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Polymorphisms of ERCC1, XPD, XRCC1 and XPG Predict Clinical Outcome in Advanced Gastric Cancer Patients Receiving Oxaliplatin-Based Chemotherapy in Chinese Population

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OBJECTIVE To investigate whether polymorphisms in ERCC1, XPD, XPG, XRCC1 genes are associated with clinical outcomes in advanced gastric cancer (AGC) patients treated with oxaliplatin-based chemotherapy.

METHODS The genetic polymorphisms in ERCC1, XPD, XPG, XRCC1 were determined in 94 advanced gastric cancer patients treated with oxaliplatin-based chemotherapy, using TaqMan-MGB probes. The clinical response of 60 patients with stage IV disease, time to progression (TTP) and overall survival (OS) of 94 patients were evaluated.

RESULTS The overall disease control rate (CR + PR + SD) of the 60 patients in stage IV was 70% (42/60). Patients with XRCC1 399 G/G, XPG 46 C/C genotypes showed enhanced response to the oxaliplatin-based chemotherapy compared to those with other genotypes ($P < 0.05$). The median OS and TTP of the patients were 5.5 months and 9.0 months, respectively. Among the 4 types of polymorphisms in the study, XRCC1 399 G/A + A/A, XPG 46 C/T + T/T genotypes were regarded to be associated with chemoresistance and poor survival ($P < 0.05$). Combination analysis of the 2 polymorphisms using the Kaplan–Meier method revealed that the TTP and OS of the patients with a number of risk genotypes were significantly shortened ($P < 0.05$). No significant association was found between the genotypes of the XPD codon 751, the ERCC1 codon 118 and the clinical outcome ($P > 0.05$).

CONCLUSION Testing for XRCC1 399, XPG 46 polymorphisms may allow identification of the gastric cancer patients who will benefit from oxaliplatin-based chemotherapy. Specific polymorphisms may influence clinical outcomes of AGC patients. Selecting specific chemotherapy based on pretreatment genotyping represents an innovative strategy that warrants prospective studies.

KEY WORDS: gastric cancer, polymorphism, oxaliplatin, chemotherapy, ERCC1, XPD, XPG, XRCC1.

Introduction

Gastric cancer is one of the most common malignant tumors accounting for approximately 930,000 new cases and 700,000 deaths annually worldwide[1,2]. Radical surgery has been the standard therapy with curative intent for the patients. Nonetheless, a relative proportion of patients present with metastasis or in advanced stages when diagnosed. A series of studies have indicated that chemotherapy can improve OS of the advanced gastric patients and quality of life in regards to the best supportive care (BSC)[3]. Recently, several ac-

tive agents such as oxaliplatin, taxanes, irinotecan, capecitabine have been introduced into gastric cancer therapy[4-6]. However, the response rates of the patients to these drugs or to the combination of the drugs are less than 50%[7-9]. Thus, the correct diagnosis of the subgroup of gastric cancer patients that truly benefit from specific regimens remains an open question. Rapid advances in genomics present the opportunity to establish a novel chemotherapeutic strategy and personalized medicine, which would allow the selection of optimal therapy and dose based on the molecular profile of a tumor and on the physical conditions of each patient. In other tumors, related biomarkers have been reported in some retrospective and perspective studies[10,11]. Nevertheless, few concerning pharmacogenetics in gastric cancer patients have been reported.

Oxaliplatin plays an important role in the treatment of gastric cancer. Those who have a poor response to oxaliplatin-based chemotherapy usually have a poor outcome. Important mechanisms of resistance to platinum drugs have been attributed to genetic polymorphisms in genes involved in DNA repair, drug metabolism and detoxification pathways. Oxaliplatin is a third-generation platinum compound which carries a 1,2-diamino-cyclohexane ring, leading to apoptosis, inhibiting cellular replication and interfering with RNA synthesis by forming intra- or inter-DNA-platinum adducts between adequate oxaliplatin derivatives and a DNA base. These can be counteracted by cellular defense mechanisms preventing DNA damage through increased detoxification of DNA-platinum adducts via DNA repair pathways leading to increased DNA-repair activity and improved removal of DNA-platinum adducts[12-14].

