Association between excision repair cross-complementation group 1 polymorphism and clinical outcome of platinum-based chemotherapy in patients with epithelial ovarian cancer

Sokbom Kang¹, Woong Ju²,
Jae Weon Kim^{3,4,5,6,7}, Noh-Hyun Park^{3,4},
Yong-Sang Song^{3,4}, Seung Cheol Kim^{2,6},
Sang-Yoon Park^{1,6}, Soon-Beom Kang^{3,4,6}
and Hyo-Pyo Lee^{3,4}

¹Research Institute and Hospital
National Cancer Center

Goyang, Gyeonggi-do 410-769, Korea

²Department of Obstetrics and Gynecology
Ewha Womans University
Seoul 158-710, Korea

³Department of Obstetrics and Gynecology

⁴Cancer Research Institute

⁵Human Genome Research Institute

Seoul National University

Seoul 151-742, Korea

⁶Clinical Research Center for Solid Tumors
Goyang, Gyeonggi-do 410-769, Korea

⁷Corresponding Author: Tel, 82-2-2072-3511;
Fax, 82-2-762-3599; E-mail, kjwksh@snu.ac.kr

Accepted 8 December 2005

Abbreviations: CI, confidence interval; EOC, epithelial ovarian cancer; *ERCC1*, excision repair cross-complementation group 1; OR, odds ratio

Abstract

ERCC1 is a DNA repair gene and has been associated with resistance to DNA damaging agents. In this study we hypothesized that a polymorphism of *ERCC1* Asn118Asn (C→T) might affect the platinum-resistance of epithelial ovarian cancer patients to platinum-taxane chemotherapy administered post-operatively. Using the SNaPshot assay, we assessed this polymorphism in *ERCC1* in 60 ovarian cancer patients. Platinum-resistance was defined as progression on platinum-based chemotherapy or recurrence within 6 months of completing therapy. Although not significant, platinum-resistance was less frequently observed in patients with the C/T + T/T genotype ($P = 0.064$). Multivariate analysis showed that the C/T + T/T genotypes constituted an independent predictive factor of reduced risk of pla-

tinum-resistance in ovarian cancer (odds ratio 0.17, 95% confidence interval 0.04-0.74, $P = 0.018$, Fisher's exact test). No significant correlation was observed between overall survival and the *ERCC1* polymorphism. Our results suggest that genotyping of the *ERCC1* polymorphism Asn118Asn may be useful for predicting the platinum-resistance of epithelial ovarian cancer patients. However, these findings require prospective confirmation.

Keywords: DNA repair; drug resistance, neoplasm; *ERCC1* protein, human; ovarian neoplasms; polymorphism, single nucleotide

Introduction

Epithelial ovarian cancer (EOC) is a leading cause of death from gynecological malignancies and the fifth most common malignancy among Korean women (Gloeckler *et al.*, 2003). It is well known that EOC patients who initially respond to platinum-based chemotherapy can achieve objective tumor regression in response to the same or similar chemotherapy programs if the disease subsequently recurs (Markman *et al.*, 1991). Because of the relevance of this biological feature and because it can significantly influence the choice of therapy, platinum-resistance was frequently defined as progression on platinum-based chemotherapy or recurrence within 6 months of completing therapy. Platinum agents are known to act through via the formation of interstrand and intrastrand DNA cross-links, which inhibit DNA replication and/or transcription (Zamble *et al.*, 1995). Thus, DNA repair capacity is clearly an important determinant of resistance to platinum agents.

ERCC1 (excision repair cross-complementation group 1) is one of the genes that have been most extensively investigated with regard to the association between DNA repair capacity and platinum-resistance. A number of studies have suggested that *ERCC1* mRNA levels are significantly correlated with cisplatin resistance in various human cancer cell lines and in a xenograft model (Britten *et al.*, 2000; Selvakumaran *et al.*, 2003). Moreover, it was reported that the expression levels of *ERCC1* in EOC tumor tissue are significantly associated with a response to platinum-based therapy (Dabholkar *et al.*, 1992). Similarly, the mRNA levels of *ERCC1*

were reported to be significantly associated with the response to cisplatin and fluorouracil chemotherapy in gastric cancer patients (Metzger *et al.*, 1998).

Recently, it was noted that the codon 118 C/T polymorphism (rs11615) is associated with differential mRNA levels (Yu *et al.*, 2000; Park *et al.*, 2003) and has been found to be associated with shorter overall survival for advanced colorectal cancer patients treated with platinum-based chemotherapy (Park *et al.*, 2003). So, we hypothesized that this polymorphism in *ERCC1* might affect the platinum-resistance of EOC patients. In this study, we retrospectively explored the possible association between this polymorphism in *ERCC1* and resistance to first-line platinum-taxane chemotherapy in EOC patients.

