의학석사 학위 논문

Prognostic role of tumor marker and XRCC1 polymorphism in advanced biliary tract cancer patients treated with S-1 and Cisplatin

S-1과 Cisplatin 항암화학요법으로 치료한 진행성 담도계암 환자에서 종양표지자와 XRCC1 유전자 다형성이 예후에 미치는 영향

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이대원

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Prognostic role of tumor marker and XRCC1 polymorphism in advanced biliary tract cancer patients treated with S-1 and Cisplatin

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ABSTRACT

Background: As biliary tract cancer is a rare malignancy, prognostic and predictive markers of advanced biliary tract cancer have not been clearly elucidated. The purpose of this study is to evaluate the prognostic and predictive role of tumor marker, tumor marker change and gene polymorphism in advanced biliary tract cancer.

Patients and methods: Patients with pathologically proven metastatic or relapsed biliary tract cancer who had undergone first line S-1 plus cisplatin chemotherapy were enrolled. Time to progression (TTP) and overall survival (OS) were compared.

Results: Among a total of 104 patients, 69 (66.3%) patients had elevated baseline CA 19-9 level and 40 (38.5%) patients had elevated baseline CEA level. Eighty patients (77%) had either elevated CEA or CA 19-9 level. Multivariate analysis revealed that patients with elevated baseline CEA level have poorer TTP and OS compared to patients with normal CEA level. Baseline CA 19-9 level did not influence TTP or OS in multivariate analysis. Eleven germline polymorphisms within 4 genes were analyzed using polymerase chain reaction–restriction fragment length polymorphism methods. Three genes were involved in DNA damage repair and other single gene was associated with fluoropyrimidine. Only XRCC1 exon 194 gene had a negative prognostic role in terms of OS. Multivariate analysis revealed that XRCC1 194 C/T and T/T had a poor OS compared to C/C (adjusted HR 1.59, $p = 0.048$). In patients with elevated baseline CA 19-9, decline ≥ 30% after first cycle of chemotherapy showed prolonged TTP (5.0 *vs.* 8.6 months, $p = 0.015$) and OS (7.9 *vs.* 18.4 months, $p < 0.001$) as well as better chemotherapy response. Similar results were also obtained with CEA level change.

Conclusions: Baseline CEA or XRCC1 codon 194 polymorphism had a prognostic role in advanced biliary tract cancer. CA 19-9 or CEA decline ≥ 30% after first cycle of chemotherapy serves as a positive predictive and prognostic marker.

Keywords: XRCC1 polymorphism, CEA, CA 19-9, biliary tract cancer

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List of abbreviation

ALP: Alkaline phosphatase

ANC: Absolute neutrophil count

CA: Carbohydrate Antigen

CEA: Carcinoembryonic antigen

ECOG: Eastern Cooperative Oncology Group

ERCC1: Excision repair cross-complementation group 1

HR: Hazard ratios

OS: Overall survival

PFS: Progression free survival

RR: Response rate

TS: Thymidylate synthase

TTP: Time to progression

ULN: Upper limit of normal

UTR: Untranslated region

XPD: Xeroderma pigmentosum group D

XRCC1: X-ray repair cross-complementing group 1

INTRODUCTION

Biliary tract cancer includes intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gall bladder cancer, and ampulla of Vater cancer. While incidence of biliary tract cancer is low in Western countries (2 to 3 persons/100,000 population per year), their incidence is relatively high in Asian countries (4 to 6 persons /100,000 population per year) (1, 2). Complete resection is the only option for the cure of biliary tract cancer but only 10% of the patients are diagnosed at early stage of disease and are considered for curative resection (3). For unresectable or metastatic biliary tract cancer, chemotherapy has shown significant benefit compared to best supportive care only (4, 5). In a randomized controlled phase III study, gemcitabine plus cisplatin showed improved Overall survival (OS) and progression free survival (PFS) compared to gemcitabine alone (6). Although gemcitabine plus cisplatin is considered as a standard of care in first-line setting, this regimen has not been compared head to head with other fluoropyrimidine-based regimen in phase III studies. In a phase II study, combination of S-1 and cisplatin showed comparable efficacy and favorable safety compared to gemcitabine plus cisplatin in advanced biliary tract cancer (7). Gemcitabine-based or fluoropyrimidine-based combinations are the standard chemotherapy regimen for advanced biliary tract cancer (6, 8-10).

