RESEARCH ARTICLE

DNA Repair Gene Associated with Clinical Outcome of Epithelial Ovarian Cancer Treated with Platinum-based Chemotherapy

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Abstract

Objective: The nucleotide excision repair (NER) and base excision repair (BER) pathways, two DNA repair pathways, are related to platinum resistance in cancer treatment. In this paper, we studied the association between single nucleotide polymorphisms (SNPs) of involved genes and response to platinum-based chemotherapy in epithelial ovarian cancer. Method: Eight SNPs in XRCC1 (BER), XPC and XPD (NER) were assessed in 213 patients with epithelial ovarian cancer using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and primer-introduced restriction analysis-polymerase chain reaction (PIRA-PCR) techniques. Results: The median progression-free survival (PFS) of patients carrying the Lys/Lys and Lys/Gln+Gln/Gln genotype of the XPC Lys/Gln polymorphism were 25 and 12 months, respectively (*P*=0.039); and the mean overall survival (OS) of patients was 31.1 and 27.8 months, respectively (*P*=0.048). Cox's multivariate analysis suggested that patients with epithelial ovarian cancer with the Gln allele had an increased risk of death (HR=1.75; 95% CI=1.06-2.91) compared to those with the Lys/Lys genotype. There are no associations between the XPC PAT+/-, XRCC1 Arg194Trp, Arg280His, Arg399Gln, and XPD Asp312Asn, Lys751Gln polymorphisms and the survival of patients with epithelial ovarian cancer when treated with platinum-based chemotherapy. Conclusion: Our results indicated that the XPC Lys939Gln polymorphism may correlate with clinical outcome of patients with epithelial ovarian cancer when treated with platinum-based chemotherapy in Northern China.

Keywords: DNA repair gene - polymorphisms - epithelial ovarian cancer - platinum-based chemotherapy

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Introduction

Within all gynecological malignancies, ovarian cancer is the leading cause of death in Chinese women (Cao, 2004). In the initial, symptom-free stage of the disease, approximately two-thirds of patients will have developed peritoneal and lymph node metastasis. The overall 5-year survival rate of advanced ovarian cancer is roughly 20-25%. Therefore, cytoreductive surgery alone is an insufficient treatment, and the prognosis for the patient mainly depends on cancer responsiveness to subsequent chemotherapy.

The standard first-line chemotherapy for advanced ovarian cancer is a combination of platinum with either cyclophosphamide or paclitaxel administered after surgery (du et al., 2004). However, 30% of patients do not respond to platinum-based chemotherapy in the first attempt. Platinum agents are known to act through the formation of interstrand and intrastrand DNA cross-links, thereby changing the DNA conformation, which may affect the replication of DNA and inhibit its synthesis (Zambl et al., 1995). One of the mechanisms by which the tumor cells develop resistance to platinum agents is by the enhanced repair of bulky DNA adducts (Reed, 1998). Therefore, DNA repair capacity (DRC) is an important determinant of the resistance to platinum agents.

The four main DNA repair pathways are: base excision repair (BER), nucleotide excision repair (NER), double strand break repair (DSBR) and mismatch repair (MMR). The BER and NER pathways contribute to repairing a broad spectrum of chemical adducts, DNA damage induced by UV and ionizing radiation as well as intra- and interstrand crosslinks. In NER pathway, the XPC participates in DNA damage-induced DNA distortion recognition and DNA repair initiation by binding to the HR23B to form the XPC-HR23B complex (Masutani et al., 1994), while the DNA helicase XPD takes part in the unwinding of DNA and formation of a complex with TFIIH for DNA repair. The XRCC1 genes in the BER pathway act as a scaffold in the removal of adducts through both single-strand break repair and base excision repair, and in the repair of other types of cisplatin-induced damage,

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Table 1. PCR Conditions for XRCC1, XPC, and XPD Restriction Fragment Length Polymorphisms

