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ORIGINAL ARTICLE

Clinicopathologic significance of ERCC1, thymidylate synthase and glutathione S-transferase P1 expression for advanced gastric cancer patients receiving adjuvant 5-FU and cisplatin chemotherapy

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Abstract

The objective of this study was to determine whether the expressions of the excision cross-complementing (ERCC1), thymidylate synthase (TS) and glutathione S-transferase P1 (GSTP1) are predictive of clinical outcomes in advanced gastric cancer (AGC) patients receiving treatment with adjuvant 5-fluorouracil (5-FU) and cisplatin (FP) chemotherapy. One hundred forty nine patients were included in this study. ERCC1 and GSTP1 expression was correlated significantly with tumor size ($p = 0.040$, $p = 0.018$, respectively). Stage and positive lymph node ratio were associated independently with disease free survival (DFS) and overall survival (OS). Both ERCC1 and GSTP1 expression had a significant impact on OS (hazard ratio = 0.069, $p = 0.021$). TS expression was not related to DFS and OS.

Keywords: *Gastric cancer; 5-fluorouracil; cisplatin; ERCC1; GSTP1*

Introduction

Gastric cancer remains a significant health problem despite declining incidence in the West. It is the 4th most common cancer worldwide, accounting for 8.6% of all new cancer diagnoses in 2002 (Parkin et al., 2005). In Korea, gastric cancer is the most common carcinoma in men, and the third most common type of cancer in women (Won et al., 2009). Surgery is the only potentially curative treatment for localized gastric cancer. However, the majority of patients will relapse after definitive surgery, and the 5-year overall survival (OS) rates of this condition remain lower than 30% to 40% in Western countries (Parkin et al., 2005).

Over the last several decades, many clinical trials have attempted to improve survival with adjuvant therapy for gastric cancer. The role of adjuvant therapy in gastric cancer has been a matter of some controversy, considering the lack of significant survival benefit noted in many randomized studies conducted thus far (Lim et al., 2005, Ng et al., 2007). A variety of chemotherapeutic agents and combinations thereof have been used. 5-fluorouracil (5-FU) is the earliest and yet one of the most important cytotoxic agents used in the management of this disease. Cisplatin, which is often used in combination with 5-FU, has also shown promise in this regard. In fact, the combination of 5-FU and cisplatin (FP) is regarded as a standard chemotherapeutic protocol for metastatic gastric cancer(Wagner et al., 2006). Advances in molecular pharmacology have refined our understanding of the mechanisms of action of these drugs, as well as the mechanisms underlying resistance to chemotherapy. Several mechanisms of resistance have been identified among the agents most commonly

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utilized for the treatment of gastric cancer patients (Park and Lenz, 2006).

Thymidylate synthase (TS) is the rate-limiting enzyme in the synthesis of the pyrimidine nucleotides required for DNA synthesis, and is also a critical target for fluoropyrimidines. Elevated TS protein levels may interfere with the mechanisms of action of fluoropyrimidines (Marsh and McLeod, 2004). The majority of studies have been conducted in colorectal cancer patients, and a recent meta-analysis confirmed the poorer overall survival of patients with enhanced TS activity as compared with the cases with low TS activity (Popat et al., 2004). However, conflicting results have been reported regarding the expression of TS in cases of gastric cancer (Kim et al., 2009, Kwon et al., 2007).

The role of cisplatin in gastric cancer was first assessed in patients with advanced disease. A previous phase II study showed a response rate of between 23% and 73% for advanced gastric cancer patients treated with a cisplatin-based combination therapy (Wagner et al., 2006). On the basis of its effects on advanced gastric cancer, cisplatin has previously and is currently being used as a basic agent in the adjuvant setting of gastric cancer (Lim et al., 2005). However, the antitumor activity of cisplatin has only been incompletely defined as an adjuvant therapy thus far (Bouche et al., 2005). The destruction of cells by cisplatin requires the binding of the drug to DNA and the creation of platinum–DNA adducts. Some of these adducts establish covalent cross-linking between DNA strands, thereby inhibiting DNA replication (Johnson et al., 1980). Nucleotide excision repair plays a central role in DNA repair and is associated with resistance to platinum-based chemotherapy (Reed, 2005).

