Pathology & Oncology Research (2020) 26:1105–1116
https://doi.org/10.1007/s12253-019-00666-5

ORIGINAL ARTICLE

Predictive Value of ERCC1, ERCC2, and XRCC Expression for Patients with Locally Advanced or Metastatic Gastric Cancer Treated with Neoadjuvant mFOLFOX-4 Chemotherapy

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Received: 13 January 2019 / Accepted: 12 April 2019 / Published online: 10 May 2019

Abstract

The dismal outcome in patients with locally advanced or metastatic gastric cancer (GC) highlights the need for effective systemic neoadjuvant chemotherapy to improve clinical results. This study evaluated the correlation between the expression of three DNA repair genes, namely the excision repair cross-complementing group 1 (ERCC1), excision repair cross-complementing group 2 (ERCC2), and X-ray repair cross-complementing protein 1 (XRCC1) and the clinical outcome of patients with locally advanced or metastatic GC treated with mFOLFOX-4 neoadjuvant chemotherapy. Fifty-eight patients with histologically confirmed locally advanced or metastatic GC following neoadjuvant mFOLFOX-4 chemotherapy were enrolled between January 2009 and January 2018. We analyzed clinicopathological features and ERCC1, ERCC2, and XRCC1 expression to identify potential predictors of clinical response. Among the 58 patients, 16 (27.6%) were categorized into the response group (partial response) and 42 into the nonresponse group (stable disease in 24 patients and progressive disease in 18 patients). A multivariate analysis showed that ERCC1 overexpression ($P = 0.003$), ERCC2 overexpression ($P = 0.049$), and either ERCC1 or ERCC2 overexpression ($P = 0.002$) were independent predictors of response following mFOLFOX-4 neoadjuvant chemotherapy. Additionally, ERCC1 and ERCC2 overexpression did not only predict the response but also progression-free survival (both $P < 0.05$) and overall survival (both $P < 0.05$). ERCC1 and ERCC2 overexpression are promising predictive biomarkers for patients with locally advanced or metastatic GC receiving neoadjuvant mFOLFOX-4 chemotherapy and the potential clinical implication is mandatory for further investigation.

Keywords locally advanced gastric cancer · metastatic gastric cancer · mFOLFOX-4, neoadjuvant · ERCC1 · ERCC2

Abstract was presented as a poster in 2017 NCRI Cancer Conference and published in Abstract in European Journal of Surgical Oncology 2017; 43: 2216.

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Introduction

Gastric cancer is one of the most common forms of cancer worldwide and remains a major public health issue. Despite earlier detection of gastric cancer and considerable advancement in treatments that provide an improved opportunity for survival, its mortality and morbidity rate remains high, with local advanced or distant metastases occurring in up to 60% of patients [1]. In patients with unresectable locally advanced or metastatic gastric cancer, the median survival time without any chemotherapy is approximately 3–4 months. Systemic chemotherapy is widely accepted as a palliative treatment for patients with unresectable locally advanced or metastatic gastric cancer, leading to objective responses and improvement in quality of life and survival time [1, 2]. Neoadjuvant platinum-based chemotherapy followed by surgery is the standard of care for patients with unresectable locally advanced or metastatic gastric cancer [3]. However, the first-line chemotherapy option that is the most suitable to an individual patient remains unclear, and the decision to treat with any chemotherapy type is subjective and remains largely an empirical decision. Preoperative chemotherapy has some theoretical benefits in comparison with postoperative chemotherapy in such patients, including down-staging that increases the possibility of subsequent R0 resection, treating micrometastatic disease early in the course of therapy, evaluating susceptibility to chemotherapy, and generally better tolerability of more intensive chemotherapy [4]. Hence, remarkable efforts are ongoing to establish a more precise treatment protocol that can enable us to appropriately select patients and provide a specific therapy for individual patients based on their specific tumor profile [5].

Since first being reported in the late 1990s, the FOLFOX regimen, which includes bolus/infusional 5-fluorouracil (5-FU), folinic acid modulation, and platinum-based oxaliplatin, has become the main treatment for patients with gastroesophageal or colorectal adenocarcinoma; in particular, this treatment has exhibited high efficacy and less toxicity than other regimens [6]. Platinum-based oxaliplatin regimens are also active and well-tolerated in patients with advanced or metastatic gastric cancer [1]. However, the prognosis remains largely unknown, and a biological parameter that can be used to evaluate whether a neoadjuvant chemotherapy should be administered in patients susceptible to the response is not available. Therefore, genetic biomarkers that can predict the response in patients with locally advanced or metastatic gastric cancer, who would greatly benefit from neoadjuvant chemotherapy, should be identified [6].

For maintaining genome stability and integrity, DNA repair systems (nucleotide excision repair (NER), base excision repair (BER), mismatch repair, and double-strand break repair) play a key role [7, 8]. In contrast to cisplatin, oxaliplatin-induced adducts are apparently not recognized or processed by mismatch repair but are predominantly repaired by the NER pathway. Resistance to oxaliplatin has been attributed to enhanced tolerance and repair of DNA damage through the NER pathway, which includes the excision repair cross-complementing group 1 (*ERCC1*) and the excision repair cross-complementing group 2 (*ERCC2*). In addition, the X-ray cross-complementing group 1 (*XRCC1*) is one of the rate-limiting members of BER.[6,8] Therefore, alteration of NER capacity may affect the clinical outcome of patients with locally advanced or metastatic gastric cancer treated with neoadjuvant modified FOLFOX-4 (mFOLFOX-4) chemotherapy [9].

This study investigated the role of the expression of these three DNA repair genes (*ERCC1*, *ERCC2,* and *XRCC1*) through protein levels. Specifically, the protein expression levels of these three genes were analyzed through immunohistochemical (IHC) staining, we determined whether ERCC1, ERCC2, and XRCC1 can serve as potential predictive biomarkers of response to neoadjuvant mFOLFOX-4 chemotherapy in patients with locally advanced or metastatic gastric cancer.

Materials and Methods

Patient Selection

We enrolled 58 patients with histologically confirmed locally advanced T4 or metastatic gastric cancer from January 2009 to January 2018. All patients underwent pretreatment evaluation procedures, including a complete history review and physical examination, gastroesophagoscopy, laboratory data analyses, and image studies (i.e., chest radiography, abdominal computed tomography [CT], and an additional magnetic resonance imaging [MRI] if the CT scan could not clarify the cancer stage). The TNM classification was defined according to the criteria of the American Joint Commission on Cancer/Union for International Cancer Control (AJCC/UICC) [10]. The study protocol was approved by the hospital's institutional review board (KMUHIRB-20130022) and was not supported by any commercial company.