Some researches have suggested that with the decrease of DNA repair in a tumor, the removal of platinum-DNA adducts declines and therefore, the clinical response of patients to the drugs is increased[15]. In contrast to cisplatin, oxaliplatin-induced adducts are apparently not recognized or processed by mismatch repair but are repaired predominantly by the nucleotide excision repair pathway and the base excision repair pathway[16]. Excision cross-complementing (ERCC1), xeroderma pigmentosum group D (XPD), xeroderma pigmentosum group G (XPG) and X-ray repair cross-complementing group 1 (XRCC1) are the key genes in the NER and BER pathways respectively. Therefore, they may have the potential to influence the sensitivity of tumor cells to oxaliplatin[17]. Their functional single nucleotide polymorphisms (SNPs), for example, the ERCC1 Asn118Asn, XPD Lys751Gln and, XPG His43His XRCC1 Arg399Gln are considered to be predictive and prognostic factors for patients receiving oxaliplatin-based chemotherapy[15].

So far, the relationship between some polymorphisms of the above genes and the clinical outcome of gastric cancer patients receiving platinum has been largely explored in other tumors throughout the world[15,18-22]. Only fragmented information is available in Chinese patients with advanced gastric cancer. To investigate whether these polymorphisms influence the response to oxaliplatin-based chemotherapy and the survival of Chinese patients with AGC, we analyzed a panel of 4 genetic polymorphisms of ERCC1, XPD, XPG, and XRCC1.

Patients and Methods

Patients

The study population included 94 patients confirmed by histologic examination as advanced gastric cancer. The characteristics of the patients are presented in Table 1. From July 2007 to July 2009, 94 patients were enrolled, including 60 men (63.83%) and 34 women (36.17%) with a median age of 55 years (ranging from 32 to 77 years). Thirty-four (36.17%) advanced gastric cancer patients were in stage III and 60 (63.83%) in stage IV when diagnosed. The median follow-up time was 13.2 months (ranging from 3 months to 24 months). The study was conducted in the treatment and research center of oncology at the Affiliated Hospital of Medical College Qing Dao University. An informed consent was signed by all patients. The research was approved by the ethics committee at our institution.

Table 1. Clinical characteristics of the 94 patients.

Characteristics	*n*	%
Sex		
Male	60	64
Female	34	36
Age (years)		
≤ 55	50	53
> 55	44	47
Histotype		
Intestinal	56	60
Diffuse	38	40
Differentiation		
Well	10	11
Moderate	14	15
Poor	60	63
Signet-ring	10	11
Performance status (ECOG)*		
≤ 1	51	54
= 2	43	46
Stage		
IIIA	14	15
IIIB	20	21
IV	60	64

* Performance status was defined according to the Eastern Cooperative Oncology Group (ECOG) Criteria for Toxicity and Response.

Chemotherapy treatment

All patients were treated with at least 4 courses of oxaliplatin-based treatment, 40 of the patients with the modified FOLFOX 4 regimen and 54 with the XELOX regimen. The modified FOLFOX 4 regimen consisted of oxaliplatin 130 mg/m^2 given through intravenous infusion lasting for 2 h on day 1; leukovorin 130 mg/m^2 followed by 5-fluorouracil 300 mg/m^2 via intravenous drip lasting for 4 h on day 1- day 5 every 3 weeks. The XELOX regimen consisted of oxaliplatin 130 mg/m^2 administered via intravenous infusion lasting for 2 h on day 1; capecitabine 1250 mg/m^2, bid orally, on day 1-day 14 every 3 weeks. The treatment was given until disease progression or unacceptable toxicity occurred or when patients opted to discontinue the treatment.

Genotyping

Blood samples were obtained from each patient before chemotherapy and genomic DNA was extracted from 200 µl of whole blood using the Axygen Genomic DNA Purification Kit (Axygen Biotechnology, China). SNPs in ERCC1 Asn118Asn, XPDLys751Gln, XPG His46His and XRCC1 Arg399Gln were assessed using TaqMan SNP Genotyping Assays using a fluorescent temperature cycler (Rotor-Gene RG-3000 PCR, Australia). Briefly, the 25 µl PCR mixture contained DNA 40 ng,12.5 µl Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 1.25 µl 20 × Taqman SNP Genotyping Assay Mix (Applied Biosystems, Foster City, CA, USA), and 5.25 µl ultrapure water. The PCR conditions were 95°C for 10 min, followed by 48 cycles at 92°C for 15 s and at 60°C for 1 min. Sequences of primers and probes were designed by Applied Biosystems. A minimum of 20 randomly selected DNA samples were genotyped at least twice to confirm the results.

