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Evaluation of effects of thymidylate synthase and excision repair cross-complementing 1 polymorphisms on chemotherapy outcome in patients with gastrointestinal tumors using peripheral venous blood

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Abstract. The aim of the present study was to evaluate the effects of thymidylate synthase (TYMS) and excision repair cross-complementing 1 (ERCC1) polymorphisms on chemotherapeutic efficacy in patients with gastrointestinal tumors using peripheral venous blood. Preoperative peripheral venous blood and tumor tissue samples of 43 patients with gastric cancer and the peripheral venous blood samples of 76 patients with cancer who underwent chemotherapy were studied. The 3R/3R and 2R/2R or 2R/3R genotypes of *TYMS* were identified in 72.09 and 27.91%, respectively (P<0.01), of untreated patients, and the C/C and T/T or C/T genotypes of *ERCC1* were present in 81.39 and 18.61%, respectively (P<0.01), of patients. The 3R/3R and 2R/2R or 2R/3R genotypes of *TYMS* were identified in 65.79 and 34.21%, respectively, of chemotherapy-treated patients. The overall response rates (ORRs) for the two aforementioned genotypes were 18.00 and 57.69%, respectively (P<0.01), and those for the C/C and T/T or C/T genotypes of *ERCC1* were 63.16 and 36.84%, respectively. The ORRs were 47.91 and 3.57%, respectively (P<0.01). In conclusion, peripheral blood samples may be used to replace tumor tissue for detecting *TYMS* and *ERCC1* polymorphisms, and may be used to evaluate the efficacy of 5-fluorouracil and platinum drugs.

Introduction

Malignant tumors represent a significant health problem, and the incidence of and mortality associated with these tumors have rapidly increased in China in recent decades. Among them, gastrointestinal tumors remain a major cause of cancer-related mortality worldwide (1). Chemotherapy serves as an essential treatment option, and several meta-analyses have demonstrated that chemotherapy has significant benefits for patients with gastric cancer (GC) (2). Although new therapeutic strategies are being developed rapidly, the prognosis of patients with GC remains poor, mainly due to interindividual variations in drug response. It has been predicted that genetic variants may account for 20-95% of interindividual variation in drug response (3). Therefore, identifying polymorphisms in xenobiotic-metabolizing enzymes prior to the administration of chemotherapy and pharmacogenetic markers influencing drug response may help doctors to make more precise and effective treatment choices for individual patients.

As first-line drugs, 5-fluorouracil (5-FU) and platinum-based drugs are being widely used clinically. Numerous studies have confirmed the close correlation between drug efficacy with metabolic enzymes and gene polymorphisms in DNA repair enzymes (4-6). Among them, thymidylate synthase (TYMS) and excision repair cross-complementing 1 (ERCC1) were the earliest identified (5,7). TYMS is an enzyme that plays a significant role in converting deoxyuridine-5'-monophosphate (dUMP) to deoxythymidine-5'-monophosphate (dTMP). Studies illustrated that the level of *TYMS* expression in patients with colorectal cancer receiving 5-FU-based chemotherapy was association with clinical responsiveness (8,9). The *TYMS* promoter comprises a 28-bp tandem repeat in the 5'-untranslated enhanced region (5'-UTR) that usually presents as a double-(2R) or triple-tandem repeat (3R).

ERCC1 is a key rate-limiting enzyme in the multistep nucleotide excision repair process that participates in single-strand annealing repair and the homologous repair of double-strand breaks. ERCC1 is highly conserved, and it is crucial for the removal of DNA adducts caused by platinum compounds (10,11). A common C→T polymorphism at codon 118 of *ERCC1* has been identified as a meaningful predictor of outcome in patients with colorectal cancer who received platinum-based chemotherapy (12). C/C, T/T and C/T are the most common genotypes.