Eligibility Criteria

Patients with histologically confirmed locally advanced or metastatic gastric cancer were considered eligible for this study. Other eligibility criteria included an Eastern Cooperative Oncology Group performance status of 0–2; sufficient hepatic, renal, and bone marrow functions; the absence of central nervous system metastases, uncontrolled or serious concurrent medical illnesses, active infections, and other primary malignancies; age > 18 years; and life expectancy > 3 months. Patients with a history of other malignant diseases or who were unable to receive neoadjuvant chemotherapy were excluded.

Clinicopathological Features

Clinicopathological features analyzed in this study included patients' age; sex; tumor size; tumor invasion depth; lymph node metastasis; clinical TNM status; vascular invasion; perineural invasion; tumor location; histological tumor differentiation grade; pretreatment metastasis site; pretreatment carcinoembryonic antigen (CEA) level; and ERCC1, ERCC2, and XRCC1 expression levels. Neoadjuvant mFOLFOX-4 chemotherapy was administered to patients with locally advanced or metastatic gastric cancer.

Treatment Schedules

On day 1, oxaliplatin (85 mg/m^2) and leucovorin (200 mg/m^2) were administered through intravenous infusion of 5-FU over a 2-h period, followed by a 48-h continuous infusion of 5-FU at a dose of 2000 mg/m^2 every 2 weeks. The primary endpoint of this study was the response rate, progression-free survival (PFS), and overall survival (OS), and the secondary endpoint was toxicity.

Toxicity

Safety and toxicity were evaluated in each cycle by using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm, 2018 access). Peripheral neuropathy was graded according to the following oxaliplatin-specific scale: grade 1, paresthesia or dysesthesia of a short duration with complete recovery before the next cycle; grade 2, paresthesia persisting between two cycles without functional impairment; and grade 3, permanent paresthesia interfering with functioning [1]. Neoadjuvant chemotherapy was discontinued in cases of unacceptable toxicity (>grade 3), disease progression, or patient refusal to continue treatment.

Evaluation and Assessment of Efficacy

Before and after every 2-week treatment course, a physical examination, hepatic and renal function tests, complete blood cell count and serum CEA level examinations, and electrocardiography were performed. Abdominal CT and additional MRI (if required) was performed every 3 months during chemotherapy, and chest radiography was performed once a year. Bone scanning or positron emission tomography was performed selectively for those images showing suspicious findings on the CT or MRI, or where specific sites of metastases were suspected. All enrolled patients were followed up at 3-month intervals until their last visit or death.

Patient responses were classified according to the Response Evaluation Criteria in Solid Tumors [1]. A complete response (CR) was defined as the disappearance of all target cancer lesions in response to treatment. A partial response (PR) was defined as an at least 30% decrease in the sum of the longest diameter of metastatic lesions, with no evidence of new lesions. A progressive disease (PD) was defined as an at least 20% increase in the sum of the longest diameter of target lesions, with the smallest sum of the longest diameter recorded before a patient started to receive treatment utilized as a reference; PD was also defined as the identification of one or more new lesions. A stable disease (SD) was defined as neither having sufficient shrinkage to qualify for a PR nor having an adequate increase to qualify for a PD. Finally, PFS was determined by measuring the time interval from the initiation of mFOLFOX-4 chemotherapy until the first documentation of progression regardless of a patient's treatment status, and OS was determined by measuring the time interval from the initiation of mFOLFOX-4 chemotherapy to the date of death or last contact.

Immunohistochemistry (IHC) Staining

Immunohistochemistry was performed using the standard streptavidin–biotin–peroxidase procedure on the formalin-fixed and paraffin-embedded (FFPE) tissue blocks of each patient's sample [11]. Furthermore, 4-μm-thick sections were serially cut from the FFPE tissue blocks of each sample. The slides were deparaffinized with two changes of xylene, rehydrated with graded alcohols, and then washed in tap water. Antigen retrieval was performed using a target retrieval solution with a pH of 9.0 (DAKO, Glostrup, Denmark). Endogenous peroxidase in the section was blocked by incubation with 3% hydrogen peroxide for 5 min. Before immunostaining, antigen retrieval was performed by immersing sections in a citrate buffer (pH 9.0). The sections were then incubated for 15 min at room temperature with antibodies to ERCC1 (1:25 dilution; clone 8 F1; Abcam, Beijing, China), ERCC2 (1:250 dilution; clone FE11; Calbiochem, Shanghai, China), and XRCC1 (1:25 dilution; clone G168-728; Pharmingen, Shanghai, China). Subsequently, the Dako Real EnVision Detection System-HRP (DAKO, Glostrup, Denmark) was applied for 30 min. Finally, sections were incubated in 3′, 3-diaminobenzidine for 5 min, followed by Mayer's hematoxylin counterstaining. Dehydration was performed through two changes of 95% ethanol and two changes of 100% ethanol, and the samples were cleared in three changes of xylene and then mounted. Negative controls were obtained by replacing the primary antibody with nonimmune serum.

Scoring of Immune Staining

The immunostaining of ERCC1, ERCC2, and XRCC1 was scored by an experienced pathologist and oncologist together, who were blinded to the clinicopathological characteristics of patients. A consensus score was agreed for each score by the investigators. The scoring of gene expressions was based on the intensity of IHC staining and the percentage of positive cancerous cells. Nuclear ERCC1 and XRCC1

immunostaining and cytoplasmic ERCC2 immunostaining were considered positive. A score of 0, 1, and 2 represents a complete lack of staining, positive staining in less than 50% of cells, and positive staining in more than 50% of cells, respectively. The overexpression of ERCC1, XRCC1, and ERCC2 is defined as a score of 2, whereas non-overexpression is defined as a score of 0 or 1 (Fig. 1).

Ethics Approval and Consent to Participate

The present study was approved by the Institutional Review Board of the Kaohsiung Medical University Hospital (KMUHIRB-20130022). Patients' clinical outcomes and survival statuses were regularly followed up.

Statistical Analysis

Continuous variables are presented as the mean ± SD, and dichotomous variables are presented as numbers and percentage values. All data were analyzed using SPSS (version 19.0, SPSS Inc., Chicago, IL, USA). The chi-square test was used to compare toxicity and response between the two groups (overexpression vs. nonoverexpression), and multivariate logistic regression models were used to evaluate independent predictors. Finally, PFS and OS were calculated and plotted according to Kaplan–Meier methods and compared using the log-rank test. A P value of less than 0.05 was considered statistically significant.