Despite the progress in chemotherapy, prognosis of biliary tract cancer remains poor with median OS of 5 to 15 month and only 15 to 40% of patient shows response to chemotherapy (11). Selection of patients who might benefit from specific chemotherapy is important. In addition, early assessment of treatment efficacy can help physician's clinical decision and can prevent patients from unnecessary treatment. Metastatic disease, intrahepatic cholangiocarcinoma, liver metastasis, Eastern Cooperative Oncology Group (ECOG) performance status and alkaline phosphatase (ALP) level were identified as a prognostic factor in advanced BTS (12). However predictive and prognostic role of other factors including carcinoembryonic antigen (CEA), carbohydrate Antigen (CA) 19-9 and gene polymorphism has not been clearly elucidated in advanced biliary tract cancer. A number of studies have investigated the relationships between genetic polymorphism and efficacy of distinct

chemotherapy agent. The aim of our study is to evaluate the predictive and prognostic role of baseline CEA, baseline CA 19-9, tumor marker changes and gene polymorphisms, using our homogenous cohort of patients who has undergone first-line S-1 plus cisplatin chemotherapy in advanced biliary tract cancer. S-1 is an oral fluoropyrimidine agent containing tegafur, gimeracil and oteracil potassium. The mechanism of action of fluoropyrimidine is through the inhibition of thymidylate synthase (TS). Cisplatin is a platinum agent, which inhibits DNA synthesis by the formation of DNA cross-links. We chose 4 genes for polymorphism analysis, three genes were associated with DNA damage repair and other gene was TS.

MATERIAL AND METHODS

Patients and treatment

Patients (N = 104) with pathologically proven unresectable, metastatic, or relapsed biliary tract adenocarcinoma participated in a phase II trial of S-1 and cisplatin were included (13). At least one measurable lesion according to the Response Evaluation Criteria in Solid tumors (RECIST) was required. Eligibility criteria included age over 18, ECOG performance status of 0 to 2, no prior chemotherapy or radiotherapy, adequate bone marrow, hepatic, and renal function [absolute neutrophil count (ANC) $\geq$ 1.5 x 10^9/l, platelet count $\geq$ 100 x 10^9/l, total bilirubin $\leq$ 2 x upper limit of normal (ULN), serum transaminases $\leq$ 2.5 x ULN, alkaline phosphatase $\leq$ 5 x ULN, serum creatinine $\leq$ 1.5 x ULN or actual or calculated creatinine clearance $\geq$ 60 ml/min]. Written informed consent was obtained from each patient before enrollment and the protocol was approved by the institutional review board of the Seoul National University Hospital, Seoul, Korea.

S-1 was administered orally at a dose of 40 mg/m^2 twice daily for 14 days, followed by a 7-day rest period. Cisplatin was given as a 90–minute infusion on day 1 of each cycle at a dose of 60 mg/m^2. Treatment was repeated every 3 weeks until disease progression, unacceptable toxicity, or withdrawal of patient consent. Drug administration was delayed until ANC $\geq$ 1.5 x 10^9/l, platelet count $\geq$ 75 x 10^9/l, and recovery from non-hematologic toxicity to baseline or less than or equal to grade 1. S-1 was reduced by 25% on all subsequent cycles for febrile neutropenia,

grade 4 neutropenia, grade 3/4 thrombocytopenia, greater than or equal to grade 3 non-hematologic toxic effects. Cisplatin was reduced by 25% for febrile neutropenia and greater than or equal to grade 3 non-hematologic toxic effects.

Best tumor response was assessed using RECIST criteria, with computed tomography (CT) scans (14). CT scan was made at baseline and every two cycles (6 weeks) thereafter. Medical history taking, physical examination, measurement of the CEA, CA 19-9 level and toxicity evaluation was made on every cycle. Toxicity was measured according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Genotype analysis

For the analysis of germline genetic polymorphisms, peripheral blood samples were obtained from patients with informed consent. Genomic DNA was extracted form peripheral blood samples using QIAmp DNA blood kit (Qiagen,Valencia, CA, USA). Eleven polymorphisms in four genes were investigated. The following polymorphisms were analyzed using polymerase chain reaction–restriction fragment length polymorphism methods: thymidylate synthase (TS; 28-bp repeat in the enhancer region, G⁄C polymorphism in the second repeat, 6-bp deletion in the 3'-untranslated region [UTR]), excision repair cross-complementation group 1 (ERCC1; Asn118Asn, C8092A), Xeroderma pigmentosum group D (XPD; Arg156Arg, Asp312Asn, Lys751Trp), and X-ray repair cross-complementing group 1 (XRCC1; Arg194Trp, Arg280His, Arg399Gln). Primer sequences and restriction enzymes are listed in Table 1.