At present, tumor tissue is often used to detect *TYMS* and *ERCC1* polymorphisms, but there are several limitations

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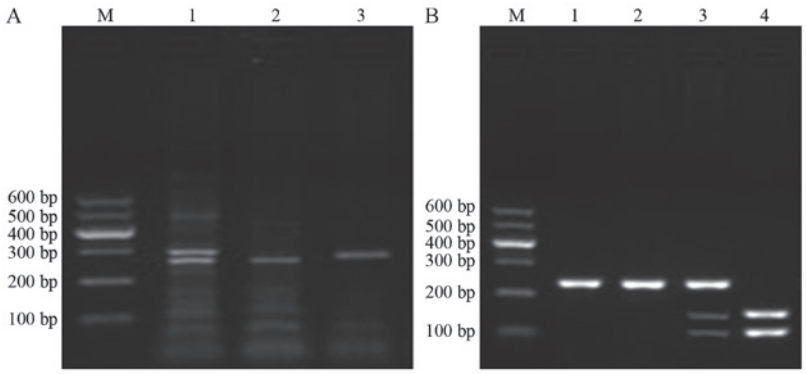

Figure 1. Thymidylate synthase (TYMS) and excision repair cross-complementing 1 (ERCC1) genotype electrophoresis. (A) TYMS genotype electrophoresis; M, marker; 1, 2R/3R genotype; 2, 2R/2R genotype; 3, 3R/3R genotype; (B) ERCC1 genotype electrophoresis; M, marker; 1, ERCC1 gene; 2, C/C genotype; 3, C/T genotype; 4, T/T genotype.

in using tissue for this purpose. It was previously reported that *TYMS* and *ERCC1* polymorphisms could be detected in the blood of patients with colorectal and rectal cancers (13), but it is unclear whether they could be detected in the blood of patients with GC and whether the polymorphisms are consistent in tumor tissue and peripheral blood. The aim of this study was to assess *TYMS* and *ERCC1* polymorphisms in the cancer tissue and peripheral blood of patients with GC to verify whether the results of these samples were consistent, thereby exploring the feasibility of using peripheral blood to detect gene polymorphisms instead of tumor tissue. On this basis, peripheral blood was collected from patients with gastrointestinal tumors who received 5-FU- and platinum-based chemotherapy to determine whether the different polymorphisms in *TYMS* and *ERCC1* may be predictive of the outcome of chemotherapy in these patients. In this study, we evaluated the effects of *TYMS* and *ERCC1* polymorphisms on the efficacy of chemotherapy in patients with gastric tumors using peripheral venous blood, in order to provide a simple laboratory evaluation measurement to individualize patient treatment.

Materials and methods

Patient information. Forty-three patients who underwent surgery in the Department of Gastrointestinal Surgery of the First Affiliated Hospital of Zhengzhou University, China, between February and August 2012 were selected, comprising 31 males and 12 females. Pathological examination confirmed that all cancer tissues were gastric adenocarcinoma, and none of the patients received radiotherapy or chemotherapy prior to surgery. Additionally, 76 patients with gastrointestinal cancer who received 5-FU- and platinum-based chemotherapy were also enrolled. This study was conducted in accordance with the declaration of Helsinki, and with approval from the Ethics Committee of Zhengzhou University. Written informed consent was obtained from all participants.

Extracting genomic DNA. Peripheral blood and tumor tissue genomic DNA was extracted using a Blood Genomic mini kit (ComWin Biotech Co, Beijing, China) and UNQ-10 Column Animal Genomic DNA isolation kit (Sangon Biotech, Shanghai, China).

Polymerase chain reaction (PCR). The primer pair used for detecting *TYMS* was as follows: F, 5'-GCGGAAGGGGTC CTGCCA-3'; and R, 5'-CGTGCGGTCGTCCTTCCTG-3'. The volume of the PCR reaction mixture (Sangon Biotech) was 25 μl, and PCR amplification was performed using the following procedure: pre-denaturation at 95˚C for 5 min, followed by 40 cycles at 95˚C for 30 sec, 63˚C for 30 sec, and 72˚C for 30 sec, with a final extension at 72˚C for 5 min. PCR products (5 μl) were subjected to electrophoresis, and the results were analyzed using a gel imaging system (UVP, Upland, CA, USA).

PCR-restriction fragment length polymorphism. The primer pair for *ERCC1* was as follows: F, 5'-TGTGGTTATCAAGGG TCATCC-3'; and R, 5'-CAGTCCAGAACACTGGGACAT-3'. The volume of the PCR reaction mixture was 25 μl, and PCR amplification was performed using the following procedure: pre-denaturation at 95˚C for 5 min, followed by 40 cycles at 95˚C for 30 sec, 63˚C for 30 sec, and 72˚C for 30 sec, with a final extension at 72˚C for 5 min. PCR products (10 μl) and *Hin*dIII incision enzyme (2 μl; Promega, Madison, WI, USA) were added to the reaction tube and incubated for 4 h in a water bath at 37˚C, and the reaction was finally terminated via 5 min of heating at 65˚C. The digested products were subjected to electrophoresis, and the results were analyzed using a gel imaging system (UVP).