Results

Patient Demographics

From January 2009 to January 2018, 58 patients with locally advanced or metastatic gastric cancer who received mFOLFOX-4 as the first-line neoadjuvant chemotherapy were enrolled. The characteristics of these patients are listed in Table 1. All 58 patients received at least six chemotherapy

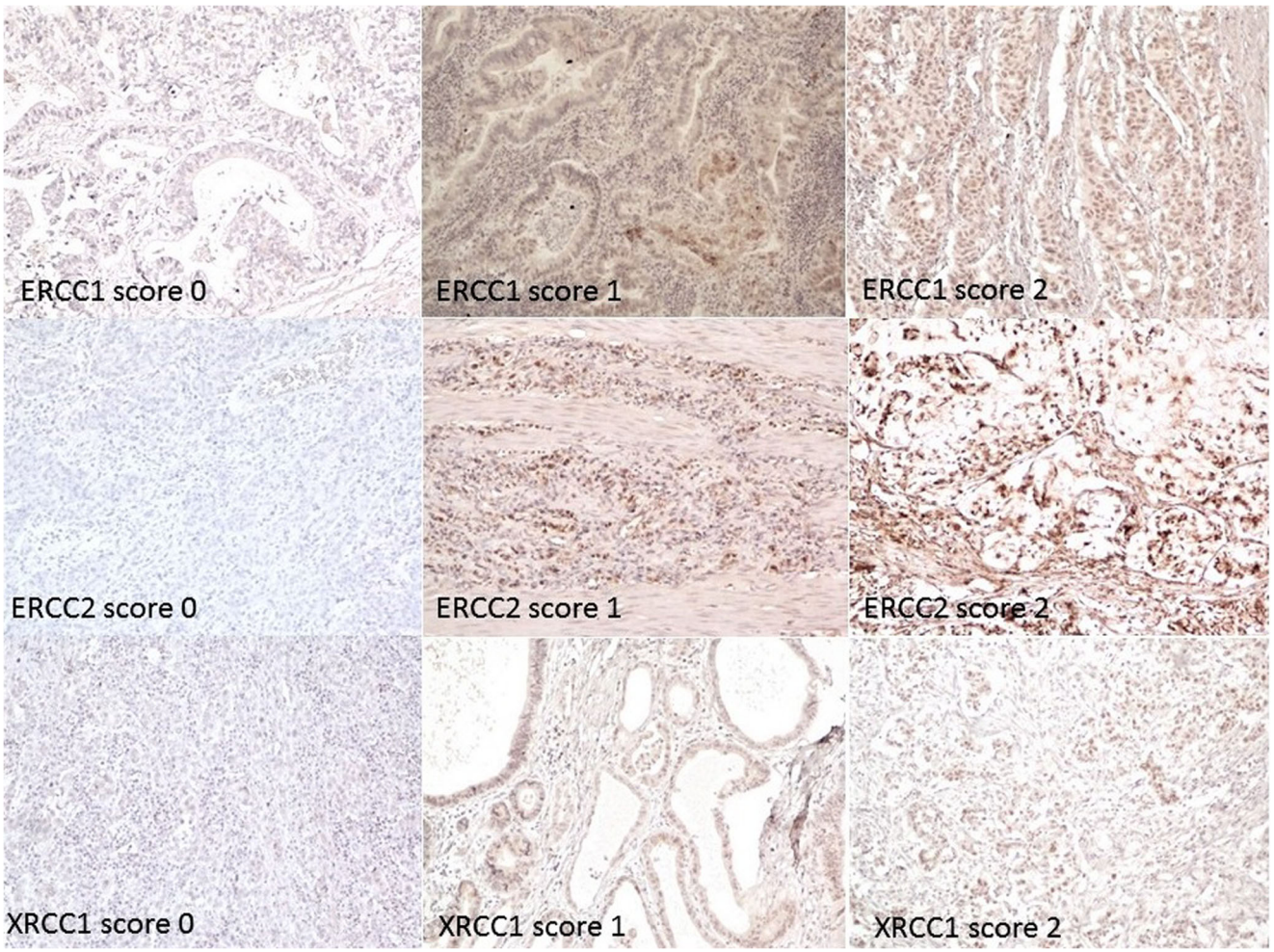


Fig. 1 Immunohistochemical staining of ERCC1, ERCC2, and XRCC1 in advanced or metastatic gastric cancer tissue. ERCC1 and XRCC1 proteins were stained in the nucleus, and ERCC2 protein was stained in the cytoplasm of tumor cells (brown color). Overexpression of ERCC1, ERCC2, and XRCC1 is defined as a score of 2 (positive staining in more than 50% cells), whereas nonoverexpression is defined as a score of 0 (no staining) or 1 (positive staining in less than 50% cells). Original magnification, 100×

Table 1 Baseline characteristics of 58 locally advanced/metastatic gastric cancer patients undergoing neoadjuvant mFOLFOX4 chemotherapy

	N=58(%)
Age, years	
Mean (range)	66 (31-85)
≧65 years	30 (51.7)
< 65 years	28 (48.3)
Gender	
Male	38 (65.5)
Female	20 (34.5)
Tumor size	
<5cm	35 (60.3)
≧5cm	23 (39.7)
Clinical T status	
T4	41 (70.7)
T3	17 (29.3)
Clinical N status	
N1	9 (15.5)
N2+N3	49 (84.5)
Vascular invasion	
Positive	10 (17.2)
Negative	13 (22.4)
ND	35 (60.4)
Perineural invasion	
Positive	11 (19.0)
Negative	12 (120.7)
ND	35 (60.4)
Clinical TNM stage	
Stage III	36(62.1)
Stage IV	22(27.9)
Tumor location	
Cardiac	18 (31.0)
Body	16 (27.6)
Antrum	27 (46.6)
Diffuse (Borrmann IV)	17 (29.3)
Stump	3(5.2)
Histology	
Well-Differentiated	0
Moderately Differentiated	6 (10.3)
Poorly Differentiated	17 (29.3)
ND	35 (60.3)
Metastasis site	
Peritoneum carcinomatosis	7 (12.1)
Liver	5 (8.6)
Lung	5 (8.6)
Locoregional lymph node	4 (6.9)
Bone	2 (3.4)
Ovary	3 (5.2)
Bladder	1 (1.7)
Pre-C/T CEA (ng/ml)	
≧5	14 (24.1)
< 5	44 (75.9)
TNM down-staging	
Yes	16 (27.6)
No	42 (72.4)
T down-staging	
Yes	20 (34.5)
No	38 (65.5)
N down-staging	
Yes	19 (32.8)
No	39 (67.2)
R0/R1 resection	
Yes	23 (39.7)
No	35 (60.3)
ERCC1 overexpression	
Yes	26 (44.8)
No	32 (55.2)
ERCC2 overexpression	
Yes	41 (70.7)
No	17 (29.3)
XRCC1 overexpression	
Yes	32 (55.2)
No	26 (44.8)

ND, Not done due to unresectable tumor; *C/T*, Chemotherapy

cycles and were eligible to be analyzed for efficacy and toxicity. The patients included 38 men and 20 women, who had a mean age of 66.0 years (range, 31–85 years). Upon baseline gastroesophagoscopy and abdominal CT scanning, 41 patients (70.7%) were shown to have T4 tumors, 22 patients were shown to have distant metastasis (37.9%) and all 58 patients were shown to have N+ disease.

