Predictive values of 5-fluorouracil pathway genes for S-1 treatment in patients with advanced gastric cancer

Hei-Cheul Jeung[a,b,c], Sun Young Rha[a,b,c], Sang Joon Shin[a,c], Seung Joon Lim[b], Jae Kyung Roh[a,b,c], Sung Hoon Noh[d] and Hyun Cheol Chung[a,b,c]

Determination of significant associations between gene expression and predefined endpoints might improve treatment tailoring for advanced gastric cancer. We investigated the mRNA expression of 5-fluorouracil (5-FU) pathway genes in prechemotherapeutic tumor samples of primary gastric cancer to try to predict the treatment outcome of S-1 monotherapy. 5-FU pathway genes, dihydropyrimidine dehydrogenase (*DPD*), orotate phosphoribosyltransferase (*OPRT*), thymidylate synthase (*TS*), and thymidine phosphorylase (*TP*), were analyzed using quantitative real-time PCR of RNA extracted from archived formalin-fixed paraffin-embedded tissues. We selected the median value for each gene as a cutoff to separate patients into high and low gene expression groups. High OPRT gene expression was significantly associated with tumor response ($P=0.014$). In a combined analysis including OPRT, patients with high OPRT and TP showed a higher overall response rate than did the remaining patients (40 vs. 10%, respectively; $P=0.002$). For survival, patients with high OPRT and low TS levels showed prolonged survival in both progression-free survival (3.4 vs. 2.4 months, $P=0.024$) and overall survival (11.0 vs. 8.2 months, $P=0.007$). In a multivariate analysis, the combinations of OPRT and TP for response and OPRT and TS for both progression-free survival and overall survival were independent variables. To conclude, mRNA expression levels of molecular markers in formalin-fixed paraffin-embedded specimens of primary gastric tumors can be useful for identifying patients with advanced gastric cancer who would most likely benefit from S-1 treatment. *Anti-Cancer Drugs* **22:801–810 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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[a]Division of Medical Oncology, Yonsei Cancer Center, [b]Cancer Metastasis Research Center, Departments of [c]Internal Medicine and [d]Surgery, Yonsei University College of Medicine, Seoul, Korea

Correspondence to Professor Hyun Cheol Chung, MD, PhD, Department of Internal Medicine, Division of Medical Oncology, Yonsei Cancer Center, Cancer Metastasis Research Center, Yonsei University College of Medicine, 250, Seongsanno (134, Shinchon-Dong), Seodaemun-Gu, Seoul 120 752, Korea
Tel: +82 2 2228 8132; fax: +82 2 362 5592;
e-mail: unchung8@yuhs.ac

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Introduction

5-Fluorouracil (5-FU), a mainstay of chemotherapeutic agents in advanced gastric cancer (AGC), improves survival and the quality of life. Bolus injection of 5-FU yields a 13–20% of response rate, and protracted continuous infusion (PCI) yields an 18–26% of response rate [1–5]. The antitumor activity of 5-FU mainly results from the inhibition of thymidylate synthase (TS) by 5-fluoro-2′-deoxyuridine-5′-monophosphate. 5-FU also incorporates into RNA and DNA in its metabolite forms, which result from the pathways of 5-fluorouridine-5′-monophosphate by orotate phosphoribosyltransferase (OPRT) and 5-fluoro-2′-deoxyuridine-5′-monophosphate by 2′-deoxy-5-fluorouridine, catalyzed by thymidine phosphorylase (TP). However, interpatient concentrations of 5-FU in the plasma vary significantly with the PCI schedule. Approximately 90% of 5-FU is catabolized to α-fluoro-β-alanine by dihydropyrimidine dehydrogenase (DPD), minimizing the antitumor effect of 5-FU [6]. Accordingly, DPD is supposed to be another factor affecting 5-FU chemosensitivity.

S-1 was developed to mimic the PCI of 5-FU. A high-5-FU level can be maintained in both the plasma and tumor without increasing gastrointestinal toxicity by combining tegafur with the two biochemical modulators 5-chloro-2,4-dihydroxypyridine and potassium oxonate [7,8]. With S-1 monotherapy, early and late phase II trials in Japan achieved remarkable overall response rates (ORR) of 54 and 45%, respectively [9–11]. Unfortunately, this initial high efficacy has not been reproduced beyond Japan. Western trials had to restrict daily dosage because of the dose-limiting toxicity of diarrhea and hyperbilirubinemia [12]. Ethnic differences have also been recorded among Asian patients. A Korean phase II study tried the highest dose intensity of S-1 ever, but the ORR was limited to 19% with anemia as a major toxicity [13]. On the basis of these outcomes, we suggest that pharmacogenetic factors may contribute to the treatment outcome.

Recently, a growing body of evidence suggests that combining information from multiple genes seems to be a rational approach for the prediction of the treatment

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outcome [14]. Determination of significant associations between gene expression and predefined endpoints might improve treatment tailoring. The methodology for selecting candidate genes from among different pathways or within a single pathway requires different clinical approaches. Such an approach has prevailed in colorectal cancer but rarely in gastric cancer. Accordingly, we investigated the mRNA expression of the 5-FU pathway genes, *DPD*, *OPRT*, *TS*, and *TP*, in formalin-fixed paraffin-embedded (FFPE) specimens of primary gastric tumor. We also evaluated the value of these genes for predicting the treatment-related clinical endpoints of response rates and survival.

