SPANDIDOS PUBLICATIONS

Comprehensive analysis of *excision repair complementation group 1*, *glutathione S-transferase*, *thymidylate synthase* and *uridine diphosphate glucuronosyl transferase 1A1* polymorphisms predictive for treatment outcome in patients with advanced gastric cancer treated with FOLFOX or FOLFIRI

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Received March 11, 2009; Accepted April 30, 2009

DOI: 10.3892/or_00000415

Abstract. Oxaliplatin and irinotecan have proven effective in the treatment of gastric cancer. We attempted to determine whether single nucleotide polymorphisms in *ERCC1*, *GST*, *TS* and *UGT1A1* predicted overall survival in gastric cancer patients receiving FOLFOX and/or FOLFIRI chemotherapy. Total genomic DNA was extracted from the whole blood of patients. The PCR-restriction fragment length polymorphism technique was applied in order to detect the known variant sites of *ERCC1*, *GST*, *TS* and *UGT1A1*. The response rate of FOLFOX (N=75) was 24%. Grade 3-4 neutropenia and neurotoxicity were observed at frequencies of 34.7 and 16%, respectively. TTP and OS of first-line administration of FOLFOX (N=35) were 3.1 months (95% CI, 0.1-6.1 months) and 13.9 months (95% CI, 12.2-15.6 months), respectively. Only the *GSTM1* positive genotype exhibited a significantly better time to progression (P=0.023). However, significant genotypic variation of *TS*, *GST* and *ERCC1*, which was assumed to affect the activity of oxaliplatin, was not observed to affect RR, toxicity and overall survival. The response rate of FOLFIRI (N=74) was 23%. Grade 3-4 neutropenia and diarrhea were observed in 55.4 and 9.5% of cases, respectively. TTP and OS of first-line administration of FOLFIRI (N=33) was 4.9 months (95% CI, 3.5-6.4 months) and 19.0 months (95% CI, 8.5-29.5months). The low expression type (2R/2R, 2R/3C and 3C/3C) of TS was associated with a high incidence of grade ≥3 neutropenia. However, significant genotypic variation of *UGT1A1*, which was assumed to affect irinotecan toxicity, was not observed to affect RR, toxicity or survival. In this study, the *GSTM1* positive genotype evidenced a significantly better time to progression in cases of advanced gastric cancer being treated with FOLFOX. The low expression type (2R/2R, 2R/3C and 3C/3C) of TS was associated with a high incidence of grade ≥3 neutropenia in cases of advanced gastric cancer treated with FOLFIRI.

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Key words: excision repair complementationgroup 1, *elutathione S-transferase*, *thymidylate synthase*, *uridine diphosphate glucuronosyl transferase 1A1*, gastric cancer, FOLFOX, FOLFIRI

Introduction

In Korea, gastric cancer is the most common cancer in men, the second most common cancer in women, and the first leading cause of cancer mortality (1), and it remains the second most frequent cause of cancer-related deaths worldwide (2). Although the incidence of gastric cancer has declined over the past three decades, it is frequently diagnosed in locally advanced or metastatic diseases. Therefore, less than 30% of patients will undergo a curative resection, and approximately 35% of the patients who do undergo surgery will survive for 5 years (3). Only 10% of patients with advanced gastric cancer who undergo chemotherapy survive for 2 years (4). Chemotherapy appears to be the only useful tool for the prolongation of survival and the maintenance of quality of life in the advanced gastric cancer.

The FOLFIRI and FOLFOX regimen, which includes bolus/infusional fluorouracil with folic acid modulation and irinotecan or oxaliplatin, has become the most useful first- or second-line treatment for patients with colorectal cancer (5-7). Since 2003, these regimens have been applied to the treatment of advanced gastric cancer.

Oxaliplatin exerts antitumor effects by virtue of its ability to form platinum-DNA adducts. Bulky platinum-DNA adducts are repaired principally by the nucleotide excision repair pathway, in which proteins of the excision repair cross-complementation 1 (*ERCC1*), xeroderma pigmentosum

group D (*XPD*, also known as *ERCC2*) and X-ray repair cross-complementing group (*XRCC*), all perform crucial functions (8,9). *ERCC*, *XPD* and *XRCC* harbor SNPs, which may confer different activities to platinum agents, thus modifying the clinical outcome (10-12). Glutathione S-transferase π 1 (*GSTP1*), which is involved in platinum detoxification, also harbors a polymorphism associated with prolonged survival in cisplatin-treated gastric cancer (13,14).