Statistical analysis

Evaluation criteria of solid tumors were adopted to evaluate the response of the patients to the treatments[14]. The time to progression (TTP) of the tumor was calculated from the start of chemotherapy to the first evidence of disease progression. The overall survival time was defined from the date of diagnosis to the date of the last follow-up or death from any cause. A combined analysis was planned if multiple genotypes showed a significant association. Each genotype was independently analyzed for correlation with response to chemotherapy and survival time. The relationship between genotype frequencies and clinical characteristics was assessed by Chi-square test or Fisher's exact tests (the *P* values for the two-sided exact significance are presented). The Kaplan-Meier method was adopted to estimate survival curves and the log-rank test was used to compare different survival time among patients with different genotypes. Univariate and multivariate analyses were conducted using Cox proportional hazard model to assess the importance of genotypes with adjustment for clinical and histopathologic features such as ECOG PS (Eastern Cooperative Oncology Group performance status), tumor stage or grade. All *P* values were two-sided. The value of $P < 0.05$ was regarded as statistically significant. All analyses were performed with the SPSS 16.0 software (SPSS Inc, Chicago, IL, USA).

Results

Genotype frequencies and polymorphisms of ERCC1, XPD, XPG and XRCC1

The genotyping results of ERCC1-118, XPD-751, XPG-46 and XRCC1-399 were available for all patients. The distribution of genotypes of the 4 polymorphisms is listed in Table 2. Genotype frequencies for ERCC1, XPD, XPG and XRCC1 polymorphisms were found consistent with the Hardy-Weinberg equilibrium. Each of these polymorphisms had no significant association in respective comparison with age, sex, ECOG status, tumor initial stage and grade. Polymorphisms of all the genes were successfully amplified in the DNA extracted from the samples.

Table 2. The distribution of genotypes.

Genotype	*n* (%)
ERCC1 codon 118 (C/C)	48 (51.06)
C/T	39 (41.49)
T/T	7 (7.45)
XPD codon 751 Lys/Lys (A/A)	77 (81.91)
Lys/Gln (A/C)	15 (15.96)
Gln/Gln (C/C)	2 (2.13)
XPG codon 46 (C/C)	48 (51.06)
C/T	36 (38.30)
T/T	10 (10.64)
XRCC1 codon 399 Arg/Arg (G/G)	51 (54.26)
Arg/Gln (G/A)	35 (37.24)
Gln/Gln (A/A)	8 (8.50)

Activity of chemotherapy and genotypes

The overall disease control rate (CR + PR + SD) of the gastric cancer patients was 70%. A statistically significant association was found between polymorphisms of XPG and *XRCC1* treated with oxaliplatin-based chemotherapy in stage IV patients. When patients were divided into responders and non-responders to the treatments (Table 3), a significantly different distribution of the XPG 46 and XRCC1 399 genotypes was observed. XPG 46C/T + T/T, XRCC1 399 G/A + A/A were overexpressed in unresponsive patients. Conversely, carriers of the XPG 46 C/C, XRCC1 399 G/G were prevalent in responsive patients (Table 3). Logistic regression analysis showed a significantly increased chance of treatment response in patients with genotypes of XPG 46 C/C, XRCC1 399 G/G (Table 3). ERCC1 Asn118Asn

and XPD Lys751Gln polymorphisms had no correlation with response. Combined analysis of polymorphisms of XRCC1 399 and XPD 751 showed that patients with at least 1 wild genotype demonstrated a significantly better response to the treatment compared with those without wild genotypes ($\chi^2 = 8.396$, $P = 0.015$). The gender, age, stage, differentiation and PS of patients were not related to the response to chemotherapy tested by logistic regression model.

Survival and genotypes

The median OS and TTP were 5.5 months and 9.0 months, respectively**.** Among the 4 studied polymorphisms, significantly inferior TTP and OS were found in XPG46C/T + T/T and XRCC1399G/A + A/A carriers. Polymorphisms of ERCC1 and XPD did not show a statistically significant survival difference between the patients with wild and variant genotypes. The Cox proportional hazards model was used to adjust for sex, stage, performance status, histotype, chemotherapy regimen and differentiation, and following this, survival differences were still found (Tables 4,5). Combinations of these risk genotypes were analyzed in the 94 patients. Forty-four patients (46.8%) did not show any of these risk genotypes (group 0), yet 10 patients (10.64%) showed 1 risk genotype (group 1), and 40 patients (42.55%) showed 2 risk genotypes (group 2). Patients with at least 1 risk genotype demonstrated significantly

Table 3. Response to chemotherapy according to genetic polymorphism.