Following neoadjuvant chemotherapy, 23 (39.7%) of the 58 patients underwent resection with curative intent, and R0 resection was performed in all of them. According to the pathological reports of these 23 patients, positive vascular invasion and positive perineural invasion were found in 10 (17.2%) and 11 (19.0%) of them, respectively. Histologically, no tumor was well-differentiated; instead, 6 tumors (10.3%) were moderately differentiated, and 17 tumors (29.3%) were poorly differentiated.

The most common location of the primary tumor was the antrum (46.6%), followed by cardiac (31.0%), diffuse (29.3%), body (27.6%), and gastric stump (5.2%). Additionally, the peritoneum (12.1%), liver (8.6%), lungs (8.6%), and ovary (5.2%) were the most common sites of metastases. Among all 58 patients, 20 (34.5%) had T down-staging, 19 (32.8%) had N down-staging, and 16 (27.6%) had TNM down-staging.

Toxicity

The most frequent grade 3/4 hematological and non-hematological toxicities are shown in Table 2. The major grade 3/4 hematological toxicities included neutropenia, febrile neutropenia, and thrombocytopenia, which appeared in four (6.9%), three (5.2%), and one (1.7%) patients, respectively. Other non-hematological toxicities reaching grade 3/4 status were nausea/vomiting (15.5%), anorexia/fatigue (13.8%), abnormal liver function (8.6%), abnormal renal function (6.9%), peripheral neuropathy (6.9%), stomatitis (6.9%), diarrhea (6.9%), and constipation (5.2%). All treatment-related

Table 2 Grade 3/4 toxicities according to the National Cancer Institute Common Terminology Criteria for Adverse Events

	Total N=58 (%)
Hematologic	
Neutropenia	4 (6.9)
Febrile neutropenia	3 (5.2)
Thrombocytopenia	1 (1.7)
Non-hematologic	
Nausea/Vomiting	9 (15.5)
Anorexia/Fatigue	8 (13.8)
Abnormal liver function	5 (8.6)
Abnormal renal function	4 (6.9)
Peripheral neuropathy	4 (6.9)
Stomatitis	4 (6.9)
Diarrhea	4 (6.9)
Constipation	3 (5.2)

toxicities were treated with proper medical care and no treatment-related deaths occurred.

IHC Analyses and Clinicopathological Correlations

ERCC1 overexpression was observed in 26 patients (44.8%), ERCC2 overexpression in 41 patients (70.7%), and XRCC1 overexpression in 32 patients (55.2%). No significant association was observed between these three biomarkers and baseline clinicopathological features, namely age, sex, tumor size, clinical T status, clinical N status, vascular invasion, perineural invasion, clinical TNM stage, and the prechemotherapy CEA level (Table 3, all $P > 0.05$).

Efficacy

All 58 patients were evaluated for their tumor response. Major responses were observed in 16 patients (27.6%) with a PR; however, no patients could achieve a CR. Additionally, 24 patients (41.4%) showed a SD, and 18 patients (31.0%) were considered to have a PD.

Correlation between Response and Clinicopathological Features

On the basis of the univariate analysis of the correlation between the response group and clinicopathological features, we found that ERCC1 overexpression (P =0.003), ERCC2 overexpression (P = 0.049), and either ERCC1 or ERCC2 overexpression (P = 0.002) were significant predictive factors of clinical response (Table 4). However, no significant differences in age, sex, tumor size, clinical T status, clinical N status, vascular invasion, perineural invasion, clinical TNM stage, prechemotherapy serum CEA level, or XRCC1 overexpression were observed. Additionally, the multivariate logistic regression analysis indicated that the presence of ERCC1 overexpression (P = 0.001; odds ratio [OR], 0.107; 95% confidence interval [CI], 0.022-0.532), ERCC2 overexpression (P = 0.041; OR, 0.273; 95% CI, 0.080-0.930), and either ERCC1 or ERCC2 overexpression (P = 0.001; OR, 0.105; 95% CI, 0.025-0.436) were independent predictive factors for response following mFOLFOX-4 neoadjuvant chemotherapy (Table 5).

Table 3 Correlation between ERCC1, ERCC2, XRCC protein overexpression and clinicopathologic features in 58 locally advanced/metastatic gastric cancer patients

		ERCC1 overexpression			ERCC2 overexpression			XRCC1 overexpression		
	Total n=58	Yes N (%)	No N (%)	*P*-value	Yes N (%)	No N (%)	*P*-value	Yes N (%)	No N (%)	*P*-value
Age, years				0.198			0.151			0.798
≧65 years	30 (51.7)	16 (61.5)	14 (43.8)		24 (58.5)	6 (35.3)		16 (50.0)	14 (53.8)	
< 65 years	28 (48.3)	10 (38.5)	18 (56.2)		17 (41.5)	11 (64.7)		16 (50.0)	12 (46.2)	
Gender				0.282			0.073			0.591
Male	38 (65.6)	15 (57.7)	23 (71.9)		30 (73.2)	8 (47.1)		22 (68.8)	16 (61.5)	
Female	20 (34.5)	11 (42.3)	9 (28.1)		11 (26.8)	9 (52.9)		10 (31.2)	10 (38.5)	
Tumor size				0.183			0.772			1.000
< 5cm	35 (60.3)	13 (50.0)	22 (68.8)		24 (58.5)	11 (64.7)		19 (59.4)	16 (61.5)	
≧5cm	23 (39.7)	13 (50.0)	10 (31.2)		17 (41.5)	6 (35.3)		13 (40.6)	10 (38.5)	
Clinical T status				1.000			0.342			0.156
T4	41 (70.7)	18 (69.2)	23 (71.9)		27 (65.9)	14 (82.4)		20 (62.5)	21 (80.8)	
T3	17 (29.3)	8 (30.8)	9 (28.1)		14 (34.1)	3 (17.6)		12 (37.5)	5 (19.2)	
Clinical N status				0.274			0.258			0.495
N1	9 (15.5)	6 (23.1)	3 (9.4)		8 (19.5)	1 (5.9)		6 (18.8)	3 (11.5)	
N2+N3	49 (84.5)	20 (76.9)	29 (90.6)		33 (80.5)	16 (94.1)		26 (81.2)	23(88.5)	
Vascular invasion				1.000			0.339			0.685
Positive	10 (43.5)	5 (41.7)	5 (45.5)		9 (50.0)	1 (20.0)		5 (38.5)	5 (50.0)	
Negative	13 (56.5)	7 (58.3)	6 (54.5)		9 (50.0)	4 (80.)		8 (61.5)	5 (50.0)	
Perineural invasion				0.684			1.000			0.214
Positive	11 (47.8)	5 (41.7)	6 (54.5)		9 (50.0)	2 (40.0)		8 (61.5)	3 (30.0)	
Negative	12 (52.2)	7 (58.3)	5 (45.5)		9 (50.0)	3 (60.0)		5 (38.5)	7 (70.0)	
Clinical TNM stage				0.416			1.000			0.594
Stage III	36 (62.1)	18 (69.2)	18 (56.2)		25 (61.0)	11 (64.7)		21 (65.6)	15 (57.7)	
Stage IV	22 (37.9)	8 (30.8)	14 (43.8)		16 (39.0)	6 (35.3)		11 (34.4)	11 (42.3)	
Pre-C/T CEA (ng/ml)				1.000			0.737			0.761
≧5	14 (24.1)	6 (23.1)	8 (25.0)		9 (22.0)	5 (29.4)		7 (21.9)	7 (26.9)	
< 5	44 (75.9)	20 (76.9)	24 (75.0)		32 (78.0)	12 (70.6)		25 (78.1)	19 (73.1)	

