RESEARCH ARTICLE

Prediction Role of Seven SNPs of DNA Repair Genes for Survival of Gastric Cancer Patients Receiving Chemotherapy

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Abstract

We aimed to investigate DNA repair gene expression of response to chemotherapy among gastric patients, and roles in the prognosis of gastric cancer. A total of 209 gastric cancer patients were included in this study between January 2007 and December 2008, all treated with chemotherapy. Polymorphisms were detected by real time PCR with TaqMan probes, and genomic DNA was extracted from peripheral blood samples. The overall response rate was 61.2%. The median progression and overall survivals were 8.5 and 18.7 months, respectively. A significant increased treatment response was found among patients with XPG C/T+T/T or XRCC1 399G/A+A/A genotypes, with the OR (95% CI) of 2.14 (1.15-4.01) and 1.75 (1.04-3.35) respectively. We found XPG C/T+T/T and XRCC1 399 G/A+A/A were associated with a longer survival among gastric cancer patients when compared with their wide type genotypes, with HRs and 95% CIs of 0.49 (0.27-0.89) and 0.56 (0.29-0.98) respectively. Selecting specific chemotherapy based on pretreatment genotyping may be an innovative strategy for further studies.

Keywords: DNA repair genes - SNPs - survival - gastric cancer - chemotherapy

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Introduction

Gastric cancer is the most frequently occurring malignancy occurring malignancy in China, and it is one of the main causes of cancer death (IARC, 2008). The prognosis of advanced gastric cancer is poor, and it is estimated the median survival time ranges from approximately from 7.5 to 12 months (Parkin, 2001). Surgery is the primary modality for managing early-stage and locally advanced diseases. However, most of the gastric cancer patients would develop local or distance recurrence after surgery. The increasing evidence show the adjuvant chemotherapy is the standard treatment for patients with resectable gastric cancer after surgery. 5-fluorouracil (5-FU) remains the main chemotherapeutic agent for the treatment of gastric cancer, and combination chemotherapy with 5-FU has shown an improved clinical outcomes (Wohrer et al., 2004). Oxaliplatin, another platinum based agent, has a more favorable tolerability profile than cisplatin, and the combination of 5-FU and oxaliplatin may show a higher efficacy and lower toxicity. A increased number of evidences suggest that inter-individual variation in drug-metabolizing enzymes, nucleotide excision repair (NER) and base excision repair (BER) system may affect anticancer drug efficacy by influencing DNA repair or related enzyme activities (Ichikawa, 2006). Excision cross-complementing (ERCC1), xeroderma pigmentosum group D (XPD), xeroderma pigmentosum group G (XPG) and X-ray repair cross complementing group 1 (XRCC1) as well as X-ray repair cross complementing group 3 (XRCC3) are the key genes in the NER and BER pathways respectively. Therefore, these genes might have a potential role in the sensitivity of tumor cells to 5-FU and oxaliplatin (Weaver et al., 2005). The functional single nucleotide polymorphisms (SNPs) of ERCC1, XPD, XPG, XRCC1 and XRCC3 genes may be considered to be predictive and prognostic factors for patients receiving 5-FU and oxaliplatin chemotherapy.

The association between polymorphisms in DNA repaired genes and clinical outcome of gastric cancer receiving 5-FU and oxaliplatin chemotherapy has been studies in various cancers worldwide (Gurubhagavatula et al., 2004; Isla et al., 2004; Quintela-Fandino et al., 2006). Few studies are available in Chinese patients with advanced gastric cancer. Therefore, our study aimed to investigate the role seven SNPs of DNA repaired genes on the response to combination effect of 5-FU and oxaliplatin chemotherapy and clinical outcome of advanced gastric cancer.

Materials and Methods

Subjects

A total of 209 patients with advanced gastric cancer were confirmed by histologically examination, and

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collected from our hospital between March 2007 and January 2009. Included patients were aged at the range of 34 and 78 years. Patients had no prior chemotherapy except adjuvant chemotherapy administered more than 6 months previously, and they had an adequate performance status (0 to 2 according to the eastern Cooperative Oncology Group scale), adequate bone marrow, hepatic, and renal functions. All patients were administered a modified FOLFOX-6 regimen composed of 2 hours infusion of oxaliplatin (100 mg/m^2) and folinic acid (100 mg/m^2), followed by 46 hour continous infusion of 5-FU (2,400 mg/m^2), as a first-line palliative chemotherapy. Treatment was repeated every 2 weeks until disease progression, patient refusal or unacceptable adverse reactions. Patients were subsequently grouped as responders (complete+partial response) or nonresponders (stable+progressive disease).

