



Correlation of ERCC1 expression in peripheral blood lymphocytes with outcomes of patients with gastric cancer treated with oxaliplatin-based adjuvant chemotherapy

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ABSTRACT. Excision repair cross-complementing gene-1 (ERCC1) is a key regulatory enzyme whose expression patterns in tumor tissues are associated with survival in gastric cancer. The present study aimed to evaluate the effects of ERCC1 expression in peripheral blood lymphocytes (PBLs) on the outcome of patients with gastric cancer treated with oxaliplatin-based adjuvant chemotherapy. Tumor and PBL samples from 48 patients treated with adjuvant oxaliplatin-based chemotherapy for gastric cancer were analyzed. Immunohistochemistry was used to assess the expression of ERCC1. After a median follow-up of 18.5 months, the median disease-free survival (DFS) and overall survival (OS) were 12 and 20 months, respectively. Expression of ERCC1 was found in 72.9% (35/48), 56.3% (27/48), and 10.0% (2/20) of tumor tissues, PBLs from gastric cancer patients, and PBLs from controls, respectively. A significant

positive correlation between ERCC1 expression in PBL and cancer tissue was found ($\chi^2 = 12.098$, $P = 0.001$, Pearson contingency coefficient = 0.502). Patients with negative expression of ERCC1 in tumor tissues had a significantly longer median DFS and median OS compared to patients with positive expression of ERCC1 (median DFS, 18 vs 10 months, $P = 0.006$; median OS, 30 vs 17 months, $P = 0.012$). In PBLs, high expression of ERCC1 was associated with decreased DFS (9 vs 18 months, $P = 0.032$), but not OS (16 vs 24 months, $P = 0.057$). Patients with gastric cancer exhibiting negative expression of ERCC1 are more likely to benefit from oxaliplatin-based adjuvant chemotherapy.

Key words: Stomach neoplasms; Human ERCC1 protein; Oxaliplatin; Adjuvant chemotherapy; Cancer survival

INTRODUCTION

Gastric cancer is one of the most frequent cancers worldwide and is the second leading cause of cancer death in China. At present, surgery remains the mainstay of cure for patients without distant metastases. However, even following radical surgery, the majority of patients develop recurrence (Macdonald, 2004). Several postoperative adjuvant trials have demonstrated that chemotherapy can have significant benefit for these patients (Carrato et al., 2005), but treatment is still not individualized. It is important that physicians avoid unnecessary toxicity and hospitalization with the aim of preserving economic and human resources. Therefore, the identification of molecular markers that are able to predict outcomes is essential for the development of adjuvant chemotherapy in patients with gastric cancer.

Platinum has significant activity as chemotherapy for gastric cancer. Among platinum-containing agents, oxaliplatin is a third generation platinum compound. Beneficial results of platinum-based adjuvant chemotherapy were recently seen in two large randomized trials. In the CLASSIC trial, an improvement of 15% was seen in the 3-year disease-free survival (DFS) of patients treated with adjuvant oxaliplatin and capecitabine compared to surgery alone (hazard ratio, 0.56; $P < 0.0001$) (Bang et al., 2012). Moreover, in the ARTIST study, the addition of capecitabine plus cisplatin (XP) plus radiotherapy to XP chemotherapy did not significantly reduce the recurrence rate after curative resection and D2 lymph node dissection in gastric cancer. However, in view of the good efficacy and safety of XP chemotherapy, XP was recommended for postoperative adjuvant chemotherapy (Lee et al., 2012).

Platinum causes the formation of platinum-DNA adducts that block transcription, leading to cytotoxicity and cell death. Studies have shown that cell amplification in response to DNA repair plays an important role in resistance to platinum drugs. Nucleotide excision repair is one of several DNA repair pathways that correct damaged DNA structures (Rabik and Dolan, 2007). Excision repair cross-complementing gene-1 (*ERCC1*) is a critical enzyme in the nucleotide excision repair pathway (Niedernhofer et al., 2004). As shown in several studies, both *ERCC1* protein and mRNA expression are negatively correlated with the efficacy of platinum-based chemotherapy in patients with gastric cancer (Matsubara et al., 2008; Ozkan et al., 2010). In particular, in two recent studies of patients who received cisplatin-based adjuvant chemotherapy after curative resection, high *ERCC1* expression was associated with decreased overall survival (OS) (Fareed et al., 2010; Kim et al., 2011).