C/T, chemotherapy; *CEA*, carcinoembryonic antigen

Table 4 Correlation between ERCC1, ERCC2 and XRCC1 and response rate of locally advanced/metastatic gastric cancer patients undergoing neoadjuvant mFOLFOX-4 chemotherapy

	Total n=58	ERCC1 overexpression			ERCC2 overexpression			XRCC1 overexpression		
		Yes (%)	No %)	*P*-value	Yes (%)	No (%)	*P*-value	Yes (%)	No (%)	*P*-value
Response rate										
PR	16 (27.6)	2 (7.7)	14 (43.8)	0.003	8 (19.5)	8 (47.1)	0.049	6 (18.8)	10 (38.5)	0.140
SD + PD	42 (72.4)	24 (92.3)	18 (56.2)		33 (80.5)	9 (52.9)		26 (81.2)	16 (61.5)	

PR, partial response; *SD*, stable disease; *PD*, progressive disease

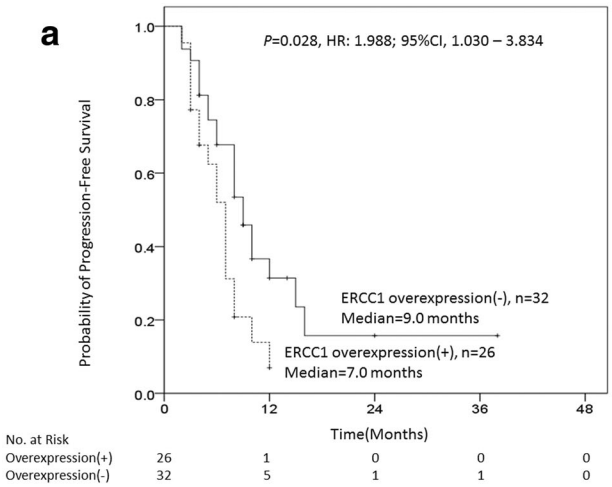
PFS and OS Based on ERCC1, ERCC2, or XRCC1 Overexpression

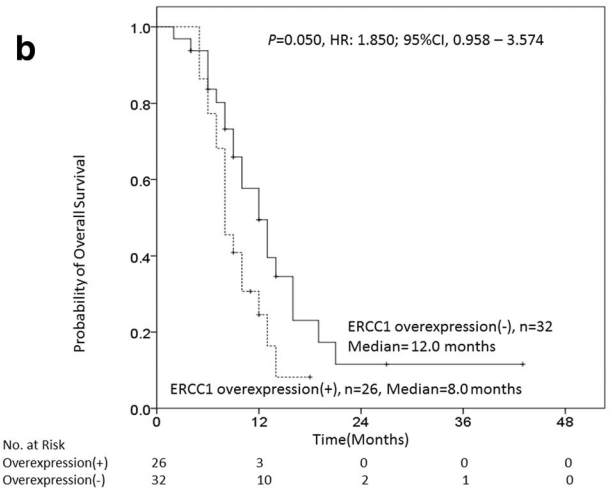
PFS and OS based on ERCC1, ERCC2, or XRCC1 overexpression were listed in Fig. 2. The median OS and PFS were 8.0 months (95% CI: 6.474–9.526) and 7.0 months (95% CI: 5.710–8.290), respectively, in patients with ERCC1 overexpression. Conversely, the median OS and PFS were 12.0 months (95% CI: 8.845–15.155) and 9.0 months (95% CI: 6.644–11.356), respectively, in patients without ERCC1 overexpression ($P = 0.049$ and $P = 0.028$ respectively). The 12-month OS rate in patients with and without ERCC1 overexpression was 19.2% and 43.8%, respectively, whereas the 12-month PFS rate in the patients with and without ERCC1 overexpression was 11.5% and 21.9%, respectively. Likewise, the median OS and PFS were 9.0 months (95% CI, 7.224–10.776) and 7.0 months (95% CI, 5.707–8.293), respectively, in patients with ERCC2 overexpression. Conversely, the median OS and PFS were 14.0 months (95% CI, 11.956–16.044) and 12.0 months (95% CI, 8.948–15.052), respectively, in patients without ERCC2 overexpression ($P = 0.020$ and $P = 0.007$, respectively). The 12-month OS rate in the patients with and without ERCC2 overexpression was 24.4% and 52.9%, respectively, whereas the 12-month PFS rate in the patients with and without ERCC2 overexpression was 7.3% and 41.2%, respectively. However, no significant differences were observed in either the OS or PFS between the patients with and without XRCC1 overexpression (Fig. 2 and Table 6).

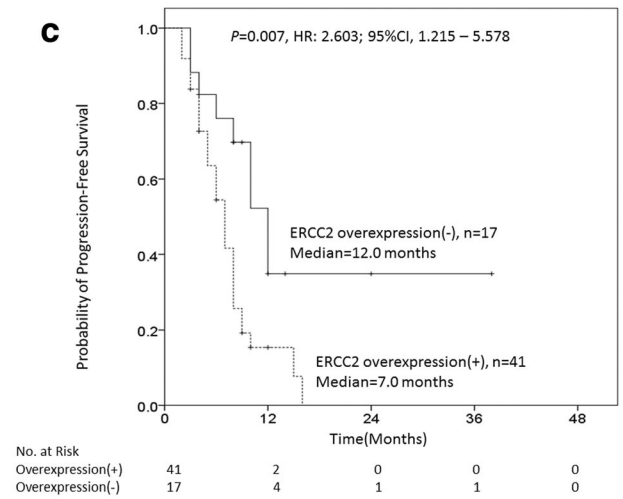
The median OS and PFS were 9.0 months (95% CI, 6.041–11.950) and 8.0 months (95% CI, 5.772–10.228), respectively, in patients with either ERCC1 or ERCC2 overexpression (Fig. 3); 8.0 months (95% CI, 6.655–9.345) and 7.0 months (95% CI, 6.170–7.811), respectively, in patients with both ERCC1 and ERCC2 overexpression; and 14.0 months (95% CI, 11.477–16.523) and 12.0 months (95% CI, 7.457–16.539), respectively, in patients without ERCC1 or ERCC2 overexpression ($P = 0.016$ and $P = 0.004$, respectively). Moreover, the 12-month OS rate in patients with either ERCC1 or ERCC2 overexpression, with both ERCC1 and ERCC2 overexpression, and without ERCC1 or ERCC2 overexpression was 36.0%, 14.3%, and 58.3%, respectively. The 12-month PFS rate in patients with either ERCC1 or ERCC2

