RESEARCH ARTICLE

Association Between Polymorphisms of XRCC1 Arg399Gln and XPD Lys751Gln Genes and Prognosis of Colorectal Cancer in a Chinese Population

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Abstract

We conducted this study to detect associations between XRCC1 Arg399Gln and XPD Lys751Gln genotypes and survival of colorectal cancer patients treated with 5-FU/oxalipatin chemotherapy. We included 289 Chinese patients with advanced colorectal cancer, who had received 5-FU/oxalipatin chemotherapy as first-line treatment from January 2005 to January 2007. All patients were followed up till Nov. 2011. Genotyping for XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms was based upon duplex polymerase-chain-reaction with the PCR-RFLP method. In our study, we found the XRCC1 399 Gln/Gln genotype to confer significantly higher rates of response to chemotherapy when compared to the Arg/Arg genotype [OR (95% CI)= 2.56(1.57-2.55)]. patients with the XPD 751 Gln/Gln genotype had significantly higher rates of response to chemotherapy [OR (95% CI)= 1.54(0.87-2.65)] and those with the XRCC1 399 Gln/Gln genotype had a longer average survival time and significantly lower risk of death than did those with the Arg/Arg genotype [HR (95% CI)= 0.66(0.36-0.95)]. Similarly, those carrying the XPD 751Gln/Gln genotype had 0.51-fold the risk of death of those with XPD 751Lys/Lys [HR (95% CI)= 0.51(0.33 -0.94)]. In conclusion, it is suggested that the XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms should be routinely assessed to determine colorectal patients who are more likely to benefit from 5-FU/oxalipatin chemotherapy.

Keywords: XRCC1 Arg399Gln - XPD Lys751Gln - polymorphisms - colorectal cancer - chemotherapy - response

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Introduction

Colorectal cancer is the third most common cancer diagnosed in the world and the leading cause of cancer death in western industrialized countries (IARC, 2008). Genetic susceptibility to this disease may result from inherited mutations in genes involved in carcinogenesis. Deficit of adequate function of DNA repair gene could accelerate genetic instability and the rate of genetic change, and thus enhance the probability of carcinogenesis (Mohrenweiser and Jones, 1998; Shilds and Harris, 2000). Moreover, these DNA repair genes are reported that they have a role in the prognosis of colorectal cancer. For advanced colorectal cancer, oxaliplatin, a cytotoxic platinum compound, is one cytotoxic platinum compound, is one of the most important chemotherapeutic agents for treating advanced colorectal cancer patients. By causing intrastrand cross-links in DNA, oxaliplatin results in structural DNA damage and apoptosis of tumor cells (Raymond et al., 1998). At least four pathways have been postulated for repairing DNA damage, among which the nucleotide excision repair (NER) pathway plays a major role in repairing platinum-DNA lesions, and counteracts against platinum effects in various tumor cells (Saldivar et al., 2007). Overexpression and polymorphisms of genes involved in the NER pathway result in resistance to platinum-based chemotherapy in variety of malignant diseases. Single nucleotide polymorphism of genes involved in the NER pathway affects DNA repair capacity, and therefore, influences the prognosis of malignant diseases (Zhou et al., 2004; Handra-Luca et al., 2007; McWilliams et al., 2008; Shore et al., 2008; Chang et al., 2009).

XRCC1 is a base excision repair and single strand break repair protein that may play an important role in resistance to variety of DNA damaging agents. In our previous studies, we found the XRCC1 is related to colorectal cancer susceptibility, and we may hypothesis this type of gene may influence the survival of colorectal cancer (Chang-Claude et al., 2005). A SNP in the XRCC1 gene, consisting of a nucleotide substitution of G to A, designated as XRCC1-01, results in an Arg to Gln amino acid change at codon 399. Although the functional consequences of this polymorphism are unknown, it may affect several protein-protein interactions (Zhao et al., 2012). In vitro, tumor cell lines homozygous for the XRCC1-01 AA genotype are more resistant to a diverse array of anti-cancer and cytotoxic drugs compared with the AG or the GG (least resistant) variants. These include alkylating agents such as busulfan, thiotepa, carboplatin, and cisplatin; DNA/RNA antimetabolites such as fluorouracil; and antimitotics such as vinblastine (Lunn

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et al., 1999).

