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ORIGINAL ARTICLE

Different relation between ERCC1 overexpression and treatment outcomes of two platinum agents in advanced biliary tract adenocarcinoma patients

I. G. Hwang · J. S. Jang · J. H. Do · J. H. Kang · G. W. Lee · S. Y. Oh · H. C. Kwon · H. J. Jun · H. Y. Lim · S. Lee · K. C. Chi · S. J. Lee

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Abstract
Purpose The aim of this study is to evaluate the effect of excision repair cross-complementation group 1 (ERCC1) expression on treatment outcomes in advanced biliary tract adenocarcinoma (ABTA) patients treated with platinum-based chemotherapy.
Methods One hundred and six patients with histologically confirmed adenocarcinoma of biliary tract were enrolled at 5 institutions in South Korea between January 2002 and September 2008. Of 106 patients, 93 were assessed by immunohistochemistry from tissue specimens. Sixty-five patients were treated with cisplatin-based regimens and the other 28 treated with oxaliplatin-based ones.
Results For total study population, no significant differences were noted in progression-free survival (PFS) and overall survival (OS) between ERCC1-negative and ERCC1-positive patients, respectively (4.2 vs. 2.9 months, $p = 0.116$; 7.0 vs. 7.8 months, $p = 0.143$). In patients treated with cisplatin-based regimens, median PFS and OS were significantly longer in ERCC1-negative group than in ERCC1-positive group, respectively (4.6 vs. 1.9 months, $p = 0.014$; 9.1 vs. 7.9 months, $p = 0.017$). Disease control rate (DCR) was better in patients with ERCC1 negative than in patients with ERCC1 positive ($p = 0.048$). On the other hand, in patients treated with oxaliplatin-containing regimens, median PFS and OS tended to be longer in ERCC1-positive group, but these did not reach statistical significances. Response rate was better in patients with ERCC1 positive ($p = 0.005$).

I. G. Hwang · J. S. Jang (✉) · J. H. Do · S. J. Lee
Department of Internal Medicine,
Chung-Ang University College of Medicine,
224 Heukseok-dong, Dongjak-gu,
Seoul 156-755, Republic of Korea
e-mail: alsaba@daum.net

J. H. Kang · G. W. Lee
Department of Medicine,
Gyeongsang National University College of Medicine,
Chilam-dong, Jinju-si, Gyeongsangnam-do 660-702,
Republic of Korea

S. Y. Oh · H. C. Kwon
Department of Medicine, Dong-A University College
of Medicine, Dongdaesin-dong 3-ga, Seo-gu,
Busan 602-715, Republic of Korea

H. J. Jun
Department of Medicine, Myongi Hopital,
Kwandong University College of Medicine,
697-24 Hwajung-dong, Deokyang-gu,
Gyeonggi-do 412-270, Republic of Korea

H. Y. Lim
Department of Medicine, Samsung Medical Center,
Sungkyunkwan University School of Medicine, 50 Ilwon-dong,
Kangnam-gu, Seoul 135-710, Republic of Korea

S. Lee
Department of Pathology, Kangwon National University Hospital,
Kangwon National University School of Medicine, 1
7-1 Hyoja-3dong, Chunchon, Kangwon 200-722,
Republic of Korea

K. C. Chi
Department of Surgery, Chung-Ang University College
of Medicine, 224 Heukseok-dong, Dongjak-gu,
Seoul 156-755, Republic of Korea

Conclusions ERCC1 shows a significant prognostic value in ABTA patients treated with cisplatin. A survival benefit was observed in ERCC1-negative patients from cisplatin-containing chemotherapy but not from oxaliplatin-containing ones. The action mechanism of ERCC1 on cisplatin may be different from that on oxaliplatin.

Keywords Advanced biliary tract adenocarcinoma · ERCC1 · Cisplatin · Oxaliplatin

Introduction

Advanced biliary tract cancer (ABTC) is an aggressive tumor characterized by unresponsiveness to chemotherapy in the vast majority of cases, and a standard palliative chemotherapy regimen has not been established yet. Still, ABTC has been known to have a response to gemcitabine-based regimens and these regimens are increasingly prescribed. Platinum agents including cisplatin and oxaliplatin have been known to be synergic in combination with gemcitabine in diverse solid tumors, and gemcitabine/platinum doublet regimens have overall response rates of about 20–60% and a median survival of 6–11 months in phase II studies in the treatment of ABTC [5, 10]. Recently, a randomized phase III trial comparing gemcitabine/cisplatin combination with gemcitabine alone showed a significant survival benefit in the cisplatin-combination arm supporting a clearly beneficial role of platinum in the treatment of biliary tract cancer [23]. Indeed, this doublet has been the most widely used regimen raising the possibility of standard regimen in the treatment of ABTC. However, compared with other tumors, poorer treatment outcome raises a need to investigate molecular markers putting a damper on the execution of antitumor activity of drugs in this tumor.