Irinotecan (CPT-11) is a water-soluble chemical derivative of camptothecin. As an inhibitor of topoisomerase I, CPT-11 interferes with DNA replication, resulting in double-strand DNA breaks and cellular death. CPT-11 is active in a broad array of malignancies, including gastric cancer. The liver is the main site at which CPT-11 is converted into its active metabolite, SN-38. This conversion is mediated by human liver carboxyesterase (CES). Oxidative metabolism of CPT-11 and SN-38 is accomplished via the cytochrome P450 isoforms 3A4 and 3A5 (CYP3A4 and CYP3A5). Uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) glucoronidates SN-38 to its inactive metabolite, SN-38G. Moreover, the elimination of CPT-11 via efflux transport is mediated by *ABCB1* (multidrug resistance protein 1) and *ABCB2* (multidrug resistance protein 2) (15). Interestingly, the most significant progress made thus far in irinotecan pharmacogenomics involves the prediction of toxicity. As stated previously, the hepatic isoform 1A1 of *UGT* is responsible for the glucuronidation and detoxification of SN-38 to its inactive form, SN-38 glucoronide (16).

As a pyrimidine analogue, 5-FU exerts its antitumor effects through anabolism, which is determined by the rate of catabolism. Thus, the genes coding for the key enzymes in 5-FU metabolism may play a pivotal role in the efficacy of 5-FU. Thymidylate synthase (TS) is the target enzyme for 5-FU and catalyzes the methylation of dUMP to dTMP, a crucial process of DNA biosynthesis. The levels of TS expression in tumor tissues are considered to affect the sensitivity of several tumors, including gastric cancer, toward 5-FU-based chemotherapy (17,18). However, the scarcity of tumor tissue, the potential biases of immunohistochemistry and mRNA quantification, and the genetic heterogeneity of clinical tumor tissue limits its clinical applications. By way of contrast, it is substantially easier to obtain DNA isolated from peripheral blood lymphocytes for polymorphism analysis. Genetic polymorphisms constitute an important mechanism influencing gene function. A great many previous studies have shown that certain TS polymorphisms may affect the response to 5-FU. The first detected functional polymorphism in the TS promoter is a variable number of tandem repeats with two or three repeats of a 28-base pair sequence in the 5-untranslated region (UTR) (*2R/3R*). The 3R allele was associated with enhanced *TS* expression when compared with the 2R allele (19,20). Another important *TS* polymorphism is a 6-bp deletion or insertion (del6/ins6) in the 3-UTR. The results of several studies appear to verify that the *TS* 3-UTR del6 allele is associated with reduced *TS* mRNA stability and lower intratumoral *TS* expression, as compared to the ins6 allele (21).

This exploratory pharmacogenomic collateral study was conducted to determine the predictive or prognostic value of germline polymorphisms of candidate genes associated with 5-FU and oxaliplatin/irinotecan.

Patients and methods

Patients. Patients with metastatic or relapsed AGC were enrolled in this clinical trial. The eligibility criteria included the following: i) pathologically confirmed gastric adenocarcinoma with a measurable lesion; ii) no prior history of other cancer; iii) ECOG performance 0-2; iv) Adequate bone marrow function (absolute neutrophil count (ANC) $\geq 1500 \times 10^3/\mu l$, platelet count $\geq 100000 \times 10^3/\mu l$), renal functions (serum creatinine ≤1.5 mg/dl or calculated creatinine clearance by Cockroft formula ≥50 ml/min), and hepatic function (aspartate aminotransferase, alanine aminotransferase ≤3 x upper limits of normal, total bilirubin ≤1.5 x upper limits of normal).

Treatment protocol

Modified-FOLFOX regimen. On day 1, oxaliplatin (85 mg/m^2) was administered via intravenous (i.v.) infusion in 500 ml of normal saline or dextrose over 2 h. On days 1 and 2, ldLV (20 mg/m^2) was administered as an i.v. bolus, immediately followed by 5-FU (400 mg/m^2) administered as a 10-min i.v. bolus, followed by 5-FU (600 mg/m^2) as a continuous 22-h infusion, with a light shield.

Modified-FOLFIRI regimen. On day 1, irinotecan (150 mg/m^2) was administered via intravenous (i.v.) infusion in 500 ml of normal saline or dextrose over 2 h. On days 1 and 2, ldLV (20 mg/m^2) was administered as an i.v. bolus, immediately followed by 5-FU (400 mg/m^2) administered as a 10-min i.v. bolus, followed by 5-FU (600 mg/m^2) as a continuous 22-h infusion, with a light shield. Each cycle of chemotherapy was administered every 2 weeks.