Polymorphism	Primer sequence	Product size (bp)	Annealing temp/ restriction enzyme/allele sizes	rs
XRCC1 Arg194Trp (C/T) Exon 6	F: GCCCCGTCCCAGGTA R: AGCCCCAAGACCCTTTCACT	491	59.1°C/MspI/ C=293, 178, 20 T=391, 178	1799782
XRCC1 Arg280His (G/A) Exon 9	F: CCAGTGGTGCTAACCTAATC R: CACTCAGCACCACTACCACA	201	58.4°C/RasI/ G=145, 56 A=201	25489
XRCC1 Arg399Gln (G/A) Exon 10	F: CCCCAAGTACAGCCAGGTC R: TGTCCCGCTCCTCTCAGTAG	242	62.8°C/MspI/ G=148, 94 A=242	25487
XPC Ala499Val (C/T) Exon 8	F: TAAGGACCCAAGCTTGCCCG R: CCCACTTTTCCTCCTGCTCACAG	152	63°C/ SacII/ C=131, 21 T=152	2228000
XPC PAT+/- Intron 9	F: TAGCACCCAGCAGTCAAAG R: TGTGAATGTGCTTAATGCTG	+: 344 –: 266	60°C	
XPC Lys939Gln (A/C) Exon 15	F: GGAGGTGGACTCTCTTCTGATG R: TAGATCCCAGCAGATGACC	765	64°C/ PvuII/ C=582, 183 A=765	2228001
XPD Asp312Asn (G/A) Exon 10	F:CTGTTGGTGGGTGCCCGTATCTG TTG GTCT R:TAATATCGGGGCTCACCCTGCAG CACTTCCT	751	69°C/StyI/ A=474, 244, 33 G=507, 244	1799793
XPD Lys751Gln (A/C) Exon 23	F: GCCCGCT CTGGATTATACG R: CTATCATCTCCTGGC CCCC	436	63°C/ PstI/ C=227, 146, 63 A=290, 146	13181

such as double-strand breaks, through a nonhomologous end-joining pathway (Lindahl et al., 1999;Weaver et al., 2005). In vitro and in vivo studies have demonstrated that those genes in the NER and BER pathway are involved in the pharmacokinetics of platinum-based drugs and platinum resistance of cancer patients (Rosell et al., 2003; Azuma et al., 2007; Walsh et al., 2008).

Single nucleotide polymorphisms (SNPs) in any of the NER and BER genes may modulate DRC and contribute to individual variations in chemotherapy response (Yu et al., 2008; Kalikali et al., 2009; Sun et al., 2009; Fleming et al., 2012). Based on previous reports, we chose 8 single nucleotide polymorphisms (SNPs) of the XPC, XPD, XRCC1 genes (Saldivar et al., 2007; Qiu et al., 2008; Sun et al., 2009; Ji et al., 2012) and investigated the association between these SNPs and the response to platinum-based chemotherapy in patients with epithelial ovarian cancer.

Materials and Methods

Study population

The study included 213 patients who had presented for treatment of ovarian cancer to the Fourth Affiliated Hospital, Hebei Medical University, between 2002 and 2008. Eligibility criteria for this cohort included newly diagnosed, histologically confirmed primary epithelial ovarian cancer in women of any age and Han nationality. Patients were excluded from this study if they had neoadjuvant chemotherapy, any chemotherapy before surgical staging, concurrent primary neoplasms, or benign ovarian disease. The mean age of patients was 54 years (range 22-75 years). All patients had been evaluated according to the FIGO surgical staging system. Patients with early-stage cancer (FIGO stage IB-IIC) received 3 to 5 cycles of platinum-based combination chemotherapy after cytoreductive surgery; the other patients with advanced-stage cancer (FIGO stage IIIA-IV) received 6 to 9 cycles. Optimal debulking surgery was defined a maximal residual tumor diameter of 1 cm or less, whereas a maximal residual tumor diameter more than 1 cm meant suboptimal debulking surgery. The first-line chemotherapy protocol included platinum compounds 1 to 2 weeks after surgery, cisplatin (75 mg/m^2) or carboplatin (AUC 5, Calvert's formula) and cyclophosphamide (700 mg/m^2) or paclitaxel (175 mg/m^2) were administered intravenously (IV) every 3-4 weeks for 6 cycles. Both overall survival (OS) and progression-free survival (PFS) were used to evaluate the survival status of patients. The study was approved by the Ethics Committee of Hebei Cancer Institute, and informed consent was obtained from all recruited subjects.