Sample collection and SNP genotyping

3 ml blood were sampled at the time before treatment and stored at -70°C until analysis. DNA was extracted from these samples by using the Qiagen Blood Kit (Qiagen, Chastworth, CA). Genomic DNA was extracted using a Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's protocol. Genotyping of the seven genes was conducted with TaqMan Gene Expression assays using the ABI PRISM®7900HT Sequence Detection System (Applied Biosystems, Poster City, CA). Briefly, the 25 μl PCR mixture contained DNA 40 ng, 12.5 μl Taqman Universal PCR Master Mix, 1.25 μl 20 × Taqman SNP Genotyping Assay Mix, 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% of DNA samples were randomly selected and were genotyped at least twice to confirm the results. These results of the quality control analysis confirmed 100% concordance.

Table 1. Characteristics of Patients with Advanced Gastric Cancer

Characteristics	Numbers of patients	%
Gender		
Male	133	63.64
Female	76	36.36
Median age (range), years	57.5 (34-78)	
Histological type		
Intestinal	116	55.5
Diffuse	87	41.63
Unknown	6	2.87
Differentiation		
Well	24	11.48
Moderate	36	17.22
Poor	129	61.72
Unknown	20	9.57
Performance status (ECOG)[1]		
≤1	121	57.89
2	88	42.11
Stage		
IIIA	37	17.7
IIIB	43	20.57
IV	130	62.2
Metastatic site		
Liver	83	39.71
Peritoneum	81	38.76
Distant M1 node	64	30.62
Other	44	21.05

[1]Performance status was defined according to the Eastern Cooperative Oncology Group (ECOG) Criteria for Toxicity and response

Statistical analysis

Descriptive data for the major characteristics of study groups were expressed as mean and percent. Pearson's 2×2 χ^2-test (gender) and independent sample t-test (mean age) were used for analysis the differences of several qualitative and quantitative data. The correlation between expression of seven NSPs and the response to chemotherapy was examined by using Chi-square test or Fisher's exact test. Logistic regression analysis was used to calculate the odds ratios and their confidence intervals (CI). The Cox proportional hazards model was used to compare overall survival (OS) with the seven SNPs. OS was calculated from the day of diagnosis to the data of death or the last contact. A primary death from gastric cancer was defined as a failure event. Logistic regression test and Cox multivariate analysis were analyzed after adjusting for age, gender, performance status, histology and number of metastatic sites. All statistical analysis was calculated by SPSS 11.0 with a significance level of P equally to 0.05.

Results

Among 209 patients in our studies, 133 patients were men (63.64%), and 76 patients were women (36.36%). The median age was 57.5 years (range 34-78 years). The patients' characteristics were presented in Table 1.

The distribution of genotypes of the seven SNPs for ERCC1, XPD, XPG, XRCC1 and XRCC3 were showed in Table 2. Genotype frequencies for the polymorphisms in the seven genes were consistent with the Hardy-Weinberg equilibrium. Among 209 patients, 128 of them showed response to chemotherapy. Individuals with XPG C/T+T/T were prevalent in responsive patients, and XRCC1 399G/A+A/A was overexposed in responsive patients. Logistic regression analysis showed a significant increased risk of treatment response in patients with XPG C/T+T/T or XRCC1 399G/A+A/A genotypes, with the OR(95% CI) of 2.14(1.15-4.01) and 1.75(1.04-3.35), respectively. However, ERCC1 Asn118Asn, ERCC1 Gln504Lys, XPD Asp312Asn, XPD Lys751Gln, XRCC1 Arg194Trp and XRCC3 Thr241Met had no correlation with response.