Statistics analysis. SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used to perform the statistical analysis. Comparisons between the two groups were performed using the Chi-squared test with correction for continuity. Correlations between sample groups were evaluated using Spearman's test, and Fisher's exact test was performed to analyze the correlation between genotype and chemotherapeutic efficacy.

Results

Correlation analysis. Among the 43 patients who underwent surgery, there was no significant correlation between *TYMS* and *ERCC1* genotypes and patient gender, tumor location, differentiation, number of metastatic sites and carcinoembryonic antigen levels (P>0.05, Table I).

Table I. Correlation of *TYMS* and *ERCC1* genotypes with clinicopathological factors in gastric cancer patients.

	TYMS			ERCC1		
Clinicopathological factors	3R/3R	2R/2R, 2R/3R	P-value	C/C	T/T, C/T	P-value
Gender			0.651			0.293
Male	22	9		25	6	
Female	9	3		11	1	
Tumor location			0.778			0.885
Stomach fundus	3	1		4	0	
Gastric body	16	7		18	5	
Antrum	11	4		13	2	
Other	1	0		1	0	
Differentiation			0.417			0.466
High/medium	9	2		10	1	
Low	22	10		26	6	
Metastatic sites			0.489			0.717
0	7	2		8	1	
1	16	9		20	5	
2	6	1		7	0	
≥3	2	0		1	1	

TYMS, thymidylate synthase; ERCC1, excision repair cross-complementing 1.

Table II. Genotype frequencies of TYMS in peripheral blood and tumor tissues.

	TYMS in peripheral blood				
TYMS in tumor tissue	3R/3R	2R/2R, 2R/3R	Total	Chi-squared	P-value
3R/3R	31	0	31	38.175	<0.01
2R/2R, 2R/3R	0	12	12		
Total	31	12	43		

TYMS, thymidylate synthase.

Table III. Genotype frequencies of ERCC1 in peripheral blood and tumor tissue.

	ERCC1 in peripheral blood				
ERCC1 in tumor tissue	C/C	T/T, C/T	Total	Chi-squared	P-value
C/C	35	0	35	38.750	<0.01
T/T, C/T	0	8	8		
Total	35	8	43		

ERCC1, excision repair cross-complementing 1.

Detection and analysis of TYMS and ERCC1 genotypes. In Fig. 1A and B, the amplification fragments produced according to the *TYMS* and *ERCC1* genotypes are presented. Tables II and III illustrate that the distributions of *TYMS* and *ERCC1* genotypes in peripheral blood and tumor tissue samples were consistent. The detection rates of the 3R/3R and 2R/2R or 2R/3R genotypes of TYMS were 72.09% (31/43) and 27.91% (12/46), respectively, and the detection rates of the C/C and T/T or C/T genotypes of ERCC1 were 81.39% (35/43) and 18.61% (8/43), respectively.

Table IV. Comparison of chemotherapy efficacy of *TYMS* and *ERCC1* genotypes.

	TYMS genotypes (n)					*ERCC1* genotypes (n)				
Chemotherapy efficacy	3R/3R	2R/3R, 2R/2R	Total	F	P-value	C/C	C/T, T/T	Total	F	P-value
Effective	9	15	24		<0.01	23	1	24	19.89	<0.01
Ineffective	41	11	52	14.53		25	27	52		
Total	50	26	76			48	28	76		

TYMS, thymidylate synthase; ERCC1, excision repair cross-complementing 1; F, F value, calculated by Fisher's exact test.