Table 1. Primer sequences and restriction enzymes

	Gene	Polymorphism	Primer (5’ - 3’)	Restriction enzyme
Fluoropyrimidine associated gene	**TS**	28-bp repeat in enhancer region	Forward: GTGGCTCCTGCGTTTCCCCC Reverse: GCTCCGAGCCGGCCACAGGCATGGCGCGG	-
		G/C SNP in second repeat	Forward: GTGGCTCCTGCGTTTCCCCC Reverse: GCTCCGAGCCGGCCACAGGCATGGCGCGG	*Hae* III
		6-bp deletion in 3’-UTR	Forward: CAAATCTGAGGGAGCTGAGT Reverse: CAGATAAGTGGCAGTACAGA	*Dra* I
DNA damage repair gene	**ERCC1**	Asn118Asn	Forward: TCATCCCTATTGATGGCTTCTGCCC Reverse: GACCATGCCCAGAGGCTTCTCATAG	*BsrD* I
		C8092A	Forward: CAGAGACAGTGCCCCAAGAG Reverse: GGGCACCTTCAGCTTTCTTT	*Mbo* II
	XPD	Arg156Arg	Forward: CACACCTGGCTCATTTTTGTAT Reverse: TCATCCAGGTTGTAGATGCCA	*Tfi* I
		Asp312Asn	Forward: CTGTTGGTGGGTGCCCGTATCTGTTGGTCT Reverse: (TAATA)TCGGGGCTCACCCTGCAGCACTTCCT	*Sty* I
		Lys751Trp	Forward: GCCCGCTCTGGATTATACG Reverse: CTATCATCTCCTGGCCCCC	*Pst* I
	XRCC1	Arg194Trp	Forward: GCCCCGTCCCAGGTA Reverse: AGCCCCAAGACCCTTTCACT	*Pvu* II
		Arg280His	Forward: TTGACCCCCAGTGGTGCTAA Reverse: AGTCTGCTGGCTCTGGGCTGG	*Rsa* I
		Arg399Gln	Forward: TTGTGCTTTCTCTGTGTCCA Reverse: TCCTCCAGCCTTTTCTGATA	*Msp* I

Abbreviations: ERCC1, Excision repair cross-complementation group 1; TS, Thymidylate synthase; XPD, Xeroderma pigmentosum group D ; XRCC1, X-ray repair cross-complementing group 1

Statistical analysis

The primary objective of this study was to investigate the effect of serum tumor marker (CEA, CA 19-9), tumor marker change and gene polymorphism status on the treatment outcome of advanced biliary tract cancer patients treated with S-1 and cisplatin chemotherapy. Categorical variables were compared using chi-square test. Time to progression (TTP) and OS were calculated using the Kaplan-Meier method and comparisons were made using the log-rank tests. Hazard ratios (HR) were calculated using the Cox proportional hazard model. In order to adjust for the baseline characteristics, we used Cox proportional hazard model in a forward stepwise manner with variable as followings: initial presentation with metastatic disease, intrahepatic cholangiocarcinoma, liver metastasis, ECOG performance status, ALP level, CEA level, CA 19-9 level and gene polymorphism. Gene polymorphisms with *p*-values of less than 0.10 in the univariate analysis were included in the multivariate analysis. Two-sided *p*-values of less than 0.05 were considered statistically significant. Statistical analysis was performed with SPSS software for Windows, version 18.0 (SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics

A total of 104 patients with advanced biliary tract cancer, treated with S-1 and cisplatin chemotherapy were included from January 2005 to December 2008. Baseline characteristics are summarized in Table 2. Tumor type was intrahepatic cholangiocarcinoma in 57, gall bladder cancer in 33, extrahepatic cholangiocarcinoma in 11 and ampulla of vater cancer in 3 patients. Presentation of disease was metastatic in 71 patients, relapsed case in 29 patients and 4 patients had unresectable disease. Baseline serum ALP level, CEA level and CA 19-9 level was above normal level in 39 (37.1%), 40 (38.5%) and 69 (66.3%) patients respectively. Eighty (77%) patients had either elevated CEA or CA 19-9 level. Most of the patients had good physical performance status. According to the inclusion criteria, none of the patients received prior systemic chemotherapy or radiotherapy.