DNA extraction

Venous blood (5 ml) from each subject was drawn into Vacutainer tubes containing EDTA and stored at 4°C. After collection, genomic DNA was extracted within one week by proteinase K (Merck, Darmstadt, Germany) digestion followed by a desalting procedure, according to the method published by Miller et al. (1988).

Determination of the Genotypes

The 8 SNPs, which included Arg194Trp, Arg280His and Arg399Gln in the XRCC1 gene, Ala499Val, Lys939Gln and PAT+/- in the XPC gene, and Asp312Asn and Lys751Gln in the XPD gene, were genotyped using the restriction fragment length polymorphism (RFLP) and the primer introduced restriction analysis (PIRA) method. Additional details, including the location of SNPs in the respective genes, the PCR conditions and restriction enzyme with product sizes are presented

in Table 1. Briefly, the DNA sequence containing the relevant polymorphic site was amplified by polymerase chain reaction (PCR), and the product was digested with an appropriate restriction enzyme that cleaves only 1 of the 2 alleles. The digests were then subjected to gel electrophoresis and visualized by ethidium bromide staining. The genotype of the XPC PAT+/- [PAT: poly (AT)] polymorphism was determined by primer-introduced restriction analysis-polymerase chain reaction (PIRA-PCR). For a negative control, distilled water, instead of DNA in the reaction system, was used in each PCR plate. For 10% of the samples, the PCR reactions were repeated for quality control.

Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software package (SPSS Company, Chicago, IL, USA). Survival analyses were performed using the Kaplan–Meier analysis with log-rank and Breslow test. The association of each SNP with the risk of recurrence and death was analyzed by the Cox proportional hazard model, adjusting for age, stage, grade, tumor residual, and histology. A probability level of 5% was considered significant.

Table 2. The Association Between Clinical Characteristics and Treatment Outcome in Ovarian Cancer Patients Treated with Platinum-based Chemotherapy

Characteristics	Survival n (%)		*P*-value	OR	95% CI
	Alive	Dead			
Age					
<50 years	53(76.8)	16(23.2)		Reference	
≥50 years	80(55.6)	64(44.4)	**0.003***	2.33	**1.35-4.03**
FIGO stage					
I-II	55(80.9)	13(19.1)		Reference	
III-IV	78 (53.8)	67(46.2)	**<0.01**	3.20	**1.76-5.80**
Grade					
1	17(85.0)	3(15.0)		Reference	
2	70(93.3)	5(6.7)	0.252	0.43	0.10-1.81
3	46(39.0)	72(61.0)	**0.003**	5.91	**1.86-18.78**
tumor residual size					
0	38(88.4)	5(11.6)		Reference	
<1cm	79 (59.4)	54(40.6)	**0.002**	4.37	**1.75-10.93**
>1cm	16(43.2)	21(56.8)	**<0.01**	7.35	**2.77-19.51**
Pathology					
serous	74(62.2)	45(37.8)		Reference	
mucinous	16(76.2)	5(23.8)	0.310	0.62	0.25-1.56
endometrioid	26(57.8)	19(42.2)	0.465	1.22	0.71-2.09
undifferentiated	17(60.7)	11(39.3)	0.566	1.21	0.63-2.35

* bold values are significant

Results

Patients' characteristics

Among all patients, 140 (65.7%) responded to the first-line therapy (median time of recurrence was 35.5 month), whereas 73 (34.3%) did not respond to the therapy (median time of recurrence was 4 months). The distribution of 8 SNP genotype frequencies did not significantly deviate from that expected for a Hardy-Weinberg equilibrium (all *P* values > 0.05). The clinical characteristics of patients with epithelial ovarian cancer and their relationship to treatment outcome are listed in Table 2. Table 3 illustrates the link between the clinical characteristics of patients and the frequencies of 8 SNP genotypes. For the XPC Ala499Val polymorphism, the frequency of the Ala/Ala genotype is significantly higher in stage III-IV patients than in stage I-II patients (*P*=0.03). For the XPD Asp312Asn polymorphism, there is a borderline significance between the distribution of genotype frequency and the histological type of patients (*P*=0.05).

