Expression of Bax Predicts Outcome in Advanced Gastric Cancer Patients Treated With 5-fluorouracil and Platinum Palliative Chemotherapy

By

Seong Hyun Jeong

Major in Medicine

Department of Medicine

The Graduate School, Ajou University

Expression of Bax Predicts Outcome in Advanced Gastric Cancer Patients Treated With 5-fluorouracil and Platinum Palliative Chemotherapy

By
Seong Hyun Jeong

A Dissertation Submitted to The Graduate Schhol of Ajou University in Partial Fulfillation of the Requirements for the Degree of Master of Medicine

Supervised by

Jin-Hyuk Choi, M.D., Ph.D.

Major in Medicine

Department of Medicine

The Graduate School, Ajou University

August, 2009

This certifies that the dissertation
of Seong Hyun Jeong is approved

SUPEVISORY COMMITTEE

Jin-Hyuk Choi

Kwang Jae Lee

Jae Ho Han

The Graduate School, Ajou University

June, 22nd, 2009

- ABSTRACT -

Expression of Bax Predicts Outcome in Advanced Gastric Cancer Patients Treated With 5-fluorouracil and Platinum Palliative Chemotherapy

Purpose: The present study evaluated the predictive role of Bax, excision repair cross-complementation group 1 (ERCC1), and thymidylate synthase (TS) on clinical outcomes in patients with advanced gastric cancer treated with 5-fluorouracil (5-FU) and platinum palliative chemotherapy.

Materials and Methods: One hundred and twenty-eight patients with metastatic or recurrent gastric cancer were treated with a chemotherapy regimen of either 5-FU and heptaplatin (FH) (56 patients) or 5-FU, leucovorin, and oxaliplatin (FOLFOX) (72 patients). Pretreatment tumor biopsy specimens were analyzed for Bax, ERCC1, and TS expression by immunohistochemistry.

Results: High expression of Bax, ERCC1 and TS was observed in 49 (38%), 60 (47%), and 48 (38%) patients, respectively. The median overall survival (OS) of patients in total was 10 months. Low expression of Bax was associated with poor OS (median, 9 months vs. 12 months; 2-year, 7% vs. 32%; p=0.0005) in univariate analysis, while expression of ERCC1 and TS was not correlated with patient outcome. The outcome of patients with low

expression of Bax was significantly worse in the FOLFOX group (median OS, 9 months vs. 18 months; p=0.0008), without significant difference in the FH group. In multivariate analysis, low expression of Bax was a significant independent predictor of poor OS (p=0.014).

Conclusions: Low expression of Bax was significantly associated with the poor survival of patients with metastatic or recurrent gastric cancer treated with 5-FU and platinum chemotherapy. Immunohistochemical staining for Bax with pretreatment biopsy specimen may be useful in selecting FOLFOX regimen as a treatment option for these patients.

Key words: Gastric cancer, Chemotherapy, Bax, Prognosis

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. INTRODUCTION

Gastric cancer is the second most common cause of cancer mortality in the world (Parkin *et al*, 2005). The overall outcome of gastric cancer is still unsatisfactory, because many patients present with unresectable or metastatic disease, while the recurrence is quite common even after radical resection, which is the only curative treatment (Chau *et al*, 2004; Wohrer *et al*, 2004; Wagner *et al*, 2006; Rivera *et al*, 2007; Van Cutsem *et al*, 2008). Palliative chemotherapy is the standard of care in patients with unresectable or metastatic or recurrent disease with some improvement of survival and quality of life (Chau *et al*, 2004; Wohrer *et al*, 2004; Wagner *et al*, 2006; Rivera *et al*, 2007; Van Cutsem *et al*, 2008).

Although there is no standard regimen in palliative chemotherapy for gastric cancer, cisplatin and 5-fluorouracil (5-FU)-based regimens, with or without another agent, have been used the most frequently (Chau *et al*, 2004; Rivera *et al*, 2007; Van Cutsem *et al*, 2008). Recently, oxaliplatin, a third-generation platinum compound, and 5-FU combination regimens have demonstrated comparable efficacy with more favorable toxicity profiles compared to 5-FU and cisplatin-based regimens (Louvet *et al*, 2002; Chao *et al*, 2004; De Vita *et al*, 2005; Lordick *et al*, 2005; Rivera *et al*, 2007; Al-Batran *et al*, 2008; Cunningham *et al*, 2008;Van Cutsem *et al*, 2008). However, despite the availability of various effective chemotherapy regimens, the overall outcome of advanced gastric cancer is still very poor, with median survival of 6-11 months (Louvet *et al*, 2002; Chao *et al*, 2004; Chau *et al*, 2004; Wohrer *et al*, 2004; De Vita *et al*, 2005; Lordick *et al*, 2005; Ichikawa & Sasaki, 2006; Wagner *et al*, 2006; Lee *et al*, 2007; Lee J, 2007; Rivera *et al*, 2007; Al-Batran *et al*, 2008;

Cunningham *et al*, 2008; Van Cutsem *et al*, 2008). Therefore, the determination of parameters that may identify those patients who would benefit from the specific chemotherapy regimen and those who would not has strong clinical implications. Drug resistance-related proteins, including apoptosis-related proteins and DNA damage repair proteins are important candidates for such parameters.

Chemotherapy kills cancer cells by triggering apoptosis, which is primarily regulated by Bcl-2 family proteins (Fulda & Debatin, 2006; Reed, 2006). Among the Bcl-2 family proteins, Bax protein has a central role in the activation of downstream apoptosis signaling pathways by permeabilizing the mitochondrial outer membrane after activation by BH3-only proteins or p53 (Fulda & Debatin, 2006; Reed, 2006). On the other hand, platinum compounds, which are an integral component of chemotherapy for gastric cancer, induce apoptosis in cancer cells by forming DNA adducts (Siddik, 2003). DNA damaged by platinum compounds is repaired by the nucleotide excision repair pathway by removing DNA adducts (Reardon *et al*, 1999; Siddik, 2003; Reed, 2005). Therefore, alterations in both apoptosis-related proteins and DNA damage repair proteins are associated with resistance to platinum-based chemotherapy, which is the most important cause of treatment failure.