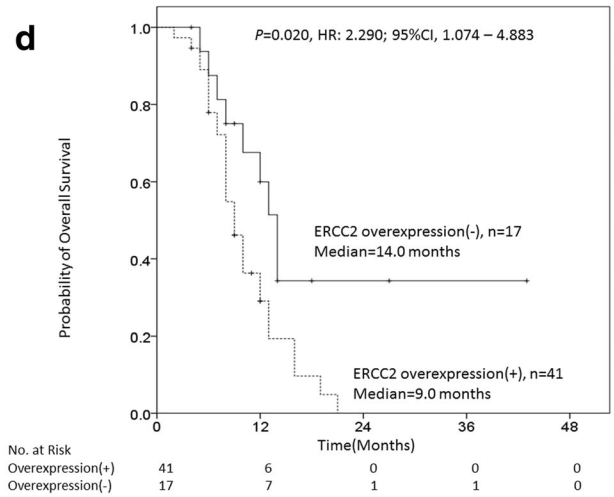
Table 5 Univariate and multivariate analysis of predictors of response status in 58 locally advanced/metastatic gastric cancer patients

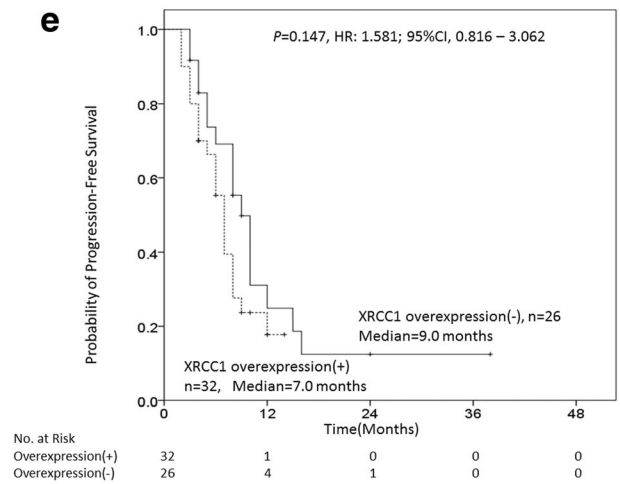
Variables	Response	Non-response	Univariate	Multivariate analysis	
	(n=16) (%)	(n=42) (%)	*p*-value	Odds ratio(95% CI)	*P*-value
Age, years (≧65/<65 years)	7 (43.8)/9 (56.2)	23 (54.8)/19 (45.2)	0.561	0.643 (0.201-2.049)	0.561
Gender (male/female)	11 (68.8)/5 (31.2)	27 (64.3)/15 (35.7)	1.000	1.222 (0.357-4.187)	1.000
Tumor size (<5/≧5 cm)	10 (62.5)/6 (37.5)	25 (59.5)/17 (40.5)	1.000	0.882 (0.270-2.886)	1.000
Clinical T status (T4/T3)	9 (56.2)/7 (43.8)	32 (76.2)/10 (23.8)	0.197	0.402 (0.119-1.356)	0.197
Clinical N status (N1/N2+N3)	4 (25.0)/12 (75.0)	5 (11.9)/37 (88.1)	0.243	0.405 (0.093-1.758)	0.243
Vascular invasion (positive/negative/miss)	4 (25.0)/5 (31.3)/7 (43.8)	6 (14.3)/8 (19.0)/28 (66.7)	1.000	1.067 (0.197-5.769)	1.000
Perineural invasion (positive/negative/miss)	4 (25.0)/5 (31.3)/7 (43.8)	7 (16.7)/7 (16.7)/28 (66.7)	1.000	0.800 (0.149-4.297)	1.000
Clinical TNM stage (III/IV)	9 (56.2)/7 (43.8)	27 (64.3)/15 (35.7)	0.763	1.400 (0.434-4.521)	0.763
Pre-C/T CEA (≧5/<5) (ng/ml)	4 (25.0)/12 (75.0)	10 (23.8)/32 (76.2)	1.000	1.067 (0.280-4.057)	1.000
ERCC1 overexpression (no/yes)	14 (87.5)/2 (12.5)	18 (42.9)/24 (57.1)	0.003	0.107 (0.022-0.532)	0.001
ERCC2 overexpression (no/yes))	8 (50.0)/8 (50.0)	9 (21.4)/33 (78.6)	0.049	0.273 (0.080-0.930)	0.041
XRCC1 overexpression (no/yes))	10 (62.5)/6 (37.5)	16 (38.1)/26 (61.9)	0.140	0.369 (0.113-1.212)	0.090
Any ERCC1 or ERCC2 overexpression (no/yes))	8 (50.0)/8 (50.0)	4 (9.5)/38 (90.5)	0.002	0.105 (0.025-0.436)	0.001

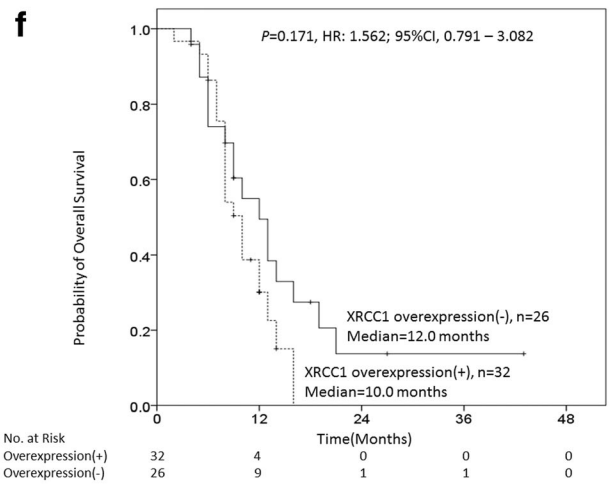

a
P=0.028, HR: 1.988; 95%CI, 1.030 – 3.834
Probability of Progression-Free Survival
ERCC1 overexpression(-), n=32
Median=9.0 months
ERCC1 overexpression(+), n=26
Median=7.0 months
Time(Months)
No. at Risk
Overexpression(+) 26 1 0 0 0
Overexpression(-) 32 5 1 1 0