Materials and Methods

Patients population and clinical data

The present study involved 60 EOC Korean patients

who were treated with platinum-based chemotherapy between April, 1996 and July, 2002 at Seoul National University Hospital. Eligible patients had a histologic diagnosis of EOC with adequate surgical staging, and received first-line platinum-taxane chemotherapy after surgery. All research was conducted with informed patient consent and the approval of the Institutional Review Board of Seoul National University Hospital. For genotyping, 10 ml of peripheral blood was obtained from all subjects. All patients were staged according to the 1987 criteria recommended by the International Federation of Gynecology and Obstetrics (FIGO). Histologic slides were reviewed by two pathologists without any knowledge of the clinical data. After primary debulking surgery, the patients received 6 cycles of chemotherapy consisting of a standard regimen of carboplatin (AUC 5 every 3 weeks) and paclitaxel (175 mg/m² every 3 weeks). All clinical data were assessed without knowledge of the genotype status. Platinum-resistance was defined as progression on platinum-based chemotherapy or recurrence within 6

Table 1. Clinical characteristics and *ERCC1* polymorphisms according to platinum-resistance (PFI < 6 months).

	Resistant (PFI < 6 months) (n = 20)	Not resistant (PFI ≥ 6 months) (n = 40)	Univariate		Multivariate [†]	
			P*	OR	95% CI	P
Age						
Median	49	52	0.298			
Range	15-73	36-67				
Stage						
I-II	2 (10.0)	13 (32.5)	0.067	1.00	0.92-33.79	0.061
III-IV	18 (90.0)	27 (67.5)		5.58		
Histology						
Serous	15 (75.0)	23 (57.5)	0.258			
Non-serous	5 (25.0)	17 (42.5)				
Grade						
I	3 (15.0)	10 (25.0)	0.513			
II-III	17 (85.0)	30 (75.0)				
Residual mass						
Less than 2 cm	8 (40.0)	32 (80.0)	0.002	1.00	1.88-29.49	0.003
2 cm and over	12 (60.0)	8 (20.0)		7.44		
<i>ERCC1</i> Asn118Asn						
C/C	15 (75.0)	20 (50.0)	0.132 [‡]	1.00	0.05-0.99	0.049
C/T	5 (25.0)	16 (40.0)		0.23		
T/T	0 (0)	4 (10.0)		-		
C/T + T/T	5 (25.0)	20 (50.0)	0.064	0.17	0.04-0.74	0.018

OR, odds ratio; CI, confidence interval. *P-value was obtained using Fisher's exact test and Mann-Whitney test, [†]Logistic regression analysis was used for multivariate analysis, [‡]Linear association was confirmed, $P_{\text{trend}} = 0.047$.

months of completing therapy. Overall survival (OS) was defined as the time interval between the date of surgery and the date of death. The platinum-free interval (PFI) was defined as the interval between treatment commencement and the date when evidence of recurrence was obtained. The detailed characteristics of the case subjects are described in Table 1.

Genotyping

Genomic DNA was extracted using conventional methods. Genotypes were assayed using a single base primer extension assay and a SNaPSHOT assay kit (ABI). Briefly, the genomic region containing the SNP Asn118Asn in *ERCC1* was amplified by PCR using the 5-AGGAGGGAGGAGGTGTGG (forward) and 5-AACACAGGGTCCCACCAA (reverse) primer pairs. The PCR conditions used were 1 cycle for 10 min at 95°C, and then 30 cycles for 30 s at 95°C, 1 min at 65°C, 1 min at 72°C, followed by 1 cycle for 7 min at 72°C. After amplification, the PCR products were treated with 2.0 U of SAP (shrimp alkaline phosphatase) and 2.0 U of EXO I (exonuclease I) for 60 min at 37°C and for 15 min at 72°C to remove the unincorporated primers and nucleotides. The SNP genotyping primers for *ERCC1* Asn118Asn (5-AC-TGAAGTTCGTGCGCAA) were added to the purified PCR products with an appropriate amount of SNaPSHOT mix and cycled on a PCR machine for 25 cycles of 10 s at 96°C, 5 s at 50°C, 30 s at 60°C. A half u of SAP was added to the product, followed by incubation for 60 min at 37°C and then 15 min at 72°C. The final products were analyzed on an ABI 3700 automated sequence analyzer.