Bax and excision repair cross-complementation group 1 (ERCC1), one of the nucleotide excision repair system's key enzymes, have been investigated as important candidates for predictors of resistance to chemotherapy in many malignancies (Krajewski *et al*, 1995; Baekelandt *et al*, 2000; Kang *et al*, 2006; Olaussen *et al*, 2006; Kang *et al*, 2007; Lee *et al*, 2008). In terms of resistance to 5-FU, thymidylate synthase (TS), which is a critical target of 5-FU, has been widely investigated. TS catalyzes the methylation of

deoxyuridine monophosphate to deoxythymidine monophosphate, which is an essential process for DNA synthesis (Pinedo & Peters, 1988). High expression of TS may be associated with 5-FU resistance in a variety of malignancies including gastric cancer (Johnston *et al*, 1995; Lenz *et al*, 1996; Boku *et al*, 1998; Metzger, 1998; Yeh *et al*, 1998).

We evaluated the predictive role of Bax, ERCC1, and TS on clinical outcomes in advanced gastric cancer patients treated with 5-FU and third-generation platinum regimens.

II. MATERIALS AND METHODS

A. MATERIALS

Advanced gastric cancer patients who underwent palliative chemotherapy with 5-FU and third-generation platinum combination regimens at Ajou University Medical Center between January 2001 and December 2006 were eligible to be included in this retrospective study if the following criteria were met: (1) histologically documented gastric cancer, (2) no previous chemotherapy except adjuvant chemotherapy, and (3) distant metastasis at initial diagnosis or recurrent disease. Patients who underwent palliative gastrectomy with or without metastasectomy of distant metastasis at initial diagnosis or who received resection of metastatic lesions after recurrence were included.

Each patient underwent the following staging procedures: physical examination, esophagogastroduodenoscopy, chest radiograph, computed tomography of abdomen and pelvis, and hematologic and biochemical profiles. This research protocol was approved by the Institutional Review Board of the Ajou University Medical Center, Suwon, Korea.

B. METHODS

1. Chemotherapy

Patients had been treated with two regimens consecutively. Between 2001 and 2003, patients had received combination chemotherapy with 5-FU and heptaplatin, which is a third generation platinum compound developed by a Korean pharmaceutical company (SK Chemicals, Gyeonggi-do, Korea) (Kim *et al*, 1999; Min, 2004). Since 2004, when oxaliplatin

was approved in Korea, 5-FU, oxaliplatin, and leucovorin (FOLFOX) regimen had been used. For the 5-FU and heptaplatin (FH) regimen, 5-FU 1000 mg/m^2/day was administered as a continuous intravenous (IV) infusion on days 1 to 3, and heptaplatin 400 mg/m^2 was given over 1 hour by IV infusion on day 1, and this cycle was repeated every 4 weeks. For the FOLFOX regimen, chemotherapy consisted of oxaliplatin 100 mg/m^2 as a 2-hour IV infusion on day 1, leucovorin 100 mg/m^2 as a 2-hour infusion followed by IV bolus 5-FU 400 mg/m^2, and a 22-hour IV infusion of 5-FU 600 mg/m^2 on days 1 and 2 every 2 weeks. In both regimens, chemotherapy was continued until disease progression, unacceptable toxicity, patient refusal, or physician's decision.

Patients were evaluated with CT scan of the abdomen and pelvis every 2 cycles for FH and every 3 cycles for FOLFOX regimen.

2. Immunohistochemical Staining for Drug Resistance-Related Proteins

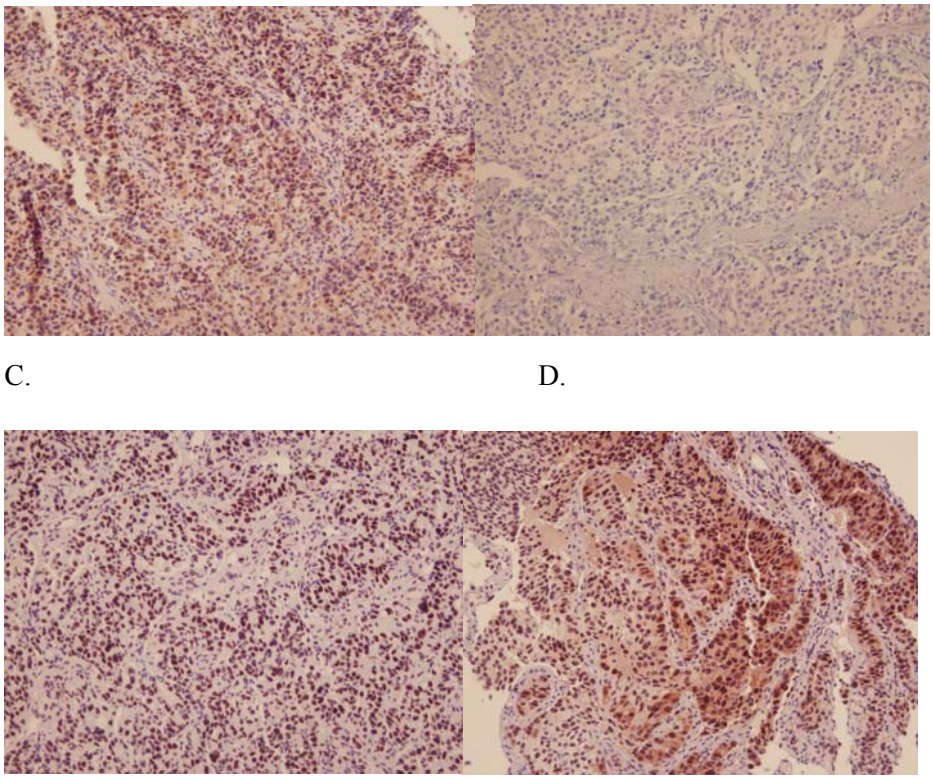
Immunohistochemical staining of formalin-fixed, paraffin-embedded tumor tissue was performed for primary tumor tissue obtained by gastroscopic biopsy or gastrectomy in cases of initially metastatic disease. In patients with recurrent disease, tumor tissue from metastatic lesions was preferentially used for staining, and if it was not available, primary tumor tissue obtained at the time of diagnosis was stained.