Patients and methods

Patient eligibility

This study was collaterally designed with two published open-label phase II trials [13,15]. In brief, patients were required to have histologically proven metastatic or recurrent gastric adenocarcinoma and were considered eligible when they met all of the following criteria: age of more than or equal to 18 years; performance status of less than or equal to 3 by Eastern Cooperative Oncology Group criteria; life expectancy of more than or equal to 3 months; either no previous chemotherapy (adjuvant chemotherapy completed at least 6 months before enrollment) or failure of first-line chemotherapy; assessable or measurable lesions; and adequate organ function. Patients were excluded if they had concurrent active cancer, brain metastasis, or uncontrolled comorbidity. The protocol was approved by the institutional review board, and written informed consent with International Conference on Harmonisation guidelines was obtained according to institutional regulations.

Treatment schedule

The starting dose of S-1 was 35 mg/m^2 twice daily. S-1 was administered for either 28 consecutive days followed by a 14-day resting period [13], or for 14 consecutive days followed by a 7-day resting period [15]. S-1 dosage was calculated based on body surface area, which was different from the Japanese guide [13]. The planned dose intensity was 327 mg/m^2 per week. The schedule was repeated until the occurrence of disease progression, unacceptable toxicities, or withdrawal of consent. Tumor was measured following the Response Evaluation Criteria in Solid Tumors (version 1.0) [16]. Patients were considered suitable for evaluation of response if they received a minimum of one cycle of treatment with at least one follow-up tumor measurement. Early progressive disease (PD) was defined as disease in patients that progressed rapidly within two cycles of treatment.

Extraction of RNA and real-time PCR of 5-fluorouracil-metabolizing enzymes

Archival FFPE specimens of primary gastric tumors obtained at the time of endoscopic biopsy or surgery were used for this study. Immediately after sampling, each tumor specimen was fixed in 10% formalin overnight and embedded in paraffin. Sections of 5-μm thickness were stained with hematoxylin and eosin for histological diagnosis. For microdissection, representative sections were stained with nuclear fast red (American MasterTech Scientific, Lodi, California, USA), and malignant cells were selectively isolated using laser-captured microdissection (PALM Microlaser Technologies, Munich, Germany).

Dissected tissue particles were transferred into a reaction tube containing 400 μl of RNA lysis buffer. The samples were homogenized and heated at 93°C for 30 min; 50 μl of sodium acetate (2 mol/l; pH 4.0) was then added, followed by 600 μl of freshly prepared phenol/chloroform/isoamyl alcohol (250 : 50 : 1). Tubes were placed on ice for 15 min and then centrifuged in a chilled (8°C) centrifuge. After the upper aqueous phase had been carefully removed, 10 μl of glycogen and 300 μl of isopropanol were added. The tubes were chilled at −20°C for 30–45 min to precipitate the RNA. The samples were washed in 500 μl of 75% ethanol and air dried for 15 min. Finally, the pellet was resuspended in 50 μl of RNAse-free water.

Four genes of interest and an internal reference gene (β-actin) were quantified using a fluorescence-based real-time detection method (ABI PRISM 7900 Sequence detection System, TaqMan, Perkin-Elmer Applied Biosystem, Foster City, California, USA). Each PCR reaction was performed in a final volume of 20 μl containing 1200 nmol/l of each primer, 200 nmol/l of probe, AmpliTaq gold polymerase (0.4 U), 200 nmol/l each of dNTPs, 3.5 mmol/l of $MgCl_2$, and 1 × TaqMan buffer A with a reference dye (all reagents from Perkin-Elmer Applied Biosystems). Cycling conditions consisted of 50°C for 2 min and 95°C for 10 min, followed by 46 cycles of 95°C for 15 s and 60°C for 1 min. The primers and probes used for detection of TS, DPD, TP, OPRT, and β-actin were designed according to the respective sequences, as previously described [17]. Gene expression values (relative mRNA levels) are expressed as ratios (differences between C_t values) between the gene of interest and β-actin.

Statistical considerations

Spearman's rank correlation was used to evaluate the correlation between measured gene expressions. Statistical differences of gene expression and clinicopathological variables were analyzed using the Mann–Whitney *U*-test. Gene expression values were dichotomized as equal/above or below the median expression values of the respective genes. Association of gene expression with response was analyzed using the Mann–Whitney *U*-test and Fisher's exact test (two sided).

The main parameters we examined were the objective tumor response, progression-free survival (PFS), and overall survival (OS). PFS was defined from the onset of chemotherapy to disease progression or death (irrespective

of the cause). OS was reckoned from the onset of chemotherapy until death. All of the survival curves were obtained using the Kaplan–Meier method and analyzed using the log-rank test.