However, analyses of *ERCC1* expression have been mainly based on tumor tissue samples from operative resection or gastroscopy, which are associated with significant patient discomfort. Therefore, there is a need to develop affordable and non-invasive methods that can detect *ERCC1* expression. Many studies have confirmed that peripheral blood lymphocytes (PBL) and tumor cells carry a homologous *ERCC1* gene (Kaspers et al., 1991; Yang et al., 2006). In a previous study, we demonstrated that the expression of *ERCC1* in PBLs might indirectly reflect *ERCC1* expression in gastric cancer tissues (Zhang and Gu, 2013). If the effects of *ERCC1* expression in PBL on outcome are confirmed, this could greatly advance clinical practice.

MATERIAL AND METHODS

Patients and treatments

Tumor specimens were collected from 48 patients with stage II-IV gastric cancer (AJCC 7th edition) who were recruited during the period from April 2010 to November 2012 and who underwent radical surgery at the First Affiliated Hospital of Anhui Medical University. Inclusion criteria were as follows: 1) histologically confirmed gastric cancer; 2) no previous chemotherapy or radiotherapy; 3) availability of clinical and pathological data; 4) good organ functions; 5) signed informed consent prior to entering the study; 6) no serious liver, kidney, pulmonary, or cardiovascular diseases; 7) Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤ 3 ; 8) no prior palliative resection of advanced metastatic disease; and 9) no obvious signs of recurrence with imaging at baseline assessment after surgery.

The study protocol was approved by the Institutional Ethics Committee of Anhui Medical University and was conducted according to the principles of the Declaration of Helsinki. All patients had signed an informed consent before enrollment.

Adjuvant chemotherapy consisted in 4-6 courses of XELOX, administered as follows: oxaliplatin (100 mg/m² D1) and capecitabine (1000 mg/m² BID D1-14) every 3 weeks.

In addition, peripheral blood from 20 non-cancer patients was collected as a control group.

Follow-up evaluation and endpoints

Results from physical examination, hematological studies, and whole-body computed tomography as well as the carcinoembryonic antigen levels were assessed every 3 months in the first year and every 6 months thereafter. Patients underwent gastroscopy every 12 months after surgery. Recurrences were documented by clinical or radiologic assessment. Follow-up was closed on May 2014.

Analyses for OS and DFS were performed. OS was the time-period from study initiation until the date of mortality, regardless of cause, or until the most recently documented follow-up. DFS was calculated from time of diagnosis to disease recurrence or last follow-up.

Separation of PBL

Peripheral blood samples were collected in heparin sodium-containing tubes from a control group and from gastric cancer patients before chemotherapy. PBLs were isolated from the whole blood on histopaque gradients (Histopaque 1077, TBD, Tianjin, China).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tumor specimens obtained by biopsy were cut into 4- μ m sections. Slides were deparaffinized in xylene for epitope retrieval, exposed to 10 mM citric acid antigen retrieval solution (pH 6.0), and heated for 30 min in a water bath. PBLs were fixed with methanol and Triton X-100 was used to permeabilize membranes.

Specimens were incubated overnight with a monoclonal antibody against human *ERCC1* protein (mouseclone 8F1, Maixin Bio, Fujian, China). Antibody binding was detected with the Maxvision kit (sheep/rabbit anti-mouse, Maixin Bio, Fujian, China) by incubation for 15 min with the substrate and counterstained with hematoxylin.

Two pathologists who were unaware of clinical data independently evaluated the percentage of positive tumor nuclei under a light microscope at a magnification of 400X. The grading system was as follows: immunoreactivity in $\geq 10\%$ of tumor cells or PBLs was considered as positive. If $< 10\%$, it was defined as negative (Wachters et al., 2005).

Statistical analysis

Data analysis was performed using SPSS 18.0 for Windows (SPSS Inc., Chicago, USA). Statistical significance was defined as $P < 0.05$. The association of *ERCC1* expression with clinicopathological features was assessed using chi-square analysis. The strength of correlation between *ERCC1* expression in tumor tissue and PBLs was assessed with the Pearson correlation test. Kaplan-Meier log-rank survival analysis was performed to evaluate the association of expression of *ERCC1* with survival.

RESULTS

Patient characteristics

Demographic and pathologic characteristics are summarized in Table 1. The cohort included 30 men (62.5%) and 18 women (37.5%), with a median age of 59 years (range: 24-77 years). AJCC staging yielded 19 stage II cases, and 29 stage III or IV cases. After surgery, all patients received adjuvant treatment. At the end of follow-up, 44 patients experienced tumor recurrence or distant metastasis, and 33 patients died of gastric cancer related complications. Disease relapse accounted for all deaths (33 events). After a median follow-up of 18.5 months (range 3-47), DFS and OS were 12 (95%CI = 8.982-15.018) and 20 (95%CI = 15.504-24.496) months, respectively.