For immunohistochemical staining, mouse anti-human monoclonal antibodies against ERCC1 (ERCC1 Ab-2, dilution 1:100) and TS (TS 106, dilution 1:100) (Neomarkers, Fremont, CA, U.S.A.), and rabbit anti-human polyclonal antibodies against Bax (dilution 1:1,000, DAKO, Carpinteria, CA, U.S.A.), were used. Sections were deparaffinized in

xylene and rehydrated in graded alcohols and water. Endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide for 10 minutes. Sections were treated with protein-blocking solution and then with primary antibodies overnight at 4°C. After several rinses in phosphate-buffered saline, the sections were incubated in the biotinylated secondary antibodies. Bound antibodies were detected by the streptavidin-biotin method with a Cap-Plus detection kit (Zymed Laboratories Inc, San Francisco, CA, U.S.A.). Slides were rinsed in phosphate-buffered saline, exposed to diaminobenzidine, and counterstained with Mayer's hematoxylin. The negative controls for these proteins were made by the omission of the primary antibody during the process of staining. For positive controls for Bax and ERCC1, lymphocytes in the germinal center of normal lymph nodes and endothelial cells in tonsil were used, respectively. A positive control for TS was formalin-fixed, paraffin-embedded sections of colon adenocarcinoma known to have high expression of TS.

The slides were examined independently by two observers blinded to both clinical and pathologic data. Expression of the drug resistance-related proteins was quantified using a visual grading system based on the extent of staining (by percentage of positive tumor cells graded on a scale of 0 to 3: 0 = none, 1 = 1%-10%, 2 = 11%-50%, 3 = 51%-100%) and the intensity of staining (graded on a scale of 0 to 3: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining) (Fig. 1). A semiquantitative H-score was obtained by multiplying the grades of extent and intensity of staining. The median value of all the H scores was chosen *a priori* as the cutoff value for dividing the expression of proteins into high and low.

A. B.



C. D.

Fig. 1. Immunohistochemical staining of drug resistance-related proteins in gastric cancer (grade in both the extent and intensity of staining, X400). (A) High expression of Bax: cytoplasmic staining (grade 3). (B) Low expression of Bax (grade 0). (C) High expression of ERCC1: nuclear staining (grade 3), (D) High expression of TS: cytoplasmic staining (grade 3).

3. Statistical Analysis

A comparison of characteristics of patients was evaluated with the Fisher's exact test and the Mann-Whitney test. Response to chemotherapy was evaluated by RECIST criteria (Therasse *et al*, 2000). Overall survival (OS) was calculated using the Kaplan-Meier method (Kaplan, 1958). OS was defined as the time from start of treatment to death; data on survivors were censored at the last follow-up. The differences between the survival curves were tested by using the log-rank test. The Cox proportional-hazards regression model was used to determine the joint effects of several variables on survival (Cox, 1972). Factors with P values <0.1 in univariate analysis were included in the Cox proportional-hazards regression model. All statistical analyses were performed two-sided, with SPSS for Windows 12.0 software.

III. RESULTS

Patient Characteristics

During the study period, 134 patients with metastatic or recurrent gastric cancer were treated with FH or FOLFOX regimen. Among them, 6 patients were excluded from the study due to the unavailability of a pathology specimen or insufficient quality of samples for immunohistochemistry, leaving 128 patients for analysis. Fifty-six patients were treated with FH, while 72 underwent chemotherapy with FOLFOX.

The characteristics of the 128 patients are listed in Table 1. Seventy-two patients presented with metastatic disease at the time of diagnosis, and 56 patients had recurrent disease. Among patients with initially metastatic disease, 29 patients underwent palliative gastrectomy with metastasectomy (12 patients) or without (17 patients), whereas 11 patients with recurrence received resection of metastatic lesions. Sixty-two patients had measurable lesions and 39 had evaluable lesions, respectively. On the other hand, 27 patients did not have definitive radiologically measurable or evaluable lesions. Fifty-four patients with recurrent disease had a history of adjuvant chemotherapy (5-FU and mitomycin-C: 28, 5-FU and cisplatin: 17, 5-FU and paclitaxel: 3, oral fluoropyrimidines: 6). There were significant differences in gender, disease status, and history of adjuvant chemotherapy between the FH and FOLFOX groups (Table 1).