Dose modification for toxicity. Dose modifications of oxaliplatin, irinotecan or 5-FU were made for hematologic or gastrointestinal toxicities based on the most severe grade of toxicity occurring during the previous cycle. The patients were evaluated prior to the initiation of each 2-week cycle, using the National Cancer Institute-Common Toxicity Criteria (NCI-CTC). Treatment was delayed for up to one week if symptomatic toxicity persisted, if the absolute neutrophil numbers were $<1500 \times 10^3/\mu l$, or if the platelet counts were $<100000 \times 10^3/\mu l$. The dosage of 5-FU was reduced by 25% for subsequent courses if NCI-CTC grade 3 stomatitis, dermatitis, thrombocytopenia and neutropenia were noted. The dose of oxaliplatin was also reduced by 25% in subsequent cycles in cases of NCI-CTC grade 3 neuropathy and neutropenia. The dose of irinotecan was also reduced by 25% in subsequent cycles in cases of NCI-CTC grade 3 diarrheas and neutropenia. Treatment was continued until signs of disease progression or unacceptable toxic effects developed or until a patient refused further treatment.

Assessment of response. Physical examination, complete blood counts, chemistry and chest X-rays were conducted after each cycle. Responses were assessed using RICIST criteria (22). Computed tomography (CT) scans were repeated every three cycles or earlier in cases of clinical deterioration. Only patients with unidimensionally measurable lesions on the CT scan were evaluated for tumor response.

rimer sequences and restriction enzymes.

	Gene primers	Enzyme	Refs.
UGT1A1			
U1F1	5'AGATACTGTTGATCCCAGTG3'	Ava II	Huang *et al* (23)
U211R	5'CTTCAAGGTGTAAAATGGTC3'		
U1F4	5'CACTGTATTCTTCTTGCATG3'	Bsr I	
U1R2	5'CGATCCAAAGTAATACATCTG3'		
U4F4	5'GCCAACATGTCCTACATTGC3'	Bcl I	
U1091R	5'GTGATAAAGGCACGGGTGAT3'		
U5F3	5'GTGGAGTTTGTGATGAGGCA3'	Ava II	
U5R1	5'GGAAATGACTAGGGAATGGT3'		
(TA)F	5'TAACTTGGTGTATCGATTGGT3'		
(TA)R	5'CTTTGCTCCTGCCAGAGGTT3'		
ERCC1			
118F	5'GCAGAGCTCACCTGAGGAAC3'	HpyCH4	Zhou *et al* (24)
118R	5'GAGGTGCAAGAAGAGGTGGA3'		
8092F	5'TGAGCCAATTCAGCCACTAGAG3'	Mbo II	
8092R	5'CTTTAGTTCCTCAGTTTCCCG3'		
GST			
P1-105F	5'ACCCCAGGGCTGTATGGGAA3'	Alw261	Harries *et al* (25)
P1-105R	5'TGAGGGCACAAGAAGCCCCT3'		
GST			
M1F	5'GAACTCCCTGAAAAGCTAAAGC3'		Arand *et al* (26)
M1R	5'GTTGGGCTCAAATATACGGTGG3'		
T1F	5'TTCCTTACTGGTCCTCACATCTC3'		
T1R	5'TCACCGGATCATGGCCAGCA3'		
TS			
TS25F	5'AGGCGCGCGGAAGGGGTCCT3'	Hae III	Kawakami *et al* (19)
TS18R	5'TCCGAGCCGGCCACAGGCAT3'		

ERCC1, excision repair complementation group 1; *GST*, glutathione S-transferase; *TS*, thymidylate synthase; *UGT1A1*, uridine diphosphate glucuronosyl transferase 1A1.

Genotyping. Total genomic DNA was extracted from 200 μl whole blood of AGC patients using a QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA, USA). The PCR-restriction fragment length polymorphism (RFLP) method was applied for the detection of the known variant sites.

After restriction enzyme analysis, the PCR fragments were detected via electrophoresis on 1.5-3.5 % agarose gels. All restriction enzymes were purchased from New England BioLabs (Hertfordshire, UK). Primer sequences, restriction enzymes, and references for the analyses are provided in Table I. An aliquot of 100 ng of total genomic DNA from each sample was utilized as a template in a PCR reaction with 200 ng each of primers, 1.25 mM of each dNTP, buffer solution [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$] and 1 U Taq DNA polymerase (Promega, Madison, USA) in a total volume of 25 μl. Each PCR reaction condition was as previously described (Fig. 1) (19,23-26).

Statistical methods. The two-tailed χ^2 test was conducted to determine the significance of the difference between the covariates. Survival durations were calculated via the Kaplan-Meier method. The log-rank test was employed to compare cumulative survival in the patient groups. In all tests, $P<0.05$ was the threshold of statistical significance. The SPSS software program (version 15.0; SPSS, Inc., Chicago, IL) was utilized in the analyses.

Results

Patients characteristics. Our analysis was conducted with 94 enrolled patients. Seventy-five and 74 patients were treated with FOLFOX and FOLFIRI, respectively. Their characteristics are provided in Table II.