Association between polymorphisms and the clinical outcome of patients treated with platinum-based chemotherapy

XPC: The median PFS of patients carrying the Lys/Lys and Lys/Gln+Gln/Gln genotype of the XPC Lys/Gln polymorphism was 25 and 12 months, respectively; and the mean OS of those patients was 31.1 and 27.8 months, respectively. Survival analysis showed that the XPC Lys/Gln polymorphism was associated with prognosis of epithelial ovarian cancer patients (Figure 1A and 1B; Table 4). Compared with the Lys/Lys genotype, patients carrying the Lys/Gln+Gln/Gln genotype had a shorter median PFS and median OS time. Kaplan-Meier plots illustrate the differences in PFS (Figure 1A; *P*=0.039)

Table 3. Clinical Characteristics of Ovarian Cancer Patients Stratified by Genotype of the 8 SNPs

Genotype	Age		*P*	FIGO stage		*P*	Grade			*P*	Residual disease			*P*	Pathology				*P*
	<50	≥50		I-II	III-IV		1-2	1-3	1-4		None	≤1cm	>1cm		Serous	Mucinous	Endometrioid	Others	
XRCC1(Arg194Trp)																			
Arg/Arg	31(31.6)	67(68.4)	0.88	32(32.7)	66(67.3)	0.88	8(8.2)	34(34.7)	56(57.1)	0.84	12(12.3)	20(20.4)	66(67.3)	0.38	55(56.1)	11(11.2)	22(22.4)	10(10.3)	0.65
Arg/Trp+Trp/Trp	38(33.0)	77(67.0)		36(31.3)	79(68.7)		12(10.4)	41(35.7)	62(53.9)		8(7.0)	28(24.3)	79(68.7)		64(55.7)	10(8.7)	23(20.0)	18(15.6)	
XRCC1(Arg280His)																			
Arg/Arg	53(31.7)	114(68.3)	0.72	53(31.7)	114(68.3)	0.91	19(11.4)	60(35.9)	88(52.7)	0.11	17(10.2)	36(21.5)	114(68.3)	0.72	99(59.2)	18(10.8)	31(18.6)	19(11.4)	0.09
Arg/His+His/His	16(34.8)	30(65.2)		15(32.6)	31(67.4)		1(2..2)	15(32.6)	30(65.2)		3(6.5)	12(26.1)	31(67.4)		20(43.5)	3(6.5)	14(30.4)	9(19.6)	
XRCC1(Arg399Gln)																			
Arg/Arg	36(32.7)	74(67.3)	0.92	35(31.8)	75(68.2)	0.97	12(10.9)	36(32.7)	62(56.4)	0.62	10(9.1)	27(24.5)	73(66.4)	0.78	57(51.8)	11(10.1)	26(23.6)	16(14.5)	0.65
Arg/Gln+ Gln/Gln	33(32.0)	70(68.0)		33(32.0)	70(68.0)		8(7.8)	39(37.9)	56(54.3)		10(9.7)	21(20.4)	72(69.9)		62(60.2)	10(9.7)	19(18.4)	12(11.7)	
XPC(Ala499Val)																			
Ala/Ala	36(34.0)	70(66.0)	0.66	26(24.5)	80(75.5)	0.03	8(7.6)	42(39.6)	56(52.8)	0.33	8(7.5)	20(18.9)	78(73.6)	0.23	57(53.8)	7(6.6)	26(24.5)	16(15.2)	0.25
Ala/Val+Val/Val	33(30.8)	74(69.2)		42(39.3)	65(60.7)		12(11.3)	33(30.8)	62(57.9)		12(11.2)	28(26.2)	67(62.6)		62(57.9)	14(13.1)	19(17.8)	12(11.2)	
XPC (PAT,+/-)																			
+/+	10(35.7)	18(64.3)	0.67	6(21.4)	22(78.6)	0.28	2(7.1)	9(32.2)	17(60.7)	0.87	1(3.6)	4(14.3)	23(82.1)	0.3	12(42.9)	2(7.1)	9(32.1)	5(17.9)	0.29
+/- + -/-	59(31.9)	126(68.1)		62(33.5)	123(66.5)		18(9.7)	66(35.7)	101(54.6)		19(10.3)	44(23.8)	122(65.9)		107(57.8)	19(10.3)	36(19.5)	23(12.4)	
XPC (Lys939Gln)																			
Lys/Lys	26(36.1)	46(63.9)	0.44	28(38.9)	44(61.1)	0.12	9(12.5)	25(34.7)	38(52.8)	0.53	10(13.9)	16(22.2)	46(63.9)	0.27	43(59.7)	9(12.5)	11(15.3)	9(12.5)	0.4
Lys/Gln+Gln/Gln	43(30.5)	98(69.5)		40(28.4)	101(71.6)		11(7.8)	50(35.5)	80(56.7)		10(7.1)	32(22.7)	99(70.2)		76(53.9)	12(8.5)	34(24.1)	19(13.5)	
XPD (Asp312Asn)																			
Asp/Asp	52(31.9)	111(68.1)	0.86	52(31.9)	111(68.1)	0.99	16(9.8)	59(36.2)	88(54.0)	0.81	18(11.0)	34(20.9)	111(68.1)	0.24	93(57.1)	11(6.7)	38(23.3)	21(12.9)	0.05
Asp/Asn+Asn/Asn	17(34.0)	33(66.0)		16(32.0)	34(68.0)		4(8.0)	16(32.0)	30(60.0)		2(4.0)	14(28.0)	34(68.0)		26(52.0)	10(20.0)	7(14.0)	7(14.0)	
XPD (Lys751Gln)																			
Lys/Lys	55(33.1)	111(66.9)	0.73	53(31.9)	113(68.1)	1	15(9.0)	58(34.9)	93(56.1)	0.88	17(10.2)	36(21.7)	113(68.1)	0.7	95(57.2)	13(7.8)	38(22.9)	20(12.1)	0.16
Lys/Gln+Gln/Gln	14(29.8)	33(70.2)		15(31.9)	32(68.1)		5(10.6)	17(36.2)	25(53.2)		3(6.4)	12(25.5)	32(68.1)		24(51.1)	8(17.0)	7(14.9)	8(17.0)	

