



Association of DNA repair gene polymorphisms with response to chemotherapy and prognosis of gastric cancer

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ABSTRACT. We investigated the correlation between the response to chemotherapy in patients and excision repair cross-complimentary group 1 gene (*ERCC1*) and xeroderma pigmentosum complementation group F gene (*XPF*) polymorphisms and the effect of these polymorphisms on the clinical outcome of gastric cancer. Samples from a total of 255 patients with newly diagnosed and histopathologically confirmed primary gastric cancer were collected in our study. The *ERCC1* rs11615, *ERCC1* rs2298881, *XPF* rs2276465, and *XPF* rs6498486 polymorphisms were genotyped. Among the 255 patients, the median follow-up time was 29.7 months. A total of 103 patients (40.4%) died from gastric cancer during the follow-up period. We observed that the *XPF* rs6498486 CC genotype and the *XPF* rs2276465 GG genotype were associated with response to chemotherapy, with odds ratios and 95% confidence intervals of 3.88 (1.23-16.07) and 2.66 (1.17-6.45), respectively. In the Cox proportional hazards model, patients carrying the *ERCC1* rs11615 AA genotype and the *XPF* rs2276465 GG genotype showed only a 0.22- and

0.30-fold increased risk of death from gastric cancer. We found that the *XPF* rs6498486 and *XPF* rs2276465 polymorphisms are markers of response to oxaliplatin/5-fluorouracil-based chemotherapy in gastric cancer patients.

Key words: ERCC1; XPF; Gastric cancer; Response; Oxaliplatin/5-Fu-survival

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide with an estimated 988,000 new cases and 736,000 deaths each year [International Agency for Research on Cancer (IARC), 2008]. In China, gastric cancer is the second-most common cause of cancer-related deaths, killing approximately 231,193 people in 2008 [International Agency for Research on Cancer (IARC), 2008]. Although the incidence of non-cardia gastric cancer has slightly decreased in the last 2 decades, the incidence of gastric cardia cancer shows a significantly increased trend in China. Surgery is the primary modality for managing early-stage disease, but most patients develop local or distant recurrence (MacDonald, 2004). 5-Fluorouracil (5-FU) remains the main chemotherapeutic agent for treating gastric cancer, and combination chemotherapy with 5-FU has shown improved clinical outcomes (Wohrer et al., 2004); however, many patients show different prognoses even when they received the same treatment. This suggests that inter-individual variation in drug metabolizing enzymes may influence the prognosis of gastric cancer.

Recently, several studies have shown that single nucleotide polymorphisms (SNPs) influence the mechanisms of DNA repair function and play a role in removing DNA adducts. Therefore, SNPs may also affect the response to chemotherapy. Repair of DNA damage is a complex process involving a series of DNA repair pathways. In humans, more than 130 genes participate in the DNA repair process (Wood et al., 2001). Nucleotide excision repair (NER) is the most versatile DNA repair mechanism pathway and is responsible for removing a wide variety of DNA lesions, such as bulky adducts, cross-links, oxidative DNA damage, alkylating damage, and thymidine dimers (De Silva et al., 2000; Friedberg, 2001; Wood et al., 2001). The excision repair cross-complimentary group 1 gene (*ERCC1*) encodes a subunit of the NER complex required for the incision step of NER, which forms a heterodimer with the xeroderma pigmentosum complementation group F (*XPF*) endonuclease to catalyze the 5' incision during excision of the DNA lesion (Wang et al., 2011). Previous studies have indicated that *ERCC1-XPF* genetic variation is associated with prognosis of various cancers, such as ovarian cancer, head and neck cancer, and breast cancer (Carles et al., 2006; Alexander et al., 2010; Vaezi et al., 2011; DeLoia et al., 2012; Fleming et al., 2012). However, only one study conducted in the United Kingdom demonstrated the role of *ERCC1* and *XPF* polymorphisms in response to chemotherapy and in the prognosis of gastric cancer.

There have been no studies conducted in China to examine whether *ERCC1-XPF* gene polymorphisms correlate with survival or response chemotherapy in cancer patients. The goal of the present study was to identify whether a correlation exists between *ERCC1-XPF* gene polymorphisms and the response to chemotherapy and the role of these 2 gene polymorphisms on the clinical outcome of gastric cancer.