Among the FOLFOX treated patients, the male-to-female ratio was 44-31, the median age was 56 years (range, 29-84),

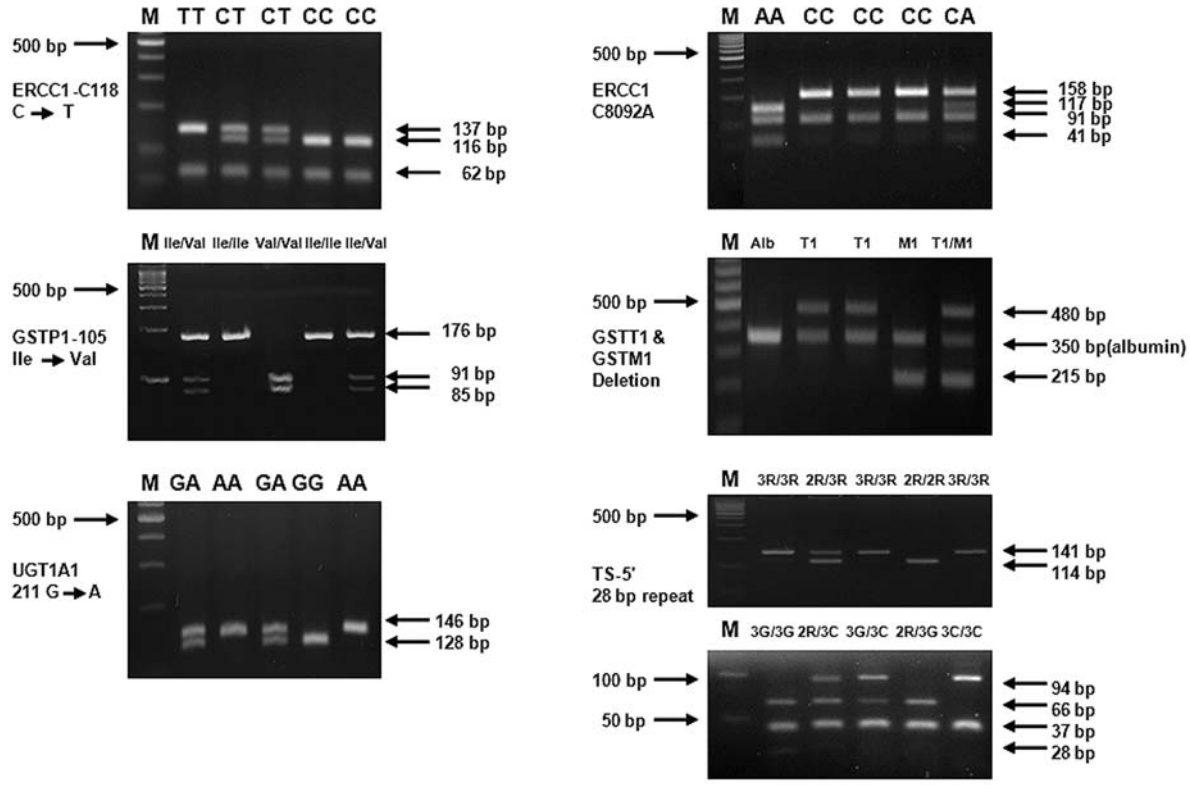

Figure 1. The PCR-restriction fragment length polymorphism (RFLP) method was applied to detect the known variant sites of *excision repair complementation group 1* (*ERCC1*), *glutathione S-transferase* (*GST*), *thymidylate synthase* (*TS*) and *uridine diphosphate glucuronosyltransferase 1A1* (*UGT1A1*).

46.7% were receiving first-line treatment, and 65.3% of the patients were initially metastatic. Among the FOLFIRI-treated patients, the male-to-female ratio was 45-29, the median age was 54 years (range, 31-84), 44.6% of patients were receiving first-line treatments, and 65.3% of patients were initially metastatic.

ERCC1, GST, TS and UGT1A1 genotypes and allele frequencies. We analyzed 7 germline polymorphisms within 4 genes. Among the FOLFOX treatment group, for the *ERCC1-C8092A* polymorphism, the frequencies of the *C/C*, *C/A* and *A/A* genotypes were 52, 41.3 and 6.7%, respectively. For the *ERCC1*-codon 118 polymorphism, the frequencies of the *C/C*, *C/T* and *T/T* genotype were 56, 40 and 4%, respectively. Thirty-nine patients (52%) evidenced the *GSTT1*-negative genotype, 49 patients (65.3%) *GSTM1*-negative, and 47 (62.7%) the *Ile/Ile* and 27 (36%) the *Ile/Val* genotype of *GSTP1*. The genotype distributions of the favorable *TS* genotype (*2R/2R*, *2R/3C* and *3C/3C*) and unfavorable *TS* genotype (*2R/3G*, *3C/3G* and *3G/3G*) were observed in 27 (36%) and 48 (64%), respectively.

