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Correlation of *UGT1A1* and *ERCC1* gene polymorphisms with the outcome of combined irinotecan plus cisplatin treatment in recurrent ovarian cancer

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ABSTRACT. The aim of this study was to define the genotypes of *UGT1A1* and *ERCC1* and to examine their relationship with the efficacy and toxicity of a combination therapy of irinotecan and cisplatin in patients with advanced ovarian cancer. The allelic frequencies of the *UGT1A1* and *ERCC1* variants in a group of 89 patients with advanced ovarian cancer were determined. The relationship between the adverse events of irinotecan-based chemotherapy and the efficacy of cisplatin in patients with advanced ovarian cancer were analyzed. For patients who carried the *UGT1A1**28 wild-type (WW) or the *UGT1A1**28 heterozygous and homozygous mutant (WM+MM) genotypes, the incidences of grade 2 or 3 tardive diarrhea were 52.2 and 72.7% respectively, and the difference was statistically significant (P = 0.031, OR = 2.1, 95%CI = 1.6-9.2). For grade 3 or 4 tardive diarrhea, the incidence rates were 7.5 and 36.4% respectively; this difference was also statistically significant (P = 0.000, OR = 4.9, 95%CI = 3.3-15.8). The response rates of *ERCC1* WW and *ERCC1* WM+MM carriers were 30.3 and 20.2% respectively; this difference was significant (P = 0.032, OR = 3.2, 95%CI = 1.4-9.1).

Together, the results from this study suggest that *UGT1A1* is a target gene for tardive diarrhea, and that the *UGT1A1**28 gene mutation might increase the risk of diarrhea with irinotecan-based chemotherapy. Furthermore, the results suggest that *ERCC1* WW carriers might obtain a better rate of clinical response from a combined irinotecan and cisplatin regimen than *ERCC1* WM+MM carriers.

Key words: UGT1A1; ERCC1; Advanced ovarian cancer; Irinotecan and cisplatin

INTRODUCTION

Combined chemotherapy with cisplatin is an important scheme for the comprehensive treatment of recurrent ovarian cancer. However, drug resistance to cisplatin usually causes the failure of chemotherapy for recurrent ovarian cancer. Therefore, irinotecan chemotherapy combined with platinum has been used clinically in recent years (An and Zheng, 2012), and has provided hope for the effective treatment of recurrent ovarian cancer. Irinotecan is a specific inhibitor of DNA topoisomerase I and causes DNA disruption resulting in tumor cell death through the formation of a stable complex between topoisomerase I and DNA. The active component of irinotecan, SN-38, is metabolized by the enzyme uridine diphosphate glucuronosyltransferase 1A (UGT1A); thus if the *UGT1A* gene becomes mutated and the enzyme activity is decreased, the risk of adverse reactions caused by irinotecan will be increased (Xu et al., 2013). Cisplatin can inhibit the replication and transcription of DNA in cancer cells through the formation of DNA/platinum complexes, which cause DNA fracture and coding errors. Drug resistance to cisplatin is closely related to the mutations and expression of the single stranded DNA endonuclease *ERCC1* gene, which is the determinant of platinum resistance (Zhou and Fu, 2009). Therefore, it is very important to study the genetic polymorphisms of *UGT1A* and *ERCC1* with respect to the rational use of irinotecan and cisplatin, which might alleviate the adverse drug reactions thereof. Accordingly, this study analyzed the single nucleotide polymorphisms (SNPs) of *UGT1A* and *ERCC1* by pyrosequencing in 89 patients with recurrent ovarian cancer who were treated with irinotecan and cisplatin. Correlation analyses were performed between the *UGT1A* gene polymorphism and adverse reactions induced by irinotecan, and the effect of the *ERCC1* gene polymorphism on the efficacy of cisplatin was determined. The results obtained are reported as follows.

MATERIAL AND METHODS

General information

We included 89 patients with recurrent ovarian cancer in this study. The mean age was 48 ± 23 years of age, and ranged from 28 to 71 years. The first operations of 48 patients were conducted in our hospital, and surgeries on the remaining 41 patients were performed in outer court. Diagnoses of recurrent ovarian epithelial carcinoma were confirmed by pathological examination, and operations were successful cytoreductive surgery. All patients had pelvic metastasis, 11 had ascites, 8 had enlarged retroperitoneal lymph nodes confirmed by computed tomography (CT), and 35 had received treatment with cisplatin plus paclitaxel or

CAP. Specimens for the detection of *UGT1A1* and *ERCC1* gene polymorphism were taken from the peripheral venous blood of patients with recurrent ovarian cancer. For the treatment regimen, 60 mg/m^2 irinotecan combined with 60 mg /m^2 cisplatin was administered to patients with recurrent ovarian cancer at 1 and 8 days; the initial doses were provided intravenously. A single cycle consisted of 21 days.