Patient-tailored chemotherapy adapted to molecular markers in the individual patient could improve response rate and survival. In addition, this would allow the identification of the patients who most likely will not benefit from the treatment thus saving them from toxicity as well as saving resources. One potential way to accomplish this goal is the finding of predictive markers that are associated with drug resistance to treatment regimens.

The resistance to cisplatin appears to be associated with the increased removal of the cisplatin-DNA adducts and interstrand cross-links [13]. Proteins of the nucleotide excision repair pathway are thought to repair DNA damage caused by platinum agents [7, 13]. The excision repair cross-complementing (ERCC) gene family protein is the 5′ endonuclease of the nucleotide excision repair complex to prevent damage to DNA by nucleotide excision and repair [7, 13]. ERCC1, which is among the most promising predictive markers, has been explored in lung, ovarian, gastric, and colorectal cancer [4, 14, 16, 20]. In vitro study showed that DNA adducts regions induced by oxaliplatin or cisplatin are removed with similar ways by enzymes of the nucleotide excision repair pathway including ERCC1 [9]. On the basis of these findings, we tested whether the expression level of ERCC1 protein can predict the clinical outcomes such as the tumor response and survivals in patients with ABTA treated with platinum-based regimens.

Materials and methods

Patients

A total of 106 patients with histologically confirmed ABTA who were treated with platinum-based palliative chemotherapy were recruited from 5 institutions in South Korea between January 2002 and September 2008. Ninety-three adequate specimens from the 106 patients for analysis of ERCC1 expression were enrolled in this study. Their Eastern Cooperative Oncology Group performance status was 0–3. No prior chemotherapy was allowed, but patients with previous adjuvant chemotherapy and/or radiation therapy administered more than 6 months away from the date of enrollment were included in the study. This trial was approved by an Institutional review board at each institute. All participating patients were required to provide written informed consent.

Treatments and evaluation

All of the patients received one of the chemotherapy regimens as follows and had at least 1 or more courses of chemotherapy. The chemotherapy regimens consisted of 5-fluorouracil/cisplatin(FP) (F; 700 mg/m^2/d as a continuous intravenous (i.v.) infusion on days(D) 1–5 and P; 70 mg/m^2 i.v. on D1 every 4 weeks), gemcitabine/cisplatin(GP) (G; 1,000 mg/m^2 i.v. on D1, 8 and P; 70 mg/m^2 i.v. on D1 every 3 weeks), capecitabine/cisplatin(XP) (X; 1250 mg/m^2 p.o. bid on D1-14 and P; 60 mg/m^2 i.v. on D1 every 3 weeks), gemcitabine/oxaliplatin(GEMOX) (G; 1000 mg/m^2 i.v. on D1, 8 and O; 100 mg/m^2 i.v. on D1 every 3 weeks or G; 1,000 mg/m^2 i.v. on D1 and O; 85 mg/m^2 i.v. on D2 every 2 weeks), and capecitabine/oxaliplatin (XELOX) (X; 1250 mg/m^2 p.o. bid on D1-14 and O; 100 mg/m^2 i.v. on D1 every 3 weeks). Response to treatment was evaluated with enhanced computed tomography after every two cycles of chemotherapy according to RECIST (Response Evaluation Criteria in Solid Tumors) criteria [21].

Immunohistochemical staining for ERCC1

The tumor samples were taken by biopsy or surgery. Immunohistochemical staining for ERCC1 was carried out on

formalin-fixed, paraffin-embedded 4-μm-thick tissue section. The tissue sections were deparaffinized in xylene and then rehydrated in serial-graded alcohol. ERCC1 antigen retrieval comprised heating in 10 mM citrate buffer at pH 6.0 in an electric cooker (15 min, 700 W) and cooling at room temperature for 20 min. The sections were washed in Tris-buffered saline (TBS), and endogenous peroxidase activity was blocked by incubating the slides in a 3% hydrogen peroxide blocking reagent (provided in kit) for 20 min. The slides were incubated at room temperature with mouse monoclonal anti-ERCC1 (8F1; Neomarkers, Fremont, CA, USA) at a dilution of 1:200 for 2 h in a humidified chamber. The primary antibody was visualized with the Polink-1 horseradish peroxidase (HRP) Detection Kit (GBI, Mukilteo, WA, USA). The sections were washed again in TBS, and color was developed by adding 3,3′-diaminobenzidine (DAB) Plus Substrate System (Thermo Scientific, Fremont, CA, USA). Finally, the sections were counterstained with hematoxylin.