With regard to the FOLFIRI treatment group, for the *UGT1A1* polymorphism, the *UGT1A1* variants typed in this study and their allele frequencies are provided in Table III. Forty-four patients (59.5%) evidenced the *UGT1A1*6 G/G* genotype, 30 patients (40.5%) exhibited the *UGT1A1*6 G/A* genotype. *UGT1A1*28*, *UGT1A1*27*, *UGT1A1*1091* was observed in only 1 patient with a heterozygous allele. The genotype distributions of the favorable *TS* genotype (*2R/2R*, *2R/3C* and *3C/3C*) and unfavorable *TS* genotype (*2R/3G*, *3C/3G* and *3G/3G*) were noted in 26 (35.1%) and 48 (64.9%) of the subjects, respectively.

Association between ERCC1, GST, TS polymorphism and FOLFOX chemotherapy

Response. In our association analysis of genotype and chemotherapy response, CR and PR patients were designated as 'responders' and SD and PD patients were designated as 'non-responders'. The overall rate of response to FOLFOX treatment was 24%. However, different *ERCC1*, *GST* and *TS* genotypes were not correlated with the response rates (Table IV-A).

Toxicity. Toxicities were evaluated using the NCI-CTC v3.0. The major toxicities of FOLFOX treatment were neutropenia and neuropathy. Grade 3 of neutropenia and neuropathy and higher were noted in 26 (34.7%) and 12 (16%) of the patients, respectively. However, no significant genetic type was observed in conjunction with the *ERCC1*, *GST* and *TS* polymorphisms (Table V-A).

Survival. In order to determine accurately the influence of genetic polymorphism, patients were limited to those who had received FOLFOX as a first-line treatment for advanced gastric cancer. The median TTP and OS values of the first-line administration of FOLFOX (N=35) were 3.1 months (95% CI, 0.1-6.1 months) and 13.9 months (95% CI, 12.2-15.6 months).

Patient characteristics.

	FOLFOX (%)	FOLFIRI (%)
Total no. of patients	75	74
Gender		
Male	44 (58.7)	45 (60.8)
Female	31 (41.3)	29 (39.2)
Age		
Median (range)	56 (29-84)	54 (31-84)
Initial presentation		
Relapse	26 (34.7)	28 (37.8)
Initial metastatic	49 (65.3)	46 (62.2)
No. of prior chemo-therapy		
0	35 (46.7)	33 (44.6)
1	32 (42.7)	27 (36.5)
≥2	8 (10.6)	14 (18.9)
No. of treatment cycles		
Median (range)	4 (1-18)	5 (1-27)
Response of treatment		
CR	0	0
PR	18 (24.0)	17 (23.0)
SD	28 (37.3)	28 (37.8)
PD	26 (34.7)	25 (33.8)
NA	3 (4.0)	4 (5.4)
Survival of first-line treatment		
No. of patients	35	33
TTP (months, 95% CI)	3.1 (0.1-6.1)	4.9 (3.5-6.4)
OS (months, 95% CI)	13.9 (12.2-15.6)	19.0 (8.5-29.5)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NA, not available; TTP, time to progression; OS, overall survival.

Only patients with the *GTPM1*-positive genotype evidenced longer TTP durations than those with the *GPTM1*-negative genotype (3 vs. 9 months, P=0.023) (Table VI-A, Fig. 2).

Table IV. A. FOLFOX response according to the genotype.

	Frequency N=75	(%)	Responder N=18	(%)	P-value
ERCC1-C8092A					
C/C	39	52.0	9	23.1	0.529
C/A or *A/A*	36	48.0	9	25.0	
ERCC1-118					
C/C	42	56.0	11	26.2	0.412
C/T or *T/T*	33	44.0	7	21.2	
GSTT1					
Positive	36	48.0	10	27.8	0.321
Negative	39	52.0	8	20.5	
GSTM1					
Positive	26	34.7	6	23.1	0.565
Negative	49	65.3	12	24.5	
GSTP1-105					
Ile/Ile	47	62.7	10	21.3	0.328
Ile/Val, *Val/Val*	28	37.3	8	28.6	
TS					
2R/2R,2R/3C,3C/3C	27	36.0	7	25.9	0.490
2R/3G,3C/3G,3G/3G	48	64.0	11	22.9	

ERCC1, excision repair complementation group 1; *GST*, glutathione S-transferase; *TS*, thymidylate synthase.