Table 1. Patient characteristics

Characteristics	Total (%)	FOLFOX (%)	FH (%)	*P*
Age				0.057
Median	54	50	59	
Range	23-74	29-71	23-74	
Gender				0.021
Male	89 (70)	44 (61)	45 (80)	
Female	39 (30)	28 (39)	11 (20)	
Performance status[a]				0.106
0, 1	96 (75)	58 (81)	38 (68)	
2	32 (25)	14 (19)	18 (32)	
Disease status				0.021
Primary metastatic	72 (56)	34 (47)	38 (68)	
Recurrent	56 (44)	38 (53)	18 (32)	
Adjuvant chemotherapy				0.020
Yes	54 (42)	37 (51)	17 (30)	
No	74 (58)	35 (49)	39 (70)	
Liver metastasis				0.420
Yes	32 (25)	16 (22)	16 (29)	
No	96 (75)	56 (78)	40 (71)	
Peritoneal metastasis				0.373
Yes	62 (48)	32 (44)	30 (54)	
No	66 (52)	40 (56)	26 (46)	
Palliative resection[b]				0.442
Yes	40 (31)	25 (35)	15 (27)	
No	88 (69)	47 (65)	41 (73)	
Cycles of chemotherapy				0.482[c]
Median	5	6	3	
Range	1-12	1-12	1-6	

FOLFOX = 5-FU, Oxaliplatin, and Leucovorin; FH = 5-FU, and Heptaplatin.
a Eastern Cooperative Oncology Group performance status grade.
b Palliative gastrectomy with or without metastasectomy, or metastasectomy.
c Two cycles of FOLFOX were regarded as 1 cycle of FH.

Association of Expression of Drug Resistance-Related Proteins With Characteristics of Patients

In patients with primary metastatic disease, 65 samples (90%) were from their primary tumor, obtained by surgical resection or gastroscopic biopsy; and 7 (10%) from metastatic sites, due to the unavailability or insufficient quality of primary tumor specimens. In patients with recurrence, 37 samples (66%) were from metastatic lesions, while 19 (34%) were from primary tumors taken during initial diagnosis.

The median values of the H-scores of Bax, ERCC1, and TS were 3, 3, and 4, respectively, and tumors with H-scores higher than these median values were classified as high expression. High expression of Bax, ERCC1 and TS was observed in 49 (38%), 60 (47%), and 48 (38%) patients, respectively. The expression status of these drug resistance-related proteins did not correlate with each other. There was no significant association between Bax expression and characteristics of patients, while TS expression correlated with several characteristics such as disease status (Table 2). In addition, patients with high expression of ERCC1 showed a lower incidence of palliative resection compared with those with low expression (22% vs. 40%, p=0.036) without any significant association with other characteristics (data not shown). In 62 patients with measurable lesions, the overall response rate was 23% (FH: 10%, FOLFOX: 33%). The response rate was not correlated with the expression of any proteins (data not shown).

Table 2. Association between expression of Bax or TS and characteristics of patients

Characteristics	Bax Expression			TS Expression		
	Low (%)	High (%)	*P*	Low (%)	High (%)	*P*
Age			0.719			0.046
<54[a]	40 (51)	23 (47)		45 (56)	18 (37)	
≥54	39 (49)	26 (53)		35 (44)	30 (63)	
Gender			0.242			0.328
Male	58 (73)	31 (63)		53 (66)	36 (75)	
Female	21 (27)	18 (37)		27 (34)	12 (25)	
Performance status[b]			0.210			0.139
0, 1	56 (71)	40 (82)		56 (70)	40 (83)	
2	23 (29)	9 (18)		24 (30)	8 (17)	
Disease status			0.205			0.001
Primary metastatic	48 (61)	24 (49)		36(45)	36 (75)	
Recurrent	31 (39)	25 (51)		44 (55)	12 (25)	
Adjuvant chemotherapy			0.270			0.003
Yes	30 (38)	24 (49)		42 (53)	12 (25)	
No	49 (62)	25 (51)		38 (47)	36 (75)	
Liver metastasis			0.296			0.019
Yes	17 (22)	15 (31)		14 (18)	18 (37)	
No	62 (78)	34 (69)		66 (82)	30 (63)	
Peritoneal metastasis			0.365			1.000
Yes	41 (52)	21 (43)		39 (49)	23 (48)	
No	38 (48)	28 (57)		41 (51)	25 (52)	
Palliative resection[c]			0.172			0.555
Yes	21 (27)	19 (39)		27 (34)	13 (27)	
No	58 (73)	30 (61)		53 (66)	35 (73)	
Regimens			0.272			0.002
FH	38 (48)	18 (37)		26 (32)	30 (63)	
FOLFOX	41(52)	31 (63)		54 (68)	18 (37)	
ERCC1 expression			1.000			0.067
High	37 (47)	23 (47)		32 (40)	28 (58)	
Low	42 (53)	26 (53)		48 (60)	20 (42)	
TS expression			0.094			–
High	25 (32)	23 (47)		-	-	
Low	54 (68)	26 (53)		-	-	
Bax expression			-			0.094
High	-	-		26 (32)	23 (48)	
Low	-	-		54 (68)	25 (52)	

FOLFOX = 5-FU, Oxaliplatin, and Leucovorin; FH = 5-FU, and Heptaplatin.
a Median value.
b Eastern Cooperative Oncology Group performance status grade.
c Palliative gastrectomy with or without metastasectomy, or metastasectomy.

Association of Expression of Drug Resistance-Related Proteins With Patient Outcome

The median follow-up duration of patients was 10 months (range: 1 – 77 months), and no patient was lost to follow-up. Sixteen patients were alive at the time of analysis. The median OS of patients in total was 10 months with a 2-year OS rate of 17%. The FOLFOX group showed better outcome with median and 2-year OS of 12 months and 26% compared to the FH group with those of 7 months and 5% (p=0.001).

The median and 2-year OS of patients with low expression of Bax were significantly inferior compared with those of patients with high expression (9 months vs. 12 months and 7% vs. 32%, p=0.0005; Fig 2A; Table 3). In addition to Bax, poor performance status, no palliative resection before chemotherapy, and the FH regimen were associated with poor OS in univariate analysis (Table 3). The outcome of patients with low expression of Bax was significantly worse in the FOLFOX group, without significant difference in the FH group (Figs. 2B and 2C). In patients with the FOLFOX regimen, the median and 2-year OS for the low Bax group compared with the high Bax group were 9 months vs. 18 months and 9% vs. 48% (p=0.0008; Fig. 2B). High expression of ERCC1 and TS was not correlated with patient outcome (Table 3). Even the combined high expression of TS and ERCC1 did not reveal any prognostic significance (data not shown). In multivariate analysis, low expression of Bax was a significant independent predictor of poor OS (p=0.014), along with poor performance status and no palliative resection before chemotherapy (Table 4).