Genotype	*n* (%)		Chi-Square Test		Logistic regression analysis		
	Responder	Nonresponder	χ^2	*P*	OR	95%CI	*P*
ERCC1 118 (C/C)	21 (67.74)	10 (32.26)					
C/T + T/T	21 (72.41)	8 (27.59)	0.156	0.782	0.792	0.253-2.486	0.690
XPD 751 (A/A)	34 (58.62)	14 (41.38)					
A/C + C/C	8 (66.67)	4 (33.33)	0.079	0.740	1.058	0.224-4.991	0.943
XPG 46 (C/C)	28 (87.50)	4 (12.50)					
C/T + T/T	14 (50.00)	14 (50.00)	10	0.002	7.000	1.940-25.255	0.003
XRCC1 399 (G/G)	27 (84.38)	5 (15.62)					
G/A + A/A	15 (53.57)	13 (46.43)	6.747	0.012	4.680	1.397-15.682	0.009

Frequency of the genotypes and their distribution in 42 patients with complete, partial response or stable disease (responders) and 18 with disease progression (nonresponders).

Table 4. The relationship between genotypes and time to progression of gastric cancer

Genotype	TTP Months (95%CI)	Log-rank test		Cox regression analysis		
		χ^2	*P*	HR	95%CI	*P*
ERCC1 118 (C/C)	5.5 (5.03, 5.97)					
C/T+ T/T	5.2 (4.78, 5.61)	0.065	0.799	1.084	0.704-1.669	0.715
XPD 751 (A/A)	5.2 (4.80, 5.60)					
A/C + C/C	6.5 (5.21, 5.79)	3.069	0.080	0.836	0.463-1.508	0.551
XPG 46 (C/C)	6.0 (5.07, 6.93)					
C/T+ T/T	5.0 (4.43, 5.57)	15.096	0.000	2.823	1.721-4.630	0.000
XRCC1 Arg399Gln (G/G)	6.4 (5.31, 7.49)					
G/A+A/A	4.7 (3.96, 5.44)	22.364	0.000	3.685	2.202-6.165	0.000

Table 5. The relationship between genotypes and overall survival of gastric cancer.

Genotype	Overall survival Months (95%CI)	Log-rank test		Cox regression analysis		
		χ^2	*P*	HR	95%CI	*P*
ERCC1 118 (C/C)	10.5 (8.01, 12.99)					
C/T+ T/T	8.0 (6.19, 9.81)	0.379	0.583	1.335	0.734-2.428	0.344
XPD 751 (A/A)	8.6 (6.84, 10.36)					
A/C +C/C	9.0 (6.86, 11.14)	1.232	0.267	0.637	0.283-1.433	0.252
XPG 46 (C/C)	11.1 (8.45, 13.75)					
C/T+ T/T	7.7 (6.46, 8.94)	5.716	0.017	2.064	1.119-3.806	0.019
XRCC1 399 (G/G)	10.5 (8.23, 12.77)					
G/A+A/A	7.0 (5.61, 8.39)	7.113	0.008	2.231	1.212-4.106	0.01

inferior OS (7.7 months) compared with those (11.1 months) without any risk genotype ($\chi^2 = 5.915$, $P = 0.015$). However, significant differences in survival were not detected between patients with 1 risk genotype and the patients with 2 risk genotypes ($\chi^2 = 0.036$, $P = 0.85$). Combined analysis of polymorphisms of XRCC1 399 and XPD 751, did not show significant differences in survival between patients with at least 1 wild genotype and the patients without a wild genotype ($\chi^2 = 4.234$, $P = 0.120$). The survival curve is shown in Figs.1,2.

Discussion

The results of the present study support the pharmacogenetic role of ERCC1, XRCC1, XPD and XPG polymorphisms in patients with advanced gastric cancer treated with oxaliplatin-based chemotherapy. To the best of our knowledge, some of the results in this study were consistent with results of previous studies and some results were not. Therefore, the development of future studies is a worthwhile undertaking.

The self-repairing capability of DNA is one of the most important factors which can affect genome stability. The DNA-repair system includes base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and DNA double-strand break repair (DDSBR) pathways[23] that can repair specific types of damage to DNA. Among a number of genes involved in the altered DNA-repair mechanisms, we selected ERCC1, XPD, XPG, and XRCC1, 3 of the key DNA-repair genes in the NER pathway and 1 in the BER pathway as the focus genes of this study because of their pivotal roles in the function of oxaliplatin.