Table 4. Gene Polymorphisms and Clinical Outcome in Ovarian Cancer Patients Treated with Platinum-based Chemotherapy

Genotype	Recurrence		HR* (95%CI) †	Survival		HR* (95%CI) †
	No n (%)	Yes n (%)		Yes n (%)	No n (%)	
XRCC1 (Arg194Trp)						
Arg/Arg	30(30.6)	68(69 d.4) [d]	Reference	60(61.2)	38(38.8)	Reference
Arg/Trp	33(35.5)	60(64.5)	0.91(0.64-1.31)	59(63.4)	34(42.5)	0.96(0.60-1.54)
Trp/Trp	7(31.8)	15(68.2)	1.41(0.79-2.51)	14(63.6)	8(36.4)	1.80(0.80-4.02)
Arg/Trp+Trp/Trp	40(34.8)	75(65.2)	1.01(0.68-1.48)	73(63.5)	42(36.5)	1.05(0.67-1.64)
XRCC1 (Arg280His)						
Arg/Arg	55(32.9)	112(67.1)	Reference	105(62.9)	62(37.1)	Reference
Arg/His	14(32.6)	29(67.4)	1.05(0.69-1.60)	26(60.5)	17(39.5)	0.92(0.54-1.60)
His/His	0(0)	2(100.0)	3.88(0.91-16.66)	1(50.0)	1(50.0)	3.45(0.44-26.76)
Arg/His+His/His	15(32.6)	31(67.4)	1.13(0.73-1.74)	28(60.9)	18(39.1)	0.96(0.56-1.63)
XRCC1 (Arg399Gln)						
Arg/Arg	39(35.5)	71(64.5)	Reference	70(63.6)	40(36.4)	Reference
Arg/Gln	25(28.4)	63(71.6)	1.22(0.85-1.74)	52(59.1)	36(40.9)	1.08(0.67-1.73)
Gln/Gln	6(40.0)	9(60.0)	1.01(0.49-2.05)	11(73.3)	4(26.7)	0.58(0.20-1.67)
Arg/Gln + Gln/Gln	31(30.1)	72(69.9)	1.12(0.76-1.65)	63(61.2)	40(38.8)	1.00(0.63-1.58)
XPC(Ala499Val)						
Ala/Ala	33(31.1)	73(68.9)	Reference	67(63.2)	39(36.8)	Reference
Ala/Val	26(34.2)	50(65.8)	0.84(0.58-1.23)	51(67.1)	25(32.9)	0.83(0.49-1.42)
Val/Val	10(33.3)	20(66.7)	0.81(0.48-1.37)	14(46.7)	16(53.3)	0.97(0.52-1.82)
Ala/Val+Val/Val	37(34.6)	70(65.4)	1.02(0.70-1.49)	66(61.7)	41(38.3)	0.88(0.55-1.42)
XPC (PAT,+/-)						
+/+	7(25.0)	21(75.0)	Reference	18(64.3)	10(35.7)	Reference
+/-	33(33.0)	67(67.0)	0.93(0.56-1.53)	57(57.0)	43(43.0)	1.43(0.71-2.90)
-/-	29(34.5)	55(65.5)	0.85(0.51-1.43)	57(67.9)	27(32.1)	0.98(0.46-2.06)