Methods

Evaluation of efficacy

According to the criteria of objective efficacy evaluation of solid tumors, formulated by the World Health Organization, the efficacy indices utilized herein consisted of the decrease in ovarian cancer epithelial antigen levels and the shrinkage of lesions. After three courses (cycles) of treatment, if the ovarian cancer epithelial antigen levels had decreased below 35,000 U/L and the clinical symptoms had disappeared, the evaluation was made of complete remission (CR); if ovarian cancer epithelial antigen levels decreased more than 50% those of the levels pre-chemotherapy, the outcome was evaluated as partial remission (PR); values ranging at approximately 50% of the original value were evaluated as stability (SD). Levels of ovarian cancer epithelial antigen above 50% of the baseline were evaluated as progress (PD). By CT evaluation, the case was determined as CR if the lesions were shown to have disappeared completely, and the clinical symptoms were completely relieved; the evaluation was PR if the tumor size was over 50% its initial size; SD was determined if the tumor size was ≤50% its original value and the increase was less than 25%; and PD was assigned if the tumor size increased more than or equal to 50% (Yang and Wu, 2004).

Analysis of gene polymorphisms

Venous blood samples (4 mL) were obtained and treated with the anticoagulant EDTA-2Na. DNA was extracted using a whole blood genomic DNA extraction kit. According to the human *UGT1A1* gene sequence listed in GenBank, we utilized the AssaY Design Software: Version 1.0.6 and the PyroMark ID system (Biotage, Uppsala, Sweden) to design primers for gene amplification and polymorphism detection, as follows: *UGT1A1**28 forward primer 5'-GCC AGT TCA ACT GTT GTT GC-3', reverse primer 5'-GTC CGT CAG CAT GAC ATC AA-3', and pyrosequencing primer 5'-TCC GTG TCT TCT GCT GAG ATG G-3'; *UGT1A1**6 forward 5'-CAC CTG ACG CCT CGT TGT A-3', reverse 5'-GAA CAG CCA GAC AAA AGC ATA G-3', and pyrosequencing primer 5'-CTC TGG GGT GAG GAC CAC TG-3'; *ERCC1* forward primer 5'-GTC ATC CCT ATT GAT GGC TTC TG-3', reverse primer 5'-TCG TGC GCA ACG TGC CCT-3', and pyrosequencing primer 5'-GGG AAT TAC GTC GCC AAA TTC-3'. Extracted DNA was amplified by polymerase chain reaction (PCR); the reaction conditions were as follows: 95°C denaturation for 4 min, 44 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 3 min, followed by an extended hold at 4°C. Products were purified by agarose gel electrophoresis.

The single stranded DNA separation medium, PCR product fixation, single stranded DNA configuration template preparation, and primer hybridization were as previously described (Xu et al., 2011). Pyrosequencing of the PCR products was performed using a PyroMark ID sequencing system and general SNP identification kit (Biotage) according to the

instrument and kit instructions. Alleles and mutation types were determined according to the detected base sequences.

Statistical analysis

All data were analyzed with the SPSS11.5 statistical software (SPSS, Chicago, IL, USA). The *UGT1A1* and *ERCC1* alleles and allele frequencies were computed with the χ^2 test. Whether the mutant allele and genotype distributions of *UGT1A1* and *ERCC1* were consistent with Hardy-Weinberg equilibrium were determined by Fisher analysis. $P < 0.05$ was taken to indicate a significant difference.

RESULTS

Genotype determination

UGT1A1 has two common polymorphic sites: one in the exonic region and the other in the promoter region. *UGT1A1** 6 (211G>A, G71R) is located in the exonic region, and yields three potential genotypes, i.e., G/G (homozygous wild-type; WW), A/G (heterozygous; WM), and A/A (homozygous mutant; MM). In the promoter region, differences in numbers of TA repeats yield the wild-type 6-repeat allele variant *UGT1A1**1 and the mutant 7-repeat variant *UGT1A1**28, giving rise to the genotypes WW (TA6/TA6 or *1/*1), MM (TA7/TA7 or *28/*28), and WM (TA6/TA7 or *1/*2). The polymorphism in *ERCC1* is found in the fourth exon at codon 118 and is synonymous (Asn118Asn) (Martinez-Balibrea et al., 2010; Shulman et al., 2011). The frequency distributions of the *UGT1A1* and *ERCC1* alleles in the 89 patients with recurrent ovarian cancer can be seen in Table 1.