The NER system is a major DNA-repair system in mammalian cells and plays a significant role in repair-

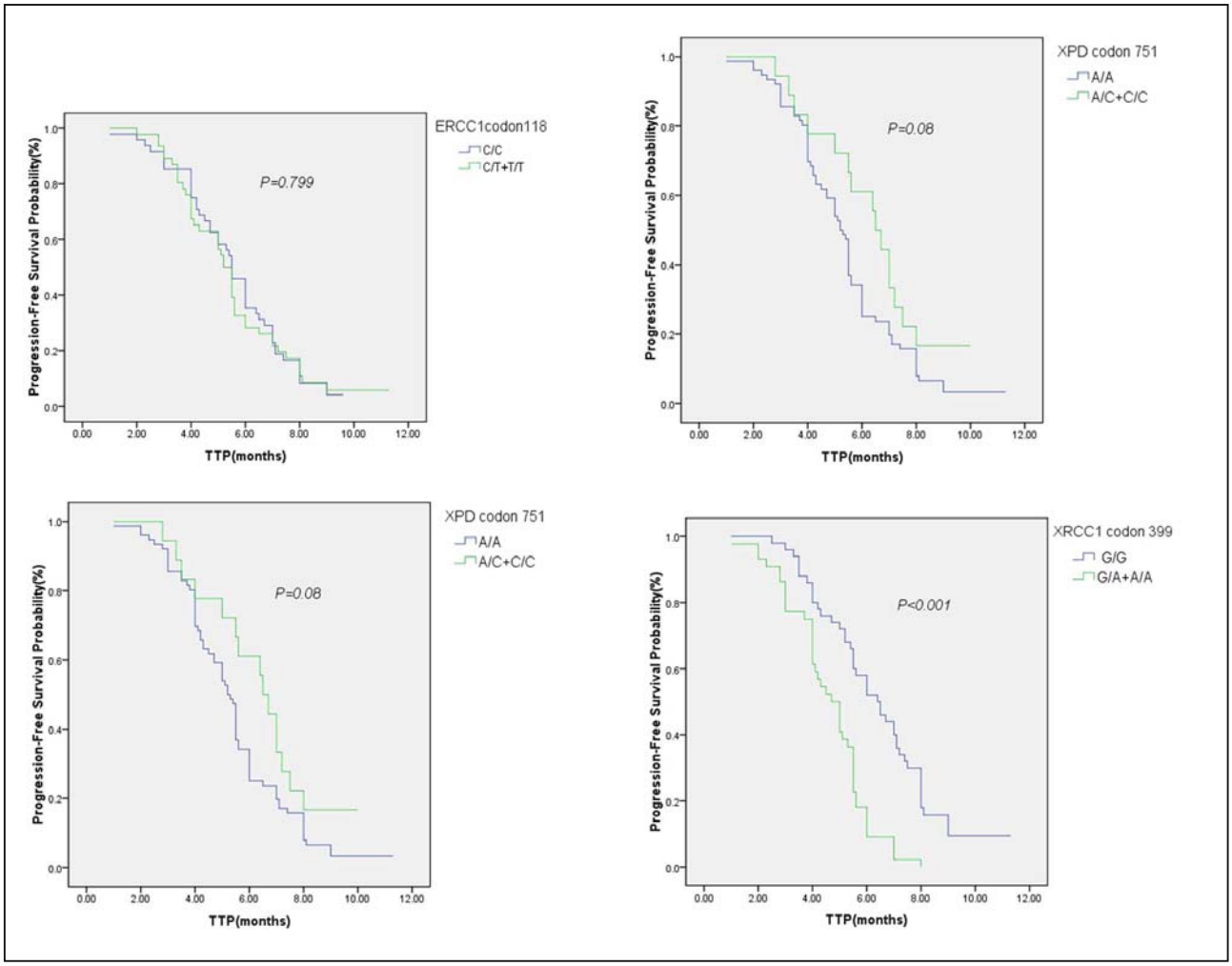

Fig.1. Kaplan–Meier curves of time to progression according to the genotypes of excision repair cross-complementation group 1 (ERCC1), xerodernm pigmentosum pomplementary group D (XPD), xerodernm pigmentosum pomplementary group G(XPG), X-ray repair cross-complementing group 1 (XRCC1). A-D Kaplan–Meier estimates of no progression by the ERCC1 codon 118, XPD codon 751,XPG codon 43, XRCC1 codon 399 genotypes, respectively.