+/- + -/-	63(34.1)	122(65.9)	0.90(0.54-1.50)	115(62.2)	70(37.8)	1.22(0.62-2.41)
XPC (Lys939Gln)						
Lys/Lys	26(36.1)	46(63.9)	Reference	51(70.8)	21(29.2)	Reference
Lys/Gln	33(31.4)	72(68.6)	1.29(0.89-1.88)	59(56.2)	46(43.8)	1.87(1.11-3.15)[‡]
Gln/Gln	11(30.6)	25(69.4)	1.25(0.75-2.07)	23(63.9)	13(36.1)	1.40(0.68-2.86)
Lys/Gln+Gln/Gln	44(31.2)	97(68.8)	1.20(0.81-1.80)	82(58.2)	59(41.8)	1.75(1.06-2.91)[‡]
XPD (Asp312Asn)						
Asp/Asp	58(35.6)	105(64.4)	Reference	101(62.0)	62(38.0)	Reference
Asp/Asn	11(23.4)	36(76.6)	1.22(0.82-1.82)	29(61.7)	18(38.3)	1.11(0.64-1.94)
Asn/Asn	0(0)	2(100.0)	1.13(0.26-4.82)	2(100.0)	0(0)	
Asp/Asn+Asn/Asn	12(24.0)	38(76.0)	0.99(0.61-1.62)	32(64.0)	18(36.0)	1.07(0.62-1.87)
XPD (Lys751Gln)						
Lys/Lys	59(35.5)	107(64.5)	Reference	103(62.0)	63(38.0)	Reference
Lys/Gln	11(25.0)	33(75.0)	1.40(0.93-2.11)	27(61.4)	17(38.6)	1.53(0.87-2.68)
Gln/Gln	0(0)	3(100.0)	1.13(0.33-3.91)	3(100.0)	0(0)	
Lys/Gln+Gln/Gln	11(23.4)	36(76.6)	1.25(0.76-2.05)	30(63.8)	17(36.2)	1.41(0.80-2.50)

*H, Hazard Ratio; †Cox proportional hazard model was used for multivariate analysis and adjusted for age, stage, grade, tumor residual, and histology; ‡bold values are significant

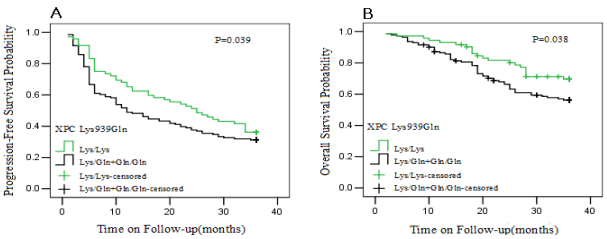

Figure 1. Kaplan–Meier Estimate of Overall Survival (A, B) in Patients Categorized by XPC939 Polymorphism

and OS (Figure 1B; *P*=0.048) distributions for patients categorized by XPC Lys/Gln polymorphisms. However, after adjusting for the prognostic factors (age, FIGO stage, grade, tumor residual size and histology), patients with the Lys/Gln+Gln/Gln genotype only had an increased risk of death (HR=1.75; 95% CI=1.06-2.91) compared with those carrying the Lys/Lys genotype (Table 4).