Evaluation of ERCC1 expression

Immunohistochemical evaluation was made by two independent pathologists, with no knowledge of the clinical data, and discrepancies were resolved by consensus. The immunohistochemical staining of ERCC1 was reviewed with appropriate positive and negative controls. The pathologists recorded whether tumor or stromal cells expressed ERCC1. The staining intensity was graded on a scale of 0–3; endothelial cells were used as the internal reference and assigned an intensity of 2. The percentage of positive nuclei was calculated, and the proportion score was assigned (0 if 0%, 0.1 if 1–9%, 0.5 if 10–49%, and 1.0 if ≥50%). The proportion score was multiplied by the staining intensity to obtain a final semiquantitative H score. The median value of the H score was chosen as the cutoff point for separating low and high levels of ERCC1 expression, as described previously [1, 8].

Statistical analysis

Clinical and pathological characteristics of the low and high levels of ERCC1 expression groups of patients were compared using the χ^2 or Fisher exact tests for categorical variables. OS was calculated from the first day of palliative chemotherapy until the date of death or the most recent documented follow-up. Patients who were alive at the last follow-up were censored at that time. PFS was calculated from the first day of palliative chemotherapy to the day when disease progression was recognized or the day of the last follow-up visit. Patients who were taken off study or who died before progression were censored at the time as they were taken off. OS and PFS were estimated using the Kaplan–Meier method. Significant between-group differences were assessed by the log-rank test. All reported *P values* are two sided, and $P < 0.05$ was considered significant. Multivariate analysis of the independent predictive or prognostic factors for survival was performed using Cox regression model with 95% confidence intervals (CIs). Factors with *P values* <0.05 in univariate analyses were examined with multivariate regression model. Analyses were carried out using SPSS version 12.0.

Results

Patients' characteristics

The clinicopathologic characteristics of 93 patients are shown in Table 1. The median age was 58 years (range, 32–87 years). Sixty-two had cholangiocarcinoma (66.6%), 23 patients had carcinoma of the gallbladder (24.7%), and 8 had extrahepatic biliary tract disease (8.6%). The chemotherapy regimens patients received were as follows: 29 patients were treated with FP-combination chemotherapy; 20 with GP; 16 with XP; 26 with GEMOX; and 2 with XELOX. Grouping by platinum drugs, 65 patients were treated with cisplatin (70%), and the remaining 28 were with oxaliplatin (30%).

Patients' characteristics in relation to platinum agents and ERCC1 expression

ERCC1 expression was localized to the nucleus (Fig. 1). The median value of H scores was 1.5 (range, 0 to 3). "ERCC1 positive" was defined as an H score >1.5 and was observed in 49.5% (46/93) of patients. No significant difference was noted among clinicopathological parameters assessed by status of ERCC 1, whether positive or negative (Table 1). Exceptionally, patients with ERCC1-positive tumors had poorer performance status than patients with ERCC1-negative tumors ($p = 0.026$; Table 1).

There were no significant differences in the clinicopathological factors including gender, age, performance status, and primary cancer site between patients with ERCC1-positive tumors and ERCC1-negative tumors with cisplatin- and oxaliplatin-based chemotherapies, respectively (Table 2).

Treatment responses in relation to platinum agents and ERCC1 expression

ERCC1 expressions in patients who achieved clinically objective responses including complete response(CR) or partial response (PR) were not different from those in patients with stable disease(SD) or progressive disease (PD) ($p = 0.621$; Table 3).

Table 1 Patients' characteristics

Characteristic	All patients ($N = 93$)	ERCC1-negative tumors ($N = 47$)	ERCC1-positive tumors ($N = 46$)	P value[†]
Sex				
Male	66	35	31	0.499
Female	27	12	15	
Age (years)				
<58	42	22	20	0.836
≥58	51	25	26	
ECOG[a]				
0–1	88	47	41	0.026
2–3	5	0	5	
Primary site				
GB[b]	23	14	9	0.368
IHD[c]	62	29	33	
EHD[d]	8	4	4	
Prior treatment				
None	73	34	39	0.374
Surgery only	10	8	2	
S + CRTx[e]	10	5	5	
Disease status				
Metastatic	80	37	43	0.070
Locally advanced	13	10	3	
Metastatic sites				
Liver	54	24	30	0.209
Lung	18	7	11	0.304
Lymph nodes	58	25	33	0.087
Peritoneum	15	11	4	0.089
Platinum				
Cisplatin	65	32	33	0.822
Oxaliplatin	28	15	13	
Tx cycles (median)	3	3	2	
(range)	(1–13)	(1–13)	(1–9)	

