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Genetic variability of *ERCC1* and *ERCC2* influences treatment outcomes in gastric cancer

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ABSTRACT. We performed a study to investigate the role of *ERCC1* (rs11615, rs2298881, and rs3212986) and *ERCC2* (rs13181, rs238406, and rs1799793) polymorphisms in the prognosis of gastric cancer. A total of 346 patients with gastric cancer were recruited between May 2009 and May 2012. Single nucleotide polymorphism genotyping was performed using the Sequenom MassARRAY platform. The GA+AA genotype of *ERCC2* rs1799793 showed significant and favorable response to chemotherapy than the wide-type GG genotype in multivariate analysis (OR = 1.78, 95%CI = 1.13-2.81). In a Cox proportional hazard model, carriers of *ERCC2* rs1799793 GA+AA genotype exhibited longer duration of survival than did those with the GG genotype (hazards ratio = 0.57, 95%CI = 0.35-0.92). In conclusion, our study suggests that *ERCC2* rs1799793 polymorphic variation could be used as a predictor for the prognosis of gastric cancer.

Key words: ERCC1; ERCC2; Polymorphism; Gastric cancer; Clinical outcome

INTRODUCTION

Gastric cancer, which exhibits aggressive behavior, is the fourth most common cancer and the second most common cause of cancer-related death world wide. Furthermore, although the frequency of gastric cancer in China has been observed to be reduced in recent years, it is still reported as the second most common cause of cancer-related deaths, after lung cancer (Stadtlander and Waderbor, 1999). Despite advanced diagnosis and treatment has been made in gastric cancer, the survival of gastric cancer still remains poor. It is estimated that the 5-year survival of gastric cancer is between 10-15% even after curative surgical reaction (Stacey, 2010). Patients with the same tumor/node/metastasis (TNM) stage might show different clinical outcomes, which suggests that genetic biomarkers play an important role in the prognosis of gastric cancer patients.

DNA repair systems, including nucleotide excision repair (NER), base excision repair, mismatch repair, and double-strand break repair, are involved in maintaining genome stability and integrity (Lindahl and Wood, 1999; Goode et al., 2002). NER is a versatile system to monitor and repair DNA damage caused by both endogenous and exogenous factors, such as therapeutic agents (de Laat et al., 1999). Polymorphisms of genes in NER pathway could influence the expression and function of proteins, and thus altering individual survival of gastric cancer patients. Therefore, polymorphisms of several NER genes could be correlated with the prognosis of gastric cancer patients. In our study, we performed a study to investigate the roles of *ERCC1* (rs11615, rs2298881 and rs3212986) and *ERCC2* (rs13181, rs238406 and rs1799793) polymorphisms in the prognosis of gastric cancer.

MATERIAL AND METHODS

Study subjects

This case-control study was approved by the Ethics Committee of the Second Hospital of Shandong University and was performed according to the Declaration of Helsinki, and written inform consents were obtained from all subjects. A total of 375 patients with gastric cancer were recruited from the Second Hospital of Shandong University between May 2009 and May 2012. Stage of tumors was defined by the 7th edition of the TNM staging system of the International Union Against Cancer/American Joint Committee on Cancer (2010) according to postoperative pathologic examination. The exclusion criteria were that patients who had other malignant tumors, or underwent preoperative radiotherapy or chemotherapy before enrolling into our study. Finally, 346 patients were included into our study, with a participation rate of 92.27%. The follow-up of the patients was complete by May 2014.

Assessment of treatment outcome

Demographic characteristics of patients with gastric cancer were obtained from a standardized questionnaire, such as age and gender. Clinical and treatment parameters were obtained from the medical records. Patients with gastric cancer received oxaliplatin, fluorouracil (5FU), and folinic acid chemotherapy after recruiting in this study until the presentation of unacceptable toxicity or progressive disease. Tumor response to chemotherapy was assessed according to World Health Organization criteria (Miller et al., 1981). Overall survival (OS) was defined as the time from the beginning of treatment to an event or death, respectively.