Table 3. Univariate analysis of overall survival

Characteristics	Median Overall Survival (months)	*P*
Age		0.287
<54[a]	10	
≥54	10	
Gender		0.188
Male	9	
Female	12	
Performance status[b]		<0.0001
0, 1	12	
2	4	
Disease status		0.952
Primary metastatic	10	
Recurrent	10	
Adjuvant chemotherapy		0.761
Yes	10	
No	10	
Liver metastasis		0.981
Yes	7	
No	10	
Peritoneal metastasis		0.157
Yes	9	
No	11	
Palliative resection[c]		0.0002
Yes	14	
No	9	
Regimens		0.001
FH	7	
FOLFOX	12	
Bax expression		0.0005
High	12	
Low	9	
ERCC1 expression		0.901
High	9	
Low	10	
TS expression		0.356
High	10	
Low	10	

FOLFOX = 5-FU, Oxaliplatin, and Leucovorin; FH = 5-FU, and Heptaplatin.

a Median value.
b Eastern Cooperative Oncology Group performance status grade.
c Palliative gastrectomy with or without metastasectomy, or metastasectomy

Table 4. Multivariate analysis of overall survival

Characteristics	Hazard Ratio (95% CI)	*P*
Performance status[a]		<0.0001
0, 1	1.00	
2	4.62 (2.91-7.33)	
Palliative resection[b]		0.006
Yes	1.00	
No	1.86 (1.20-2.89)	
Regimens		0.102
FOLFOX	1.00	
FH	1.38 (0.94-2.02)	
Bax expression		0.014
High	1.00	
Low	1.68 (1.11-2.54)	

CI = confidence interval; FOLFOX = 5-FU, Oxaliplatin and Leucovorin; FH = 5-FU, and Heptaplatin.

a Eastern Cooperative Oncology Group performance status grade.

b Palliative gastrectomy with or without metastasectomy, or metastasectomy.

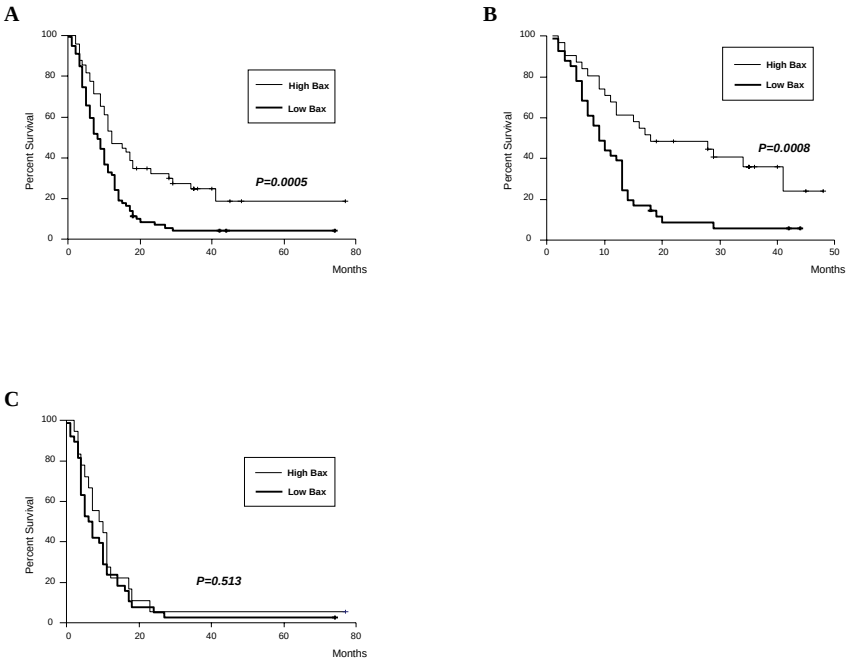


Fig. 2. (A) Overall survival of advanced gastric cancer patients treated with 5-FU and platinum palliative chemotherapy according to Bax expression. (B) Overall survival of patients according to Bax expression in the FOLFOX group. (C) Overall survival of patients according to Bax expression in the FH group.

IV. DISCUSSION

We evaluated the expression of Bax, ERCC1, and TS under the assumption that abnormalities in these drug resistance-related proteins may be associated with resistance to palliative chemotherapy with 5-FU and third-generation platinum regimens, ultimately leading to poor survival in patients with advanced gastric cancer. The median survival of 10 months in the present patient cohort is comparable to the outcomes in large phase III clinical trials for palliative chemotherapy of advanced gastric cancer (Chau *et al*, 2004; Wohrer *et al*, 2004; Wagner *et al*, 2006; Rivera *et al*, 2007; Cunningham *et al*, 2008; Van Cutsem *et al*, 2008). Patients treated with FOLFOX regimen demonstrated a better outcome compared to those with FH. Differences in patient characteristics such as disease status may explain this result. In addition, a phase II trial with FH regimen for advanced gastric cancer suggested that the efficacy of FH seemed to be inferior to other regimens, which may help explain the difference in outcomes (Min YJ, 2004).

In terms of the relationship between the expression of drug resistance-related proteins and the characteristics of patients, Bax and ERCC1 did not demonstrate a significant correlation, while low expression of TS was more frequently observed in patients with recurrent disease. The explanation for these findings is beyond the scope of the present study. However, since almost all recurrent patients previously received 5-FU-based adjuvant chemotherapy, there is a possibility that exposure to 5-FU might induce the change in TS expression.