To explore the effects of covariates on response and survival, multivariate logistic regression and Cox's proportional hazard models were used to detect the independent effects of genetic expression on response and survival. Estimates of hazard ratios with 95% confidence intervals (CIs) were used to provide quantitative summaries. All reported *P* values are two sided, and the level of significance was at a *P* value of less than 0.05.

Results

Patient characteristics

A total of 75 patients were eligible for the study. The median body surface area of all patients was 1.58 m^2 (range: 1.16–2.09 m^2). Forty-five patients (60%) received S-1 as a first-line treatment. Forty-one patients (55%) underwent gastrectomy earlier. Abdominal lymph nodes and liver were common sites for measurable lesions, and a primary gastric mass was the main nonmeasurable lesion. Baseline characteristics of these patients are presented in Table 1.

Table 1 Patient characteristics

Characteristics	Patients (%)
Sex	
Male	50 (67)
Female	25 (33)
Age (years)	
Median (range)	60 (23–81)
<70	61 (81)
≥ 70	14 (19)
BSA [m^2, median (range)]	1.58 (1.16–2.09)
Performance status	
0	5 (7)
1	17 (23)
2	29 (39)
3	24 (31)
Histological type	
Differentiated	22 (29)
Undifferentiated	53 (71)
Previous gastrectomy	
No	34 (45)
Yes	41 (55)
Overall response (by RECIST 1.0)	
CR/PR	15 (20)
SD	28 (38)
PD	31 (41)
NE	1 (1)
Measurable lesion[a]	
Lymph node	36 (55)
Liver	17 (26)
Abdominal mass	6 (9)
Lung	3 (5)
Others	3 (5)
Unmeasurable lesion[a]	
Gastric mass or anastomosis site	34 (33)
Peritoneal seeding	28 (27)
Colon	14 (14)
Bone	6 (6)
Others	21 (20)
Dose of S-1 (mg; median, range)	120 (80–150)
Dose of S-1 per BSA (mg/day; median, range)	71 (56–88)
Treatment cycle (median, range)	4 (1–20)
Treatment week (median, range)	12 (2–58)
ADI (mg/m^2/week; median, range)	315 (162–373)
RDI (median, range)	0.96 (0.50–1.14)

ADI, actual dose intensity; BSA, body surface area; CR, complete response; NE, not evaluable; RDI, relative dose intensity; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

[a]As patients could have lesions at multiple sites, the total number of lesions is greater than the number of patients.

Expression levels of 5-fluorouracil pathway genes and their associations with clinicopathological parameters

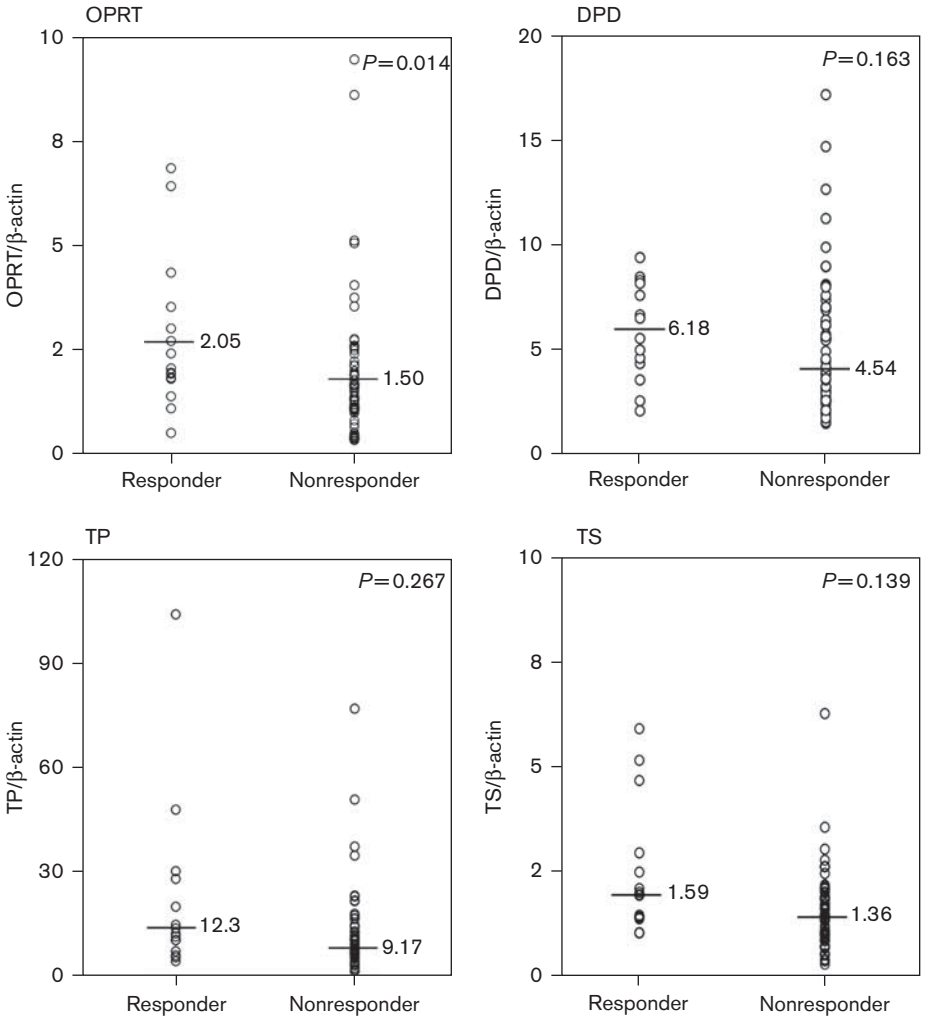
Expression levels of the four genes were determined using real-time RT-PCR. Gene expression levels relative to β-actin ($\times 10^{-3}$) were as follows: DPD, 4.975 (range, 1.83–16.90); OPRT, 1.66 (range, 0.32–9.47); TP, 10.36 (range, 1.43–103.57); and TS, 1.36 (0.27–8.64). Patients were separated into high-expression and low-expression groups using median cutoff values for each gene. *TP* gene expression moderately correlated with DPD expression (Spearman's rank correlation coefficient: 0.233, $P = 0.046$), and also with TS expression (Spearman's rank correlation coefficient: 0.298, $P = 0.009$). mRNA levels of the four genes did not show any association with age (<70 vs. ≥ 70), sex, timing of metastasis (recurrent vs. metastatic), pathology (differentiated vs. undifferentiated), earlier treatment (first line vs. second line), or metastatic pattern (peritoneal vs. hematogenous).