DNA extraction and genotyping

Two milliliters peripheral blood was taken from each patient with gastric cancer for DNA extraction using the TIANamp Blood DNA Kit (Tiangen, Beijing, China), according to the manufacturer instructions. The extracted DNA samples were stored at -80°C before genotyping. The DNA samples were placed randomly onto 384-well plates and were blinded for disease status. The design of the assay and single nucleotide polymorphism genotyping were performed by Bio Miao Biological Technology (Beijing, China) using the Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA) according to the manufacturer instructions.

Statistical analysis

All the statistical analyses were conducted with the SPSS 16.0 statistical software (SPSS, Chicago, IL, USA). Frequencies of demographic and clinical characteristics of patients with gastric cancer were used to describe the distribution of categorical variables, and median and standard deviation were used for continuous variables. Logistic regression analysis was taken to analyze the association between ERCC1 and ERCC2 genetic polymorphisms and response to chemotherapy, and the results were expressed by ORs and their 95%CIs. The significances of ERCC1 and ERCC2 gene polymorphisms in survival were analyzed using multivariate Cox proportional hazard modeling, and the results were calculated using the hazards ratio (HR) and the 95%CI. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. A P value of less than 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of patients with gastric cancer were shown in Table 1. A total of 346 patients with gastric cancer were enrolled in the present study, including 149 women and 197 men. The mean age of patients with gastric cancer was 64.5 ± 9.2 years. Among these 346 patients, the cancers in 224 (64.74%) were diffuse, 122 (35.26%) were intestinal, 200 (57.80%) exhibited tumor size ≥5 cm, and 212 (61.27%) showed lymphovascular invasion.

The effects of the clinicopathological characteristics on the response to chemotherapy in patients with gastric cancer are shown in Table 2. Of the six investigated polymorphisms in this study, patients carrying the GA+AA genotype of *ERCC2* rs1799793 showed significant favorable response to chemotherapy compared to those with the wide-type GG genotype in multivariate analysis (OR = 1.78, 95%CI = 1.13-2.81). However, variants in *ERCC1* rs11615, rs229881, and rs3212986, and in *ERCC2* rs13181 and rs238406 did not show statistical association with the response to chemotherapy in patients with gastric cancer.

The results of the relationship between the six gene polymorphisms of the NER pathway and gastric cancer survival are summarized in Table 3. By the Cox proportional hazards model, carriers of the *ERCC2* rs1799793 GA+AA genotype exhibited longer survival time than did those with the GG genotype (HR = 0.57, 95%CI = 0.35-0.92) (Figure 1). However, the *ERCC1* rs11615, rs229881, and rs3212986, and the *ERCC2* rs13181 and rs238406 polymorphisms could not predict the survival of patients with gastric cancer (P > 0.05).

Table 1. Characteristics of patients with gastric cancer included in this study.

Variable	N	%
Age (years)		
<60	144	41.62
≥60	202	58.38
Gender		
Female	149	43.06
Male	197	56.94
Lauren's type		
Intestinal	122	35.26
Diffuse	224	64.74
Tumor size (cm)		
<5 cm	146	42.20
≥5 cm	200	57.80
Lymphovascular invasion		
Absent	134	38.73
Present	212	61.27

Table 2. Association between *ERCC1* and *ERCC2* gene polymorphisms and response to chemotherapy.