B. FOLFIRI response according to the genotype (N=74).

	Frequency N=74	(%)	Responder N=17	(%)	P-value
*UGT1A1*6*					
G/G	44	59.5	10	25.0	0.583
G/A	30	40.5	7	26.1	
TS					
2R/2R,2R/3C,3C/3C	26	35.1	7	26.9	0.375
2R/3G,3C/3G,3G/3G	48	64.9	10	20.8	

UGT1A1, uridine diphosphate glucuronosyl transferase 1A1; *TS*, thymidylate synthase.

Table III. *UGT1A1* expression in FOLFILI treated patients (N=74).

	*UGT1A1*6*		*UGT1A1*28*		*UGT1A1*27*		*UGT1A*7*		*UGT1A1*1091*	
	G/G	*G/A*	6/6	7/6	*C/C*	*C/A*	*T/T*	*T/G*	*C/C*	*C/T*
No.	44	30	73	1	73	1	74	0	73	1
(%)	58.2	41.8	98.6	1.4	98.6	1.4	100	0	98.6	1.4

UGT1A1, uridine diphosphate glucuronosyl transferase 1A1.

Table V. A. FOLFOX toxicity according to the genotype (N=75).

	Grade 3-4 neutropenia				Grade 3-4 neuropathy		
	N	N	(%)	P-value	N	(%)	P-value
ERCC1-C8092A							
C/C	39	14	36	0.504	4	10	0.136
C/A or *A/A*	36	12	33		8	22	
ERCC1-118							
C/C	42	14	33	0.487	7	17	0.559
C/T or *T/T*	33	12	36		5	15	
GSTT1							
Positive	36	11	31	0.318	6	17	0.564
Negative	39	15	38		6	15	
GSTM1							
Positive	26	9	35	0.601	2	7	0.135
Negative	49	17	35		10	20	
GSTP1-105							
Ile/Ile	47	18	38	0.274	9	19	0.266
Ile/Val, *Val/Val*	28	8	29		3	11	
TS							
2R/2R,2R/3C,3C/3C	27	12	44	0.140	5	19	0.445
2R/3G,3C/3G,3G/3G	48	14	29		7	15	

ERCC1, excision repair complementation group 1; *GST*, glutathione S-transferase; *TS*, thymidylate synthase.

B. FOLFIRI toxicity according to the genotype (N=74).

	Grade 3-4 neutropenia				Grade 3-4 diarrhea		
	N	N	(%)	P-value	N	(%)	P-value
*UGT1A1*6*							
G/G	44	24	55	0.524	5	11	0.401
G/A	30	17	57		2	7	
TS							
2R/2R,2R/3C,3C/3C	26	19	73	0.021	3	12	0.473
2R/3G,3C/3G,3G/3G	48	22	49		4	8	

UGT1A1, uridine diphosphate glucuronosyltransferase 1A1; *TS*, thymidylate synthase.

Association between UGT1A1, TS polymorphism and FOLFIRI chemotherapy

Response. The overall response rate of FOLFIRI treatment was 23%. However, unlike *UGT1A1*, *TS* genotypes were not correlated with response rate (Table IV-B).

Toxicity. The major toxicities associated with FOLFIRI treatment were neutropenia and diarrhea. Grade 3 of higher of neutropenia and diarrhea were noted in 41 (55.4%) and 7 (9.5%) of patients, respectively. Patients with the *TS* (*2R/2R*, *2R/3C* and *3C/3C*) genotype frequently exhibited grade ≥3 neutropenia. However, no significant genetic type in terms of the *UGT1A1*, *TS* polymorphisms (Table V-A) was found to be causative of grade ≥3 diarrhea (Table V-B).

Survival. The median TTP and OS values of first-line administration of FOLFIRI (N=34) were 4.9 months (95% CI, 3.5-6.4 months) and 19.0 months (95% CI, 8.5-29.5 months).

A. First-line FOLFOX survival according to the genotype (N=35).

	Frequency N	TTP Months	P-value	OS Months	P-value
ERCC1-C8092A					
C/C	18	2.8	0.489	13.9	0.441
C/A or *A/A*	17	5.2		13.7	
ERCC1-118					
C/C	23	3.9	0.362	14.7	0.323
C/T or *T/T*	12	1.7		13.7	
GSTT1					
Positive	19	3.9	0.783	13.7	0.229
Negative	16	3.0		20.5	
GSTM1					
Positive	12	9.5	0.023	34.5	0.262
Negative	23	3.0			13.7
GSTP1-105					
Ile/Ile	22	3.0	0.599	13.2	0.669
Ile/Val, *Val/Val*	13	3.9		13.9	
TS					
2R/2R,2R/3C,3C/3C	12	3.0	0.422	10.8	0.492
2R/3G,3C/3G,3G/3G	23	3.9		13.9	

ERCC1, excision repair complementation group 1; *GST*, glutathione S-transferase; *TS*, thymidylate synthase; *TTP*, time to progression; *OS*, overall survival.