[†] P values from the χ^2 or Fisher's exact tests; [a] ECOG scores for performance status; [b]Gall bladder; [c] Intrahepatic duct; [d] Extrahepatic duct; [e] Postoperative chemoradiation therapy

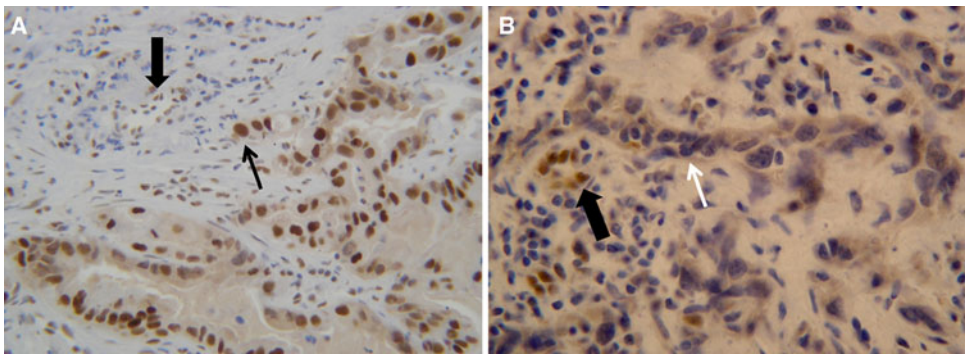

Fig. 1 Representative immunohistochemical staining for ERCC1 in biliary tract cancer. The *black arrow* in (**a**): positive nuclear staining for ERCC1. The *white arrow* (**b**): negative for ERCC1. The *bold arrows* in (**a**, **b**) show positive endothelial cells

In 65 patients treated with cisplatin, overall response rate was 22% (14 patients with PR and no CR), and disease control rate including SD was 46%. Among 14 patients who were responsive to cisplatin, 9 (64%) patients were ERCC1 negative while 5 (36%) were ERCC1 positive ($p = 0.240$; Table 3). Among 30 patients with cisplatin who obtained clinical benefits assessed by disease control rate, 19 (63%) patients were ERCC1 negative while 11 (37%) were ERCC1 positive ($p = 0.048$; Table 3). In oxaliplatin-treated patients, overall response rate was 21% (6 patients with PR

Table 2 Patients' characteristics according to ERCC1 status in patients with platinum agents

Characteristic	Cisplatin-based chemotherapy (*N* = 65)		Oxaliplatin-based chemotherapy (*N* = 28)	
	ERCC1 negative (*N* = 32) No. (%)	ERCC1 positive (*N* = 33) No. (%)	ERCC1 negative (*N* = 15) No. (%)	ERCC1 positive (*N* = 13) No. (%)
Sex				
Male	26 (53)	23 (47)	9 (53)	8 (47)
Female	6 (37)	10 (63)	6 (55)	5 (45)
Age (years)				
<58	14 (48)	15 (52)	8 (62)	5 (38)
≥58	18 (50)	18 (50)	7 (47)	8 (53)
ECOG[a]				
0–1	32 (52)	30 (48)	15 (58)	11 (42)
2–3	0 (0)	3 (100)	0 (0)	2 (100)
Primary site				
GB[b]	8 (57)	6 (43)	6 (67)	3 (33)
IHD[c]	20 (44)	25 (56)	9 (53)	8 (47)
EHD[d]	4 (67)	2 (33)	0 (0)	2 (100)
Prior treatment				
None	24 (46)	28 (54)	10 (48)	11 (52)
Surgery only	3 (75)	1 (25)	5 (83)	1 (17)
S + CRTx[e]	5 (56)	4 (44)	0 (0)	1 (100)
Disease status				
Metastatic	25 (45)	31 (55)	12 (50)	12 (50)
Locally advanced	7 (78)	2 (22)	3 (75)	1 (25)
Metastatic sites				
Liver	16 (46)	19 (54)	8 (42)	11 (58)
Lung	7 (44)	9 (56)	0 (0)	2 (100)
Lymph nodes	15 (40)	23 (60)	10 (50)	10 (50)
Peritoneum	6 (75)	2 (25)	5 (71)	2 (29)
Regimen				
FU[f]	23 (51)	22 (49)	2 (100)	0 (0)
Gemcitabine	9 (45)	11 (55)	13 (50)	13 (50)
Chemotherapy dose				
Complete	21 (45)	26 (55)	14 (52)	13 (48)
Incomplete	11 (61)	7 (39)	1 (100)	0 (0)