Several studies have shown that expression of TS and ERCC1 has an inverse

relationship with response to chemotherapy and survival in advanced gastric cancer patients receiving systemic chemotherapy with 5-FU-containing regimens (Johnston *et al*, 1995; Lenz *et al*, 1996; Boku *et al*, 1998; Yeh *et al*, 1998 Metzger, 1998; Kwon *et al*, 2007; Wei *et al*, 2008). Recently, Kwon et al. reported a significant correlation between high expression of ERCC1 and poor survival in 64 advanced gastric cancer patients treated with FOLFOX using immunohistochemical staining (Kwon *et al*, 2007). Moreover, a study by Wei et al. demonstrated that ERCC1 mRNA level was a significant independent predictor of poor OS in 76 advanced gastric cancer patients who underwent FOLFOX chemotherapy (Wei *et al*, 2008). However, in the present study, expression of ERCC1 and TS was not associated with outcome of patients. It is difficult to explain the lack of prognostic significance of ERCC1 and TS in the present study. In terms of TS, one possible explanation is that concurrent administration of oxaliplatin or heptaplatin with 5-FU might have played a role in the failure of high TS expression to predict poor survival of patients. In the report of Kwon et al., high expression of TS did not correlate with the clinical outcome of patients, either (Kwon *et al*, 2007). In terms of ERCC1, the present study revealed contradictory results compared with the previous studies using FOLFOX regimen showing prognostic significance of high expression of ERCC1. The study of Kwon et al. investigated ERCC1 expression only in patients with measurable lesions unlike the present report that included patients with both measurable and evaluable lesions and even those without definitive radiological lesions (Kwon *et al*, 2007). Another report by Wei et al. included a significant proportion of patients with potentially resectable stage III disease (37 out of 76 patients) (Wei *et al*, 2008). These differences in the study population might explain the discrepant findings.

The most important finding of the current study was the prognostic significance of Bax expression in patients treated with palliative chemotherapy. In the present study, low expression of Bax was an independent predictor of poor OS in multivariate analysis, along with poor performance status and relatively high tumor burden, which have been suggested as important predictive factors in palliative chemotherapy for gastric cancer (Chau *et al*, 2004; Lee, 2007; Lee *et al*, 2007). The role of low expression of Bax as a predictor of poor clinical outcomes has been reported in several malignancies treated with chemotherapy, such as breast, ovarian, head and neck cancers (Krajewski *et al*, 1995; Baekelandt *et al*, 2000; Kang *et al*, 2006). In addition, we recently reported that low expression of Bax was a significant independent predictor of poor OS in patients with locally advanced esophageal cancer treated with definitive chemoradiotherapy using 5-FU and cisplatin (Kang *et al*, 2007).

To our knowledge, the present study is the first report demonstrating the role of Bax expression as a predictor of clinical outcome in patients with advanced gastric cancer treated with palliative chemotherapy. However, the present study has several potential limitations, despite its demonstration of the predictive value of Bax expression. First, this study is a retrospective analysis from single institution with the patient cohort treated with two different regimens. Second, both initially metastatic and recurrent diseases were included in the study population. Third, in patients with recurrent disease, not all specimens for immunohistochemistry were obtained from recurrent lesions, while almost all these patients had previously undergone adjuvant chemotherapy. Nonetheless, Bax expression status did not show a significant difference between primary tumors and metastatic lesions ($p=0.849$; data now shown). Fourth, the response rate to chemotherapy did not correlate with Bax

expression in patients with measurable lesions. However, in a recent comprehensive review, there was no significant association between response to first-line chemotherapy and the OS of advanced gastric cancer patients treated with regimens that included new drugs such as oxaliplatin (Ichikawa & Sasaki, 2006). Finally, the prognostic significance of Bax expression was demonstrated only in patients treated with FOLFOX regimen. Difference in characteristics of patients (such as initially metastatic vs. recurrent) or the relatively poor outcome of patients with FH regimen could explain this finding. Alternatively, there is a possibility that Bax expression might have a predictive role exclusively for the FOLFOX regimen, although further studies including patients treated with other regimens are essential to prove this speculation.

Although further prospective studies with large numbers of patients are warranted to confirm the role of Bax expression as a predictive factor, the present results may have significant clinical implications, since there is no established predictive marker except for pretreatment performance status and tumor burden in palliative chemotherapy for gastric cancer (Chau *et al*, 2004; Lee, 2007; Lee *et al*, 2007). The outcome of patients with high expression of Bax in the FOLFOX group of the present study is encouraging, with a median OS of 18 months and a 2-year OS rate of 48%, respectively, considering the data of large phase III trials (Chau *et al*, 2004; Wohrer *et al*, 2004; Wagner *et al*, 2006; Rivera *et al*, 2007; Cunningham *et al*, 2008; Van Cutsem *et al*, 2008). Therefore, the results of the current study suggest that advanced gastric cancer patients with high expression of Bax might benefit from FOLFOX chemotherapy.

V. CONCLUSION

Low expression of Bax was significantly associated with the poor survival of patients with metastatic or recurrent gastric cancer treated with 5-FU and platinum chemotherapy. If the predictive role of Bax expression is validated by further prospective studies, a relatively simple immunohistochemical staining for Bax with a pretreatment gastroscopic biopsy specimen may provide valuable information to oncologists in selecting FOLFOX regimen as a treatment option for advanced gastric cancer patients.