B. First-line FOLFIRI survival according to the genotype (N=33).

	Frequency N	TTP Months	P-value	OS Months	P-value
*UGT1A1*6*					
G/G	20	4.9	0.945	19.0	0.579
G/A or *A/A*	13	5.1		NR	
TS					
R/2R,2R/3C,3C/3C	14	4.7	0.896	26.6	0.250
R/3G,3C/3G,3G/3G	19	5.0		11.9	

UGT1A1, uridine diphosphate glucuronosyltransferase 1A1; *TS*, thymidylate synthase; *TTP*, time to progression; *OS*, overall survival.

However, no significant genetic type was observed in terms of the *UGT1A1* and *TS* polymorphisms (Table VI-B).

Discussion

In this study, we assessed four common polymorphisms of the *ERCC1*, *GST*, *TS* and *UGT1A1* genes and assessed their association with response, toxicity and survival in response to FOLFOX or FOLFIRI chemotherapy in gastric cancer patients.

We have demonstrated that the *GSTM1* positive genotype was associated with a significantly better time to progression in advanced gastric cancer patients treated with FOLFOX, and a low expression type (*2R/2R*, *2R/3C* and *3C/3C*) of *TS* was associated with a high incidence of grade ≥3 neutropenia in advanced gastric cancer treated with FOLFIRI. It remains unclear, however, as to how the effects of *ERCC1*, *GST*, *TS* and *UGT1A1* genes relate to chemotherapy in cases of gastric cancer. *ERCC1* is a highly conserved protein and an essential member of the NER pathway (27). The *ERCC1-XPF*

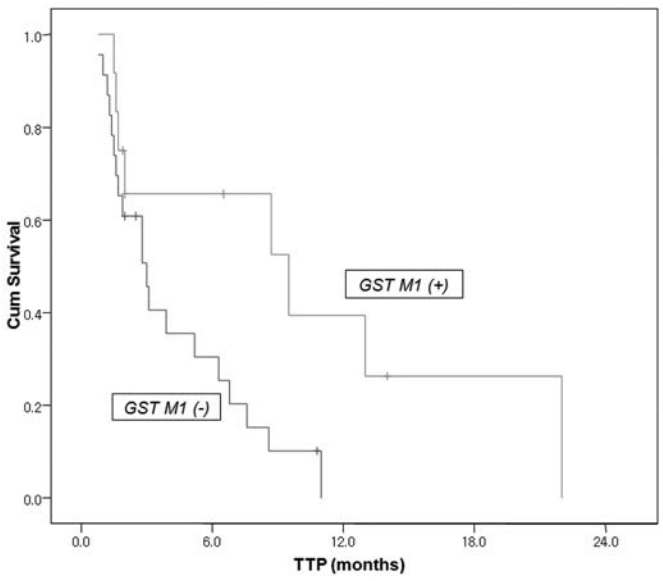

Figure. 2. *Glutathione S-transferase* (*GST*) *M1*-positive genotype showed longer time to progression (TTP) duration than *GSTM1*-negative (3 vs. 9 months, P=0.023).

(xeroderma pigmentosum group F) complex is known to be involved in the cleavage of damaged DNA strand 50 to DNA lesions. Several studies have reported an association between the expression of *ERCC1* and clinical outcomes to platinum-based chemotherapy, including gastrointestinal malignancies (8,28,29). Two common polymorphisms of the *ERCC1* gene have been identified thus far. The first SNP at codon 118 induces a C→T change coding for the same amino acid, asparagine, and might affect codon usage. An earlier analysis by Park *et al* (30), on a small number of patients (N=31) demonstrated that as the number of T alleles increased, a trend toward higher intratumoral *ERCC1* mRNA was observed. The second *ERCC1* SNP induces a C→A change and is located in position 8092 on the 30-UTR. The function of these polymorphisms in platinum sensitivity and toxicity has been retrospectively assessed in a variety of neoplasms, including melanoma (31), lung (12,24,32) and colon (30,33,34) cancers with variable and often contradictory results. Differences in populations, tumor types, therapeutic regimens, and assessments of clinical outcome may have led to diverging results.

However, in the current study, none of the polymorphisms with influence DNA-repair functions from oxaliplatin damage were clearly correlated with clinical outcomes. One possible reason for these controversial findings might be the small sample size of the study. Another salient limitation of this study is that we analyzed only the germ-line genotype. In order to more clearly determine the influence of *ERCC1*, we also identified the mRNA level and somatic genotype in tumor tissue.