Xeroderma pigmentosum group D(XPD), also known as the excision repair cross-complementing group 2(ERCC2), possessed both single- single-strand DNA-dependant adenosine triphosphate enzyme (ATPase) and 5'-3' DNA helicase activities and is thought to participate in DNA unwinding during NER and transcription (Sung et al., 1993; Hoeijmakers et al., 1996). XPD is an important component of the NER pathway and is capable of reversing ioizing radiation-induced damage and DNA damage by chemotherapy (Parshad et al., 1993; Schaeffer et al., 1994). One common nucleotide polymorphism at codon 751 of XPD results in lysine to glutamine substitution has been proposed to predict responses as well as survival to platinum-based chemotherapy in colorectal cancer risk (Park et al., 2001).

However, there were inconsistence results in the influence of XRCC1 Arg399Gln and XPD Lys751Gln on the survival of several cancers (Chang-Claude et al., 2005; Zhao et al., 2012). Therefore, whether these polymorphisms may influence the susceptibility to 5-FU/oxalipatin chemotherapy in colorectal cancer is interested. Therefore, we conducted this study to detect the association between the association of XRCC1 Arg399Gln and XPD Lys751Gln genotypes and survival of colorectal cancer patients treated with 5-FU/oxalipatin chemotherapy.

Materials and Methods

Patients

We included 327 Chinese patients with advanced colorectal cancer, who had received 5-FU/oxalipatin chemotherapy as first-line treatment from January 2005 to January 2007. Among them, 289 patients were enrolled and analyzed. The remainders were excluded due to dying before blood sampling, unwilling to participate or loss of follow-up. The FOLFOX regimen consisted of a 2-week cycle of oxaliplatin (85mg/m^2) and leucovorin (LV) (200 mg/m^2), before bolus 5-FU (300mg/m^2), and continuous infusion of 5-FU (600mg/m^2).

The response of treatment were evaluated on the basis of standard response evaluation criteria in Solid Tumors(RECIST) criteria. Patients were subsequently grouped as responders (complete+partial response) or nonresponders (stable+progressive disease). Cases with secondary or recurrent tumors were excluded. All patients were followed up till Nov. 2011. The institutional review board approved this study and informed consent was given by all patients before blood testing for genotyping.

Examination of the XRCC1 and XPD gene polymorphisms

The DNA samples were obtained from stored blood samples using the Qiagen Blood Kit (Qiagen, Chastworth, CA). Genotyping for XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms was based upon duplex polymerase-chain-reaction with the PCR-RFLP method. The primer sequences of XRCC1 Arg399Gln gene were 5'-GAACTCCCTGAAAAGCTAAAGC-3' and 5'-GTTGGGCTCAAATATACGGTGG-3'. The primers for the XPD Lys751Gln gene were 5' GCC CGC TCT GGA TTA TAC G 3' and 5' CTA TCA TCT CCT GGC CCC C 3'. Polymerase chain reaction conditions were used as follows: an initial melting step of 5 min at 94°C; 35 cycles of denaturation for 30 s at 94°C; annealing for 30 s at 55°C; extension for 45 s at 72°C, followed by a 5 min final extension at 72°C. We also performed the genotyping of internal positive control samples, use of no template controls, and use of replicates for 10% samples for quality control. These results of the quality control analysis confirmed 100% concordance.

Statistical analysis

Statistical analysis was performed by using SPSS version 16.0 statistical software (SPSS, Chicago, IL, USA). The 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 association of polymorphisms of XRCC1 Arg399Gln and XPD Lys751Gln with response to chemotherapy in colorectal cancer patients were calculated by odds ratios(OR). The odds ratio was expressed with a corresponding 95% confidence interval (CI). The relative risk [hazard ratio (HR)] and 95% CI were calculated with the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). A primary death from colorectal cancer was defined as a failure event, and the survival time was defined as the time between diagnosis and death. The cause of death was defined by specialists based on clinical documents and reports by patients' family members. If a patient died from a cause other than colorectal cancer, her data was censored at the date of death. Statistical significance was set at $P<0.05$ and all tests were two-sides.