Association between tumor response and 5-fluorouracil pathway gene expression

We categorized the patients as either responders (complete response or partial response) or nonresponders (stable disease or PD) to the S-1 treatment and compared each genetic expression level between the two groups (Fig. 1, Table 2). One patient not suitable for evaluation was excluded from this analysis. A good association was observed between *OPRT* gene expression and tumor response ($P = 0.014$ for the Mann–Whitney *U*-test; $P = 0.010$ for Fisher's exact test). There were marginally more responders in the high-TP group compared with the low-TP group ($P = 0.082$ for Fisher's exact test), but there was no statistically significant difference in TP expression in terms of response ($P = 0.267$ for the Mann–Whitney *U*-test). The other genes were not associated with tumor response. Among patients who received S-1 as a first-line treatment, only OPRT was again found to have a good association with tumor response ($P = 0.026$ for the Mann–Whitney *U*-test; $P = 0.028$ for Fisher's exact test).

In the combined analysis involving OPRT, patients with high-OPRT and high-TP levels ($n = 25$) showed a higher ORR than did the remaining patients ($n = 50$; 40 vs. 10%, respectively; $P = 0.002$ for Fisher's exact test). In a stepwise logistic regression analysis, high OPRT and high TP were the only selected independent factors in relation to response; the odds ratio for poor response was 0.08 for these patients (95% CI: 0.02–0.43; $P = 0.003$; Table 3).

Fig. 1



Expression of 5-fluorouracil pathway genes grouped by responders and nonresponders. Median values for the expression of each gene are shown by solid lines. *P* values from the Mann–Whitney test are indicated. DPD, dihydropyrimidine dehydrogenase; OPRT, orotate phosphoribosyltransferase; TP, thymidine phosphorylase; TS, thymidylate synthase.

Table 2 Association of gene expression level with response

Relative gene expression	Responders (%)	Nonresponders (%)	*P* value	OR (95% CI)
OPRT				
≥ 1.66	12 (32)	25 (68)	0.010	0.179 (0.046–0.699)
<1.66	3 (8)	35 (92)		
DPD				
≥ 4.975	10 (27)	27 (73)	0.133	0.409 (0.125–1.342)
<4.975	5 (13)	33 (87)		
TP				
≥ 10.36	11 (29)	27 (71)	0.082	0.298 (0.085–1.041)
<10.36	4 (11)	33 (89)		
TS				
≥ 1.36	9 (23)	30 (77)	0.488	0.667 (0.211–2.106)
<1.36	6 (17)	39 (83)		
TP and OPRT				
≥ 10.36 and ≥ 1.66	10 (40)	15 (60)	0.002	0.167 (0.049–0.566)
<10.36 or <1.66 or both	5 (10)	45 (90)		

CI, confidence interval; DPD, dihydropyrimidine dehydrogenase; OPRT, orotate phosphoribosyltransferase; OR, odds ratio; TP, thymidine phosphorylase; TS, thymidylate synthase.

Table 3 Univariate and multivariate analyses of factors influencing tumor response

		Univariate analysis		Multivariate analysis	
Factor	*N*	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Sex					
Male	50	1			
Female	25	1.00 (0.30–3.32)	1.000		
Age					
<70	61	1			
≥ 70	14	0.898 (0.22–3.73)	1.000		
Previous chemotherapy					
No	45	1		1	
Yes	30	13.10 (1.62–106.0)	0.003	8.82 (0.89–87.5)	0.063
Performance status					
0–1	22	1		1	
2–3	53	3.76 (1.16–12.19)	0.022	4.32 (0.76–24.5)	0.098
Histology					
Differentiated	53	1			
Undifferentiated	22	0.77 (0.23–2.62)	0.677		
DPD					
Below median	38	1			
Above median	37	0.41 (0.13∼1.34)	0.133		
OPRT					
Below median	38	1			
Above median	37	0.18 (0.05–0.70)	0.010		
TP					
Below median	37	1			
Above median	38	0.30 (0.09–1.04)	0.082		
TS					
Below median	37	1			
Above median	38	0.68 (0.21–2.11)	0.488		
TP and OPRT					
Others	50	1		1	
High OPRT and high TP	25	0.17 (0.05–0.56)	0.002	0.08 (0.02–0.43)	0.003

CI, confidence interval; DPD, dihydropyrimidine dehydrogenase; OPRT, orotate phosphoribosyltransferase; OR, odds ratio; TP, thymidine phosphorylase; TS, thymidylate synthase.