The excision repair cross-complementing 1 (ERCC1) gene encodes for a 297-amino acid protein, which appears to harbor a putative nuclear localization signal and a postulated helix-turn-helix motif--characteristic of a DNA-binding protein (Van Duin et al., 1986). The ERCC1 protein is considered to be a component of a functional complex with the ERCC4, ERCC, and XPF proteins, which may be required both for nucleotide excision repair and recombinational repair (Wilson et al., 2001). The ERCC1 gene is associated with responses to FP chemotherapy in cases of gastric cancer. Metzger *et al.*(Metzger et al., 1998) has also reported that high levels of ERCC1 expression in gastric cancer might be associated with poor survival and no response to cisplatin. We have previously published a study on the prognostic value of immunohistochemically-measured expression of the ERCC1, TS, and GSTP1 proteins for FOLFOX chemotherapy in metastatic gastric cancer (Kwon et al., 2007). We also noted an association between reduced survival and the relative overexpression of ERCC1. However, in another report, ERCC1 expression was not associated with prognosis (Kim et al., 2009), or low ERCC1 expression was correlated with poor outcome (Baek et al., 2006). Therefore, the role of ERCC1 has yet to be definitively elucidated in cases of advanced gastric cancer treated with cisplatin and 5-FU based adjuvant chemotherapy.

Glutathione is a ubiquitous tripeptide, which has been hypothesized to protect against the DNA-damaging effects of agents by conjugating toxic moieties, including metal compounds, in the cytoplasm and preventing their interaction with DNA (Tew, 1994). Glutathione S-transferase π (GSTπ) is a member of a family of isozymes that perform an important function in the detoxification of many xenobiotic substances via conjugation to glutathione (Moscow et al., 1989), and the subclass GSTP1 (Glutathione S-transferase pi 1) is expressed broadly in normal human epithelial tissues and has been shown to be highly overexpressed in colon cancer (Tsuchida and Sato, 1992). GSTP1 directly participates in the detoxification of platinum compounds, and is an important mediator of both intrinsic and acquired platinum resistance (Goto et al., 1999). In fact, some immunohistochemical studies have demonstrated that the expression of GSTP1 may predict responses to cisplatin-based chemotherapy (Harpole et al., 2001).

The principal objective of our study was to determine whether the expression of ERCC1, TS, and GSTP1 has an effect on the survival of gastric cancer patients who received FP adjuvant therapy following curative resection.

Patients and Methods

Patients' characteristics

Between January 2002 and January 2006, 149 patients were included in this study. All patients had histologically confirmed adenocarcinoma of the stomach, and had undergone a potentially curative gastrectomy with D2 dissection, with neither gross nor microscopic evidence of residual disease; a ECOG performance status < 2; adequate hematological (neutrophils ≥ 3×10^9/L; platelets ≥ 100×10^9/L), hepatic (bilirubin ≤ 1.5 mg/dL; aspartate aminotransferase and alanine aminotransferase ≤ 5× the upper normal limit), renal (creatinine ≤ 2.0 mg/dL) and cardiac function; no post-operative complications. The exclusion criteria were concurrent active malignancy other than superficial skin cancer or *in situ* cervical carcinoma. Tumor samples were obtained from these patients, and each sample was fixed in formalin and embedded in paraffin wax. Clinical outcomes were monitored from the date of surgery to either the date of death or August 2009. This

study was approved by the institutional review board of the Dong-A University Medical Center.

Administration of adjuvant 5-FU and cisplatin combination chemotherapy

After curative resection, in the patients with stage II disease or higher, according to the TNM staging system (*AJCC* Cancer Staging, 6th edition), with good performance status and adequate laboratory findings, we administered adjuvant FP combination chemotherapy. The FP regimen was as follows: 60 mg/m^2 of cisplatin was intravenously administered on day 1, and 1,000 mg/m^2 of 5-FU was intravenously administered on days 1 to 5. This regimen was repeated every three weeks. In the event of NCI-CTC toxicity, the following dose reductions and treatment delays were planned. In cases of insufficient hematological function (neutrophil count $< 1.5 \times 10^9$/L or platelet count $< 100 \times 10^9$/L) on day 21, treatment was delayed. For grade 3–4 gastrointestinal toxicities, thrombocytopenia and neutropenia, there were 25% 5-FU and cisplatin dose reductions. For grade 2 or greater cardiotoxicity, 5-FU treatment was discontinued. Cisplatin administration was discontinued in cases of grade 2 or greater neurological toxicity or if creatinine levels were > 2.0 mg/dL.