MATERIAL AND METHODS

Patient eligibility

A total of 255 patients with newly diagnosed and histopathologically confirmed primary gastric cancer were recruited for our study, including gastric cardia adenocarcinoma and non-gastric cardia adenocarcinoma cases. All cases were selected from patients of the Traditional Chinese Medicine of Chongqing and Chongqing Medical University between January 2007 and January 2008. Cases with secondary or recurrent tumors were excluded. Patients were ineligible if they had inadequate organ function, were pregnant, or breast-feeding. All patients provided written informed consent prior to chemotherapy. The protocol of our study was approved by The First Affiliated Hospital of Jinan University.

Chemotherapy

The 255 patients were treated with one of the following fluorouracil-based chemotherapy programs: 500 mg/m² 5-FU for five days and 2500 mg/m² capecitabine for 2 weeks. Conjunctive chemotherapy included 25 mg/m² cisplatin for 3 days, 60 mg/m² oxaliplatin for 8 days, 75 mg/m² docetaxel for 1 day, or 60 mg/m² paclitaxel for 8 days. The response to treatment was evaluated according to the World Health Organization (WHO) criteria (Miller et al., 1981). Complete remission (CR) and partial remission (PR) were considered to be responsive, and stable disease (SD) and progressive disease (PD) were considered as non-responsive. Overall survival (OS) was calculated from the date of chemotherapy to the date of death or last clinical follow-up.

Genotyping

Potential *ERCC1* and *XPF* SNPs were identified in the National Center for Biotechnology Information (NCBI) dbSNP database according to the following criteria: (1) minor allele frequency of selected SNPs was $\geq 10\%$ in the Chinese population; (2) affected microRNA binding site activity; (3) not included in the published genome-wide association study. A total of 2 SNPs in *ERCC1* (rs11615 and rs2298881) and 2 SNPs in *XPF* (rs2276465 and rs6498486) were selected. Genomic DNA was extracted from the buffy-coat fraction of the blood sample with the Qiagen Blood DNA Mini Kit (Qiagen; Hilden, Germany) according to manufacturer instructions. DNA purity and concentration were determined by spectrophotometric absorbance measurements at 260 nm and 280 nm using an ultraviolet spectrophotometer (Nano Drop Technologies, Inc.; Wilmington, DE, USA).

ERCC1 rs11615, *ERCC1* rs2298881, *XPF* rs2276465, and *XPF* rs6498486 were genotyped using Taqman assays in the ABI Prism 7911HT Sequence Detection System (Applied Biosystems; Foster City, CA, USA). Primers, probes, and reaction conditions are available upon request. Genotyping was conducted by laboratory personnel who were blinded to the status of cases. For quality control, we randomly selected 10% of the samples for repeated genotyping, and the results showed 100% concordance.

Statistical analysis

All analyses were performed using the SPSS version 16.0 statistical software (SPSS;

Chicago, IL, USA). Continuous variables are expressed as the mean \pm standard deviation (SD), whereas categorical variables are shown as frequencies and percentages. Demographic characteristics were compared between cases and controls using χ^2 and Student *t*-tests. Survival distributions were estimated using the Kaplan-Meier method and assessed using the log-rank test. The association between genotype and survival was estimated based on the hazard ratios (HRs) and their confidence intervals (CIs) from a multivariate Cox proportional hazards model. A two-sided P value <0.05 was considered to be significant.

RESULTS

There were 255 patients (85 females/170 males). The median age was 55.7 ± 9.3 years (Table 1).

Table 1. Demographic and clinical characteristics of subjects included.

Characteristics	Cases (N = 255)	%
Age (means \pm SD)	55.7 \pm 9.3	
Gender		
Female	85	33.3
Male	170	66.7
Smoking		
Never	169	66.3
Ever	86	33.7
Drinking		
Never	167	65.5
Ever	88	34.5
Family history of gastric cancer in first-relatives		
No	234	91.8
Yes	21	8.2
Tumor histology		
Intestinal	142	55.7
Diffuse	104	40.8
Other	9	3.5
TNM stage		
I-II	100	39.2
III-IV	155	60.8

All patients were followed up from the date of diagnosis until the end of December 2012. The median follow-up time was 29.7 months, and ranged from 2-60 months. During the follow-up period, 103 patients (40.4%) died from gastric cancer.