Results

The clinical features of 289 colorectal cancer patients are summarized in Table 1. The median age at diagnosis

Table 1. Characteristics of the Cases and Control in Our Study

Characteristics	Responders n=183	%	Nonresponders n=106	%	P value
Sex					
Male	116	63.5	68	64.4	
Female	67	36.5	38	35.6	0.93
Mean age (years)	51.5±8.7		57.5±9.4		<0.05
BMI					
<18.5	55	30.3	33	31.5	
18.5-23.9	59	32.5	36	33.7	
≥24	68	37.2	37	34.8	0.91
UICC TNM stage					
I	44	24.2	27	25.3	
II	50	27.5	29	27.6	
III	49	26.6	27	25.7	
IV	40	21.7	23	21.4	0.993
Distant metastasis					
M-	140	76.4	79	74.3	
M+	43	23.6	27	25.7	0.689
Localisation					
Rectum	95	51.7	50	47.3	
Colon	88	48.3	56	52.7	0.44

Table 2. Genotype Characteristics of the Two SNPs

Single nucleotide polymorphism	Major/minor Alleles	MAF[a] Case	MAF[a] From dbSNP	HWE (P value)[b] Case
XRCC1 Arg399Gln (rs25487)	Arg/Gln	0.33	0.26	0.17
XPD Lys751Gln (rs13181)	Lys/Gln	0.2	0.24	0.23

Table 3. Distribution of XPD and XRCC1 in Responders and Non-responders to Chemotherapy for Colorectal Cancer

Single nucleotide polymorphism	Responders n=183	%	Nonresponders n=106	%	Odds ratio (95% CI)[1]
XRCC1 Arg399Gln(rs25487)					
Arg/Arg	95	51.7	59	55.5	1
Arg/Gln	56	30.6	35	33.3	1.03(0.56-1.71)
Gln/Gln	32	17.7	12	11.2	1.84(1.07-3.98)
Gln allele	60	33	30	27.9	2.56(1.57-2.55)
XPD Lys751Gln (rs13181)					
Lys/Lys	87	47.6	58	54.7	1
Lys/Gln	79	43.3	43	40.4	1.31(0.77-2.06)
Gln/Gln	17	9.1	5	4.9	2.67(1.21-8.05)
Gln allele	56	30.75	27	25.1	1.54(0.87-2.65)

[1]Adjusted for age, sex, BMI, UICC TNM stage, Distant metastasis and Localisation

Table 4. Hazard Ratios for Overall Survival in Colorectal Cancer Patients with Chemotherapy

Genotypes	N	%	Median Survival time (months)	HR (95% CI)[1]
XRCC1 Arg399Gln(rs25487)				
Arg/Arg	149	51.7	32.3	1
Arg/Gln	88	30.6	33.6	0.85(0.51-1.23)
Gln/Gln	51	17.7	37.7	0.66(0.36-0.95)
Gln allele	191	3335.60.73(0.42-1.43)		
XPD Lys751Gln (rs13181)				
Lys/Lys	138	47.6	31.4	1
Lys/Gln	125	43.3	34.5	0.91(0.66-1.87)
Gln/Gln	26	9.1	36.1	0.51(0.33 -0.94)
Gln allele	89	30.75	34.2	0.56(0.35-2.85)

[1]Adjusted for age, sex, BMI, UICC TNM stage, Distant metastasis and Localisation

was 51.5±8.7 years. Among 289 patients, 183 patients were responders and 106 were nonresponders to chemotherapy. Among the responders, 71 showed a complete response and 112 showed a partial response. Patients had higher age and lower response to chemotherapy (P<0.05).

The allele and genotype distribution of polymorphisms in XRCC1 Arg399Gln and XPD Lys751Gln were showed in table 2. The minor allele frequencies among selected cases were consistent with the MAF from NCBI SNP databases. Moreover, all the SNPs were in line with the Hardy-Weinberg equilibrium among cases (All the p value >0.05).

Among 183 responders, the percentages of XRCC1 399Arg/Arg, Arg/Gln and Gln/Gln were 51.7%, 30.5% and 17.7%, respectively (Table 3). XRCC1 399 Gln/Gln genotype had significantly higher rates of response to chemotherapy when compared to the Arg/Arg genotype [OR (95% CI) = 2.56(1.57-2.55)]. In the case of XPD Lys751Gln, the percentages of XPD 751Lys/Lys, Lys/Gln and Gln/Gln were 47.6%, 43.3% and 9.1%, respectively. XPD 751 Gln/Gln genotype had significantly higher rates of response to chemotherapy [OR (95% CI) = 1.54(0.87-2.65)].