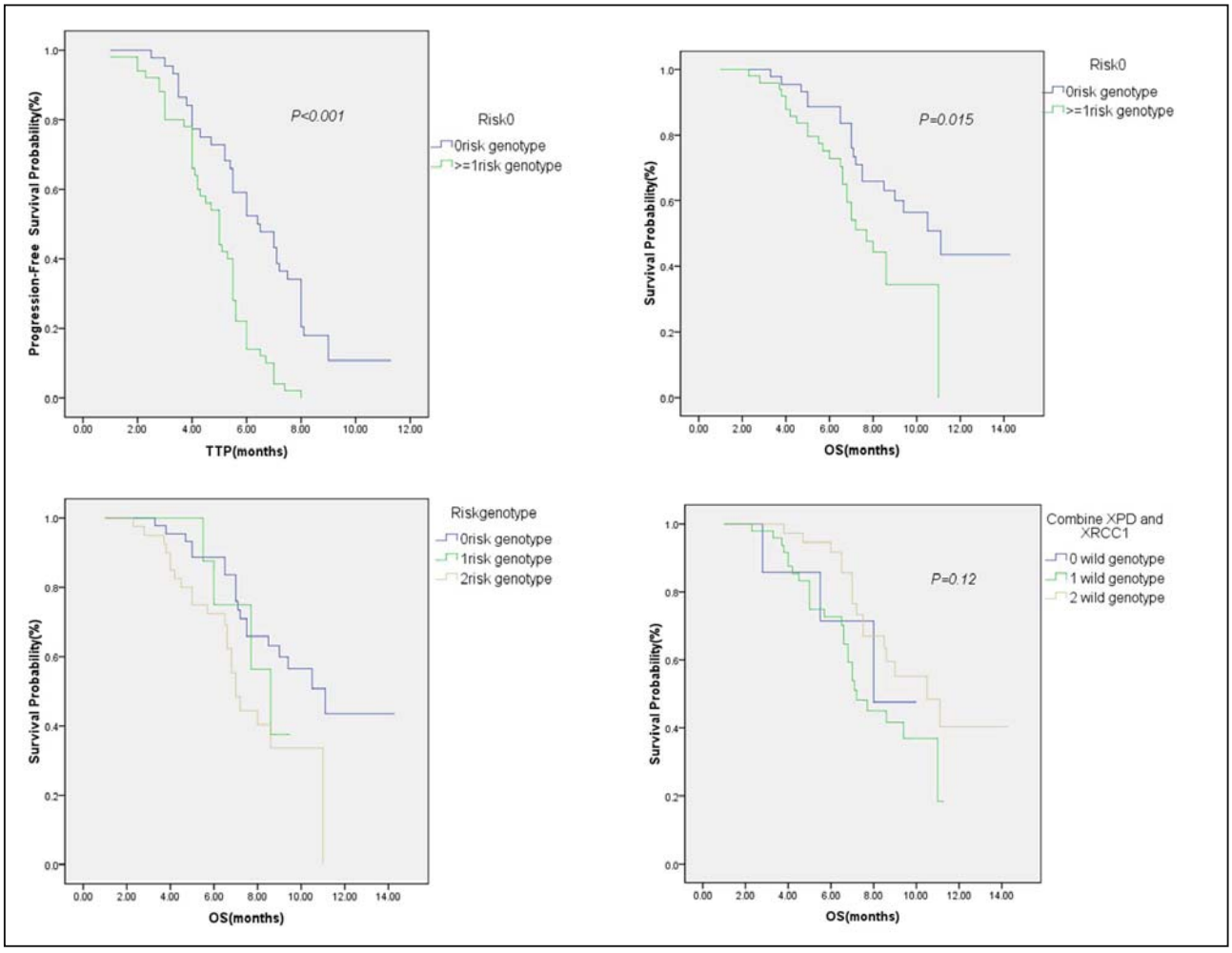

Fig.2. Survival time curves in carriers of 0 risk genotypes (44 patients), 1 risk genotype (10 patients), 2 risk genotypes (40 patients). Risk genotypes include XRCC1 399 G/A+A/A, XPG 46 C/T+T/T. A, TTP in patient carriers of at least 1 risk genotype and 0 risk genotype; B, OS in patient carriers of 1 risk genotype and 2 risk genotypes. C, OS of all the patients grouped into 0, 1, and 2 risk genotypes. D, Combined analysis OSs of XRCC1 399 and XPD 751, the patients grouped according to numbers carrying wild genotype.

ing a variety of damage in DNA, including platinum-induced DNA adducts. In fact, NER is the only known mechanism in mammalian cells for the removal of bulky, helix-distorting DNA adducts produced by platinum agents. ERCC1, XPD and XPG genes participate in the NER pathway.