Prognostic Impact of Clinicopathologic Variables and Tumor Marker

After a median follow-up duration of 32 months, 90 death events and 80 progression events occurred. The median TTP was 6.8 months (95% CI, 5.6 - 8.0) and the median OS was 13.8 months (95% CI, 10.9 - 16.7). Overall response rate (RR) was 27.9% and 42.3% of patients showed stable disease. Baseline ECOG performance status 2 (4.7 months *vs.* 8.9 months in ECOG performance status 0, $p = 0.078$), elevated CEA level (5.1 months *vs.* 7.9 months, $p = 0.015$) and elevated ALP level (5.4 months *vs.* 7.6 months, $p = 0.039$) were associated with poor TTP (Table 3). Liver metastasis and Initial metastatic presentation was not associated with TTP. In the multivariate analysis using Cox proportional hazard model, elevated baseline CEA level (adjusted HR 1.71, $p = 0.025$) and ALP level (adjusted HR 1.60, $p = 0.050$) were associated with poor TTP. There was no predictive role of CEA or CA 19-9 level based on best response assessed by RECIST criteria. In terms of OS, poor ECOG performance status, initial metastatic presentation, elevated ALP level, elevated CEA level and elevated CA 19-9 level (above 370 U/mL) were associated with worse prognosis in univariate analysis (Table 3).

Table 2. Patient characteristics

	Number of patients (%) (*N* = 104)
Age	
Median (range)	59 (31 - 76)
≥ 65 years	29 (27.9%)
Sex	
Male	60 (57.7%)
Female	44 (42.3%)
Primary site	
Intrahepatic cholangiocarcinoma	57 (54.8%)
Gall bladder cancer	33 (31.7%)
Extrahepatic cholangiocarcinoma	11 (10.6%)
Ampulla of vater cancer	3 (2.9%)
Initial presentation	
Metastatic	71 (68.3%)
Relapsed	29 (27.9%)
Unresectable	4 (3.8%)
Metastasis	
Liver	59 (56.7%)
Lymph node	53 (51.0%)
Peritoneal seeding	20 (19.2%)
Lung	9 (8.7%)
Histologic grade	
Well differentiated	5 (4.8%)
Moderately differentiated	20 (19.2%)
Poorly differentiated	6 (5.8%)
Unknown	73 (70.2%)
ECOG PS	
0	11 (10.6%)
1	89 (85.6%)
2	4 (3.8%)
ALP (*N* = 103)	
< 115 IU/L	64 (62.1%)
≥ 115 IU/L	39 (37.9%)

CEA	
< 5 ng/mL	64 (61.5%)
≥ 5 ng/mL	40 (38.5%)
CA 19-9 (*N* = 103)	
< 37 U/mL	34 (33.0%)
≥ 37 U/mL	69 (67.0%)
≥ 370 U/mL	46 (44.7%)

Abbreviations: PS, performance status

Prognostic Implication of Gene polymorphism

We analyzed 11 germline polymorphisms within 4 genes. Among variable gene polymorphism, only XRCC1 polymorphism in codons 194 had a prognostic role in OS (Table 4). The genotype of XRCC1 codons 194 C/T and T/T showed poorer OS compared to C/C (17.1 months *vs.* 10.6 months, $p = 0.053$). However, XRCC1 194 polymorphism did not have a prognostic role in terms of TTP and response rate was similar between each group. To examine whether XRCC1 194 polymorphism was associated with poor OS, we used Cox proportional hazard model in a forward stepwise manner. Multivariate analysis revealed that XRCC1 194 polymorphism had a prognostic role in OS (Table 5). XRCC1 194 C/T and T/T had a poor OS compared to C/C (adjusted HR 1.59, $p = 0.048$). Baseline elevated CEA level and ALP level was also a negative prognostic factor for OS. CA 19-9 level was not a prognostic factor for OS in multivariate analysis.