Phase II detoxification enzymes, such as the *GSTs*, are also believed to be involved in platinum resistance. The *GSTP1* isoform is overexpressed in gastrointestinal malignancies, and the results of *in vitro* studies have demonstrated its significant participation to detoxification and, thus, resistance to platinum agents (35). An SNP substitution of A→C, which causes an Ile→Val change at codon 105, has been associated with a significant reduction in enzymatic activity. Patients with the valine allele were demonstrated to have superior clinical outcomes when receiving oxaliplatin-based chemotherapy for colorectal cancer (36). A recent study has assessed the role of *GST* (*P1*, *T1*, *M1*) polymorphisms, as well as polymorphisms of four other genes, as clinical predictors of 5-FU-cisplatin chemotherapy outcomes in cases of advanced gastric cancer. This retrospective study of 52 patients demonstrated that patients homozygous for the *GSTP1* valine allele evidenced significantly higher response rates (67 vs. 21%) and median survival durations (15 vs. 6 months) (13). Interestingly, a similar pattern was observed in patients with Hodgkin's lymphoma (37) and breast cancer (38) treated with cytotoxic chemotherapy.

In our study, except for the finding that the *GSTM1* positive genotype was associated with longer TTP durations in first-line FOLFOX treatment, no associations were detected between the different genotypes of *GST* (*P1*, *M1*, *T1*) genes and response, toxicity, and survival.

Consistently with our observation, patients with low drug metabolizing genotypes with acute myeloid leukemia, esophageal cancer, lung cancer, or breast cancer exhibited reduced survival rates in some studies (39-42). This might be attributable to a higher rate of toxicity-related deaths. As all of the patients included in this study received more than 50% of the projected dose of chemotherapy and no toxicity-related deaths were observed; this fact cannot explain the worse prognosis of the *GSTM1-null* genotype as observed in our study. The exact mechanisms by which the various *GST* genotypes affect survival appear to be highly complex, and are not understood in detail.

A common polymorphism of the *UGT1A1* gene results in an additional TA repeat in the TATA sequence of the *UGT1A1* promoter, with the longer repeat (7 vs. 6) associated with a significant reduction in SN-38 glucoronidation, potentially resulting in increased toxicity. Up to 33% of Caucasians harbor the variant allele with seven repeats (43). The ability of the *UGT1A1* polymorphism to predict severe gastrointestinal and bone marrow toxicity was observed in an earlier study with a small number of patients (N=20), all of whom had solid tumors and were treated with irinotecan. Patients with the seven TA repeat allele (either 6/7 or 7/7) were more likely to experience severe grades of neutropenia and diarrhea (44). Most recently, large-scale prospective pharmacogenetic data from patients with metastatic colorectal cancer treated with irinotecan-based chemotherapy (North Central Cancer Treatment Group N9741) showed that those homozygous for the variant allele (seven repeats) had significantly higher rates of grade 4 neutropenia (36%) compared with those harboring the more common variant (8.6%) (45). This has led to an update of the package insert of irinotecan, which warns that patients with the variant *UGT1A1* polymorphism might be at higher risk for severe neutropenia.

Although several articles have reported a correlation between irinotecan and toxicities, our data did not confirm such a relationship. This might be attributable to differing percentages of the *UGT1A1* polymorphism (46).

The active metabolites of fluoropyrimidines exert their antitumor activity principally via through *TS* inhibition. This

s facilitated by the formation of a ternary complex of *TS*, 5,10-methylene THF and 5-fluoro-dUMP (47). There is a growing body of evidence to suggest the presence of functionally important polymorphic variations within the *TS* gene. The 28-bp repeat and the G/C SNP within the 50 UTR of the gene have been demonstrated to alter the expression of *TS* (21,48). In the current study, patients who possessed a *TS* 50 genotype associated with low *TS* mRNA expression levels (49) demonstrated a trend for superior survival time as compared with patients harboring *TS* genotypes known to be associated with high levels of *TS* mRNA expression. This observation is consistent with recent findings by Marcuello *et al* (50) who revealed a significant association between clinical outcomes of 5-FU-based chemotherapy in colorectal cancer and *TS* polymorphisms only if both *TS* polymorphisms within the 50 UTR were analyzed in conjunction. A trend between *TS* polymorphisms and clinical outcomes was noted in gastric cancer patients who received oral fluoropyrimidine therapy (51).

However, in our study, *TS* polymorphisms did not result in response and survival differences in accordance with genotype. Only the low expression type (2R/2R, 2R/3C and 3C/3C) of TS was associated with a high incidence of grade ≥3 neutropenia.

Acknowledgements

This study was supported by the Dong-A University Research Fund.

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