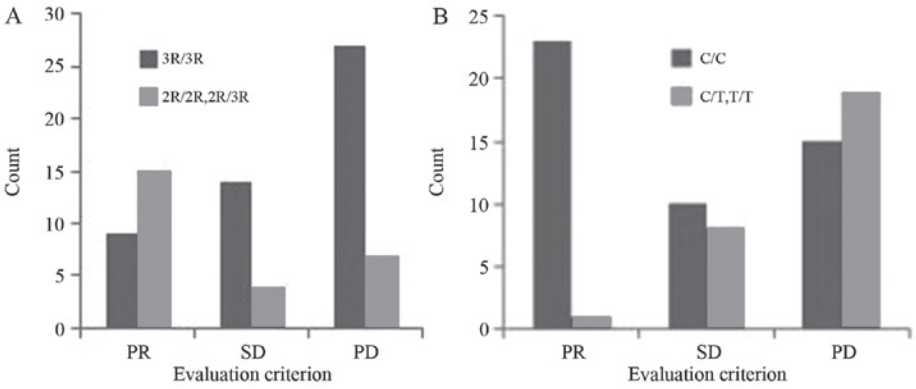

Figure 2. Evaluation results of chemotherapy efficacy of thymidylate synthase (*TYMS*) and excision repair cross-complementing 1 (*ERCC1*) genotypes. (A) Efficacy of TYMS; (B) Efficacy of ERCC1. PR, partial response; SD, stable disease; PD, progressive disease.

Correlation between TYMS polymorphisms and chemotherapeutic efficacy. Among the patients who received chemotherapy, the 3R/3R genotype was present in 65.79% of patients (50/76), vs. 34.21% for the 2R/2R and 2R/3R genotypes (26/76). In total, no complete responses (CRs), 9 partial responses (PRs), 14 cases of stable disease (SD) and 27 cases of progressive disease (PD) were recorded among the patients with the 3R/3R genotype, producing an overall response rate (ORR) of 18.00% (9/50). Conversely, 0 CRs, 4 PRs, 4 cases of SD and 7 cases of PD were noted among the patients with the 2R/2R or 2R/3R genotype, producing an ORR of 57.69% (15/26; Fig. 2A). Spearman's analysis uncovered a correlation between *TYMS* genotypes (5'-UTR) and chemotherapeutic efficacy. Fisher's exact test demonstrated that the 2R/3R and 2R/2R genotypes were associated with better chemotherapeutic efficacy than the 3R/3R genotype (Table IV).

Correlation between ERCC1 polymorphisms and chemotherapeutic efficacy. Among the patients who received chemotherapy, 63.16% (48/76) carried the C/C genotype, compared with 36.84% (28/76) for the T/T and C/T genotypes. In total, 0 CRs, 23 PRs, 10 cases of SD and 15 cases of PD were recorded among patients with the C/C genotype, giving an ORR of 47.91% (23/48). No CRs, 1 PR, 8 cases of SD and 19 cases of PD were noted among patients with the T/T or C/T genotype, resulting in an ORR of 3.57% (1/28; Fig. 2B). Spearman's analysis revealed a correlation between *ERCC1* genotypes and chemotherapeutic efficacy. Fisher's exact test demonstrated that the C/C genotype was associated with better chemotherapeutic efficacy than the C/T and T/T genotypes (Table IV).

Discussion

The *TYMS* gene is located on the 18th chromosome (18p 11.32). The gene is 16 kb long, and its main transcription initiation site is located 160-180 bp upstream of the initiation codon sequence. Previous studies have revealed that *TYMS* contains a polymorphism, which is a 28-bp tandem repeat in the 5'-UTR, generally 2R or 3R. Thus, the most common genotypes are 3R/3R, 2R/3R and 2R/2R (14). *TYMS* influences chemotherapeutic efficacy through its protein product, which regulates folic acid circulation and converts dUMP into dTMP, with the latter serving as the unique source of nucleotides for DNA synthesis and repair. Thus, restraining *TYMS* could lead to a decrease in dTMP levels and promote chromosome breakage in cells, resulting in cell death. In addition, *TYMS* is a significant target of 5-FU, and the expression level of this enzyme in the body influences the drug's antitumor effect. The 5'-UTR polymorphism of *TYMS* influences the stability and translation efficiency of mRNA, in turn influencing the expression level of *TYMS* and promoting interindividual variation in sensitivity to chemotherapeutics.

With regard to gastrointestinal tumors, previous studies have demonstrated that *TYMS* expression was higher in patients carrying the 3R/3R genotype than in those carrying the 2R/2R or 2R/3R genotype. In addition, clinical manifestations were more pronounced in the former than the latter (15), which suggested that 3R/3R was a poor prognostic factor in adjuvant chemotherapy for tumors. A study by Morganti *et al* (16) of 48 patients with gastrointestinal cancer demonstrated that the

mRNA expression level of *TYMS* in patients carrying the 3R/3R genotype was notably higher than that of 2R/2R or 2R/3R carriers. In 2005, Yawata *et al* (17) determined that forward copies of *TYMS* could be regarded as a predictive index for evaluating sensitivity to 5-FU. Subsequently, Brody *et al* (14) and Watson *et al* (15) suggested that the 5'-UTR 28-bp repeat nucleotide fragment polymorphism in *TYMS* could influence the efficacy of 5-FU *in vivo*. Huang *et al* (18) studied 116 patients with GC who received 5-FU-based chemotherapy, and drew the same conclusion.