Statistical analysis

Fisher's exact test and the Mann-Whitney test were used to compare the genotypes and clinicopathological variables of resistant and non-resistant patients. Then, multivariate logistic regression analysis was performed to adjust for confounders and age. Kaplan-Meier curves were constructed in order to investigate survival difference between the wild-type and variant genotypes. Differences were tested for statistical significance using the log-rank test.

Results

A total of 60 patients with a median age of 50 yrs (range: 15-73 yrs) were evaluated in this study. The median follow-up period for these 60 patients was 33.8 months (range 3.1-60.8). The allele frequencies of the *ERCC1* gene among the case subjects were C, 0.76; T, 0.34, and these were in Hardy-Weinberg

Figure 1. Comparison of overall survival in the *ERCC1* codon 118 C/C genotype group and the C/T + T/T genotype group in the 60 ovarian cancer patients who received first-line postoperative platinum-taxane combination chemotherapy.

equilibrium. The frequency distribution of the genotypes and the clinicopathological characteristics of the patients are shown in Table 1.

No significant associations were observed between this polymorphism and any of the clinicopathological variables (age, tumor stage, tumor histology, tumor grade, size of residual mass). The strength of the association between platinum-resistance, as represented by a PFI of less than 6 months, and the *ERCC1* polymorphism is presented in Table 1. Although marginally significant, stage and the presence of variant genotypes (C/T or T/T) were both associated with platinum-resistance. Moreover, we observed a significant linear trend among these genotypes ($P_{\text{trend}} = 0.047$). Multivariate analysis showed that those with the C/T + T/T genotype were significantly associated with a reduced risk of platinum-resistance (OR 0.17, 95% CI 0.04-0.74).

OS was compared between those with C/C and those with C/T + T/T, but no significant difference in OS was observed between patients in these two genotype groups ($P = 0.622$, Log rank test; Figure 1). In addition, it was also observed that overall PFI was not significantly different for these two patient groups ($P = 0.268$, Log rank test).

Discussion

Optimal chemotherapeutic treatment would allow clinicians to maximize the benefits of cancer chemotherapy. Since successful first-line chemotherapy following initial surgery is crucial for a favorable outcome in ovarian cancer, many prognostic and predictive markers have been investigated to individualize treatment, maximize therapeutic effect,

and minimize treatment toxicity. In the present study, resistance to platinum-based chemotherapy is more frequently observed in ovarian cancer patients with the *ERCC1* C/C genotype.

The precise mechanism underlying the association between this polymorphism of *ERCC1* codon 118 with platinum-based chemotherapy is unclear. This mutation was a C→T transition, coding the same amino acid: asparagine. Previously, Yu *et al.* reported that this transition converts a common codon usage (AAC) to an infrequent codon usage (AAT), whereas frequency of use is reduced two-fold (1997). They also described that the ovarian cancer cells with codon 118 C→T polymorphism showed markedly reduced *ERCC1* mRNA compared with cells without C→T transition (Yu *et al.*, 2000). This could be possible explanation of the association between the *ERCC1* codon 118 C→T transition and the *ERCC1* activity. Since much evidence has been presented concerning the association between low *ERCC1* mRNA expression and increased platinum-sensitivity, those studies support our finding that EOC patients with the C/C genotype have a higher risk of platinum-resistance (Dabholkar *et al.*, 1992; Metzger *et al.*, 1998). However, the functional importance of *ERCC1* codon 118 polymorphism is still at debate. Recently, it was reported a trend toward higher intratumoral *ERCC1* mRNA levels with an increasing number of T alleles in a study of 31 advanced colorectal cancer patients (Stoehlmacher *et al.*, 2004). Therefore, this association between the *ERCC1* polymorphism and its mRNA levels needs further confirmation.

In our study, *ERCC1* codon 118 polymorphism was not associated with OS for advanced EOC patients. It is consistent with the result of recent study describing that the *ERCC1* codon 118 polymorphism was not associated with the overall survival of advanced NSCLC patients treated with platinum-based chemotherapy (Zhou *et al.*, 2004). However, there were a number of studies describing that this polymorphism of *ERCC1* is associated with the overall survival of cancer patients treated with chemotherapy. In NSCLC, the *ERCC1* codon 118 C/C genotype was reported to be associated with improved survival in patients with lung cancer (Ryu *et al.*, 2004). In colon tumors, the *ERCC1* codon 118 C/C genotype was associated with a favorable survival outcome (Stoehlmacher *et al.*, 2004).

This discrepancy might be due to insufficient sample size of the present study. However, it might be speculated that the conflicting data can be due to the diverse roles of platinum chemotherapy in the patients' hospital course. For patients who receive salvage chemotherapy, *i.e.*, patients who are at the end of their hospital course, response to chemo-

therapy has a crucial impact on survival. On the other hand, as in the case of EOC, other factors have a greater opportunity to interfere with survival outcome because patients receive platinum-based chemotherapy as their 1st line treatment.