Among all patients, the median median survival time was 34.2 month. Patients with XRCC1 399 Gln/Gln genotype had a longer average survival time and significantly lower risk of death than did those with Arg/Arg genotype [HR (95% CI)= 0.66(0.36-0.95)] (Table 4). Similarly, those carrying XPD 751Gln/Gln genotype had 0.51-fold the risk of death of those with XPD 751Lys/Lys [HR (95% CI)= 0.51(0.33 -0.94)] (Table 4).

Discussion

To our best of our knowledge, no studies have investigated the role of DNA-repair gene, XRCC1 and XPD, on the response to chemotherapy among patients suffering colorectal cancer. Our results showed a significant association between XRCC1 399Gln/Gln and XPD 751Gln/Gln genotype and response to chemotherapy among colorectal cancer patients, moreover, the two genotypes could influence the survival of colorectal cancer.

Since this is the first study on the association between XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms and the response to chemotherapy. Previous evidences showed the XRCC1 Arg399Gln and XPD Lys751Gln are involved in response to chemotherapy in various cancers, such as breast cancer, thyroid carcinoma and lung cancer (Fard-Esfahani et al., 2011; Raabe et al., 2012). However, there are few studies in Chinese colorectal cancer patients on these two genes. Only several studies conducted in China investigation the association of XRCC1 with chemotherapy response and survival of colorectal cancer, but the results are conflicting (Grimminger et al., 2010; Lamas et al., 2012; Lv et al., 2012). A study conducted in China reported XRCC1 Arg399Gln polymorphisms is associated with the response to oxaliplantin-based chemotherapy and time to progression in advanced colorectal cancer in Chinese population, and patients with G/G genotype showed enhanced respond to chemotherapy compared to those with G/A and A/A genotypes. Individuals with the G/G genotype had a TTP of 10.0 (8.88-11.12) months, those with the G/A+A/A genotype had an TTP of 5.0 (4.26-5.74) months Lv et al. (2012). While another study reported patients with A/A had better respond to chemotherapy, and had a higher survival time than those with G/A and G/G genotypes. Our study finds a significant association of XRCC1 399Gln/Gln genotype with increased survival and higher response to chemotherapy among patients suffering colorectal cancer. These inconsistency results might be due to source of patients, disease stages, sample size and by chance. Further multicenter studies are warranted to establish the impact of XRCC1 and XPD genotypes on chemotherapy.

There are few studies reporting the association between XPD genotypes and response to chemotherapy among colorectal cancer patients. XPD protein, encoded by XPD gene, plays a role in NER pathway. During the NER, XPD participates in the opening of the DNA helix to allow the excision of the DNA fragment containing the damaged base (Manuguerra et al., 2006). 751 (Lys to Gln) were the main polymorphism that induce amino acid changes in the proteins (Shen et al., 1998). Previous

experimental studies showed the XPD codon Lys751Gln could modify the DNA repair ability in the NER capacity, and XPD 751Gln alleles had lower NER capacity than the wide-type genotypes (Rzeszowska-Wolny et al., 2005). In previous epidemiologic studies, only three western studies reported the XPD was associated with the progression-free survival. A study conducted in Spain reported XPD 751Gln/Gln was significantly associated with a favorable survival of colorectal cancer when compared with XPD 751Lys/Lys genotype (Lamas et al., 2011). While another study in Taiwan reported an reverse results of XPD Lys751Gln for colorectal survival (Lai et al., 2009). Our study showed modern increased survival in XPD 751Gln/Gln carriers, which is obvious because XPD 751Gln/Gln have reduced the activity and thus may have decreased DNA repair captivities. The chemotherapy for colorectal cancer is to induce the DNA damage of cancer cells, the low activity of XRCC1 and XPD polymorphisms would strengthen susceptibility to chemotherapy.

In summary, we found polymorphisms of XRCC1 399Gln/Gln and XPD 751Gln/Gln in Chinese population might be greatly strengthen the susceptibility to 5-FU/oxalipatin chemotherapy among Chinese population, it is suggested that the XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms should be routine detected to colorectal patients who are more likely benefit from 5-FU/oxalipatin chemotherapy.

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