Table 1. *UGT1A1* and *ERCC1* allele frequencies in 89 patients with recurrent ovarian cancer.

Genotype	Genotype distribution			Allele distribution	
	WW	WM	MM	W	M
UGT1A1*6	64 (71.9)	19 (21.4)	6 (6.7)	82.6	17.4
UGT1A1*28	67 (75.3)	20 (22.5)	2 (2.2)	86.5	13.5
ERCC1	44 (59.4)	36 (40.5)	9 (10.1)	69.6	30.6

WW = wild-type homozygote; WM = heterozygous mutations; MM = homozygous mutant.

Correlation of *UGT1A1* gene polymorphism with adverse reactions induced by irinotecan

Based on the importance of UGT1A1 protease activity in the metabolism of irinotecan, we analyzed the relevance of *UGT1A1* genotypes on the adverse reaction to irinotecan (Satoh et al., 2011). The occurrence rate of degree II to IV delayed diarrhea in *UGT1A1**28 WW carriers was 52.2% (National Cancer Institute, 2013), and the incidence rate in WM+MM carriers was 72.7%. Comparison between groups yielded an OR = 2.1 (95%CI = 1.6-9.2); the difference was statistically significant (P = 0.000). Similarly, the incidence rate of III to IV delayed diarrhea in *UGT1A1**28 WW carrier was 7.5%, and the incidence rate in WM+MM carriers was 36.4%, with OR = 4.9 (95%CI = 3.3-15.8); the difference was statistically sig-

nificant (P = 0.000). The incidence rate of III or IV grade leukopenia and neutropenia declined was 59.7% in *UGT1A1**28 WW carriers, whereas the incidence rate in WM+MM carriers was 59.1%. The difference between the two groups was not significant. No correlations were identified between the *UGT1A1**6 gene polymorphism and delayed diarrhea, leukopenia, neutropenia, or other adverse reactions induced by irinotecan (Table 2).

Table 2. *UGT1A1* gene polymorphism and adverse reactions induced by irinotecan.

Genotype	N	II-IV degree delayed diarrhea	III-IV degree delayed diarrhea	III-IV leucopenia or neutropenia
*UGT1A*1*6	89			
WW	64	40 (59.7)	5 (7.5)	37 (57.5)
WM+MM	25	13 (52.0)	3 (12.0)	11 (44.0)
*UGT1A1**28	89			
WW	67	35 (52.2)	5 (7.5)	40 (59.7)
WM+MM	22	16 (72.7)*	8 (36.4)**	13 (59.1)

Compared with WW, *P = 0.031, **P = 0.000; WW = wild-type homozygote; WM = heterozygous mutations; MM = homozygous mutant.

Correlation of the *ERCC1* genotype of patients with recurrent ovarian cancer with treatment efficacy

Following analysis of the treatment efficacy in 89 patients after three cycles of therapy, the clinical benefit rate of treatment as measured by the evaluation index of ovarian cancer epithelial antigen decline was found to be 48.31%, and by the CT evaluation index (lesion decrease), it was 50.6%; the results of the two evaluation indices for the clinical benefit rate were therefore roughly the same (Table 3). We performed a statistical analysis between the disease control group (CR + PR + SD) and the PD group by using the clinical benefit indicators evaluated by CT imaging. The clinical benefit rate of *ERCC1* WW carriers was 30.3% after three cycles of treatment, and 20.2% for *ERCC1* WM+MM carriers. Comparison of the two groups gave an OR = 3.2 (95%CI = 1.4-9.1) that was statistically significant (P = 0.032). For the clinical benefit rate by the evaluation index of ovarian cancer epithelial antigen decline, the effective rate (ER) (%) in *ERCC1* WW carriers was 29.2%, whereas the ER (%) in *ERCC1* WM+MM carriers was 19.1%. When the two groups were compared, values of P = 0.034, and OR (95%CI) of 3.5 (1.7-9.6) were obtained. These results demonstrated that the treatment efficacies of irinotecan and cisplatin combination therapy in patients with recurrent ovarian cancer who carried the *ERCC1* WW genotype were better than those in patients who carried *ERCC1* WM+MM (Reed et al., 2007).