Table 3. Univariate analysis of progression free survival and overall survival

Variable		TTP (95% CI)	*p*-value	OS (95% CI)	*p*-value
Age	≥65 years	7.4 (5.2 - 9.5)	0.54	12.9 (8.1 - 17.8)	0.72
	<65 years	6.7 (5.2 - 8.2)		13.8 (10.4 - 17.3)	
Sex	Male	6.9 (5.4 - 8.5)	0.80	13.7 (9.8 - 17.6)	0.71
	Female	6.6 (4.7 - 8.5)		13.5 (9.6 - 17.3)	
Primary site	Intrahepatic	6.6 (5.0 - 8.2)	0.64	12.0 (8.8 - 15.3)	0.15
	Others	7.0 (5.2 - 8.8)		16.1 (11.0 - 21.3)	
Initial presentation	Metastatic	6.2 (5.0 - 7.5)	0.24	11.1 (8.5 - 13.8)	0.020
	Others	7.8 (5.4 - 10.2)		18.5 (12.3 - 24.6)	
Liver metastasis	Present	6.4 (5.1 - 7.8)	0.50	12.2 (8.8 - 15.5)	0.22
	Absent	7.3 (5.3 - 9.3)		15.3 (10.8 - 19.8)	
ECOG PS	0	8.9 (5.4 - 12.3)	0.078 *vs. 2*	24.8 (14.7 - 34.9)	0.048 *vs.* 2
	1	6.7 (5.4 - 8.1)	0.34 *vs.* 0	12.5 (9.6 - 15.4)	0.024 *vs.* 0
	2	4.7 (1.9 - 7.5)	0.45 *vs.* 1	7.2 (4.3 - 10.2)	0.68 *vs.* 1
ALP	≥ 115 IU/L	5.4 (3.4 - 7.4)	0.039	9.4 (5.9 - 12.9)	0.006
	< 115 IU/L	7.6 (6.2 - 9.0)		16.2 (12.3 - 20.2)	
CEA	≥ 5 ng/mL	5.1 (4.0 - 6.2)	0.015	8.5 (6.1 - 11.0)	0.002
	< 5 ng/ml	7.9 (6.1 - 9.7)		17.3 (13.0 - 21.6)	
CA 19-9	≥ 37 U/mL	6.7 (5.1 - 8.3)	0.49	12.2 (9.1 - 15.3)	0.15
	< 37 U/mL	7.3 (5.5 - 9.1)		17.1 (11.0 - 23.2)	
CA 19-9	≥ 370 U/mL	6.4 (4.8 - 7.9)	0.51	10.2 (7.7 - 12.7)	0.043
	< 370 U/mL	7.2 (5.5 - 9.0)		16.7 (12.0 - 21.4)	

Abbreviations: HR, hazard ratio; CI, confidence interval; PS, performance status

Table 4. Genetic polymorphism and treatment outcomes

Gene Polymorphism (*N*)		TTP (95%CI)	*p* -value	OS (95%CI)	*p* -value
XRCC1 194	CC (51)	7.4 (5.5 - 9.3)	0.32	17.1 (12.1 - 22.1)	0.053
	CT + TT (53)	6.0 (4.8 - 7.3)		10.6 (7.7 - 13.5)	
XRCC1 280	GG (82)	6.4 (5.3 - 7.7)	0.36	12.5 (9.7 - 15.3)	0.19
	GA (22)	8.2 (4.9 - 11.5)		18.5 (9.8 - 27.1)	
XRCC1 399	GG (60)	7.0 (5.5 - 8.5)	0.67	14.4 (10.1 - 18.6)	0.91
	GA + AA (44)	6.6 (4.8 - 8.4)		13.1 (9.3 - 16.9)	
TS 5'UTR	High expression* (74)	6.9 (5.5 - 8.3)	0.90	14.5 (11.0 - 18.1)	0.44
	Low expression* (29)	7.0 (4.6 - 9.4)		12.0 (7.3 - 16.8)	
TS 6-bp deletion	+6/+6 or +6/-6 (25)	6.1 (3.4 - 8.7)	0.27	12.5 (6.2 - 18.9)	0.64
	-6/-6 (79)	7.1 (5.8 - 8.5)		14.0 (10.8 - 17.2)	
ERCC1 118	CC (53)	7.3 (5.3 - 9.2)	0.50	15.4 (10.8 - 19.9)	0.44
	CT + TT (51)	6.4 (4.9 - 7.9)		12.1 (8.6 - 15.5)	
ERCC1 8092	CC (59)	6.8 (5.5 - 8.1)	0.82	14.4 (10.5 - 18.4)	0.62
	CA + AA (45)	6.7 (4.7 - 8.8)		12.6 (8.7 - 16.5)	
XPD 156	CC (29)	7.5 (5.0 - 10.0)	0.54	16.2 (9.3 - 23.0)	0.58
	CA + AA (75)	6.6 (5.2 - 8.0)		12.5 (9.8 - 15.2)	
XPD 312	GG (99)	7.0 (5.8 - 8.3)	0.087	13.7 (10.8 - 16.7)	0.79
	GA (5)	3.5 (0.0 - 7.5)		14.7 (0.0 - 32.7)	
XPD 751	AA (96)	7.1 (5.8 - 8.4)	0.12	13.6 (10.7 - 16.5)	0.90
	AC (8)	4.4 (2.4 - 6.5)		15.1 (3.7 - 26.5)	