Gene	Total	%	CR+PR	%	SD+PD	%	OR (95%CI)	P value
ERCC1 rs11615								
TT	195	56.36	114	54.9	81	58.27	1.0 (Ref.)	-
TC+CC	151	43.64	93	45.1	58	41.73	1.14 (0.72-1.80)	0.56
ERCC1 rs2298881								
CC	137	39.60	77	37.4	60	43.17	1.0 (Ref.)	-
CA+AA	209	60.40	130	62.6	79	56.83	1.28 (0.81-2.03)	0.27
ERCC1 rs3212986								
GG	153	44.22	88	42.5	65	46.76	1.0 (Ref.)	-
GT+TT	193	55.78	119	57.5	74	53.24	1.19 (0.75-1.87)	0.44
ERCC2 rs13181								
AA	248	71.68	141	68.2	107	76.98	1.0 (Ref.)	-
AC+CC	98	28.32	66	31.8	32	23.02	1.57 (0.93-2.65)	0.07
ERCC2 rs238406								
GG	146	42.20	84	40.7	62	44.60	1.0 (Ref.)	-
GT+TT	200	57.80	123	59.3	77	55.40	1.18 (0.74-1.86)	0.46
ERCC2 rs1799793								
GG	167	48.27	88	42.5	79	56.83	1.0 (Ref.)	-
GA+AA	179	51.73	119	57.5	60	43.17	1.78 (1.13-2.81)	<0.05

[1]Adjusted for age, gender, Lauren's type, tumor size, and lymphovascular invasion.

Table 3. Association between *ERCC1* and *ERCC2* gene polymorphisms and overall survival in gastric cancer.

Gene	Total	%	Event	%	OR (95%CI)[1]	P value
ERCC1 rs11615						
CC	195	56.36	62	59.05	1.0 (Ref.)	-
CT+TT	151	43.64	43	40.95	0.85 (0.52-1.39)	0.51
ERCC1 rs2298881						
CC	137	39.60	45	42.86	1.0 (Ref.)	-
CA+AA	209	60.40	60	57.14	0.82 (0.50-1.35)	0.41
ERCC1 rs3212986						
GG	153	44.22	51	48.57	1.0 (Ref.)	-
GT+TT	193	55.78	54	51.43	0.78 (0.48-1.26)	0.28
ERCC2 rs13181						
TT	248	71.68	79	75.24	1.0 (Ref.)	-
TG+GG	98	28.32	26	24.76	0.77 (0.44-1.34)	0.33
ERCC2 rs238406						
GG	146	42.20	48	45.71	1.0 (Ref.)	-
GT+TT	200	57.80	57	54.29	0.81 (0.50-1.33)	0.38
ERCC2 rs1799793						
GG	167	48.27	61	58.10	1.0 (Ref.)	-
GA+AA	179	51.73	44	41.90	0.57 (0.35-0.92)	0.02

[1]Adjusted for age, gender, Lauren's type, tumor size, and lymphovascular invasion.

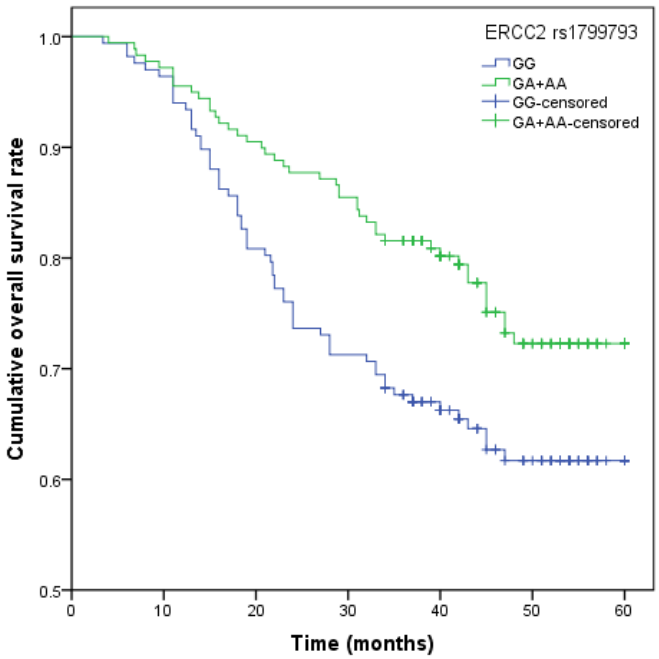

Figure 1. Influence of *ERCC2* rs1799793 polymorphism on the overall survival of patients with gastric cancer.