One hundred and fifty six patients showed responsive to chemotherapy (CR or PR), with a response rate of 61.2% (Table 2). Patients were classified into responders and non-responders, and a significantly different genetic distributions of *XPF* rs6498486 and *XPF* rs2276465 were observed between these groups, and significantly higher *XPF* rs6498486 CC and *XPF* rs6498486 CC frequencies were observed compared to the wild-type genotype, with ORs (95%CI) of 3.88 (1.23-16.07) and 2.66 (1.17-6.45), respectively. However, we observed no significant between-group differences in the frequencies of *ERCC1* rs11615 and *ERCC1* rs2298881.

Patients carrying the *XPF* rs6498486 CC genotype showed a significantly longer median survival time (34.7 months) than those carrying the AA genotype (26.4 months). By the log-rank test, a significantly difference was found in survival times (Table 3).

In the Cox proportional hazards model, after adjusting for potential confounding

factors, the HR (95%CI) for OS in patients carrying the *XPF* rs6498486 CC genotype was 0.22 (0.05-0.69) using the AA genotype as the reference variable. Patients carrying the *XPF* rs2276465 GG genotype was associate with a significantly decreased risk of death from gastric cancer when compared with those carrying the CC genotype, and the HR (95%CI) of overall survival was 0.30 (0.12-0.69).

However, we observed no significant association between the *ERCC1* rs11615 and *ERCC1* rs2298881 polymorphisms and survival time in gastric cancer patients.

Table 2. ERCC1-XPF polymorphism response to chemotherapy.

	Total frequencies	%	Responders N = 156	%	Non-responders N = 99	%	P	OR (95%CI)
ERCC1 rs11615								
GG	111	43.5	65	41.6	46	46.5	-	1.0 (Reference)
AG	97	38.1	58	37.3	39	39.4	0.86	1.05 (0.58-1.90)
AA	47	18.4	33	21.1	14	14.1	0.17	1.67 (0.76-3.76)
ERCC1 rs2298881								
AA	165	64.7	98	62.9	67	67.5	-	1.0 (Reference)
AC	58	22.8	34	21.7	24	24.5	0.92	0.97 (0.51-1.87)
CC	32	12.5	24	15.4	8	7.9	0.1	2.05 (0.83-5.59)
XPF rs6498486								
AA	174	68.2	102	65.4	72	72.6	-	1.0 (Reference)
AC	55	21.6	32	20.5	23	23.3	0.95	0.98 (0.51-1.91)
CC	26	10.2	22	14.1	4	4.1	0.01	3.88 (1.23-16.07)
XPF rs2276465								
CC	157	61.5	87	55.8	70	70.5	-	1.0 (Reference)
CG	55	21.6	36	23.1	19	19.2	0.19	1.52 (0.77-3.07)
GG	43	16.9	33	21.1	10	10.3	0.01	2.66 (1.17-6.45)

Table 3. Univariate analysis of ERCC1 and XPF in relation to overall survival of gastric cancer.

Gene	Cases		Events			Overall Survival		
	N = 331	%	N = 103		%	Median (month)	Log-rank	HR (95%CI)
ERCC1 rs11615								
GG	111	43.5	51	60	49.5	28.4		1.0 (Reference)
AG	97	38.1	36	61	35	30.2		0.69 (0.38-1.26)
AA	47	18.4	16	31	15.5	33.2	0.16	0.60 (0.28-1.30)
ERCC1 rs2298881								
AA	165	64.7	69	96	67	29.1		1.0 (Reference)
AC	58	22.8	22	36	21.3	30.7		0.85 (0.44-1.64)
CC	32	12.5	12	20	11.7	31.5	0.25	0.83 (0.35-1.93)
XPF rs6498486								
AA	174	68.2	79	95	76.7	26.4		1.0 (Reference)
AC	55	21.6	20	35	19.5	31.6		0.69 (0.35-1.34)
CC	26	10.2	4	22	3.8	34.7	0.007	0.22 (0.05-0.69)
XPF rs2276465								
CC	157	61.5	74	83	71.8	27.7		1.0 (Reference)
CG	55	21.6	20	35	19.4	30.5		0.64 (0.32-1.26)
GG	43	16.9	9	34	8.8	33.6	0.01	0.30 (0.12-0.69)