XRCC1 and XPD: There were no significant relationships between the genotype distributions of five SNPs (XRCC1 Arg194Trp, Arg280His, Arg399Gln and XPD Asp312Asn, Lys751Gln) and the clinical outcome of ovarian cancer patients treated with platinum-based chemotherapy. Compared with the genotypes of wild-type homozygous, the variant homozygous and heterozygous genotypes were not associated with disease progression or death (Table 4).

Discussion

Clinical cancer outcomes and responsiveness to platinum-based chemotherapy are attributable to some SNPs of the NER and BER pathway genes (Yu et al., 2008; Kalikali et al., 2009; Sun et al., 2009). However, reports on these polymorphisms and their effects on epithelial ovarian cancer are limited in the literature.

Previous studies showed that polymorphisms of Arg194Trp and Arg399Gln in the XRCC1 gene, PAT+/- in the XPC gene, and Asp312Asn and Lys751Gln in the XPD gene were not associated with the response rate of epithelial ovarian cancer to platinum-based chemotherapy (Saldivar et al., 2007; Kim et al., 2009). Our data also showed no significant link between genotype frequencies of these five polymorphisms and the clinical outcome of Chinese ovarian cancer patients treated with platinum-based chemotherapy. However, our study suggested that the XPC Lys/Gln polymorphism may be associated with the PFS and OS of ovarian cancer patients treated with platinum-based chemotherapy, i.e., patients carrying the Gln allele may have a shorter OS than those with the Lys/Lys genotype. To the best of our knowledge, this is the first study to assess genetic polymorphisms of Arg280His in the XRCC1 gene and Ala499Val and Lys939Gln in the XPC gene as predictive biomarkers of platinum-based chemotherapy response in EOC.

The XPC gene encodes a 125 kDa protein, which binds to HR23B to form the XPC-HR23B complex. The complex is involved in DNA damage recognition and DNA repair initiation in the NER pathway, a key pathway, which helps mediate resistance or sensitivity to platinum chemotherapeutic agents (Masutani et al., 1994). Polymorphisms in the coding and regulatory regions of the XPC gene may alter gene expression and thereby modulate the DNA repair function. The three most common polymorphisms are Ala499Val (C→T), PAT (-/+), and Lys939Gln (A→C), which have been associated with increased risks for many human malignancies. A recent meta-analysis study (Qiu et al., 2008) showed that, compared to their corresponding wild-type homozygous genotypes, the variant 939Gln homozygous genotype was a risk factor for developing lung cancer (OR=1.28, 95% CI=1.07-1.53), whereas the 499Val variant homozygous genotype was a risk factor for developing bladder cancer (OR=1.33, 95% CI=1.06-1.68). However, there were few studies about the association between three SNPs and platinum-based chemotherapy for cancer. Saldivar et al. (2007) reported that the polymorphism of XPC PAT (-/+) was not associated with responsiveness to platinum-based chemotherapy for ovarian cancer. However, a higher response rate was found in patients with advanced non-small cell lung cancer with the XPC PAT +/+ genotype compared to those that had the XPC PAT -/- genotype (Yuan et al., 2005). In this paper, Kaplan-Meier analysis showed that EOC patients carrying the 939Gln allele of the XPC Lys939Gln polymorphism may increase the risk of disease recurrence and death. Further, the Cox regression model adjusting clinical characteristics (include age, stage, grade, residual tumor size, and histology) analysis suggested that the survival rate in ovarian cancer patients with the 939Gln allele is lower than in those with the Lys/Lys genotype. However, no association was found between Ala499Val, PAT (-/+) polymorphisms and clinical outcomes in epithelial ovarian cancer treated with platinum-base chemotherapy. The Ala499Val, PAT (-/+), and Lys939Gln polymorphisms are located at exon 8, intron 9, and exon 15 of the XPC gene, respectively. The functions of these three polymorphisms are unclear, although some studies on the link between XPC protein expression and Lys939Gln polymorphism have been reported (Khan et al., 2000;Khan et al., 2002). Thus, our study was unable to unravel the mechanism of action by which Lys939Gln influences the response rate of EOC to platinum-based chemotherapy. Because our study samples were limited, more studies are needed to examine a greater sample size and breadth of cancers.