Expression of ERCC1

ERCC1 expression was positive in 72.9% (35/48), 56.3% (27/48), and 10.0% (2/20) of tumor tissues, PBLs from gastric cancer patients, and PBLs in the control group, respectively (Figure 1). A significant positive correlation between *ERCC1* expression in PBL and cancer tissue was found ($\chi^2 = 12.098$, $P = 0.001$, Pearson contingency coefficient = 0.502). There was no relationship between *ERCC1* expression and clinicopathological parameters including age, sex, clinical stage, T stage, tumor location, presence of lymph node metastasis, or degree of differentiation (Table 1).

Table 1. Clinical and demographic characteristics of gastric cancer patients assessed for the ERCC1 status.

Characteristics	Number	(%)	ERCC1-positive cases in PBLs	χ^2 (P)	ERCC1-positive cases in tumor tissues	χ^2 (P)
Age (years)						
≥59	26	54.2	19	0.978	14	0.715
<59	22	45.8	16	(0.001)	13	(0.133)
Gender						
Male	30	62.5	20	0.851	16	0.277
Female	18	37.5	15	(0.356)	11	(0.599)
Stage						
II	19	39.6	15	0.579	11	0.035
III + IV	29	60.4	20	(0.447)	16	(0.853)
T stage						
T1 + T2	9	18.8	9	2.599	7	1.148
T3 + T4	39	81.3	26	(0.107)	20	(0.284)
Tumor location						
Distal	27	56.3	20	0.042	16	0.227
Proximal	21	43.8	15	(0.838)	11	(0.634)
Lymph node metastasis	0-24					
Yes	35	72.9	25	0.000	19	0.203
No	13	27.1	10	(0.988)	8	(0.653)
Differentiation						
Poor	25	52.1	16	2.101	13	0.383
Moderate or well	23	47.9	19	(0.147)	14	(0.536)

ERCC1 = excision repair cross-complementing gene-1; PBL = peripheral blood lymphocyte.

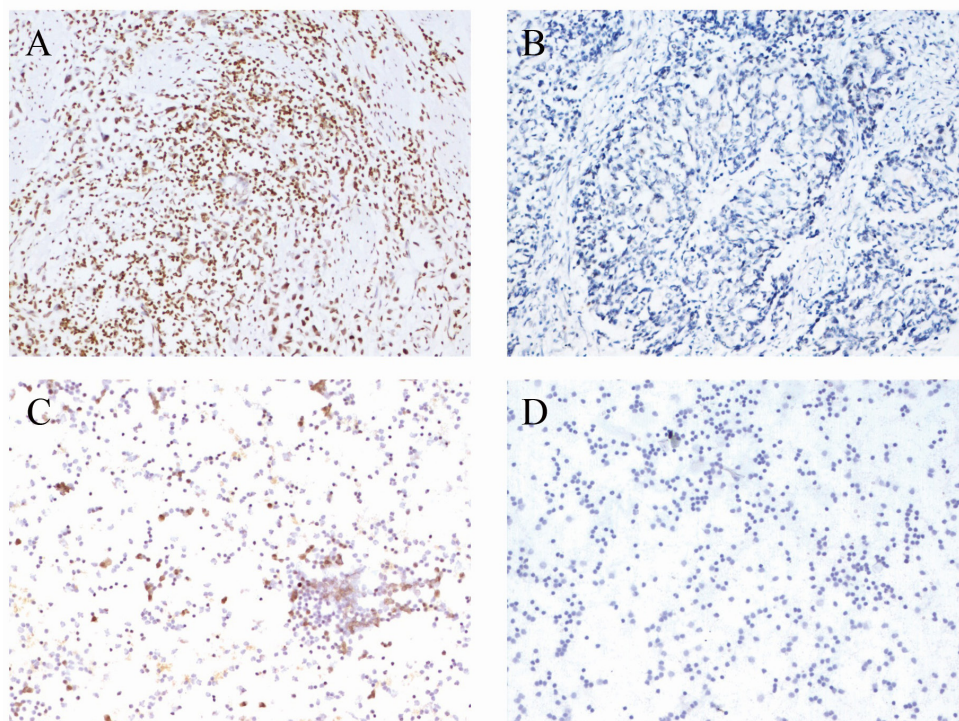


Figure 1. Staining of ERCC1 showing positive immunoreactivity in tumor tissue (A) and PBLs (C). ERCC1 with negative immunoreactivity in tumor tissue (B) and PBLs (D). (All figures, 100X). ERCC1 = excision repair cross-complementing gene-1; PBL = peripheral blood lymphocyte.