† *P* values from the χ^2 or Fisher's exact tests; [a] ECOG scores for performance status; [b] Gallbladder; [c] Intrahepatic duct; [d] Extrahepatic duct; [e] Postoperative chemoradiation therapy, [f] 5-fluorouracil

and no CR) and disease control rate 68%. Among 6 patients who achieved responses, all six (100%) were ERCC1 positive and none was ERCC1 negative ($p = 0.005$; Table 3) contrasting to the patients with cisplatin. Among 19 patients with oxaliplatin who obtained clinical benefits, 8(42%) patients had ERCC1 negative while 11 (58%) had ERCC1 positive ($p = 0.114$; Table 3).

Survivals in relation to ERCC1 expression

For total study population, a median PFS was 3.6 months (95% CI, 2.6–4.6 months) and a median OS was 7.3 months (95% CI, 5.9-8.7 months) with median follow-up period of 7.3 months (range, 0.8–47.5 months). Among ninety-three, 91 observed progression, 83 were died, and 3 were unknown for death at the time of analysis. About PFS and OS, there was a tendency toward longer survivals in patients with ERCC1 negative but the differences did not reach statistical significances (FPS: 4.2 vs. 2.9 months, $p = 0.116$, OS: 7.8 vs. 7.0 months, $p = 0.143$, Table 4, Fig. 2A, 2B).

In patients treated with cisplatin, median PFS and OS were significantly longer in ERCC1-negative group than in ERCC1-positive group (PFS, 4.6 vs. 1.9 months, $p = 0.014$;

Table 3 Treatment responses according to administrated agents and ERCC1 status

	All patients	ERCC1 negative patients	ERCC1 positive patients	*P* value†
	No. (%)	No. (%)	No. (%)	
Total patients	93 (100)	47 (51)	46 (49)	
OR[a]				
(+)	20 (22)	9 (45)	11 (55)	0.621
(−)	73 (78)	38 (52)	35 (48)	0.409
DCR[b]				
(+)	49 (53)	27 (55)	22 (45)	
(−)	44 (47)	20 (44)	25 (56)	
Cisplatin	65 (100)	32 (49)	33 (51)	
OR[a]				
(+)	14 (22)	9 (64)	5 (36)	0.240
(−)	51 (78)	23 (45)	28 (55)	0.048
DCR[b]				
(+)	30 (46)	19 (63)	11 (37)	
(−)	35 (54)	13 (37)	22 (63)	
Oxaliplatin	28 (100)	15 (54)	13(46)	
OR[a]				
(+)	6 (21)	0 (0)	6 (100)	0.005
(−)	22 (79)	15 (68)	7 (32)	0.114
DCR[b]				
(+)	19 (68)	8 (42)	11 (58)	
(−)	9 (32)	7 (78)	2 (22)	

† *P* values from the χ^2 or Fisher's exact tests; [a] Objective response (included CR complete response and PR partial response); [b] Disease control rate (include CR, PR, and SD stable disease)