Follow-up of patients

The postoperative baseline and follow-up investigations were standardized. Prior to adjuvant chemotherapy, the baseline assessments included a medical history and physical examination, a complete blood count (CBC), renal and liver function tests, urinalysis, ECG, chest X-rays, radionuclide bone scan, and abdominal CT. The patients were followed-up at 3-month intervals for 2 years, then at 6-month intervals for 3 years, and annually thereafter. This follow-up exam consisted of a physical examination, CBC, renal and liver function tests, determination of CEA, and abdominal ultrasonography or CT scan. Gastroscopy was repeated once per year.

Tissue Microarray Methods

Two cores of 2 mm in diameter of formalin-fixed, paraffin-embedded tissue blocks were obtained from each case of gastric cancer and arranged in new recipient paraffin blocks using an UNITMA apparatus (UNITMA, Seoul, Korea) after careful histological assessment. All cases, except for one endoscopic biopsy sample and a normal control tissue sample, were embedded in a 6-tissue microarray block. Tissue microarray blocks were serially sliced into 4 μm sections and stained for routine hematoxylin and eosin staining and immunohistochemistry.

Immunohistochemistry

All immunohistochemistry processes were automatically conducted using a Ventana autostainer (Benchmark; Ventana Medical Systems, Tuscon, Arizona, USA). For antigen retrieval, retrieval solution (Ventana) was automatically poured onto the sections, and was heated for 60 minutes at 100°C. Endogenous peroxide activity was blocked via 4 minutes of immersion in 3% hydrogen peroxide. With diluted primary antibodies for ERCC (mouse monoclonal antibody, product No. #MS-671-P0, 1:25, Neomarker, Fremont, CA, USA), TS (mouse monoclonal antibody, product No. #18–7319, 1:25, Invistrogen, Camarillo, CA, USA), and GSTP1 (mouse monoclonal antibody, product No. #3369, 1:800, Cell Signaling, Denvers, MA, USA), the tissue sections were incubated for 32 minutes at 36°C. Immunoperoxidase staining was conducted using the DAB system (iView DAB Detection Kit, Ventana) and the sections were counterstained lightly with hematoxylin.

Interpretation of Immunohistochemical Staining

Staining was graded for staining intensity (1, weak; 2, moderate; 3, strong) and percentage of cells stained (1, 0 to <10%; 2, 10 to <50%; 3, 50–100%). Staining for ERCC1 was considered positive when the tumor cells evidenced nuclei reactivity and both scores were 2 or above. Stainings for TS and GSTP1 were considered positive when the tumor cells evidenced nuclei or cytoplasmic reactivity and both scores were 2 or above; negative otherwise.

Statistical Analysis

The associations between the expressions of ERCC1, TS, GSTP1 and the clinicopathologic parameters (gender, age, tumor size, histological grade, Lauren's classification, Borrmann type, vascular invasion, depth of invasion, positive lymph nodes, positive lymph node ratio, type of operation, stage, CEA) were assessed via χ^2 test or Fisher's exact test. Disease-free survival (DFS) was defined as the length of time from surgery to initial disease recurrence. OS was defined as the length of time from surgery to death. The Kaplan-Meier method was utilized to construct the DFS and OS curves. Data on patients who died without evidence of disease recurrence were censored at the time of death for the DFS calculations. The log-rank test was utilized to compare distributions. To identify independent factors related significantly to patient prognosis we used Cox's proportional hazard analysis with a stepwise procedure. All tests were two-sided, and p values of < 0.05 were considered statistically significant. Analyses were conducted using SPSS version 14.0 (SPSS Inc, Chicago, IL, USA).