High expression levels of the ERCC1 gene are associated with enhanced DNA-repair capacity, yielding decreased response and an impaired tolerance to platinum-based therapy[24]. In experimental models, the ERCC1 118T allele variant showed potential functional consequences, with a trend toward higher ERCC1mRNA levels than those observed in the presence of the ERCC1 118 C allele[24,25]. Further, the common C > T polymorphism in ERCC1 was associated with altered ERCC1 protein expression levels. An increasing number of T alleles is linked with the increased ERCC1 expression levels[26]. However, the clinical reports regarding the assumed relationship between the ERCC1 codon 118 polymorphism and clinical outcome after oxaliplatin-based therapy are controversial[12,21,23,27-33]. Studies[26,33] have shown that the ERCC1-118 T allele has been associated with an adverse prognostic effect, with the risk of progression[23] or with an insignificant yet increased risk of progression[33] in addition to a significant increase in response to chemotherapy[27]. In this study, no significant association was found between the ERCC1 118 polymorphism and response or survival, which is consistent with the results of other studies in advanced gastric cancer[34-36] and lung cancer[29]. As it still remains unclear what effect the T allele has on expression levels, further investigation is necessary to clarify this association.

XPD (also called ERCC2) is an ATP-dependent helicase that contributes to DNA unwinding, allowing for the removal of the damaged DNA. XPD levels correlate with deriving resistance to platinum in human tumor cell lines. The polymorphism of the XPD gene that is largely explored is located in exon 23 (A > C) and results in a

Lys751Gln polymorphism. At present, most of reports suggest that carrying XPD751Lys/Lys is associated with a better response and a longer survival[19,33]. Our results show that the XPD Lys751Gln polymorphism does not correlate with clinical outcome, which coincides with the conclusion of some prior studies of advanced NSCLC (non-small cell lung cancer)[37], colorectal cancer[38] and gastric cancer[34,36]. Intriguingly, Rosell et al.[20] reported no significant association between the XPD-751 polymorphism and overall survival in NSCLC patients in 2004. But recently a large sample study[31] showed a significantly high risk of death in patients with the C/C genotype. However, a few C/C genotypes were detected in 94 patients. The distribution of XPD genotypes among the population of the USA, Europe and Asia is not equal[39]. These data imply that the polymorphisms might differ according to racial background, which was presumed to explain the different conclusions in the previous reports.

XPG (also called ERCC5) is responsible for a 1186 amino acid structure-specific endonuclease, the activity of which is essential for the 2 incision steps in NER. In human cells, XPG catalyzes an incision approximately 5 nucleotides 3' to the site of damage and is also involved non-enzymatically in the subsequent 5' incision[40]. It is further involved in the stabilization of a pre-incision complex on the damaged DNA. The association between the risk of lung cancer and polymorphisms of the XPG His46His has been reported[41]. Nevertheless, reports regarding the relationship between XPG His46His and clinical outcome are quite sparse. Saldivar et al.[42] et al genotyped 146 cases of advanced epithelial ovarian cancer in women with the XPG 46 T/T allele and found they had significantly shorter median survival (8.3 months, $P = 0.006$) compared with women with the homozygous XPG 46C/C allele (24.6 months). Monzo et al.[43] examined SNPs in 42 advanced colorectal cancer patients treated with first-line oxaliplatin/fluoropyrimidine. Patients with XPG 46 C/C genotype had a longer survival ($P = 0.001$) and TTP ($P = 0.009$) than patients with XPG 46 C/T or T/T genotypes. We have found that the XPG 46C/C genotype is associated with superior response and longer TTP in gastric cancer for the first time, which is agreement with the results of previous studies.

The XRCC1 gene encodes a protein that plays a prominent role in BER to efficiently repair DNA damage caused by ionizing radiation, oxidative stress, and DNA alkylating agents. XRCC1 acts as a facilitator or coordinator in BER through its interaction with poly adenosine diphosphate ribose polymerase (PARP), DNA polymerase β and DNA ligase III. Several studies have reported the association of XRCC1 with the risk in lung cancer[44], colorectal cancer[45] and prostate cancer[46]. The most commonly coding polymorphisms in the XRCC1 gene is at codon 399 (Arg to Gln), which affects drug sensitivity to oxaliplatin.