Interestingly, although we observed a statistically significant relation between the C/C genotype and a PFI of less than 6 months, we found that overall PFI was not statistically different between those with C/C and those with C/T or T/T, which may be due to an association between this *ERCC1* polymorphism and resistance to platinum chemotherapy, but not with the maintenance of a prolonged disease-free state. Another possible explanation is that platinum-taxane synergism may interfere with the relationship between overall PFI and the *ERCC1* polymorphism.

The present study has several limitations. First, the small sample size of cases made it difficult to exclude the possible association between *ERCC1* polymorphism and OS of EOC patients. Second, since platinum-paclitaxel combination treatment is the standard treatment for managing ovarian cancer patients, the synergistic effect of paclitaxel may have biased our results. And finally, functional data supporting the association between the *ERCC1* polymorphism and its activity are still controversial and insufficient.

In conclusion, our findings can be the one of preliminary data suggesting that the *ERCC1* Asn118Asn polymorphism predicts resistance to platinum-based chemotherapy in EOC patients. Because, to our knowledge, this is the first study to report an association between the *ERCC1* polymorphism and resistance to platinum-based chemotherapy in EOC, a large-scale prospective clinical trial is needed to validate the possible relationship between the *ERCC1* polymorphism and response to platinum agents.

Acknowledgement

This work was supported partly by a grant from the Korea Science and Engineering Foundation (R00-2004-000-10561-0), partly by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (0412-CR01-0704-0001) and partly by a grant from the Seoul National University Hospital Research Fund (#21-2005-007).

References

- Britten RA, Liu D, Tessier A, Hutchison MJ, Murray D. ERCC1 expression as a molecular marker of cisplatin resistance in human cervical tumor cells. *Int J Cancer* 2000;89:453-7
- Dabholkar M, Bostick-Bruton F, Weber C, Bohr VA, Egwuagu

C, Reed E. ERCC1 and ERCC2 expression in malignant tissues from ovarian cancer patients. *J Natl Cancer Inst* 1992;84:1512-7

Gloeckler Ries LA, Reichman ME, Lewis DR, Hankey BF, Edwards BK. Cancer survival and incidence from the Surveillance, Epidemiology, and End Results (SEER) program. *Oncologist* 2003;8:541-52

Markman M, Rothman R, Hakes T, Reichman B, Hoskins W, Rubin S, Jones W, Almadrones L, Lewis JL Jr. Second-line platinum therapy in patients with ovarian cancer previously treated with cisplatin. *J Clin Oncol* 1991;9:389-93

Metzger R, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groshen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Konda B, Leichman L. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 1998;16:309-16

Park DJ, Stoehlmacher J, Zhang W. ERCC1 polymorphism is associated with differential ERCC1 gene expression. *Proc Am Assoc Cancer* 2002;1591

Park DJ, Zhang W, Stoehlmacher J. ERCC1 gene polymorphism as a predictor for clinical outcome in advanced colorectal cancer patients treated with platinum-based chemotherapy. *Clin Adv Hematol Oncol* 2003;1:162-6

Ryu JS, Hong YC, Han HS, Lee JE, Kim S, Park YM, Kim YC, Hwang TS. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer

patients treated with cisplatin combination chemotherapy. *Lung Cancer* 2004;44:311-6

Selvakumaran M, Pisarcik DA, Bao R, Yeung AT, Hamilton TC. Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. *Cancer Res* 2003;63:1311-6

Stoehlmacher J, Park DJ, Zhang W, Yang D, Groshen S, Zahedy S, Lenz HJ. A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. *Br J Cancer*. 2004;91:344-54

Yu JJ, Lee KB, Mu C, Li Q, Abernathy TV, Bostick-Bruton F, Reed E. A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues. *Mutat Res* 1997;382:13-20

Yu JJ, Lee KB, Mu C, Li Q, Abernathy TV, Bostick-Bruton F, Reed E. Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are equally resistant to platinum, but differ at codon 118 of the ERCC1 gene. *Int J Oncol* 2000;16:555-60

Zamble DB, Lippard SJ. Cisplatin and DNA repair in cancer chemotherapy. *Trends Biochem Sci* 1995;20:435-9

Zhou W, Gurubhagavatula S, Liu G, Park S, Neuberg DS, Wain JC, Lynch TJ, Su L, Christiani DC. Excision repair cross-complementation group 1 polymorphism predicts overall survival in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. *Clin Cancer Res* 2004;10:4939-43