Results

Patients' Characteristics

Demographic details on the patients included in this study are shown in Table 1. The mean age of the patients was 51 years (range, 24–75 years), and the study population included 48 (32.2%) females and 101 (67.8%) males. With regard to the histopathologic classification, 59 (39.6%) were of the intestinal, 69 (46.4%) were of the diffuse type, and 21 (14.1%) were mixed-type. The majority of the tumors were located in the distal stomach. The patients were at high risk for recurrence; 64.4% had stage pT3 or pT4 tumors, 95.3% had lymph node metastases and 46 patients (30.9%) had a positive lymph node ratio of > 0.3. The postoperative stages of the patients were II, IIIA, IIIB, and IV (M0) in 51 (34.2%), 47 (31.5%), 26 (17.4%), and 25 (16.8%), respectively. The median follow-up duration of the patients was 65.2 months (range, 9.3-88.8 months). Eighty patients (53%) received 6 cycles, and 124 patients (83%) received more than 3 cycles of adjuvant FP chemotherapy.

Table 1. Patients' characteristics.

	No. of patients	%
Age (years)		
< 60	104	69.8
≥ 60	45	30.2
Gender		
Male	101	67.8
Female	48	32.2
Type of operation		
Subtotal gastrectomy	96	64.4
Total gastrectomy	53	35.6
Location		
Upper	24	16.1
Middle	19	12.8
Lower	93	62.4
Diffuse	13	8.7
Differentiation		
Well	17	11.4
Moderate	32	21.5
Poor	84	56.4
Mucinous	9	6.0
Signet ring cell	7	4.7
Lauren classification		
Intestinal	59	39.6
Diffuse	69	46.4
Mixed	21	14.1
Borrmann type		
1	8	5.4
2	10	6.7
3	126	71.1
4	25	16.8
Vascular invasion		
-	62	41.6
+	87	58.4
Stage*		
II	51	34.2
IIIA	47	31.5
IIIB	26	17.4
IV	25	16.8

* American Joint Committee on Cancer Staging manual, 6th edition

Expressions of ERCC1, TS, and GSTP1.

Of the 149 archival specimens, 94 (63.1%), 114 (76.5%), and 60 (40.3%) were positive for ERCC1, TS and GSTP1 expression, respectively. The immunostaining patterns for TS and GSTP1 were mixed nuclear and cytoplasmic, whereas the ERCC1 expression patterns in the tumor cells were nuclear (Figure 1). ERCC1 and TS expression were correlated positively with GSTP1 expression ($p = 0.039$, $p = 0.006$, respectively), however, no association between ERCC1 and TS was detected ($p = 0.692$).

Correlations between the expressions of ERCC1, TS, GSTP1 and clinicopathologic parameters

ERCC1 and GSTP1 expression was found to be correlated significantly with size. ERCC1 and GSTP1 expression were more common in cases of small-sized (less than 5 cm) gastric cancer ($p = 0.040$, $p = 0.018$, respectively). However, no significant correlations were noted between ERCC1 or GSTP1 expression and other parameters. TS expression was correlated significantly with Borrmann type ($p = 0.030$) and stage ($p = 0.041$). The positive rate of TS expression was higher in more advanced stages and Borrmann type 3.

Expressions of ERCC1, TS, GSTP1 and clinical outcomes

The median follow-up duration of the patients was 65.2 months (range, 9.3-88.8 months). Univariate analysis of clinicopathologic parameters and DFS and OS are shown in Table 2. Lauren's classification ($p = 0.009$), stage ($p < 0.001$), positive lymph node ratio ($p < 0.001$), and type of operation ($p = 0.032$) were associated significantly with 5-year DFS. There were positive correlation between immunostaining of ERCC1, TS, GSTP1, and DFS, though statistically insignificant. Lauren's classification ($p = 0.020$), stage ($p < 0.001$), lymph node ratio ($p < 0.001$), and type of operation ($p = 0.005$) were all also associated significantly with 5-year OS. TS expression was not found to be correlated with 5-year OS ($p = 0.319$, Figure 2). Patients with an ERCC1-expressing or GSTP1-expressing cancer had a significantly longer 5-year OS than those without ($p = 0.005$, Figure 3; $p = 0.024$, Figure 4; respectively). Based on these observations, we performed a second analysis to elucidate whether combined expression of ERCC1 and GSTP1 could be used to