Suh et al.[47] reported that an improved survival was seen in patients with XRCC1-399 G allele receiving 5-FU/oxaplatin chemotherapy. Similar results were demonstrated in lung cancer[31], breast cancer[48] and esophageal cancer[49]. Huang et al.[36] found that in patients with gastric cancer treated with oxaliplatin-based adjuvant chemotherapy, those with the XRCC1-399 G/G genotype had longer relapse-free survival (RFS) and overall survival (OS) than those with XRCC1 A/A and A/G genotypes. However, Ruzzo et al.[34] and Keam et al.[35]found that the XRCC1 codon 399 polymorphism did not have any correlation with the response to treatment and overall survival in gastric cancer. The results in our study are in agreement with the current understanding of XRCC1 involvement in platinum compound-based chemotherapy. Our findings showed that XRCC1-399 G/G enhanced cancer chemosensitivity to oxaliplatin-based chemotherapy and prolonged TTP in advanced gastric cancer patients. Although it is still unclear how the change of amino acid at codon 399 of the XRCC1 gene polymorphism influences clinical outcome to oxaliplatin-based chemotherapy in gastric cancer patients, the results support the hypothesis that the improved clinical outcome is due to the enhancement of the DNA repair capacity of the tumor cells.

In brief, the studies about the relationship between the polymorphisms of DNA-repair genes mentioned previously and clinical outcome are not completely consistent. Among the 4 pathways of DNA repair operating on damaged DNA, base excision repair (BER) operates on small lesions, while the NER pathway repairs bulk lesions. From this aspect, it seems that the BER mechanism should be less involved than the NER in platinum damage repair. However, in the present study, XPG and XRCC1 have been found to have an association with clinical outcome whereas the polymorphisms in ERCC1 and XPD, the important genes in NER, failed to show any significant relationship with the response chemotherapy. Possible explanations are listed as follows: *i*) There are multiple genes participating in DNA repair, and they act with each other. A single gene does not complete the entire repair, which can explain why examining 2 gene polymorphisms simultaneously can indicate the chemosensitivity. *ii*) DNA repair has been termed a double-edged sword because decreased DNA repair may increase the risk of developing cancer, although it might simultaneously improve survival in patients already diagnosed with cancer and treated with platinum agents. *iii*) As SNPs display ethnic variations, the response and survival might differ in different racial backgrounds. *iv*) Our study was developed using peripheral blood, so the difference of genetic polymorphisms between blood and solid tissue specimens may also be contributive to the conflicting results.

From a genetic perspective, in most multifactorial diseases, single polymorphisms in a single gene are unlikely to alter the expression or function of specific pro-

teins to the extent of leading to a pathologic phenotype. It is more likely that the combined effect of different SNPs in a gene result in changes in expression or protein function. Based on individual results of these polymorphisms, we further analyzed clinical outcomes according to the numbers of favorable genotypes. We demonstrate that a patient's benefit from oxaliplatin-based chemotherapy is significantly increased with the number of favorable genotypes. A combined analysis may more accurately identify patients who will attain the maximum benefit from oxaliplatin-based chemotherapy.

Several limitations of our study must be acknowledged. First of all, as a retrospective study, the evaluation of clinical response and time to progression is often imprecise. However, measuring clinical response and time to progression may be critical for further elucidating the mechanisms by which DNA repair affects outcome. These parameters can distinguish whether these gene polymorphisms are the prognostic factors that can predict the response of the patients to the treatment or are prognostic by determining outcome. Ideally, prospective validation studies should be carried out to measure these additional endpoints. Second, the number of the cases in this study was small, and it may be a limitation in generalizing to advanced gastric cancers as a whole. Third, chemotherapeutic regimens were not equal. If the same chemotherapeutic regimen had been used in treatment, the predictive role of XRCC1 polymorphism would have been attainable.

In conclusion, our study shows that the polymorphisms of XRCC1-399 and XPG-46 appear to be independent predictive and prognostic factors in gastric cancer patients treated with oxaliplatin-based chemotherapy. The new trend emerging from current studies is to adopt a multigene strategy, in which a more complex interplay of different allelic variants harbored by the patients, as opposed to a single genetic variation, will give a complete outline for better therapy tailored to each individual patient with gastric cancer. Ultimately, the benefits of pharmacogenetic profiling of patients need to be proven in prospective clinical trials.

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