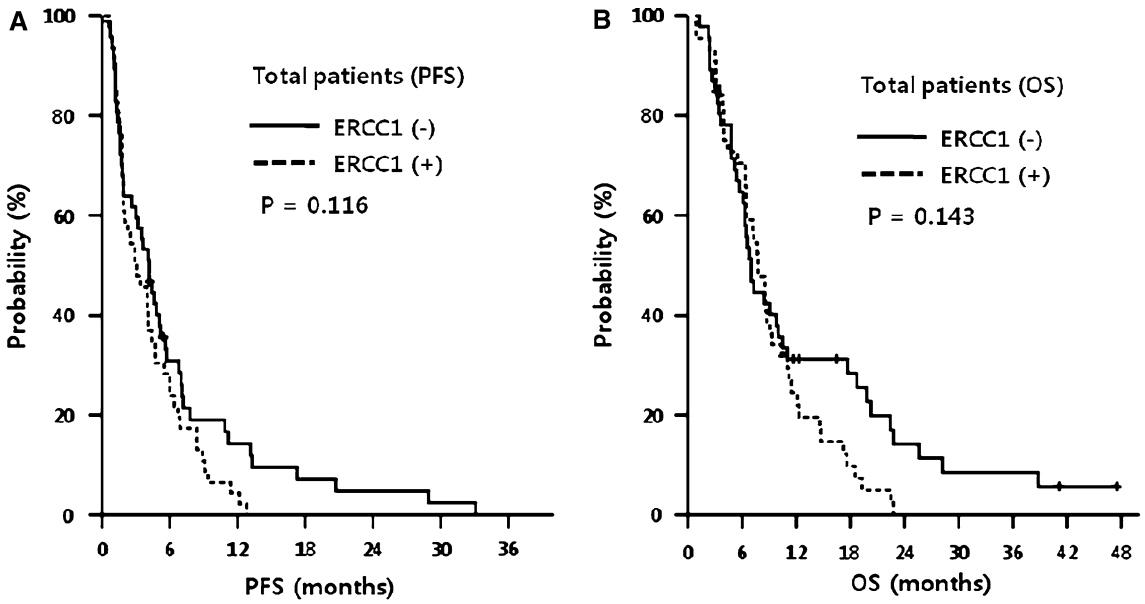

Fig. 2 Progression-free survival (**a**) and overall survival (**b**) curves for total study population according to ERCC1 expression

OS, 9.1 vs. 7.3 months, $p = 0.017$; Table 4, Fig. 3A, B). On the other hand, in patients treated with oxaliplatin, median PFS and OS were not significantly different, respectively, between the ERCC1-negative group and the ERCC1-positive group, although there was a tendency toward longer median OS and PFS in the ERCC1-positive patients (PFS, 2.6 vs. 4.7 months, $p = 0.181$; OS, 6.1 vs. 11.0 months, $p = 0.222$; Table 4, Fig. 3C, D).

Table 4 PFS and OS according to administrated agents and ERCC1 status

	All patients ($N = 93$) No. (%)	ERCC1 (−) ($N = 47$) No. (%)	ERCC1 (+) ($N = 46$) No. (%)	P value†
PFS[a]				
All patients: PFS (mo)	3.6	4.2	2.9	0.116
Cisplatin: PFS (mo)	3.0	4.6	1.9	0.014
Oxaliplatin: PFS (mo)	4.1	2.6	4.7	0.181
OS[b]				
All patients: OS (mo)	7.3	7.0	7.8	0.143
Cisplatin: OS (mo)	7.8	9.1	7.3	0.017
Oxaliplatin: OS (mo)	7.2	6.1	11.0	0.222

† Log-rank analysis; * Median progression-free survival; ** Median overall survival