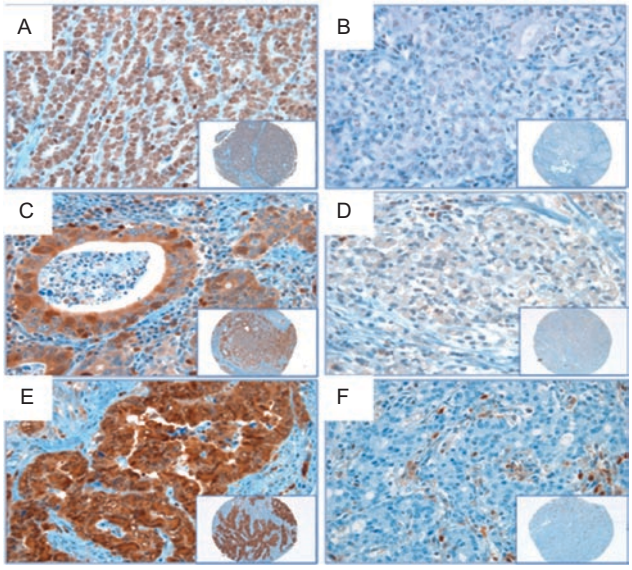

Figure 1. Typical examples of immunohistochemical staining of gastric carcinoma in representative cases. IHC for ERCC (A) shows intense nuclear staining, and that of TS (C) and GST P1(E) shows nuclear and cytoplasmic staining. In some cases, ERCC (B), TS (D) and GST P1(F) show negative immunoreactivity. (all figures, x400; inset, x40)

determine differences of clinical outcome. Patients who were ERCC1-positive combined with GSTP1-positive had a better OS (p = 0.003, Figure 5). In order to determine the independent prognostic value of these markers, we utilized a multivariate Cox proportional hazard analysis to control for other prognostic factors (Table 3). Accordingly, stage was identified as an independent prognostic factor of both DFS (HR [hazard ratio] = 1.401; 95% CI [confidence interval], 1.045-1.879; p = 0.024), and OS (HR = 1.362; 95% CI, 1.039-1.784; p = 0.025), positive lymph node ratio was also identified as significant predictors of both DFS (HR= 2.129; 95% CI, 1.138-3.982; p = 0.018), and OS (HR= 2.311; 95% CI, 1.311-4.076; p = 0.004), after controlling for the other clinicopathologic parameters. The expressions of both ERCC1 and GSTP1 significantly affected OS on multivariate analysis (HR = 0.069, 95% CI, 0.475-0.941; p = 0.021).

Discussion

Gastric cancer is one of the most commonly diagnosed tumors worldwide (Parkin et al., 2005), and is the most common cancer in Korea (Won et al., 2009). The prognosis of this disease is quite poor. Globally, it is the second and fourth leading cause of cancer-related death (Crew and Neugut, 2006). Postoperative adjuvant chemotherapy was used commonly in daily practice, but there remains some controversy as to its potential benefits. Despite the marginal benefits identified in recent meta-analyses (Sun et al., 2009), the majority of individual studies of adjuvant chemotherapy in gastric cancer have yielded negative results, owing to the lack of efficacy of traditional chemotherapy combinations (Lim et al., 2005). Therefore, there is a clear need to identify molecular markers of more aggressive gastric cancer in order to select patients for adjuvant systemic therapies. In this regard, many studies have focused on the determinants of chemosensitivity in gastric cancer (Park and Lenz, 2006).

5-FU performs an important role in the management of gastrointestinal malignancies, including gastric cancer. TS overexpression appears to be a major method of resistance against 5-FU, and the data obtained from colorectal cancer patients suggests an association with TS and resistance to 5-FU (Popat et al., 2004). The role of TS in predicting chemosensitivity remains controversial in treated metastatic gastric cancer patients (Kim et al., 2009, Kwon et al., 2007, Matsubara et al., 2008). TS expression in stage III-IV gastric cancer patients who received curative surgery followed by adjuvant FP chemotherapy was not associated with clinical outcomes (Kim et al., 2009). In this study, TS expression was associated with advanced stage, but was not associated with DFS or OS (p = 0.145, p = 0.319, respectively). These results are consistent with Kim's study.