DISCUSSION

In this study, we investigated the influence of the *ERCC1* (rs11615, rs2298881, and rs3212986) and *ERCC2* (rs13181, rs238406, and rs1799793) polymorphic variants in DNA repair mechanisms on the treatment response and survival in patients with gastric cancer. Our study found that patients carrying the GA+AA genotype of *ERCC2* rs1799793 showed significant favorable response to chemotherapy and exhibited longer survival time than did those with the wide-type GG genotype in multivariate analysis.

Oxaliplatin-based chemotherapy is a commonly used chemotherapeutic strategy that exerts its cytotoxic effect primarily through the formation of different kinds of DNA lesions. Therefore, DNA repair mechanisms might play a critical role in the response to oxaliplatin-based chemotherapy. NER enzymes are involved in damage recognition, and demarcation, and in DNA unwinding, damage incision, and new strand ligation (Ng et al., 2003; Kamileri et al., 2012). ERCC1 participates in the DNA damage incision step, and ERCC2 is responsible for the damage unwinding process.

Many previous studies have reported the role of *ERCC1* and *ERCC2* polymorphisms in the clinical outcome of many cancers (Li et al., 2012; Yang et al., 2012a,b; Rumiato et al., 2013; Yang and Xian, 2014; Goričar et al., 2015). Li et al. (2012) conducted a prospective study to investigate the role of *GSTP1*, *ERCC1*, and *ERCC2* polymorphisms in the OS of patients with colorectal cancer receiving oxaliplatin-based chemotherapy, and they found that *ERCC1* rs11615 and *ERCC2* rs1799793 might influence the clinical outcome of these patients. Rumiato et al. (2013) conducted a study with 143 patients with esophageal cancer, and found that the *ERCC1* rs3212986 was associated with chemotherapy response to 5FU-based chemotherapy. Yang et al. (2012a) reported that the *ERCC2* rs1799793 polymorphism was associated with a better response

to chemotherapy and longer OS of bone tumors. Goričar et al. (2015) reported that the *ERCC2* rs1799793 polymorphism was associated with a longer event-free survival of osteosarcoma. Yang et al. (2012b) conducted a meta-analysis with 46 studies, and they reported that *ERCC1* rs11615 and *ERCC2* rs1799793 might be useful biomarkers to influence the clinical outcomes of platinum-based chemotherapy in patients with non small cell lung carcinoma. However, the results of these studies are inconsistent: the primary reasons for this might be the differences in study populations, types of tumors, study designs, and sample sizes.

Three previous studies have reported an association between *ERCC1* and *ERCC2* variants and the clinical outcome of gastric cancer (Chu et al., 2013; Li et al., 2013; Zhou et al., 2014). Chu et al. (2013) reported that the *ERCC2* rs13181 polymorphism could be associated with a decreased risk of death compared with patients carrying the wide-type genotype, but they found no association between the *ERCC1* rs11615 and *ERCC2* rs1799793 polymorphisms and the OS of gastric cancer. Zhou et al. (2014) found that the *ERCC1* rs11615 and *ERCC2* rs1799793 polymorphisms might influence the response to chemotherapy and the OS of gastric cancer. Li et al. (2013) reported that *ERCC2* rs1799793 might affect the OS of gastric cancer. A recent meta-analysis with 17 published studies reported that the *ERCC1* rs11615 and *ERCC2* rs13181 polymorphisms are useful prognostic factors for patients with gastric cancer receiving oxaliplatin-based treatment (Yin et al., 2011). In our study, we found that only the *ERCC2* rs1799793 polymorphism was associated with a better response to chemotherapy and a longer OS of gastric cancer. Further large sample size studies are greatly needed to confirm our results.