ERCC1 and clinical outcomes

Patients with negative expression of ERCC1 in tumor tissues had significantly longer median DFS and median OS compared to patients with positive expression of *ERCC1* (median DFS, 18 vs 10 months, $P = 0.006$; median OS, 30 vs 17 months, $P = 0.012$, respectively) (Figure 2).

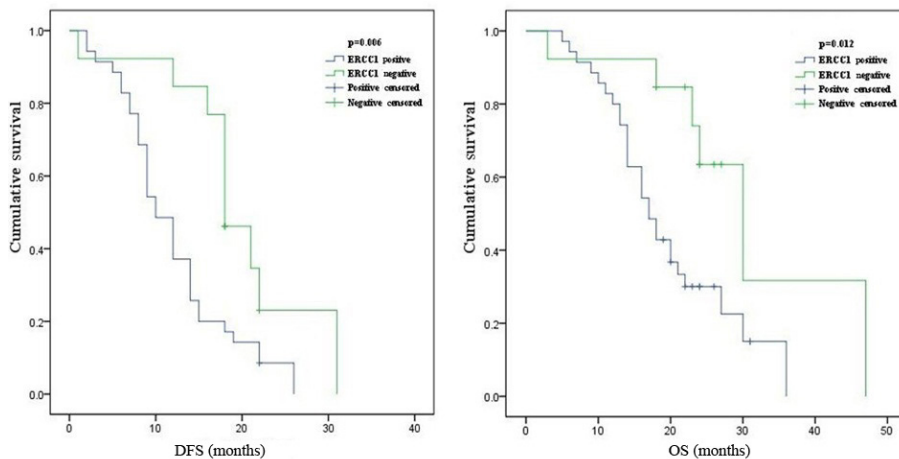


Figure 2. Kaplan-Meier estimates of DFS and OS according to ERCC1 expression in tumor tissues. DFS = disease-free survival; OS = overall survival; ERCC1 = excision repair cross-complementing gene-1.

Moreover, in PBLs from patients with gastric cancer, positive expression of *ERCC1* was associated with significantly decreased DFS (9 vs 18 months, $P = 0.032$). However, positive expression of *ERCC1* was not associated with OS (16 vs 24 months, $P = 0.057$) (Figure 3).

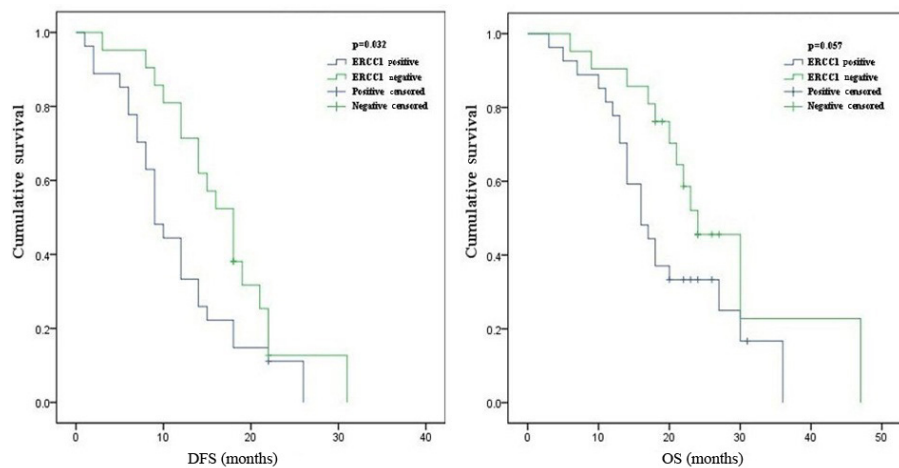


Figure 3. Kaplan-Meier estimates of DFS and OS according to ERCC1 expression in PBLs. DFS = disease-free survival; OS = overall survival; ERCC1 = excision repair cross-complementing gene-1.

DISCUSSION

Given the high rates of recurrence and poor survival associated with resected gastric cancer, molecular biomarkers that can guide treatment decisions are needed. Platinum-based adjuvant chemotherapy remains the backbone of therapy in gastric cancer. Oxaliplatin, a third generation platinum analog, is widely used in this regard. Research has shown that the mechanisms of resistance of tumor to platinum drugs include reduction of dosage accumulation, enhanced tolerance of platinum-DNA complexes, and enhancement of DNA repair. It is worth noting that *ERCC1* plays an important role in the enhancement of DNA repair (Metzger et al., 1998).