Platinum agents have also proven effective in the treatment of gastric cancer. Both cisplatin and its third-generation analogue oxaliplatin-based therapies exert significant clinical impacts (Kwon et al., 2007, Matsubara et al., 2008). ERCC1 (excision repair cross-complementation group 1) is a highly conserved protein and an essential member of the nuclear excision repair (NER) pathway (Wilson et al., 2001). The ERCC1–XPF (xeroderma pigmentosum group F) complex is involved in the cleavage of the damaged 5′ DNA strand to the DNA lesion. Several studies have revealed an association between the expression of ERCC1 and clinical outcomes of platinum-based chemotherapy (Kwon et al., 2007, Metzger et al., 1998, Olaussen et al., 2006). The other study demonstrated that median survival time with low ERCC1 expression was significantly lower in metastatic gastric cancer patients treated with the FOLFOX regimen (Matsubara et al., 2008). However, there was no association between the polymorphism of the ERCC1 – C118T and clinical outcomes in FP-treated gastric cancer patients (Goekkurt et al., 2006).

This study demonstrated that the 5-year OS for the gastric cancer patients treated with FP chemotherapy after a curative resection were higher for those patients who evidenced ERCC1 gene overexpression (59.5% vs. 41.1%, p = 0.005). The same result was noted in another report (Baek et al., 2006). The causes of the opposite results were thought to be as follows. First, this may be attributable to a limited role for cisplatin as adjuvant chemotherapy for gastric cancer. In French's study no statistically significant survival benefit was associated with toxic cisplatin-based

Table 2. Univariate analysis of disease free survival and overall survival.

	5 year DFS (%)	*p* value	5 year OS (%)	*p* value
Lauren classification		0.009		0.020
Intestinal (n = 59)	61.4		63.2	
Diffuse (n = 69)	39.9		45.3	
Mixed (n = 21)	62.0		65.3	
N stage		< 0.001		< 0.001
0 (n = 7)	57.1		71.4	
1 (n = 80)	66.4		59.5	
2 (n = 42)	54.6		59.2	
3 (n = 20)	15.4		7.5	
Positive lymph node ratio		< 0.001		< 0.001
< 0.3 (n = 103)	67.1		65.0	
≥ 0.3 (n = 46)	27.8		25.7	
Stage*		< 0.001		< 0.001
II (n = 51)	70.3		64.8	
IIIA (n = 47)	56.8		58.1	
IIIB (n = 26)	61.0		57.8	
IV (n = 25)	22.6		13.3	
Type of operation		0.032		0.005
Subtotal gastrectomy (n = 96)	62.5		59.6	
Total gastrectomy (n = 53)	43.7		40.2	
ERCC1		0.112		0.005
- (n = 55)	46.7		42.1	
+ (n = 94)	62.5		59.5	
TS		0.145		0.319
- (n = 35)	45.3		48.4	
+ (n = 114)	60.7		55.6	
GSTP1		0.399		0.024
- (n = 89)	54.8		44.5	
+ (n = 60)	60.6		64.3	
ERCC1 and GSTP1		0.287		0.003
None (n = 39)	49.0		35.9	
One (n = 66)	55.5		53.1	
Both (n = 44)	62.4		64.0	

DFS = disease free survival; OS = overall survival; * American Joint Committee on Cancer Staging manual, 6th edition; ERCC1 = excision repair cross-complementing gene 1; TS= thymidylate synthase; GSTP1 = glutathione S-transferase P1

adjuvant chemotherapy (Bouche et al., 2005). Because of the inefficacy of the FP regimen as adjuvant chemotherapy in gastric cancer, the expression of ERCC1 may not play a role as a biomarker. In a previous report it was shown that non-small cell lung cancer patients who had ERCC1 negative tumors evidenced shorter OS than did patients with ERCC1-positive tumor (Olaussen et al., 2006). Second, factors other than the ERCC1 for NER may have affected the resistance to chemotherapy. Third, the study population differed from those of previous studies. We have evaluated gastric cancer patients treated with D2 resection followed by adjuvant FP chemotherapy, whereas most other relevant studies have involved metastatic or inoperable gastric cancer patients. Fourth, there is a discrepancy between real-time quantitative PCR and immunohistochemistry for the detection of the ERCC1 gene. Further studies will be necessary to confirm these results.