b
P=0.050, HR: 1.850; 95%CI, 0.958 – 3.574
Probability of Overall Survival
ERCC1 overexpression(-), n=32
Median= 12.0 months
ERCC1 overexpression(+), n=26, Median=8.0 months
Time(Months)
No. at Risk
Overexpression(+) 26 3 0 0 0
Overexpression(-) 32 10 2 1 0


c
P=0.007, HR: 2.603; 95%CI, 1.215 – 5.578
Probability of Progression-Free Survival
ERCC2 overexpression(-), n=17
Median=12.0 months
ERCC2 overexpression(+), n=41
Median=7.0 months
Time(Months)
No. at Risk
Overexpression(+) 41 2 0 0 0
Overexpression(-) 17 4 1 1 0


d
P=0.020, HR: 2.290; 95%CI, 1.074 – 4.883
Probability of Overall Survival
ERCC2 overexpression(-), n=17
Median=14.0 months
ERCC2 overexpression(+), n=41
Median=9.0 months
Time(Months)
No. at Risk
Overexpression(+) 41 6 0 0 0
Overexpression(-) 17 7 1 1 0


e
P=0.147, HR: 1.581; 95%CI, 0.816 – 3.062
Probability of Progression-Free Survival
XRCC1 overexpression(-), n=26
Median=9.0 months
XRCC1 overexpression(+)
n=32, Median=7.0 months
Time(Months)
No. at Risk
Overexpression(+) 32 1 0 0 0
Overexpression(-) 26 4 1 0 0


f
P=0.171, HR: 1.562; 95%CI, 0.791 – 3.082
Probability of Overall Survival
XRCC1 overexpression(-), n=26
Median=12.0 months
XRCC1 overexpression(+), n=32
Median=10.0 months
Time(Months)
No. at Risk
Overexpression(+) 32 4 0 0 0
Overexpression(-) 26 9 1 1 0

◀ **Fig. 2** Cumulative survival rates of the 58 enrolled patients with advanced or metastatic gastric cancer (GC) undergoing curative resection and treated with neoadjuvant FOLFOX-4 chemotherapy, as assessed using the Kaplan–Meier method. The differences in survival rates were analyzed using the log-rank test. **(A)** The progression-free survival of GC patients without ERCC1 overexpression was significantly better than that of GC patients with ERCC1 overexpression ($P = 0.049$); **(B)** The overall survival of GC patients without ERCC1 overexpression was better than that of GC patients with ERCC1 overexpression ($P = 0.028$); **(C)** The progression -free survival of GC patients without ERCC2 overexpression was significantly better than that of GC patients with ERCC2 overexpression ($P = 0.020$); **(D)** The overall survival of GC patients without ERCC2 overexpression was better than that of GC patients with ERCC2 overexpression ($P = 0.007$); **(E)** The progression -free survival was not prominently different in GC patients with or without XRCC1 overexpression ($P = 0.171$); **(F)** The overall survival was not prominently different in GC patients with or without XRCC1 overexpression ($P = 0.147$)

overexpression, with both ERCC1 and ERCC2 overexpression, and without ERCC1 or ERCC2 overexpression was 16.0%, 54.8%, and 41.7% respectively. Notably, both the OS and PFS in patients with ERCC1 overexpression, ERCC2 overexpression, and either ERCC1 or ERCC2 overexpression were shorter than those in patients without ERCC1 or ERCC2 overexpression (all $P < 0.05$).

Discussion

Gastric cancer remains the leading malignancy worldwide, and the management of patients with locally advanced or metastatic gastric cancer has not substantially altered in the last few decades. In our previous study, we observed that the survival and quality of life of patients with locally advanced or metastatic gastric cancer who received neoadjuvant chemotherapy was superior to that of patients who received only the best supportive care [1]. Given that the high rates of poor survival are associated with locally advanced or recurrent gastric cancer, molecular biomarkers that can guide neoadjuvant treatment are required [12].

Oxaliplatin remains the backbone of treatment in gastric cancer. It is a third-generation diaminocyclohexane platinum-based compound that inhibits replication and transcription by the formation of DNA adducts with guanines that are converted into diadducts over time. Oxaliplatin, 5-FU, and leucovorin combination chemotherapy (mFOLFOX-4 regimen) has been accepted in patients with gastric cancer and was demonstrated to be an effective and tolerable first-line treatment regimen for patients with advanced or metastatic gastric cancer [12, 13].

DNA repair is required to prevent the propagation of errors and maintain genomic stability. The repair of DNA damage involves several molecular pathways, including NER, BER, homologous recombination, and nonhomologous end-joining [14–16]. Among these pathways, NER is responsible for the removal of a DNA segment with its associated bulky adduct, followed by the restoration of that DNA segment. Therefore, the alteration of NER capacity may play a crucial role in the individualized treatment outcome of patients with gastric cancer receiving platinum-based chemotherapy. Compared with cisplatin, oxaliplatin-induced adducts are clearly not recognized or processed by mismatch repair, but are mainly repaired by the NER pathway [8, 14–16]. By focusing on key DNA repair and damage signaling factors, we investigated potential biomarkers that may have the ability to predict the prognosis of patients with locally advanced or metastatic gastric cancer who receive neoadjuvant mFOLFOX-4 chemotherapy. For platinum-based chemotherapy, the anti-tumor activity is largely determined by the DNA repair capacity of cancer cells.

ERCC1 or ERCC2 overexpression has been shown to predict response to chemotherapy in gastroesophageal tumors [17, 18]. The ability of ERCC1 levels to predict response

Table 6 Median time of overall survival and progression-free survival in 58 locally advanced/metastatic gastric cancer patients according to ERCC1, ERCC2, XRCC1 overexpression status

	Patients	Events in OS	Median PFS [95% CI], months	*P*-value	Patients	Events in PFS	Median OS [95% CI], months	*P*-value
ERCC1								
Overexpression	26	18	7.0 [5.710-8.290]	0.028	26	18	8.0 [6.474-9.526]	0.049
Non-overexpression	32	21	9.0 [6.644-11.356]		32	21	12.0 [8.845-15.155]	
ERCC2								
Overexpression	41	30	7.0 [5.707-8.293]	0.007	41	30	9.0 [7.224-10.776]	0.020
Non-overexpression	17	9	12.0 [8.948-15.052]		17	9	14.0 [11.956-16.044]	
XRCC1								
Overexpression	32	22	7.0 [5.845-8.155]	0.147	32	22	10.0 [8.508-11.492]	0.171
Non-overexpression	26	17	9.0 [7.245-10.755]		26	17	12.0 [8.083-15.917]	
ERCC1 and ERCC2								
Non-overexpression	12	5	12.0 [7.457-16.539]	0.004	12	5	14.0 [11.477-16.523]	0.016
Any1 overexpression	25	20	8.0 [5.772-10.228]		25	20	9.0 [6.041-11.950]	
Both 2 overexpression	21	14	7.0 [6.170-7.811]		21	14	8.0 [6.655-9.345]	