DISCUSSION

In this case-control study, we investigated whether the *ERCC1* and *XPF* gene polymorphisms in the NER pathway were associated with the clinical outcome of gastric cancer treated with 5-Fu-based chemotherapy. We observed that the *XPF* rs6498486 CC genotype

and the *XPF* rs2276465 GG genotype were associated with a better response to chemotherapy and longer survival time in gastric cancer patients treated with 5-Fu-based chemotherapy. Our results demonstrated that the *XPF* rs6498486 and *XPF* rs2276465 polymorphisms can be used as predictive markers of response and expected to be valuable for individualizing patient treatment as this method enables discrimination of patients most likely to respond to combination therapy from those likely to be non-responsive.

ERCC1 is part of the NER complex involved in the repair of platinum/5-Fu-based induced interstrand and intrastrand cross-links (Gustavsson et al., 2009; Liang et al., 2010; Wang et al., 2012). Since ERCC1 acts on larger lesions covering 20-25 nucleotides, the efficient DNA repair capacity of ERCC1 appears to be a critical mechanism of the resistance to platinum/5-Fu-based drugs and radiation (Reed, 1998; Wood et al., 2001; Goode et al., 2002). Several previous studies indicated that the *ERCC1* rs11615 polymorphism could be used as a prognostic factor in oxaliplatin-based treatment of this cancer (Matsubara et al., 2008; Wang et al., 2008; Ozkan et al., 2010; Liang et al., 2010; Yun et al., 2010; Yin et al., 2011). Two previous studies conducted in China indicated that the *ERCC1* rs11615 polymorphism is associated with time to progression and OS of gastric cancer patients treated with oxaliplatin/5-Fu-based chemotherapy (Liang et al., 2010; Yin et al., 2011). However, the results are inconsistent. A recent study conducted in Korea indicated that ERCC1 protein expression does not appear to be associated with the prognosis of gastric cancer (Yun et al., 2010). Our study also indicated that there was no association between the *ERCC1* rs11615 polymorphism and prognosis of gastric cancer.

XPF, also known as ERCC4, is also involved in the NER pathway and is associated with susceptibility to xeroderma pigmentosum and a rare recessive syndrome that includes photosensitivity, malignant tumor development, and response to chemotherapy (Zhu et al., 2003). A previous study indicated that XPF is critical for recombination repair, mismatch repair, and possibly, immunoglobulin class switching, owing to its function in identifying damage sites (Kornguth et al., 2005). Therefore, polymorphisms of *XPF* may change the function of this gene and influence the response to chemotherapy. A previous study indicated that variations in *XPF* were associated with treatment response in ovarian cancer, esophageal cancer, and gastric cancer (Fareed et al., 2010; DeLoia et al., 2012; Alexander et al., 2012). Only one study conducted in the United Kingdom had investigated the association between *XPF* polymorphisms and the response to platinum-based chemotherapy (Fareed et al., 2010). However, this study did not identify the *XPF* polymorphism as a predictor of response. In our study, we found that the *XPF* rs6498486 CC genotype and the *XPF* rs2276465 GG genotype were correlated with good response to chemotherapy. The inconsistency of these studies may be explained by differences in population backgrounds, and further studies with different populations are needed to confirm our findings.

This is the first study reporting an association between the polymorphisms *XPF* rs6498486 and *XPF* rs2276465 and response to oxaliplatin/5-Fu-based chemotherapy. *ERCC1* rs11615 and *XPF* rs2276465 may substantially contribute to the future design of individualized cancer treatment in gastric cancer patients. Therefore, further multicenter studies involving various populations are required to confirm our results.

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