GSTP is the predominant GST in the majority of tumors, but its concentration has been shown to be significantly increased in lung, colon, and stomach cancer tissues (Howie et al., 1990). There have been a few biochemical studies and clinical reports that have provided strong evidence for the direct involvement of GSTP1 in resistance to platinum compounds (Goto et al., 1999, Harpole et al., 2001). The majority of patients who evidenced low GSTP protein expression levels responded to platinum-based treatment and exhibited better survival rates as compared to patients with high tumor GSTP expression profiles. Monden *et al.* (Monden et al., 1997) also revealed that GSTP1 overexpression was associated with lower 5-year DFS in gastric cancer patients. However, in this study, patients overexpressing GSP1 evidenced higher OS (64.3% vs. 44.5%, $p = 0.024$). The reason for this discrepancy may involve differences in patient populations and adjuvant chemotherapy

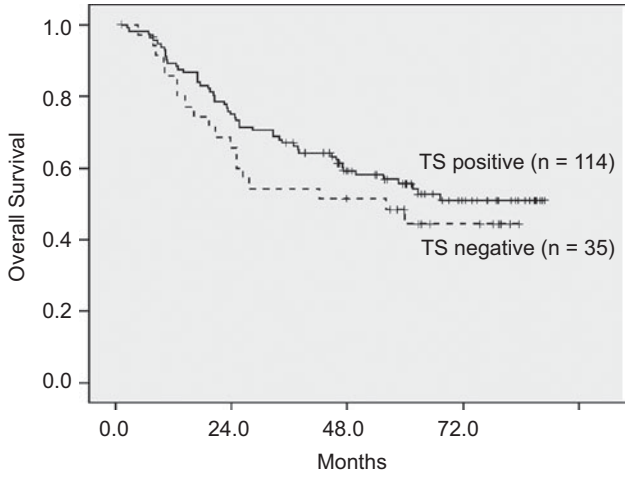

Figure 2. Overall survival curve according to TS expression ($p = 0.319$)

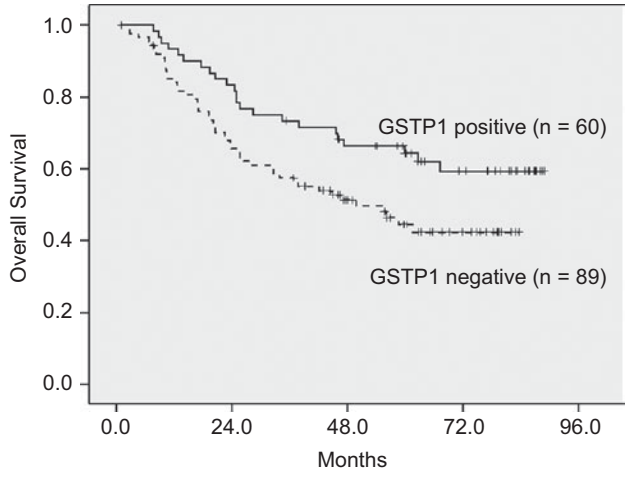

Figure 4. Overall survival curve according to GSTP1 expression ($p = 0.024$)

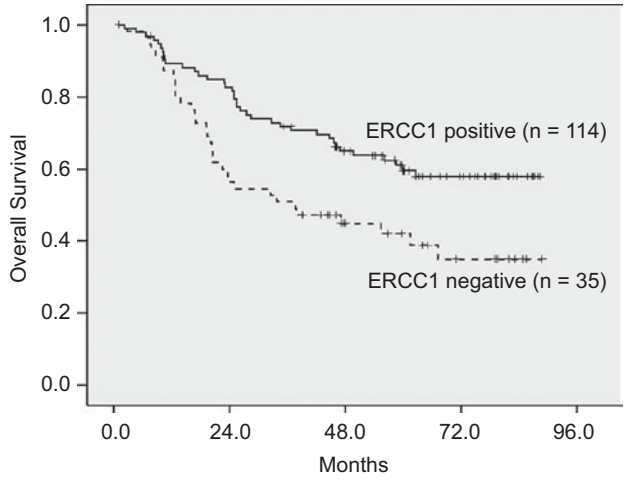

Figure 3. Overall survival curve according to ERCC1 expression ($p = 0.005$)