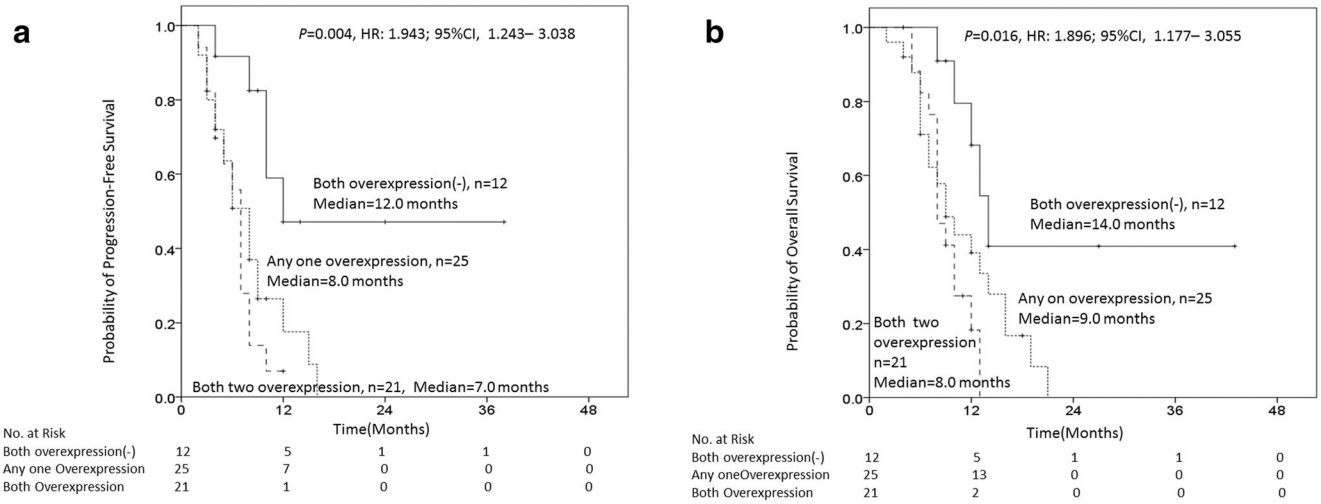


Fig. 3 Cumulative survival rates of the 58 enrolled patients with advanced or metastatic gastric cancer (GC) undergoing curative resection and treated with neoadjuvant FOLFOX-4 chemotherapy, as assessed using the Kaplan–Meier method. The differences in survival rates were analyzed using the log-rank test. **(A)** The disease-free survival of GC patients without ERCC1 or ERCC2 overexpression was significantly better than that of GC patients with ERCC1 or ERCC2 overexpression, and overexpression of both ERCC1 and ERCC2 (P = 0.016); **(B)** The overall survival of GC patients without ERCC1 or ERCC2 overexpression was significantly better than that of GC patients with overexpression of ERCC1 or ERCC2, and overexpression of both ERCC1 and ERCC2 (P = 0.004)

has also been reported in colorectal [6, 8, 19, 20], lung [21], ovarian [22], and bladder [23] cancers. A recent study used immunohistochemistry to examine ERCC1 expression in patients with advanced gastric cancer treated with 5-FU/oxaliplatin chemotherapy, and reported that patients without ERCC1 overexpression were more likely to respond to chemotherapy and have a significantly longer median OS [24].

It is generally assumed that low resectability is responsible for the poor prognosis of unresectable locally advanced or metastatic gastric cancer patients. A number of clinical trials have shown that preoperative neoagjuvant chemotherapy is feasible and able to increase the rate of R0 resection [4, 25].

In literature of previous neoadjuvant chemotherapy trials, the response rate showed modest to moderate activity (40–60 % response rate) [25–27]. Accordingly, there is a need to improve the response rate to achieve a further increase in R0 resection rates with treatment for advanced gastric cancer. We have currently shown a similar correlation in patients with locally advanced or metastatic gastric cancer; ERCC1 and ERCC2 overexpression was associated with poor survival and a trend toward platinum resistance. In our study, 16 (27.6%) of the 58 patients were categorized into the response group (PR) and the remaining 42 patients into the nonresponse group (SD in 24 patients and PD in 18 patients). We showed that the overexpression of ERCC1 and ERCC2 is significantly associated with response in patients with locally advanced or metastatic gastric cancer treated with neoadjuvant mFOLFOX-4 chemotherapy. At the same time, patients with locally advanced or metastatic gastric cancer without ERCC1 or ERCC2 overexpression tended to have longer PFS and OS than did patients with ERCC1 or ERCC2 overexpression. Therefore, patients with ERCC1 overexpression may have a higher DNA repair capacity that can effectively reduce the anticancer effect of oxaliplatin, leading to the poor prognosis of these patients. This implies that ERCC1 and ERCC2 expression is a promising predictive marker for and can be adopted as the precision medicine.

In conclusion, we demonstrated that ERCC1 and ERCC2 overexpression are promising predictive markers in patients with locally advanced or metastatic gastric cancer receiving oxaliplatin-based neoadjuvant mFOLFOX-4 chemotherapy; the PFS and OS were also found to be markedly poorer in patients showing ERCC1 or ERCC2 overexpression. These two biomarkers can potentially help clinicians to identify patients who would benefit from these therapeutic strategies; however, they cannot provide strong evidence for our limited sample size. Thus, future prospective studies that recruit multiethnic groups and enroll more patients, that also use the ERCC1/ERCC2 gene expression as predictive markers for DNA repair activity, are still needed to verify our findings.

Acknowledgements This work was supported by grants through funding from the Ministry of Science and Technology (MOST108-2321-B-037-001, MOST107-2321-B-037-003, MOST107-2314-B-037-116, MOST107-2314-B-037-022-MY2, MOST107-2314-B-037-023-MY2) and the Ministry of Health and Welfare (MOHW107-TDU-B-212-123006, MOHW107-TDU-B-212-114026B, MOHW108-TDU-B-212-

133006) funded by Health and welfare surcharge of tobacco products), and the Kaohsiung Medical University Hospital (KMUH107-7R28, KMUH107-7R29, KMUH107-7R30, KMUH107-7M22, KMUH107-7M23, KMUHS10701, KMUHS10706, KMUHS10710). In addition, this study was supported by the Grant of Biosignature in Colorectal Cancers, Academia Sinica, Taiwan, R.O.C.

Compliance with ethical standards

Conflicts of Interest The authors declare that they have no conflicts of interests.

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