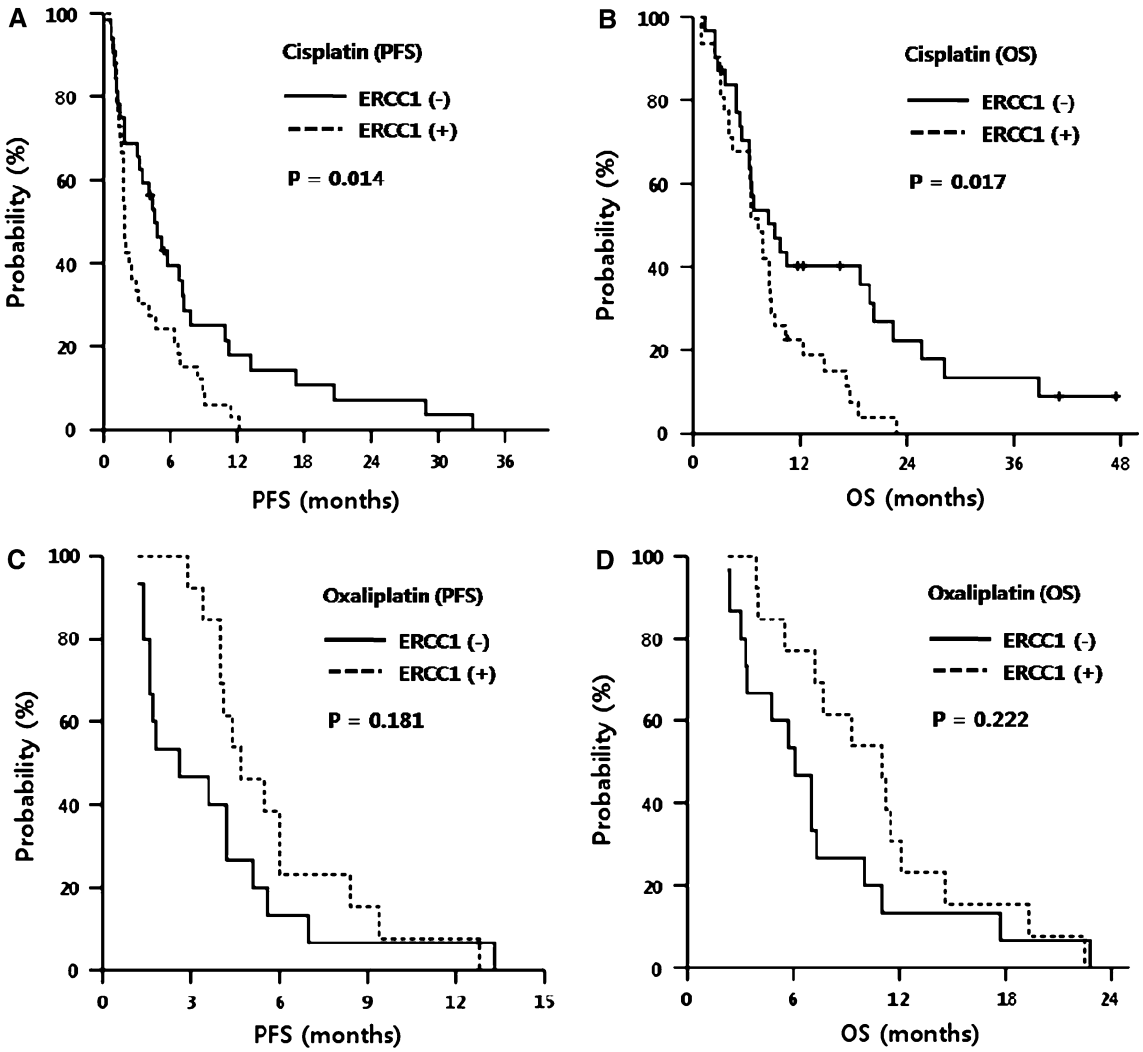

Fig. 3 Progression-free survival (**a**, **c**) and overall survival (**b**, **d**) curves for patients treated with cisplatin and oxaliplatin, respectively, according to ERCC1 expression

A Cox proportional hazard model showed that negative ERCC1 expression (hazard ratio, 0.429; 95% CI, 0.225–0.820; $P = 0.010$), existence of peritoneal metastasis (hazard ratio, 0.299; 95% CI, 0.125–0.718; $P = 0.007$), and achievement of clinical response after cisplatin-based chemotherapy (hazard ratio, 0.290; 95% CI, 0.133–0.630;

Table 5 Multivariate analysis for survival in patients treated with cisplatin-based chemotherapy

Factor		HR[b] for PFS (95% CI)	*P* value[†]	HR for OS (95% CI)	*P* value[†]
Metastatic status	Distant meta	0.250	0.002		
	Locally	(0.105–0.593)			
Peritoneum meta[b]	No	NI[c]		0.299	0.007
	Yes			(0.125–0.718)	
Clinical response	PR	0.306	0.001	0.290	0.002
	SD + PD	(0.161–0.581)		(0.133–0.630)	
ERCC1	Negative	NI[c]		0.429	0.010
	Positive			(0.225–0.820)	
Primary cancer site				NI[c]	

[†] forward stepwise (conditional LR) method of Cox proportional hazard regression model; [a] HR, hazard ratio[b] Peritoneum metastasis, [c] NI, not included in the equation, *PR* partial response; *SD* stable disease; *PD* progression disease, primary cancer site: gallbladder; intrahepatic duct; extrahepatic duct

$P = 0.002$) were independent prognostic factors for prolongation of survival (Table 5).

Discussion

In current study, we originally tried to evaluate whether ERCC1 protein expression level could predict the clinical outcome of palliative platinum-based chemotherapy in patients with biliary tract cancer as well as other tumors such as lung cancer. Instead, we found low level of ERCC1 is associated with improved clinical benefit and prolonged PFS and OS but not with response rates in patients treated with cisplatin-containing regimens. This finding is somewhat far from the reports in which a predictive role of ERCC1 is defined but as a prognostic marker it is in accordance with some of earlier reports that identified ERCC1 level for survival in lung cancers treated with chemotherapy [12, 16]. We also observed the status of ERCC1 has a different influence on patients' survival depending on whether administered regimen has cisplatin or oxaliplatin. In patients with oxaliplatin-containing regimens, high ERCC1 levels were correlated with improved clinical response and prolonged PFS and OS contrasting to the data of cisplatin-containing regimens. On this result, the issue of sample number would be raised, and therefore, we need to be careful in interpreting the data because of sample size imbalance between two groups in that sample number in oxaliplatin-containing group is not enough. Thus, it leaves room for further study with enough samples on this matter.

