genotype, HR (95% CI) of 1.98 (1.09-3.93) (Figure 2). However, no significant association was found between association of XRCC1Arg280His gene polymorphism and ovarian cancer death risk.

Discussion

To the best of our knowledge, few studies have investigated the role of polymorphism in DNA-repair genes for ovarian cancer patients treated by adjuvant chemotherapy. In this prospective study, we found polymorphisms in the inactive polymorphism of the DNA-repair genes associated with an elevated risk of death in ovarian cancer patients. In addition, a gene-dosage effect was found for ovarian cancer. These finding suggest that those genes involved in different DNA repair pathways may be associated with overall survival.

Previous study showed increased risk of death from cancer after chemotherapy, including gastric, cervical, colorectal, breast cancer and ovarian cancer (Britten et al., 2000; Shirota et al., 2001; Ren et al., 2010; Krivak et al., 2011). However, the evidence of DNA repair gene on ovarian cancer patients with adjuvant chemotherapy in lacking. Several experimental studies reported the association between XRCC1 gene polymorphism and ovarian cancer risk, and the results are conflicting (Jakubowska et al., 2010; Siddiqui-Jain et al., 2012). Two studies conducted in Korea and Russian reported the association of XRCC1 Arg194Trp and XRCC1Arg399Gln gene polymorphisms with survival of ovarian cancer with chemotherapy (Kim et al., 2009; Khrunin et al., 2010). However, both of them did not found a significant association between these two genes and survival of ovarian cancer. Our results suggested XRCC1 194 Trp/Trp and XRCC1 399Arg/Arg genotypes had a significant risk of death from ovarian cancer among patients with adjuvant chemotherapy when compared with individuals with Arg/Arg.

It is still unclear how the change of amino acid at codon 194 and 399 of the XRCC1 gene polymorphism influences clinical outcome to chemotherapy in ovarian cancer patients. One possible explanation is the enhancement of DNA repair capacity. XRCC1 is thought to be involved in DNA single-strand break repair (Brem et al., 2005), and it also plays an important role in the BER pathway (Nazarkina et al., 2007). An SNP in codon 194 and 399 was considered to be related to the DNA repair and was likely to exhibit an effect on the protein function (Savas et al., 2004). Several studies have reported the association of XRCC1- 194 and 399 with the risk in non-small-cell lung cancer (NSCLC) (Kiyohara et al., 2006), colorectal cancer (Stern et al., 2006), gastric cancer (Huang et al., 2005) and prostate cancer (Hirata et al., 2007). Only two recent studies have reported on the pharmacogenetic role of XRCC1-399 and -194 polymorphisms in ovarian cancer patients (Kim et al., 2009; Khrunin et al., 2010). Our results contradict the current understanding of XRCC1 involvement in platinum compound-based chemotherapy. Since people in different parts of China have different living standards and lifestyles, the apparent inconsistency of these reports may be due to differences in environment factors. Since we did not collect the lifestyle and living standard information on these patients, we did not explore the association between these factors and XRCC1 polymorphisms. Further studies are warranted to explore their association.

In conclusion, this study is the first time to reported the XRCC1 gene polymorphisms would well be useful as a surrogate marker of clinical outcome in ovarian cancer with adjuvant chemotherapy. Further, prospective studies incorporating larger numbers of patients would be necessary to validate its predicted value.

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