With regard to gastric cancer, Cui *et al* (19) noted that Chinese patients with gastrointestinal tumors who carry the 2R/3R genotype exhibited greater sensitivity to 5-FU than their counterparts who carried the 3R/3R genotype.

Villafranca *et al* (20) studied 65 patients with rectal cancer and demonstrated that the downstaging rate was higher for patients with the 2R/3R or 2R/2R genotype than for those with the 3R/3R genotype, and the three-year survival rates for the 2R/3R or 2R/2R genotype and the 3R/3R genotype were 81 and 41%, respectively. Pullarkat *et al* (21), Marsh *et al* (22), Park *et al* (23) and Matasui *et al* (24) observed that 2R/2R or 2R/3R carriers were more sensitive to 5-FU than 3R/3R carriers. In the present study, the ORR for 3R/3R carriers was 18%, which was significantly lower than the rate of 57.69% observed for 2R/3R or 2R/2R carriers. This illustrated that the repetitive elements polymorphism of *TYMS* could be regarded as a predictor of tumor downstaging and a new modality for predicting the effect of 5-FU-based chemotherapy.

ERCC1 is located on the 19th chromosome (19q 13.2). The gene, which is 16 kb long, contains 10 exons, and the most common and meaningful polymorphism is a C→T transition at the 118th codon on the fourth exon. The three resulting genotypes are C/C, C/T and T/T. Substantial research has demonstrated that patients carrying the C/C genotype were more sensitive to platinum drugs, which means that this genotype could reduce the transcriptional efficiency and protein expression level of ERCC1 in cells and weaken the protein's DNA repair activity (25-28). Furthermore, individual sensitivity to platinum drugs was affected. Moreover, DNA repair capacity is the molecular basis by which the effects of platinum drugs are altered, and it plays a significant part in platinum resistance mechanisms. ERCC1 is a key enzyme involved in DNA damage repair, which is significantly correlated with resistance to platinum drugs.

With regard to gastrointestinal tumors, Won *et al* demonstrated that using the *ERCC1* C118T polymorphism to predict the toxicity of chemotherapy was feasible (29). Liu *et al* also confirmed that *ERCC1* polymorphisms could predict the effect based on oxaliplatin-based therapy (30). In 2011, Yin *et al* noted in their study that *ERCC1* C118T could be a predictor of the efficacy of oxaliplatin-based therapy (12).

Liu *et al* stated that patients with GC who carry the C/C genotype could receive a survival benefit from platinum-based chemotherapy (30). Ruzzo *et al* suggested that the C/T genotype is associated with better chemotherapeutic efficacy than the T/T genotype; however, in that article the author identified the small sample size of the study, retrospective nature of the experiment, and heterogeneity of clinical situations as possible explanations for the divergent conclusion (31).

Ruzzo *et al* also observed that patients with colorectal cancer who carry the C/C genotype exhibit greater chemosensitivity than those who carry the C/T or T/T genotype (32). However, several studies contradict this finding, and Viguier *et al* noted that patients with colorectal cancer who carry the T/T genotype are more sensitive to 5-FU and platinum (33).

By studying the correlation between *ERCC1* genotypes and platinum drugs, the present study revealed that the ORR of C/C carriers (47.91%) was notably lower than that of T/T or T/C carriers (57.69%). A significant association existed between *ERCC1* genotypes and the efficacy of platinum-based chemotherapy, which indicated that chemosensitizing genotypes could be used to evaluate the effects of platinum-based chemotherapy. Therefore, adjusting the dosage of chemotherapeutics according to patients' genotypes and selectively using agents to overcome drug resistance associated with high gene expression may greatly improve chemotherapeutic efficacy.

However, in the clinic, the efficacy of drug treatment in certain 2R/3R or 2R/2R and C/C carriers was not fully consistent with the expected effect, which suggested that other possible factors including age, diet and organ function may influence chemotherapeutic efficacy.

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