REFERENCES

1. Al-Batran SE, Hartmann JT, Probst S, Schmalenberg H, Hollerbach S, Hofheinz R, Rethwisch V, Seipelt G, Homann N, Wilhelm G, Schuch G, Stoehlmacher J, Derigs HG, Hegewisch-Becker S, Grossmann J, Pauligk C, Atmaca A, Bokemeyer C, Knuth A, Jager E: Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin plus either oxaliplatin or cisplatin: a study of the Arbeitsgemeinschaft Internistische Onkologie. *J Clin Oncol* 26: 1435-42, 2008

2. Baekelandt M, Holm R, Nesland JM, Trope CG, Kristensen GB: Expression of apoptosis-related proteins is an independent determinant of patient prognosis in advanced ovarian cancer. *J Clin Oncol* 18: 3775-81, 2000

3. Boku N, Chin K, Hosokawa K, Ohtsu A, Tajiri H, Yoshida S, Yamao T, Kondo H, Shirao K, Shimada Y, Saito D, Hasebe T, Mukai K, Seki S, Saito H, Johnston PG: Biological markers as a predictor for response and prognosis of unresectable gastric cancer patients treated with 5-fluorouracil and cis-platinum. *Clin Cancer Res* 4: 1469-74, 1998

4. Chao Y, Yeh KH, Chang CJ, Chen LT, Chao TY, Wu MF, Chang CS, Chang JY, Chung CY, Kao WY, Hsieh RK, Cheng AL: Phase II study of weekly oxaliplatin and 24-h infusion of high-dose 5-fluorouracil and folinic acid in the treatment of advanced gastric cancer. *Br J Cancer* 91: 453-8, 2004

5. Chau I, Norman AR, Cunningham D, Waters JS, Oates J, Ross PJ: Multivariate prognostic factor analysis in locally advanced and metastatic esophago-gastric cancer--pooled analysis from three multicenter, randomized, controlled trials using individual patient data. *J Clin Oncol* 22: 2395-403, 2004

6. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR: Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 358: 36-46, 2008

7. De Vita F, Orditura M, Matano E, Bianco R, Carlomagno C, Infusino S, Damiano V, Simeone E, Diadema MR, Lieto E, Castellano P, Pepe S, De Placido S, Galizia G, Di Martino N, Ciardiello F, Catalano G, Bianco AR: A phase II study of biweekly oxaliplatin plus infusional 5-fluorouracil and folinic acid (FOLFOX-4) as first-line treatment of advanced gastric cancer patients. *Br J Cancer* 92: 1644-9, 2005

8. Cox DR: Regression model and life-tables. *J Roy Stat Soc B* 34: 187-220, 1972

9. Fulda S, Debatin KM: Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene* 25: 4798-811, 2006

10. Ichikawa W, Sasaki Y: Correlation between tumor response to first-line chemotherapy and prognosis in advanced gastric cancer patients. *Ann Oncol* 17: 1665-72, 2006

11. Johnston PG, Lenz HJ, Leichman CG, Danenberg KD, Allegra CJ, Danenberg PV, Leichman L: Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. *Cancer Res* 55: 1407-12, 1995

12. Kang SY, Han JH, Lee KJ, Choi JH, Park JI, Kim HI, Lee HW, Jang JH, Park JS, Kim HC, Kang S, Oh YT, Chun M, Kim JH, Sheen SS, Lim HY: Low expression of Bax predicts poor prognosis in patients with locally advanced esophageal cancer treated with definitive chemoradiotherapy. *Clin Cancer Res* 13: 4146-53, 2007

13. Kang SY, Oh YT, Han JH, Choi JH, Lim HY, Kim HI, Lee HW, Jang JH, Park JS, Kim HC, Kang S, Chun M, Kim CH, Joo HJ: Concurrent chemoradiotherapy in patients with nasopharyngeal cancer: prognostic significance of low expression of bax. *Neoplasma* 53: 450-6, 2006

14. Kaplan E MP: Non-parametric estimations from incomplete observations. *J Am Statistical Assoc* 53: 457-481, 1958

15. Kim NK, Im SA, Kim DW, Lee MH, Jung CW, Cho EK, Lee JT, Ahn JS, Heo DS, Bang YJ: Phase II clinical trial of SKI-2053R, a new platinum analog, in the treatment of patients with advanced gastric adenocarcinoma. *Cancer* 86: 1109-15, 1999

16. Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E, Nordling S, Reed JC: Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. *Cancer Res* 55: 4471-8, 1995

17. Kwon HC, Roh MS, Oh SY, Kim SH, Kim MC, Kim JS, Kim HJ: Prognostic value of expression of ERCC1, thymidylate synthase, and glutathione S-transferase P1 for 5-fluorouracil/oxaliplatin chemotherapy in advanced gastric cancer. *Ann Oncol* 18: 504-9, 2007

18. Lee HW, Han JH, Kim JH, Lee MH, Jeong SH, Kang SY, Choi JH, Oh YT, Park KJ, Hwang SC, Sheen SS, Lim HY: Expression of excision repair cross-complementation group 1 protein predicts poor outcome in patients with small cell lung cancer. *Lung Cancer* 59: 95-104, 2008

19. Lee J LT, Uhm JE, Park KW, Park SH, Lee SC, Park JO, Park YS, Lim HY, Sohn TS, Noh JH, Heo JS, Park CK, Kim S, Kang WK: Prognostic model to predict survival following first-line chemotherapy in patients with metastatic gastric adenocarcinoma. *Ann Oncol* 18: 886-91, 2007

20. Lee SS, Lee JL, Ryu MH, Chang HM, Kim TW, Kang HJ, Kim WK, Lee JS, Kang YK: Combination chemotherapy with capecitabine (X) and Cisplatin (P) as first line treatment in advanced gastric cancer: experience of 223 patients with prognostic factor analysis. *Jpn J Clin Oncol* 37: 30-7, 2007

21. Lenz HJ, Leichman CG, Danenberg KD, Danenberg PV, Groshen S, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Garcia Y, Li J, Leichman L:

Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. *J Clin Oncol* 14: 176-82, 1996

22. Lordick F, Lorenzen S, Stollfuss J, Vehling-Kaiser U, Kullmann F, Hentrich M, Zumschlinge R, Dietzfelbinger H, Thoedtmann J, Hennig M, Seroneit T, Bredenkamp R, Duyster J, Peschel C: Phase II study of weekly oxaliplatin plus infusional fluorouracil and folinic acid (FUFOX regimen) as first-line treatment in metastatic gastric cancer. *Br J Cancer* 93: 190-4, 2005