When we investigated genetic markers to predict patients of early PD, a significant association was observed between high TS and early PD ($P = 0.046$). This finding was more pronounced in the combined analysis with low OPRT; patients with high TS and low OPRT had an increased risk of early PD (odds ratio = 3.33; 95% CI: 1.04–10.66) in the multivariate analysis, which was statistically significant ($P = 0.042$).

Association between survival and 5-fluorouracil pathway gene expression

The Kaplan–Meier curves of PFS and OS according to each gene expression level are presented in Fig. 2. Patients whose tumors had high OPRT expression showed better PFS ($P = 0.034$) and OS ($P < 0.001$) than did the remaining patients. There were no differences in survival according to the gene expression of DPD, TP, or TS. When we combined the expression of OPRT and TS, patients with high-OPRT and low-TS levels showed longer PFS (3.4 vs. 2.4 months, respectively; $P = 0.024$) and OS (11.0 vs. 8.2 months; $P = 0.007$).

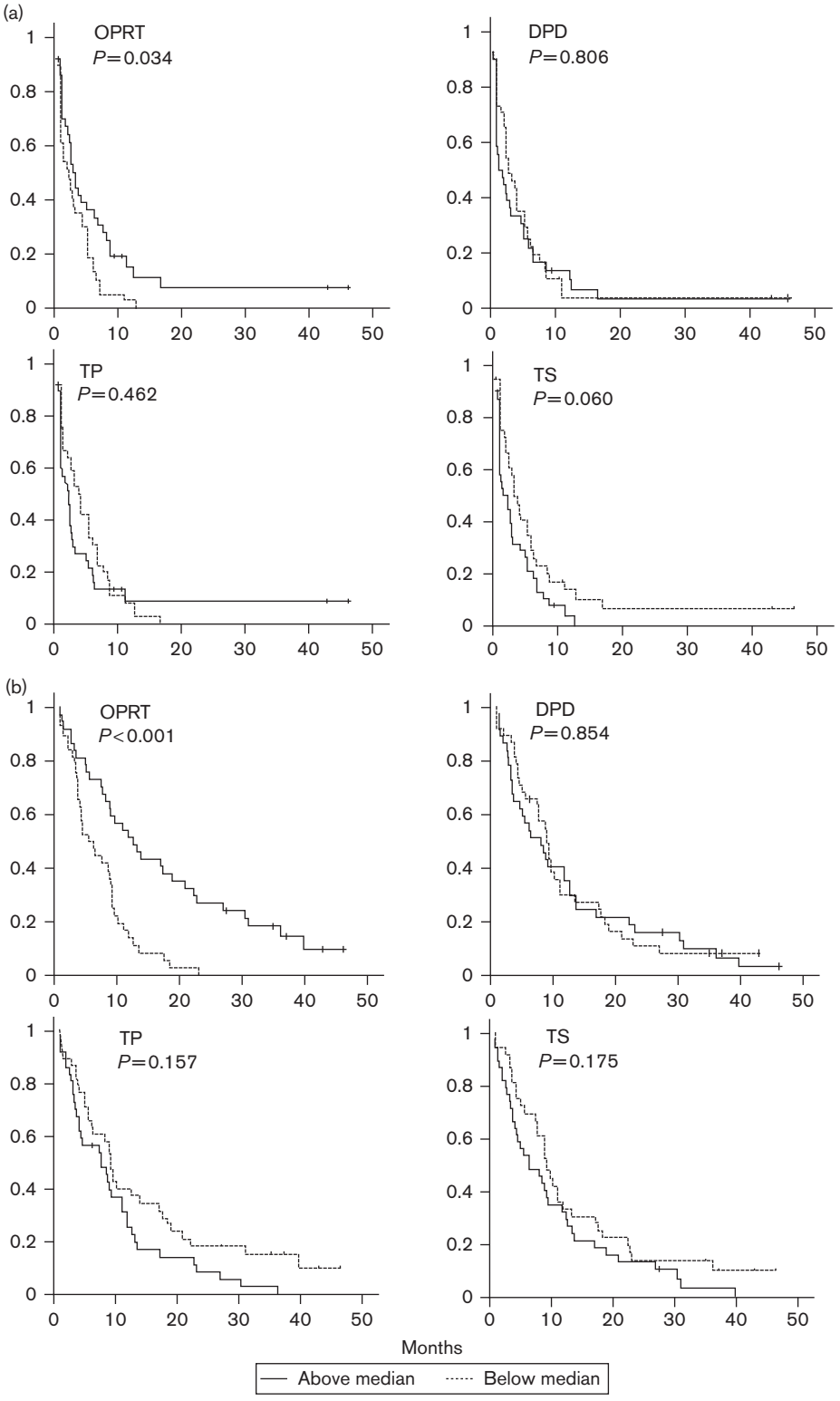
For the purpose of treatment individualization, we categorized patients into three groups: high OPRT/low TS, low OPRT/high TS, and other. The curve of PFS was clearly separated, with a median PFS of 3.9 months for high-OPRT/low-TS patients (95% CI: 2.4–5.4 months) and 2.7 months for the others. Low-OPRT/high-TS patients had the worst PFS of only 1.5 months (95% CI: 0.9–2.1 months; $P = 0.013$; Fig. 3a). For OS, high-OPRT/low-TS patients had the best OS of 13.2 months (95% CI: 3.0–25.4 months), whereas survival in the low-OPRT/high-TS group was only 4.5 months (95% CI: 3.2–5.8 months) ($P < 0.001$; Fig. 3b). When we analyzed OS separately by treatment group (first-line and second-line treatment), the survival curves were also divided clearly. For first-line treatment, high-OPRT/low-TS patients had an exceptionally prolonged survival of up to 22.3 months (95% CI: 7.3–37.3 months), whereas survival in the low-OPRT/high-TS group was only 6.3 months (95% CI: 0.3–12.3 months) ($P = 0.004$; Fig. 3c). For second-line treatment, high-OPRT/low-TS patients had an OS of 9.7 months (95% CI: 5.5–13.9 months), whereas survival in the low-OPRT/high-TS group was only 3.4 months (95% CI: 2.1–4.7 months; $P = 0.026$; Fig. 3d).