The XPD (also known as ERCC2) gene encodes for a DNA helicase, which is involved in the unwinding of DNA and forms a complex with transcription factor IIH during DNA repair. Mutations in XPD cause a severe but variable suppression of NER. Two nonsynonymous SNPs were described in the XPD gene and were located at codons 312 (exon 10 G→A, Asp→Asn) and 751 (exon 23 A→C, Lys→Gln). It is not known whether these polymorphisms have functional effects. However, it is suggested that the Lys/Lys genotype of the XPD Lys/Gln polymorphism is susceptible to X-ray-induced chromatid aberrations, while no effect has been noted on lymphocyte sister chromatid exchanges. The associations between XPD Asp312Asn and Lys751Gln SNPs and platinum-based chemotherapy have been reported in some cancers, including lung cancer (Giachino et al., 2007) and colorectal carcinoma(Park et al., 2001), but the results were inconsistent. Saldivar et al., (2007) showed that the carriers of at least one variant allele of the exon10 (Asp312Asn) SNP had a significantly reduced risk of death in epithelial ovarian cancer patients; the association was similar for exon23 (Lys751Gln) SNP. However, no association was found between these variant alleles and responsiveness to platinum-based chemotherapy. The results of our analysis on the XPD polymorphisms show that there are no associations between carriers of different genotypes and the clinical outcome of epithelial ovarian cancer treated with platinum-based chemotherapy in Chinese women.

The XRCC1 is an important gene in the base excision repair pathway. However, the study showed that the XRCC1 protein physically interacts with ligase III and poly (ADP-ribose) polymerase, which is thought to act as a scaffold in the removal of adducts through both single-strand break repair and base excision repair (Lindahl et al., 1999). The XRCC1 protein also acts in the repair of other types of cisplatin-induced damage, including double-strand breaks, through a nonhomologous end-joining pathway, which is an alternative to the predominant ATM-XRCC4-DNA ligase IV pathway (Weaver et al., 2005). More than 60 SNPs were identified within the human population. Considering the amino acid substitutions and relatively high frequency, the Arg194Trp (R194W), Arg280His (R280H) and Arg399Gln (R399Q) SNPs have been studied more extensively. XRCC1 Arg194Trp and Arg399Gln may be associated with clinical responses to platinum-based chemotherapy in advanced non-small cell lung cancer, but the results are inconsistent (Kalikali et al., 2009; Sun et al., 2009). Kim et al. (2009) showed that among the Korean population, XRCC1 Arg194Trp and Arg399Gln polymorphisms may not affect drug response, toxicity and survival in patients with EOC who received taxane- and platinum-based chemotherapy after surgery (Yu et al., 2008). Our study results also suggested that

among the Chinese population, the XRCC1 Arg194Trp, Arg280His and Arg399Gln polymorphisms were not associated with survival rates in EOC patients treated with platinum-based chemotherapy.

Our study included a very heterogeneous population of patients. For example, in our sample, patients had early stage (31.9%) and late stage (68.1%) cancers of various grades and histologies. However, the study's results were still useful in evaluating the clinical outcomes of patients who were treated with platinum-based chemotherapy. Firstly, statistical analysis demonstrated a significant relationship between the genotype distributions and the FIGO stage of patients with the XPC (Ala499Val) polymorphism. Secondly, the association between the Gln allele of the XPC Lys939Gln polymorphism and the risk of disease recurrence and death was confirmed using both univariate and multivariate analysis.

In conclusion, our study indicated that the XPC Lys939Gln polymorphisms may correlate to the clinical outcome of EOC patients treated with platinum-based chemotherapy. If confirmed in larger samples and further perspective studies, the XPC SNP might serve as biomarkers for EOC patient's personalized chemotherapy of platinum-based anticancer drugs. Therefore, evaluation of the genetic polymorphisms, especially those on DNA repair gene, in clinical outcome of cancer patients may help us to identify the individuals at higher risk of developing resistance to platinum-based chemotherapy.

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