23. Louvet C, Andre T, Tigaud JM, Gamelin E, Douillard JY, Brunet R, Francois E, Jacob JH, Levoir D, Taamma A, Rougier P, Cvitkovic E, de Gramont A: Phase II study of oxaliplatin, fluorouracil, and folinic acid in locally advanced or metastatic gastric cancer patients. *J Clin Oncol* 20: 4543-8, 2002

24. Metzger R LC, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groshen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Konda B, Leichman L: ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 16: 309-16, 1998

25. Min YJ BS, Shin JW, Kim DH, Park JH, Kim GY, Ko BK, Choi DH, Cho HR: Combination chemotherapy with 5-fluorouracil and heptaplatin as first-line treatment in patients with advanced gastric cancer. *J Korean Med Sci* 19: 369-73, 2004

26. Olaussen KA, Dunant A, Fouret P, Brambilla E, Andre F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, Le Chevalier T, Soria JC: DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med* 355: 983-91, 2006

27. Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. *CA Cancer J Clin* 55: 74-108, 2005

28. Pinedo HM, Peters GF: Fluorouracil: biochemistry and pharmacology. *J Clin*

Oncol 6: 1653-64, 1988

29. Reardon JT, Vaisman A, Chaney SG, Sancar A: Efficient nucleotide excision repair of cisplatin, oxaliplatin, and Bis-aceto-ammine-dichloro-cyclohexylamine-platinum(IV) (JM216) platinum intrastrand DNA diadducts. *Cancer Res* 59: 3968-71, 1999

30. Reed E: ERCC1 and clinical resistance to platinum-based therapy. *Clin Cancer Res* 11: 6100-2, 2005

31. Reed JC: Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. *Cell Death Differ* 13: 1378-86, 2006

32. Rivera F, Vega-Villegas ME, Lopez-Brea MF: Chemotherapy of advanced gastric cancer. *Cancer Treat Rev* 33: 315-24, 2007

33. Siddik ZH: Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene* 22: 7265-79, 2003

34. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 92: 205-16, 2000

35. Van Cutsem E, Van de Velde C, Roth A, Lordick F, Kohne CH, Cascinu S, Aapro M: Expert opinion on management of gastric and gastro-oesophageal junction adenocarcinoma on behalf of the European Organisation for Research and Treatment of Cancer (EORTC)-gastrointestinal cancer group. *Eur J Cancer* 44: 182-94, 2008

36. Wagner AD, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE:

Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. *J Clin Oncol* 24: 2903-9, 2006

37. Wei J, Zou Z, Qian X, Ding Y, Xie L, Sanchez JJ, Zhao Y, Feng J, Ling Y, Liu Y, Yu L, Rosell R, Liu B: ERCC1 mRNA levels and survival of advanced gastric cancer patients treated with a modified FOLFOX regimen. *Br J Cancer* 98: 1398-402, 2008

38. Wohrer SS, Raderer M, Hejna M: Palliative chemotherapy for advanced gastric cancer. *Ann Oncol* 15: 1585-95, 2004

39. Yeh KH, Shun CT, Chen CL, Lin JT, Lee WJ, Lee PH, Chen YC, Cheng AL: High expression of thymidylate synthase is associated with the drug resistance of gastric carcinoma to high dose 5-fluorouracil-based systemic chemotherapy. *Cancer* 82: 1626-31, 1998

– 국문요약

항암화학요법을 시행받은 위암환자의 예후인자 분석

아주대학교 대학원의학과

정성현

(지도교수: 최진혁)

본 연구는 5-fluorouracil (5-FU)과 platinum 제제로 고식적 항암화학요법을 시행 받은 진행성 위암환자들에 있어서 Bax, excisional cross-complementation 1 (ERCC1)과 thymidylate synthase (TS)의 임상결과에 대한 예측인자로써의 역할에 대해 조사하였다. 전이성 혹은 재발성 위암환자 128 명 중 56 명은 5-FU 와 heptaplatin (HP)으로 치료를 받았고 72 명의 환자는 5-FU, leucovorin, 그리고 oxaliplatin (FOLFOX)로 치료를 받았다. 치료 전 종양 생검조직으로 Bax, ERCC1, TS 의 면역조직학검사를 시행하였다. Bax, ERCC1, 그리고 TS 의 고발현은 각각 49 명(38%), 60 명(47%), 그리고 48 명(38%)에서 관찰되었다. 전체환자의 중앙 생존값은 10 개월 이었다. 단변량 분석에서 Bax 의 낮은 발현은 불량한 생존과 연관이 있었으나 (중앙생존기간, 9 개월 대 12 개월; 2 년 생존기간, 7% 대 32%; p=0.0005) ERCC1 이나 TS 의 발현은 환자의 치료성적과 관련이 없었다. Bax 의 낮은 발현을 보인 환자들의 치료성적은 FOLFOX 군의 환자에서 유의하게 불량하였으며 (중앙생존기간, 9 개월 대

18 개월; p=0.0008) FH 군의 환자에서는 유의한 차이가 없었다. 다변량분석에서 Bax 의 낮은 발현은 짧은 생존의 유의한 예측인자였다 (p=0.014). Bax 의 낮은 발현은 5-FU 와 platinum 으로 항암화학요법을 시행받은 전이성 혹은 재발성 위암환자의 불량한 생존과 연관이 있었다. 따라서, 치료전의 생검조직으로 시행하는 Bax 에 대한 면역조직학적 검사가 이러한 환자들의 치료로 FOLFOX 제제를 선택하는데 유용하게 사용될 수 있다.

핵심어: 위암, 항암화학요법, Bax, 예후