On multivariate analysis, high-OPRT and low-TS level was significantly associated with PFS and OS, whereas none of the clinicopathological variables were. High-OPRT and low-TS level was the only one selected in the multivariate model, which decreased the risk of death (hazard ratio = 0.416; 95% CI: 0.223–0.778; $P = 0.006$; Table 4).

Discussion

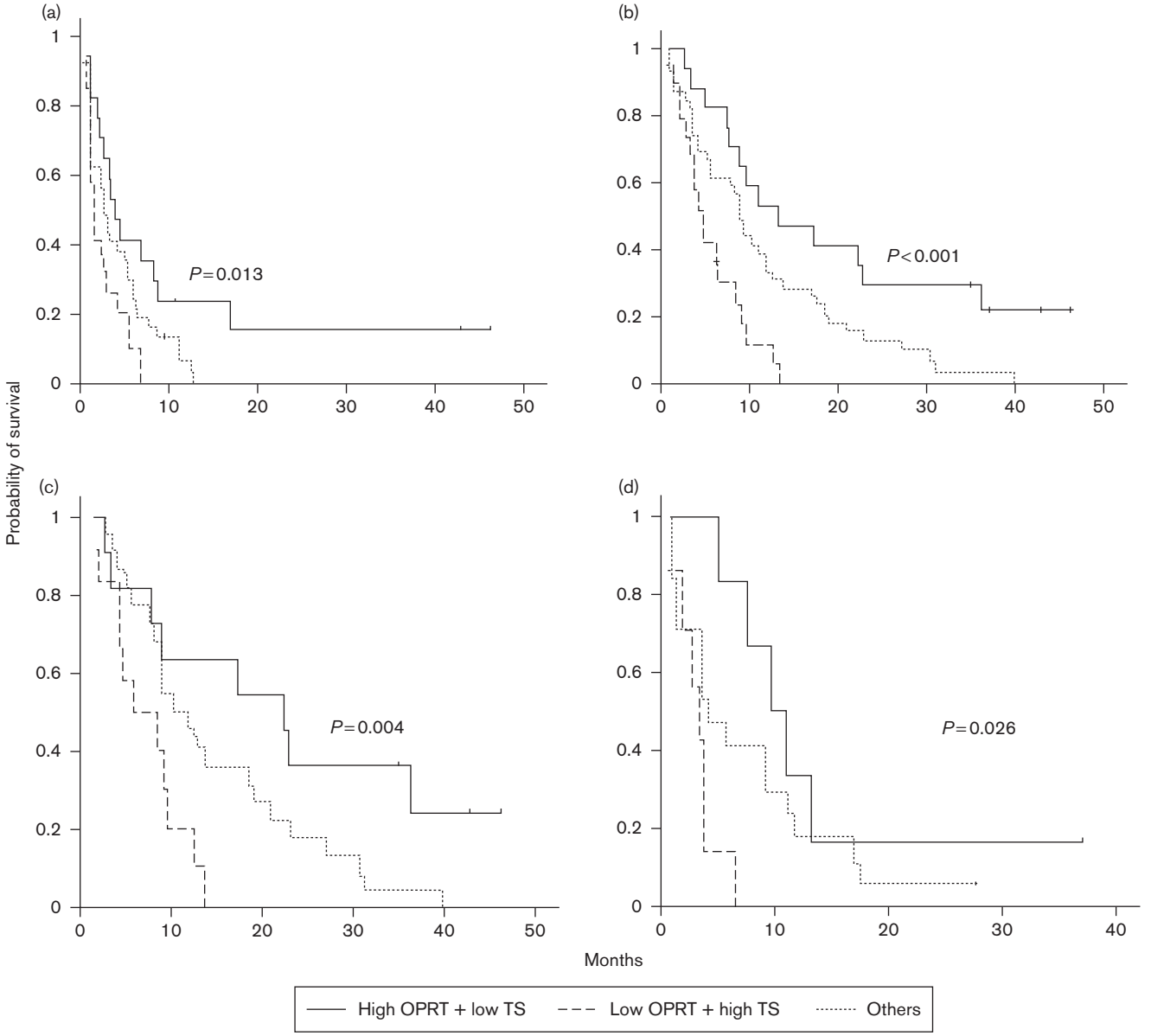
For decades, continuous attempts have been made to identify genetic factors affecting the extent of prodrug