In conclusion, our finding demonstrated that the GA+AA genotype of *ERCC2* rs1799793 was significantly associated with a favorable response to chemotherapy and a longer survival time than was the wide-type GG genotype in multivariate analysis. Our study suggests that *ERCC2* rs1799793 polymorphic variation could be used as a predictor for the prognosis of gastric cancer.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Chu H, Gu D, Xu M, Xu Z, et al. (2013). A genetic variant in *ERCC2* is associated with gastric cancer prognosis in a Chinese population. *Mutagenesis* 28: 441-446.

de Laat WL, Jaspers NG and Hoeijmakers JH (1999). Molecular mechanism of nucleotide excision repair. *Genes Dev.* 13: 768-785.

Goode EL, Ulrich CM and Potter JD (2002). Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 11: 1513-1530.

Goričar K, Kovač V, Jazbec J, Zakotnik B, et al. (2015). Genetic variability of DNA repair mechanisms and glutathione-S-transferase genes influences treatment outcome in osteosarcoma. *Cancer Epidemiol*. 39: 182-188.

Kamileri I, Karakasilioti I and Garinis GA (2012). Nucleotide excision repair: new tricks with old bricks. *Trends Genet.* 28: 566-573.

Li HY, Ge X, Huang GM, Li KY, et al. (2012). *GSTP1*, *ERCC1* and *ERCC2* polymorphisms, expression and clinical outcome of oxaliplatin-based adjuvant chemotherapy in colorectal cancer in Chinese population. *Asian Pac. J. Cancer Prev.* 13: 3465-3469.

Li Y, Liu Z, Liu H, Wang LE, et al. (2013). *ERCC1* and *ERCC2* variants predict survival in gastric cancer patients. *PLoS One* 8: e71994.

Lindahl T and Wood RD (1999). Quality control by DNA repair. *Science* 286: 1897-1905.

Miller AB, Hoogstraten B, Staquet M and Winkler A (1981). Reporting results of cancer treatment. *Cancer* 47: 207-214.

Ng JM, Vermeulen W, van der Horst GT, Bergink S, et al. (2003). A novel regulation mechanism of DNA repair by damage-induced and RAD23-dependent stabilization of xeroderma pigmentosum group C protein. *Genes Dev.* 17: 1630-1645.

Rumiato E, Cavallin F, Boldrin E, Cagol M, et al. (2013). *ERCC1* C8092A (rs3212986) polymorphism as a predictive marker in esophageal cancer patients treated with cisplatin/5-FU-based neoadjuvant therapy. *Pharmacogenet. Genomics* 23: 597-604.

Stacey EM (2010). Sternberg's Diagnostic Surgical Pathology. 5th edn, Volume two. (Carter D, Greenson JK, Reuter VE, Stoler MH et al., eds.) Wolters Kluver, Lippincott Williams Wilkins, 1297-1201.

Stadtlander CT and Waderbor JW (1999). Molecular epidemiology, pathogenesis and prevention of gastric cancer. *Carcinogenesis* 20: 2195-2108.

Yang LM, Li XH and Bao CF (2012a). Glutathione S-transferase P1 and DNA polymorphisms influence response to chemotherapy and prognosis of bone tumors. *Asian Pac. J. Cancer Prev.* 13: 5883-5886.

Yang X, Takano Y and Zheng HC (2012b). The pathobiological features of gastrointestinal cancers (Review). *Oncol. Lett.* 3: 961-969.

Yang Y and Xian L (2014). The association between the *ERCC1/2* polymorphisms and the clinical outcomes of the platinum-based chemotherapy in non-small cell lung cancer (NSCLC): a systematic review and meta-analysis. *Tumour Biol.* 35: 2905-2921.

Yin M, Yan J, Martinez-Balibrea E, Graziano F, et al. (2011). *ERCC1* and *ERCC2* polymorphisms predict clinical outcomes of oxaliplatin-based chemotherapies in gastric and colorectal cancer: a systemic review and meta-analysis. *Clin. Cancer Res.* 17: 1632-1640.

Zhou J, Liu ZY, Li CB, Gao S, et al. (2014). Genetic polymorphisms of DNA repair pathways influence the response to chemotherapy and overall survival of gastric cancer. *Tumour Biol.* 36: 3017-3023.