*High expression: 2R/3G, 3C/3G, 3G/3G; Low expression: 2R/2R,2R3C, 3C/3C

Table 5. Multivariate analysis of overall survival

Variable		Adjusted HR (95% CI)	*p*-value
CEA	< 5 ng/ml	1	0.003
	≥ 5 ng/mL	1.95 (1.26 - 2.99)	
ALP	< 115 IU/L	1	0.003
	≥ 115 IU/L	1.94 (1.26 - 3.01)	
XRCC1 194	CC	1	0.037
	CT + TT	1.59 (1.03 - 2.44)	

Abbreviations: HR, hazard ratio; CI, confidence interval

CEA and CA 19-9 Change

We next evaluated whether decrease in serum CEA and CA 19-9 level after first cycle of chemotherapy can predict treatment outcomes. Among 69 patients with baseline elevated CA 19-9 level, CA 19-9 level after first cycle of chemotherapy was measured in 67 patients (97%). 36 patients among 40 patients (90%) with baseline elevated CEA level had CEA level after first cycle of chemotherapy. In patients with baseline CA 19-9 above normal range, decrease ≥ 30% was associated with favorable prognosis, respectively for TTP (5.0 months *vs.* 8.6 months, $p = 0.015$) and OS (7.9 months *vs.* 18.4 months, $p < 0.001$) (Figure 1). Similar results were obtained with CEA level, patients with CEA decrease ≥ 30% showed favorable TTP (4.2 months *vs.* 7.7 months, $p = 0.012$) and OS (6.8 months *vs.* 14.7 months, $p = 0.024$) (Figure 2). Patients with tumor marker decline ≥ 30% was also associated with better responses to chemotherapy (Table 6). Patients with CA 19-9 decrease ≥ 30% showed RR in 55% compared to 8% in patients with CA 19-9 decrease < 30%. RR was 89% for patients who had CEA decline ≥ 30% compared to 15% in patients who did not.