Table 3. Clinical benefits of the *ERCC1* gene polymorphism with combined irinotecan and cisplatin treatment.

Genotype	N	Evaluation of ovarian cancer epithelial antigen				Evaluation of lesions by CT imaging (N = 89)			
		CR+PR	SD	PD (%)	ER (%)	CR+PR	SD	PD (%)	ER (%)
ERCC1	89	20	23	46 (51.7)	43 (48.3)	23	22	44 (49.3)	45 (50.6)
WW	44	15	11	18 (20.2)*	26 (29.2)**	17	10	17 (19.1)	27 (30.3)
WM+MM	45	5	12	28 (31.5)	17 (19.1)	6	12	27 (30.3)	18 (20.2)

Compared with WW, *P = 0.032, **P = 0.034; WW = wild-type homozygote; WM = heterozygous mutations; MM = homozygous mutant; CT = computed tomography; CR = complete remission; PR = partial remission; SD = stability; PD = progress; ER = effective rate.

DISCUSSION

Delayed diarrhea and neutropenia are specific and serious adverse reactions of irinotecan treatment. In this study, 89 patients with recurrent ovarian cancer were treated by irinotecan and cisplatin chemotherapy. The incidence of II to IV degree delayed diarrhea in *UGT1A1**28 WW genotype carriers was 52.2%; that of the WM+MM carriers was 72.7%, with a statistically significant difference between the groups ($P < 0.05$). This finding demonstrated that *UGT1A1**28 is a target gene for delayed diarrhea induced by irinotecan. In *UGT1A1**28 WM+MM carriers, the mutation is predicted to lead to a decreased UGT1A enzyme activity, blocking or inactivating the metabolism of SN38-G, resulting in the accumulation of SN-38 in the large intestine; this causes dysfunctions in water and electrolyte absorption and mucus hypersecretion, leading to the onset of delayed diarrhea (Rouits et al., 2004; Takano et al., 2009). In contrast, no association was found between *UGT1A1**6 gene polymorphism and irinotecan-related delayed diarrhea.

The incidence rate of III or IV leukopenia and neutropenia in *UGT1A1**28 WW carriers was 59.7%, and in WM+MM carriers was 59.1%; group comparison showed that there was no significant difference ($P = 0.853$). Therefore, the *UGT1A1**28 gene polymorphism was not found to be associated with III or IV degree leukopenia and neutropenia.

Cisplatin can kill tumor cells through the formation of platinum-DNA complexes, which inhibit the replication and transcription of cancer cell DNA. The nucleotide excision repair system is one of the major DNA repair systems in mammalian cells, and is also the primary means to repair DNA damage induced by platinum drugs (Arriagada et al., 2004; Fautrel et al., 2005). Polymorphism of the *ERCC1* gene plays an important role in this process; it is also an important cause of platinum resistance. Eighty-nine patients with recurrent ovarian cancer were studied, with results demonstrating that the *ERCC1* Asn118Asn wild-type genotype (WW) was associated with a better tumor control rate than were the mutant genotypes (WM+MM), suggesting that the *ERCC1* gene polymorphism was related to the efficacy of cisplatin treatment in patients.

This research examined the clinical efficacy of irinotecan combined with cisplatin therapy in recurrent ovarian cancer, and demonstrated a clinical benefit rate of 48.3% as assessed by the ovarian cancer epithelial antigen decline index, and of 50.6% as assessed by the CT evaluation index (lesion reduction). These findings suggested a good therapeutic effect of irinotecan and cisplatin chemotherapy in the treatment of recurrent ovarian cancer. Utilization of the information regarding the *UGT1A1**28 and *ERCC1* genotype status or *ERCC1* gene expression level to adjust the dosage of irinotecan and cisplatin could help in developing individualized treatment regimens for tumor patients. Appropriate alterations of the dosage of irinotecan and cisplatin in *UGT1A1**28 WM+MM or *ERCC1* WM+MM carriers might reduce the associated adverse drug reactions or improve the clinical efficacy of these treatments, and thus provide a new approach for cancer chemotherapy as well as gene therapy.

Conflicts of interest

The authors declare no conflict of interest.

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