Many clinical studies have extensively investigated the association between *ERCC1* expression and outcomes after adjuvant chemotherapy in gastric cancer. In the study by Squires et al., 109 patients who underwent resection for gastric cancer had tissue available for analysis of expression of *ERCC1* using immunohistochemistry. High tumoral expression of *ERCC1* was associated with decreased OS in patients who received platinum-based adjuvant chemotherapy (16.7 months vs 53.8 months; $P = 0.03$) (Squires et al., 2013). In the investigation by De Dosso et al. (2013), the expression of *ERCC1* expression in tumor samples obtained from surgical specimens of 68 patients treated with adjuvant cisplatin-based chemotherapy for gastric cancer were analyzed by immunohistochemistry. Significantly longer OS ($P = 0.04$) was found in patients categorized as *ERCC1* negative by immunohistochemistry. Thus, *ERCC1* may help in predicting which patients are more likely to benefit from adjuvant cisplatin-based chemotherapy in curatively resected gastric cancer (De Dosso et al., 2013). In a study from China, the effects of *ERCC1* expression on survival in were evaluated in 57 gastric cancer patients treated with surgery followed by oxaliplatin-based adjuvant chemotherapy. *ERCC1* expression levels were measured by using qRT-PCR. Univariate analyses revealed that patients with low *ERCC1* levels had longer relapse-free survival (RFS) and OS than those with high *ERCC1* levels (median RFS, 18 vs 7 months, $P = 0.001$; median OS, 27 vs 11 months, $P = 0.001$). It was concluded that gastric cancer patients with low levels of *ERCC1* expression demonstrated a benefit from oxaliplatin-based adjuvant chemotherapy (Liu et al., 2013). In our study, the association between *ERCC1* expression in tumor tissues and outcome in resected patients treated with oxaliplatin-based regimens confirms what has been described by other authors. Patients with negative expression of *ERCC1* had a significantly longer median DFS and median OS compared with patients with positive expression of *ERCC1* (median DFS, 18 vs 10 months, $P = 0.006$; median OS, 30 vs 17 months, $P = 0.012$, respectively).

However, analyses of *ERCC1* expression have been mainly based on primary tumor samples, and obtaining them is associated with substantial patient discomfort. Moreover, detection of *ERCC1* expression using tumor tissue is troublesome. Thus, a simple method of detection is desirable for routine clinical practice.

Several studies have confirmed that both PBL and tumor cells carry a homologous gene for *ERCC1* (Kaspers et al., 1991; Yang et al., 2006). In patients with non-small cell lung cancer and those with head and neck squamous cell carcinoma, the levels of *ERCC1* in PBLs may be considered prognostic (Schena et al., 2012). In a previous study, we also detected *ERCC1* expression in 53 gastric cancer patients in both tumor tissue and PBLs. We found that the *ERCC1* expression in PBLs indirectly reflected *ERCC1* expression in cancer tissues (Zhang and Gu, 2013).

However, it remains unclear if *ERCC1* expression in PBLs predicts outcome in patients with gastric cancer treated with platinum-based chemotherapy. In epithelial ovarian cancer, Darcy et al. (2007) analyzed whether mRNA expression of *ERCC1* in PBLs was associated with clinical

outcomes in patients treated with platinum-taxane chemotherapy, although negative results were reported. Herein, we assessed correlations between expression of *ERCC1* in PBLs and outcomes in 48 gastric cancer patients treated with oxaliplatin-based adjuvant chemotherapy. We found that positive *ERCC1* expression was associated with significantly decreased DFS (9 vs 18 months, $P = 0.032$). However, positive *ERCC1* expression was not associated with OS (16 vs 24 months, $P = 0.057$).

In addition, there are some potential limitations of our study. First, the sample size was relatively small, especially for survival analyses. In addition, the follow-up time was relatively short, although it was problematic to obtain OS in all patients. Lastly, the potential effect that platinum-based chemotherapy may have on the expression of *ERCC1* in tumor tissue and PBL was clear. However, after chemotherapy is administered, repeated detection of *ERCC1* expression using tumor tissue is troublesome. For this reason, repeated detection of *ERCC1* expression in PBL may be helpful in individualized treatment. Therefore, if *ERCC1* expression in PBL was detected during the entire course of chemotherapy, it could reveal resistance to platinum-based chemotherapy in gastric cancer in a timely manner.

In conclusion, we demonstrate that patients with negative *ERCC1* expression are more likely to benefit from oxaliplatin-based adjuvant chemotherapy. To further confirm the prognostic value of *ERCC1* expression of tumor tissue and PBLs in gastric cancer, a multicenter prospective study with a large sample size is required.

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