Figure 1. CA 19-9 change and Survival

A: CA 19-9 change and TTP

B: CA 19-9 change and OS

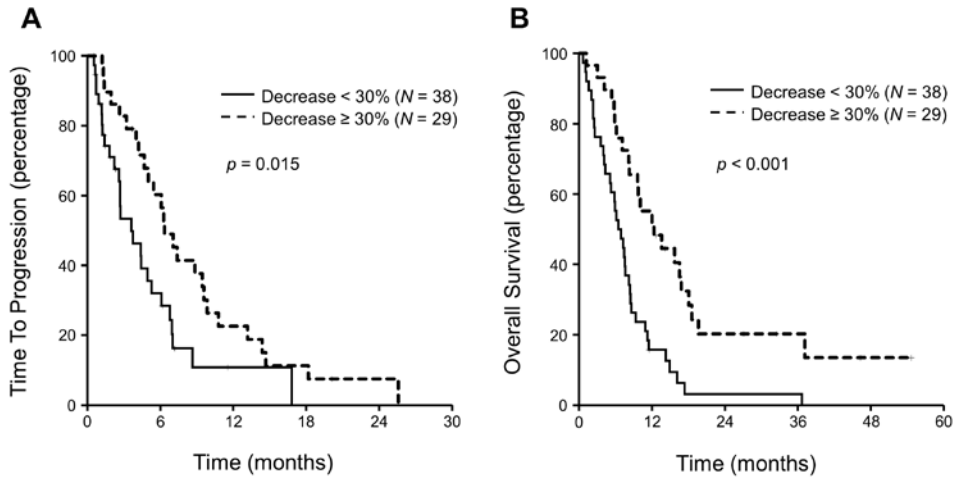

Figure 2. CEA change and Survival

A: CEA change and TTP

B: CEA change and OS

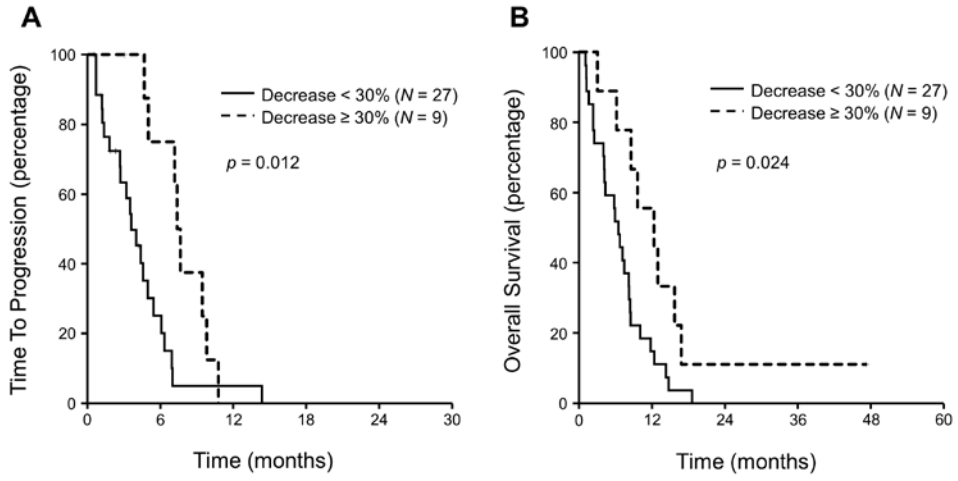

Table 6. CA19-9 ratio, CEA ratio and response rate in patients with elevated baseline tumor marker

Response	CA 19-9 decrease < 30%	CA 19-9 decrease ≥ 30%	*P*-value	CEA decrease < 30%	CEA decrease ≥ 30%	*P*-value
CR	1 (2.6%)	0 (0.0%)	<0.001	0 (0.0%)	1 (11.1%)	<0.001
PR	2 (5.3%)	16 (55.2%)		4 (14.8%)	7 (77.8%)	
SD	19 (50.0%)	9 (31.0%)		13 (48.1%)	1 (11.1%)	
PD	8 (21.1%)	3 (10.3%)		6 (22.2%)	0 (0.0%)	
N/A	8 (21.1%)	1 (3.4%)		4 (14.8%)	0 (0.0%)	

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; N/A, Not available

DISCUSSION

The aim of our study is to evaluate the predictive and prognostic role of tumor markers (CEA and CA 19-9) and gene polymorphism in patients with advanced biliary tract cancer. As biliary tract cancer is a rare cancer worldwide, predictive and prognostic factors has not been clearly defined nor have been validated. Older age, large tumor volume, metastatic disease, intrahepatic cholagiocarcinoma, liver metastasis, ECOG performance status and ALP level have been proposed as a prognostic factor in advanced biliary tract cancer (12, 15). Using our homogenous cohort of patients treated with first-line S-1 plus cisplatin, we found that baseline CEA level and XRCC1 exon 194 polymorphism is associated with treatment outcome. In addition, we also evaluated that CEA and CA 19-9 decline after first cycle of chemotherapy has both predictive and prognostic role.

Among variable tumor markers, CEA and CA 19-9 are the two markers that have been most studied in biliary tract cancer. Serum CEA level and CA 19-9 have a role in diagnosis of

cholangiocarcinoma in patients with primary sclerosing cholangitis (16-19). While diagnostic value of tumor markers have been widely evaluated and are clinically used, their predictive and prognostic roles have not been well studied. In our study, patients with baseline CEA ≥ 5ng/ml had poor TTP and OS compared to patients with CEA < 5ng/ml. Although CA 19-9 did not have a prognostic role in TTP, patients with CA 19-9 ≥ 370U/ml had a worse OS compared to patients with CA 19-9 < 370U/ml. In multivariate analysis only elevated CEA was associated with poor OS and there was no prognostic role of CA 19-9. These results are in line with studies performed in colorectal cancer and pancreatic cancer. In colorectal cancer, patients with CEA ≥ 5.0ng/mL had an adverse impact on survival independently of tumor stage (20, 21). Elevated CA 19-9 level was associated with poorer survival in patients with pancreatic cancer (22-24).

We also found out that decline in tumor marker after first cycle of chemotherapy can predict chemotherapy response and patients prognosis. CA 19-9 decrease ≥ 30% after first cycle of chemotherapy was associated with improved TTP and OS in patients with elevated baseline CA 19-9. CA 19-9 decline was also associated with better tumor response. Similar results were obtained using CEA level instead of CA 19-9 level. In patients with elevated baseline CEA level, CEA decrease ≥ 30% after first cycle of chemotherapy was associated with improved TTP, prolonged OS and better tumor response to chemotherapy. Clinical significance of tumor marker change was studied in pancreatic cancer. In advanced pancreatic cancer, decline in CA 19-9 level after chemotherapy was associated with improved survival (25, 26). CA 19-9 rise after second cycle of chemotherapy also served as a negative predictive factor (27). Tumor response assessment using RECIST criteria is usually measured after two to three months after the start of the chemotherapy because earlier changes are seldom significant. Our result shows that tumor maker measurement after the first cycle of chemotherapy (3 weeks after the start of the chemotherapy) can predict tumor response as well as survival. Earlier prediction of treatment efficacy and prognosis by tumor marker measurement can help physician’s decision and can prevent patients from unnecessary, ineffective treatment.