Fig. 2



Expressions of 5-fluorouracil (5-FU) pathway genes and progression-free survival (PFS) and overall survival (OS). Kaplan–Meier curves are shown according to the respective cutoff value for the expression of each gene. *P* values by the log-rank test are indicated. (a) PFS and (b) OS. DPD, dihydropyrimidine dehydrogenase; OPRT, orotate phosphoribosyltransferase; TP, thymidine phosphorylase; TS, thymidylate synthase.

Fig. 3



Survival analysis according to orotate phosphoribosyltransferase (OPRT) and thymidylate synthase (TS) levels. Kaplan–Meier curves are shown according to the respective cutoff values for the expression of each gene. *P* values by the log-rank test are indicated. (a) Progression-free survival; (b) overall survival (OS); (c) OS analyzed in the first-line treatment; and (d) OS analyzed in the second-line treatment.

activation of fluoropyrimidines and, as a result, influencing the treatment outcome. However, despite some promising preclinical results, conflicting results and individual differences have been reported in clinical studies and no definite conclusions have yet been drawn. Studies of small, heterogenous patient populations may be one cause of this inconsistency. The applied methodology could add another confounding factor. Initial studies focused on protein or enzymatic activity, which is unstable and requires a large amount of tissue. Immunohistochemistry is a well-established methodology for protein expression and localization, but the interpretation is subjective and hard to quantify. Obtaining fresh-frozen tissue on biopsy or surgery is a rational approach, but it is not routinely carried out in clinical practice. Ignorance of cancer cell-to-normal stroma composition is another weakness. Accordingly, we thought that FFPE tissue would be a good source for a specimen, because it is a universal way by which tissue is stored in most hospitals and is easily accessible as a tissue bank. We acknowledge that the RNA extraction from FFPE tissues requires more skill, and that the RNA is

Table 4 Univariate and multivariate analyses of factors influencing survival

		Progression-free survival					Overall survival				
			Univariate		Multivariate			Univariate		Multivariate	
Factor	*N*	Median (m) (95% CI)	HR (95% CI)	*P* value	HR (95% CI)	*P* value	Median (m) (95% CI)	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Sex											
Male	50	2.6 (1.1–4.1)	1	0.688			7.7 (4.8–10.6)	1	0.425		
Female	25	3.0 (1.7–4.3)	0.903 (0.549–1.485)				11.1 (5.8–16.4)	0.814 (0.491–1.350)			
Age											
<70	61	2.6 (2.0–3.2)	1	0.501			9.0 (8.0–10.1)	1	0.712		
≥ 70	14	3.3 (1.8–4.8)	0.812 (0.444–1.487)				4.7 (0.0–10.5)	1.125 (0.691–2.105)			
Functional status											
0–1	22	3.4 (1.8–5.0)	1	0.534			9.0 (8.2–9.8)	1	0.764		
2–3	53	2.3 (1.2–3.4)	1.090 (0.830–1.432)				7.7 (4.4–11.0)	0.961 (0.743–1.243)			
Previous gastrectomy											
No	34	2.6 (1.4–3.8)	1	0.334			8.2 (2.9–13.5)	1	0.152		
Yes	41	2.8 (1.6–4.0)	0.792 (0.493–1.272)				9.3 (6.2–12.4)	0.705 (0.437–1.138)			
Histology											
Poorly differentiated	51	2.6 (1.2–4.0)	1	0.688			8.6 (4.9–12.3)	1	0.679		
Well differentiated	23	2.6 (1.3–4.0)	0.898 (0.532–1.516)				8.9 (7.4–10.4)	1.117 (0.661–1.888)			
Weight loss											
<10%	41	2.4 (0.5–4.2)	1	0.850			9.7 (6.8–12.6)	1	0.095	1	0.061
>10%	30	3.0 (2.1–3.9)	1.050 (0.634–1.738)				7.7 (2.9–12.5)	1.524 (0.930–2.499)		1.617 (0.978–2.674)	
Genetic expression											
High OPRT and low TS	18	3.4 (2.6–4.2)	1	0.024	1	0.031	11.0 (3.7–18.3)	1	0.007	1	0.006
Others	57	2.4 (1.3–3.5)	1.991 (1.095–3.261)		1.942 (1.064–3.544)		8.2 (5.1–11.3)	2.331 (1.276–4.259)		2.402 (1.286–4.486)	

CI, confidence interval; DPD, dihydropyrimidine dehydrogenase; HR, hazard ratio; OPRT, orotate phosphoribosyltransferase; TP, thymidine phosphorylase; TS, thymidylate synthase.

prone to be extensively degraded or modified compared with that from fresh-frozen tissues. We show here, however, that mRNA from FFPE tissues and utilization of laser-captured microscopy can provide sufficient information for identifying biomarkers.