By far, the role of ERCC1 on the action of oxaliplatin is far less explored than on cisplatin, and the literatures regarding the effects of ERCC1 on the response of oxaliplatin are sometimes inconsistent with each other. In preclinical setting, in common with cisplatin-induced DNA lesion, ERCC1 would have a similar role on oxaliplatin-induced lesion because it is likely that these two platinum compounds share the same mechanisms of resistance [6, 9, 18]. In gastric and colon cancer patients treated with oxaliplatin-containing regimens, low level of ERCC1 expression was associated with a favorable overall survival suggesting a similar mechanism working on both platinum agents [11, 20, 24]. But in a study on colorectal cancer, ERCC1 did not have any significant interaction with 5-FU/oxaliplatin treatment [3]. Furthermore, our data show that positive expression of ERCC1 seems to confer a survival benefit to patients with oxaliplatin. Interestingly, a cisplatin-resistant gastric cancer cell line MKN45, in which ERCC1 was increased, showed susceptibility to oxaliplatin, raising the possibility of somewhat different reaction of ERCC1 between cisplatin and oxaliplatin [22]. In addition, other proteins, such as ERCC2 and XPF, should also be taken into account in the interpretation of our data because these proteins interact with ERCC1 and thus have a potential to modify the pathway of removing DNA adducts caused by platinum drugs, even though ERCC1 is a key enzyme of the nucleotide excision repair pathway.

In lung cancer with cisplatin-based chemotherapy, ERCC1 has positive predictive value in palliative setting with tumor burden but few or even negative prognostic role in adjuvant setting [16]. This result gives us a suggestion that ERCC1 may fall into double-bladed sword of predictive or prognostic marker for patients with cisplatin-based chemotherapy depending on the circumstances.

In the present study, we investigated the role of RRM1 in parallel with ERCC1 considering that RRM1, a key enzyme involved in DNA synthesis, is known to be associated with chemotherapy resistance especially for gemcitabine. We could not find any significant differences in responses and survivals depending on expression of RRM1 in 45 patients with gemcitabine/cisplatin or gemcitabine/oxaliplatin contrasting to the results from NSCLC treated

with gemcitabine/carboplatin or cisplatin in which RRM1 levels have predictive role in response and survival [19]. Based on these results, RRM1 level interpreted by immunohistochemistry seems to be no longer a messenger for the prediction of response and survival in patients with biliary tract cancer (data not shown here).

In this study, the attributable limitations of immunohistochemical staining should not be left out from our data interpretation because of its semiquantitative nature, tissue-aging effects, the staining technique and the enzyme antibody used, and interobserver variation. Among them, the specificity of the antibody used is an important one we should concern.

Recently, ERCC1 protein expression levels using immunohistochemical method are the subject of controversy [2, 15, 17]. Bhagwat and colleagues raised doubts about the specificity of the monoclonal mouse antibody-8F1, which is the most commonly used monoclonal antibody for the detection of ERCC1 [2, 15]. They suggested that antibody 8F1 is not specific antibody to detect ERCC1 in certain cells.

Because an additional spurious band, which observed in immunoblotting, is of equal intensity in normal and ERCC1–XPF-deficient cells and the equal staining of the nuclei of normal and ERCC1 deficient cells shows its inability to distinguish between ERCC1 positive and ERCC1-negative specimens by immunohistochemistry [2, 15]. Nonetheless, Olaussen et al. did not observe any additional band in the carcinoma cells, and the 8F1 signal was correlated with ERCC1 mRNA expression in the cell. Also, they suggested that the ERCC1 downregulation study clearly led to a decrease in ERCC1 signal. Based on their study, they suggested that the 8F1 antibody is an acceptable tool to determine nuclear ERCC1 protein expression in formalin-fixed paraffin-embedded human tumors tissue [17]. In this study, we used the same scoring system by Olaussen et al., and we had an apparently distinct difference in the results. So the authors consider our system worked appropriately in the situation we dealt. Generally, it is necessary to pay attention to a possible problem of antibody specificity when this antibody is used.

The role of ERCC1 in biliary tract cancer is far from being firmly established yet. Prospectively controlled study on ERCC1 is positively needed to elucidate its role in biliary tract cancer, and thus, ERCC1 could be translated into chemotherapy customizing.

In this study, our data demonstrate intratumoral expression of ERCC1 protein determined by immunohistochemistry has a significant prognostic role in advanced biliary tract cancer patients treated with cisplatin. This role confines to cisplatin-treated patients but not to oxaliplatin-treated ones. A predictive value of ERCC1 expression on clinical benefits determined as DCR also confined to cisplatin-treated patients rather than oxaliplatin-treated ones. These results suggest that the action mechanism of ERCC1 on cisplatin may be different from that on oxaliplatin.

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