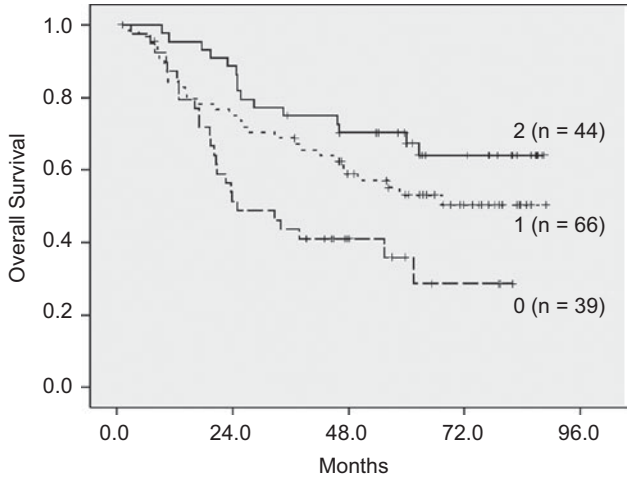

Figure 5. Overall survival curve according to ERCC1 and GSTP1 expression (0, none; 1, one, 2, both; $p = 0.003$)

Table 3. Multivariate analysis of disease free survival and overall survival.

	5 year Disease Free Survival			5 year Overall Survival		
	HR	95% CI	*p* value	HR	95% CI	*p* value
ERCC1 and GSTP1	0.987	0.578-1.430	0.321	0.669	0.475-0.941	0.021
Stage	1.401	1.045-1.879	0.024	1.362	1.039-1.784	0.025
Type of operation	0.678	0.389-1.181	0.170	0.688	0.418-1.133	0.142
Positive lymph node ratio	2.129	1.138-3.982	0.018	2.311	1.311-4.076	0.004
Lauren's classification	1.073	0.730-1.578	0.720	1.137	0.799-1.617	0.476

HR= hazard ratio; CI = confidence interval; ERCC1 = excision repair cross-complementing gene 1; TS= thymidylate synthase; GSTP1 = glutathione S-transferase P1.

regimens, as well as the limitations of immunohistochemistry. Although immunohistochemistry is useful in determining the location of the marker of interest, immunohistochemistry conducted on formalin-fixed, paraffin-embedded tissue samples is hindered by some obstacles: the semiquantitative nature of the immunohistochemical technique, the age of the stained tissues, the staining technique, the enzyme antibody used for enzyme analysis, interobserver variation for the evaluation of immunohistochemical markers, and less accurate evaluations of genetic polymorphisms.

In univariate analysis, Lauren's classification, stage, lymph node status, positive lymph node ratio, and operation type were associated with both DFS and OS. ERCC1 and GSTP1 were related to OS. When we evaluate the expression of ERCC1 combined with GSTP1, the positivity score

was related significantly to the OS ($p = 0.003$). Multivariate analysis demonstrated that stage, positive lymph node ratio, and ERCC1 and GSTP1 expression were independent prognostic factors. Positive lymph node ratio was the most significant factor in terms of both DFS (HR=2.129, p=0.018) and OS (HR=2.311, p=0.004). ERCC1 and GSTP1 expression was related to OS (HR=0.669, $p = 0.021$). The patients who were positive for both ERCC1 and GSTP1 evidenced a higher OS than other patients.

Although this study was limited in terms of its sample size, and involved a retrospective review of medical records, we detected a correlation between clinical outcomes and the expression of ERCC1 and GSTP1. Therefore, we suggest that immunohistochemical studies for ERCC1 and GSTP1 may prove useful for the prediction of clinical outcomes in advanced gastric cancer patients. The results of our analysis will hopefully provide a more rational basis for clinical decision-making, risk stratification of patients, and the selection of management strategies, and should also help in the establishment of benchmarks for future randomized controlled trials.

Conclusion

In conclusion, immunohistochemical studies of ERCC1 and GSTP1 proved useful predictive factors in advanced gastric cancer patients treated with adjuvant FP. When two independent proteins are both present, the best overall survival rates are expected in gastric cancer patients who underwent curative gastrectomy.

Declaration of interest

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