The median overall survival time was 18.7 months with a median follow-up period of 28.47 months (range 2 months to 39 months). Among the seven SNPs, significant better overall survival was found in XPG C/T+T/T and XRCC1 399 G/A+A/A carriers. However, polymorphisms in ERCC1 Asn118Asn, XPD Asp312Asn, XPD Lys751Gln, XRCC1 Arg194Trp and XRCC3 Thr241Met did not show a statistically significant survival difference between the patients with wild and variant genotypes. The Cox proportional hazards model was used to adjusted the age, sex, histological type, ECOG, stage and metastatic site as well as differentiation, and the HRs

Table 2. Response to Chemotherapy According to Eight SNPs

Genotype	Total frequencies		Responder		Nonresponder		Logistic regression analysis	
	N=209	%	N=128	%	N=81	%	OR (95%CI)[1]	P value
ERCC1 Asn118Asn								
C/C	93	44.5	62	48.44	41	50.62	-	-
C/T+ T/T	116	55.5	66	51.56	40	49.38	1.09(0.60-1.98)	0.75
ERCC1 Gln504Lys								
G/G	112	53.59	72	56.25	45	55.56	-	-
G/T+T/T	97	46.41	56	43.75	36	44.44	0.97(0.53-1.77)	0.92
XPD Asp312Asn								
G/G	112	53.59	72	56.25	47	58.02	-	-
G/T+T/T	97	46.41	56	43.75	34	41.98	1.07(0.59-1.96)	0.81
XPD Lys751Gln								
G/G	107	51.2	57	44.53	42	51.85	-	-
G/A+A/A	102	48.8	71	55.47	39	48.15	1.42(0.78-2.56)	0.3
XPG His1104Asp								
C/C	89	42.58	36	28.13	37	45.68	-	-
C/T+T/T	120	57.42	92	71.88	44	54.32	2.14(1.15-4.01)	<0.05
XRCC1 Arg194Trp								
C/C	132	63.16	79	61.72	50	61.73	-	-
C/T+T/T	77	36.84	49	38.28	31	38.27	1.0(0.54-1.85)	0.99
XRCC1 Arg399Gln								
G/G	103	49.28	51	39.84	42	51.85	-	-
G/A+A/A	106	50.72	77	60.16	39	48.15	1.75(1.04-3.35)	<0.05
XRCC3 Thr241Met								
C/C	99	47.37	54	42.19	40	49.38	-	-
C/T+T/T	110	52.63	74	57.81	41	50.62	1.24(0.74-2.43)	0.31

[1]Adjusted for sex, age, histological type, differentiation, stage and metastatic site

(95% CI) of XPG C/T+T/T and XRCC1 399 G/A+A/A carriers were 0.49 (0.27-0.89) and 0.56 (0.29-0.98), respectively.

Discussion

The results of our study support the pharmacogenetic role of seven SNPs of ERCC1, XPD, XPG, XRCC1 and XRCC3 in patients with advanced gastric cancer treated with 5-FU and oxaliplatin chemotherapy.

It is reported that XPG (ERCC5) is responsible for an 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 (Kiyohara and Yoshimasu, 2007). XPD is involved in the stabilization of a pre-incision complex of the damaged DNA. Previous studies reported the association between polymorphisms in XPG and various cancers (Yoon et al., 2011; He et al., 2012; Ma et al., 2012). However, few studies were reported on the relationship between XPG His46His and clinical outcome are quite sparse. Saldivar et al. reported 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) (Saldivar et al., 2007). Monzo et al. examined SNPs in 42 advanced colorectal cancer patients treated with first-line oxaliplatin/fluoropyrimidine (Monzo et al., 2007). 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+T/T genotypes (Monzo et al., 2007). We have found that the XPG C/T+T/T genotype is associated with superior response and longer overall survival in gastric cancer, which is agreement with the results of previous studies.

The present study indicated XRCC1 399 G/A+A/A was significantly associated with increased survival of advanced gastric cancer with chemotherapy. Similar results were found in various cancers with chemotherapy, including lung cancer (de las Peñas et al., 2006), breast cancer (Bewick et al., 2006) and esophageal cancer (Wu et al., 2006). Liu et al. (2011) 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. (2006) and Keam et al.(2008) found that the XRCC1 codon 399 polymorphism did not have any correlation with the response to treatment and overall survival in gastric cancer. Our findings indicated XRCC1-399 G/G enhanced cancer chemosensitivity 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 conclusion, our study shows that the polymorphisms of XPG His1104Asp and XRCC1 Arg399Gln appear to be independent predictive and prognostic factors in gastric cancer patients treated chemotherapy. It is suggested that selecting specific chemotherapy based on pretreatment

genotyping is an innovative strategy for further studies.

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