We found that the combined expression of 5-FU-metabolizing genes had some predictive value in Korean patients: high OPRT and high TP for response, and high OPRT and low TS for survival. Two earlier Japanese studies focused on 5-FU-metabolizing genes related to S-1 treatment. Ichikawa *et al.* [14] examined fresh-frozen tissues of primary tumors in 59 patients, and reported that simple combinations of the genes had predictive values: OPRT and TS for response and TS and TP for survival. Koizumi *et al.* [18], in a study similar to ours with respect to the use of archival FFPE tissues and laser-capture microscopy, suggested that patients with simultaneous low TP, low TS, and high OPRT had longer OS with S-1. Although our study reaffirms the value of FFPE primary gastric tissues for the prediction of S-1-related outcome in Korean patients with greater patient numbers, the selected genes showed some discrepancy with the Japanese reports. We feel that another factor may be attributable to the ethnic aspects of the candidate genes themselves. In addition, the remaining lack of knowledge about the exact mechanism of genetic regulation at the transcriptional, translational, or even posttranslational level is another confounding element.

In contrast to the Japanese studies, we found that high TP expression favored a good response. In gastric cancer, TP is expressed more in differentiated carcinoma compared with undifferentiated carcinoma, and mainly in infiltrating cells in the stroma [19]. In this study, only 29% of patients had differentiated carcinoma, whereas 56 and 39% of patients in the two Japanese studies had differentiated carcinoma, respectively. Shimaoka *et al.* [19] reported that the predictive and prognostic values of TP were applicable only for differentiated carcinoma. Similar studies with a low proportion of differentiated carcinoma have not found a significant association between TP and prognosis [20,21]. Therefore, we surmise that the incidence of differentiated carcinoma in our study population may be one explanation for the discrepancy.

The characterization of TP in modulating 5-FU sensitivity has also been mixed. Initial in-vitro experiments using TP overexpression found an increased sensitivity to 5-FU [22]. A retrospective analysis of TP mRNA expression in colorectal tumors, however, indicated that high TP-expressing tumors show low response to 5-FU [23].

These contradictory findings might be explained by the fact that TP is also an angiogenic factor and that TP expression reflects an aggressive tumor phenotype. We used only isolated tumor portions for the experiment. We assume that tumor cells do not benefit from increased angiogenic potential, and we assume that TP-mediated activation of 5-FU might predominate in this study.

Consistent with another study of Korean patients [24], we found that high-OPRT levels were consistently useful for predicting response, early progression, and long-term survival. OPRT is involved in the reaction that adds ribose-5′-phosphate to orotic acid, and 5-FU phosphorylation by OPRT is considered the most predominant in tumor tissue, implying that OPRT is the main rate-limiting step for the activation of 5-FU [25]. OPRT has a higher expression in cancer than in normal tissue. This study did not show any association between histological characteristics and OPRT, but some reports have correlated OPRT expression with tumor grade or location, implying that part of OPRT regulation is posttranslational [26]. Another hypothesis is that individual variations in drug response are because of genetic polymorphisms that influence the drug-metabolizing enzymes that determine pharmacodynamics. Ichikawa *et al.* [27], investigating the Ala allele in the OPRT G213A polymorphism in colorectal cancer, found that the Ala/Ala genotype had four times more OPRT activity than the Gly/Gly genotype and was an independent predictor of severe diarrhea after 5-FU treatment. Further study is warranted to identify ethnic differences in OPRT polymorphisms in Koreans, which could also help to clarify the role of OPRT in predicting the treatment outcome.

Our results may provide a more logical basis for risk stratification of patients. On the basis of our findings, S-1 monotherapy may be a rational option for patients with high OPRT/low TS in terms of survival. For patients with low-OPRT/high-TS expression, however, consideration of other agents or combination chemotherapy may be preferable. This study is limited by its small patient number and retrospective analysis. In fact, eight patients enrolled in the phase II trials were excluded from this genetic study, all of whom had biopsied specimens in which the tumor proportion was too small (only $< 1\,mm^2$ in 10 slides) or scattered to extract sufficient amount of RNA. It could preclude drawing firm conclusions. A comprehensive approach with a large population is needed to define more sophisticated marker sets. We did find that the genetic information in gastric cancer cells isolated from FFPE specimens of primary tumors offers a relevant approach for identifying biomarkers.

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Conflicts of interest

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