XRCC1 is a DNA repair gene involved in the repair of single strand breaks and base excision repair (28). We analyzed the prognostic role of XRCC1 polymorphism in codon 194, 280 and

399. Only polymorphism in XRCC1 codon 194 had a prognostic role and there was no prognostic role for codon 280 and 399. Patients with XRCC1 codon 194 C/T and T/T had a poor OS compared to C/C. In line with our study, preclinical studies showed that cells with XRCC1 194 C/C received higher damage to genotoxics compared to C/T and T/T (29, 30). Biliary tract cancer in XRCC1 194 C/C patients may have higher damage to chemotherapy agent. However the studies used NNK (nicotine-derived nitrosamine ketone) and bleomycin for genotoxic agent, and there is no preclinical data on relationship between XRCC1 codon polymorphism and S-1 or cisplatin chemotherapy.

The major limitation of this study is that only patients with measurable tumor lesion and those treated with first line S-1 and cisplatin were included. As XRCC1 is associated with DNA repair, prognostic role of XRCC1 polymorphism may be limited to specific chemotherapy agent. However, there is no relationship between specific chemotherapy and decline of tumor marker. This is the first study to evaluate the effect of tumor marker change in patients with biliary tract cancer. Our findings need further validation in an independent cohort, especially for patients treated with gemcitabine based chemotherapy and patients without measurable tumor lesion.

In conclusion, baseline CEA level and polymorphism of XRCC1 has a prognostic role in advanced biliary tract cancer. While there was no prognostic role for baseline CA 19-9 level, CA 19-9 decline $\geq$ 30% after first cycle of chemotherapy can be used as a positive predictive and prognostic marker.

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국문 초록

서론: 담도계암은 발생빈도가 낮은 악성종양으로, 진행성 담도계암에서의 예측인자와 예후인자는 확립되지 않았다. 우리는 진행성 담도계암에서 종양인자, 종양인자의 변화, 유전자 다형성 (genetic polymorphism)의 예측 인자 혹은 예후 인자로서의 역할을 확인하려고 한다.

대상 및 방법: 일차 항암치료제로 S-1과 cisplatin의 병용요법을 치료받은, 병리적으로 확진된 전이성 혹은 재발성 담도암 환자를 대상으로 하였다. 우리는 각 인자에 따른 진행 소요기간 (TTP) 및 생존기간 (OS)을 비교하려고 하였다.

결과: 전체 104명의 환자 중 69 (66.3%)명에서 기저 CA 19-9 수치가 상승되어 있었으며 40 (38.5%)명의 환자에서 기저 CEA 수치가 상승되어 있었다. 전체 환자 중 80 (77%)명에서 기저 CA 19-9 수치 혹은 CEA 수치가 상승되어 있었다. 다변량 분석결과 기저 CEA 수치가 높은 환자에서 진행 소요기간 및 생존 기간이 짧았다. 다변량 분석 결과 기저 CA 19-9 수치는 진행 소요기간과 생존 기간에 영향을 미치지 않았다. PCR- RFLP (Restriction Fragment Length Polymorphism)을 이용하여 4개의 유전자에서 11개의 유전자 다형성을 확인하였다. 이들 중 XRCC1 exon 194번의 유전자 다형성만이 생존기간에 영향을 미치는 예후 인자였다. 다변량 분석결과 XRCC1 194의 C/T와 T/T가 C/C에 비해 불량한 생존기간을 나타냄을 확인하였다 (adjusted HR 1.59, $p = 0.048$). 기저 CA 19-9 수치가 높은 환자를 대상으로, 1회 항암치료 후 CA 19-9 수치가 30% 이상 감소한 환자에서 그렇지 않은 환자에 비해 진행 소요기간 (5.0 *vs.* 8.6 months, $p = 0.015$) 및 생존기간 (7.9 *vs.* 18.4 months, $p < 0.001$)의 연장을 확인 하였으며 반응률이 더 좋았다. CEA 수치 변화를 이용하여도 비슷한 결과를 얻을 수 있었다.

결론: 기저 CEA 수치와 XRCC1 유전자 다형성은 진행성 담도계암 환자에서 예후 인자였다. CA 19-9 혹은 CEA 수치가 1회 항암치료 후 30% 이상 감소하는 것은 좋은 예후인자 및 예측인자였다.

Keywords: XRCC1 유전자 다형성, CEA, CA 19-9, 담도계암

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