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RESEARCH

Genetic Polymorphism of XRCC1 Correlated with Response to Oxaliplatin-Based Chemotherapy in Advanced Colorectal Cancer

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Abstract To examine the association between genetic polymorphisms of XRCC1 Arg399Gln(G→A) and response to oxaliplatin-based chemotherapy in advanced colorectal cancer. XRCC1 genotypes of totally 99 patients(37 stage III, 62 stage IV)with advanced colorectal cancer treated with oxaliplatin-based chemotherapy were detected by TaqMan-MGB probe allelic discrimination method. And clinical response of 62 patients in stage IVafter 2 to 3 cycles of chemotherapy were evaluated. Also time to progress (TTP) of all patients were evaluated. Of the genotype frequencies in all patients, up to 52.53 % were G/G genotype, 9.09 % were A/A genotype, and 38.38 % were G/A genotype. The response rate (CR+PR) of 62 patients in stage IV was 61.29 % (19/31). Patients with G/G genotype showed enhanced respond to chemotherapy compared to those with G/A+A/A (x^2=5.6, P=0.029; OR=3.845, 95 %CI=1.231~12.01, P=0.018). 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. The log-rank test was marginally significant (x^2=29.20, P<0.01). The Cox proportional hazards model, adjusted for stage, performance status, and chemotherapy regimen, showed that only XRCC1 G/G genotypes increases the OR significantly (OR=3.555; 95 % CI, 2.119~5.963; P<0.01). The results suggest that XRCC1 Arg399Gln polymorphisms is associated with the response to oxaliplantin-based chemotherapy and time to progression in advanced colorectal cancer in Chinese population. It is proposed that the XRCC1 Arg399Gln polymorphism should be routinely detected to screen patients who are more likely benefit from oxaliplantin-based treatment.

Keywords Colorectal neoplasms/genetics · Polymorphism · Oxaliplatin/drug therapy · X-ray repair cross complementing 1/genetics

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Introduction

Colorectal cancer is one of the most common malignant tumors and ranks the third morbility and the fourth mortality worldwide [1]. Up to 30 % of patients present with metastatic disease and 50–60 % ultimately develop metastatic or advanced disease. Some patients may be cured with surgery, but most of them need a chemotherapeutic treatment. Oxaliplatin is one of the most promising new drugs in the treatment of colorectal cancer [2]. Nevertheless, those who have a poor response to oxaliplatin-based chemotherapy usually have poor outcome. Therefore, it is important to select appropriate patients before chemotherapy. Unfortunately, few studies have investigated predictive factors of response to oxaliplatin-based chemotherapy in Chinese population.

Oxaliplatin carries a 1, 2-diamino-cyclohexane ring leading to DNA damage by forming DNA-platinum monoadducts with guanines that are converted in diadducts overtime. Oxaliplatin-induced adducts are not recognized or processed by mismatch repair, being predominantly repaired by the nucleotide excision repair pathway [3]. Accumulating body of

evidence suggests that functional genomic polymorphisms in drug target genes, metabolizing enzymes, and DNA-repair enzymes may be involved in drug sensitivity [4]. X-ray repair cross complementing 1(XRCC1) gene, one of the key genes in the nucleotide excision repair pathway, encodes a protein that functions in repair of single-strand breaks. XRCC1 acts as a facilitator or coordinator in base excision repair through its interaction with poly adenosinediphosphate-ribose polymerase, DNA polymerase β and DNA ligase III. The most commonly coding polymorphisms in the XRCC1 gene is at the codons 399 (Arg to Gln), which affects drug sensitivity to Oxaliplatin [5]. The aim of our study was to investigate whether the XRCC1 codon 399 polymorphism influenced the response to treatment with oxaliplatin-based chemotherapy in Chinese patients with advanced colorectal cancer. Yet, the possibility of individualizing DNA repair profiles is becoming a central issue to improve chemotherapy strategies.

Patients and Methods

Patients

The study population consisted of patients with histologically documented metastatic colorectal cancer. Characteristics of the patients are listed in Table 1. From 2005.09.01 to 2007.06.30, 99 patients consisting of 54 men (73 %) and 45 women (27 %) with a median age of 59 years (range 34–80 years) were enrolled. Thirty-seven (37.37 %) patients had stage III and 62 (62.63 %) stage IVadvanced colorectal cancer at the time of diagnosis. Forty cases suffered colon carcinoma and the others suffered rectal carcinoma. The median follow-up time was 8.9 month (range: 2.4 to 16.1 month). The study was conducted in oncology center of The Affiliated Hospital of Medical College Qing Dao University. Also informed consent was obtained from all the patients. The ethical committee of our institute approved the research protocol for this study.

Table 1 Characteristics of the 99 patients

Gender	
Male	54
Female	45
ECOG performance status	
0	24
1	48
2	27
Age, years	
Median	59
Range	34–80
Stage at diagnostic	
III	37
IV	62
Primary tumor	
Colon	40
Rectal	59
Chemotherapy	
Modified FOLFOX4 regimen	75
Xelox regimen	24
Genotypes	
G/G	52
G/A	38
A/A	9

Chemotherapy Treatment

Seventy-five cases of the patients were administered modified FOLFOX4 regiment and 24 with Xelox regiment for at least 2 cycles. Modified FOLFOX 4 regiment consists of oxaliplatin (L-OHP, JiangSu HengRui medicine corporation)130 mg/m^2, iv 2 hday 1; leukovorin(CF, JiangSu HengRui medicine corporation) 130 mg/m^2, iv 2 hday 1 to day 5; 5-fluorouracil (5-Fu, NanJing JinYao amino acids corporation) 300 mg/m^2, iv 4 hday 1 to day 5 every 3 weeks. Xelox regiment consists of oxaliplatin (L-OHP, JiangSu HengRui medicine corporation) 130 mg/m^2, iv 2 hday1; Capecitabine(Xeloda, ShangHai LuoShi medicine corporation) 1,250 mg/m^2, bid po, day1 to day 14 every 3 weeks. The treatment was given until disease progression, unacceptable toxicity, or patient's refusal to continue treatment. The average cycles of chemotherapy per patient were 2.5.

Therapeutic Evaluation

Curative effects in patients were evaluated after at least 2 cycles of treatment. The end point was the tumor response to chemotherapy evaluated according to the WHO criteria, including complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD). TTP was calculated from the date of diagnosis to the date of disease progression. Patients with CR or PR were classified as responders. Those with SD or PD were classified as nonresponders.

Genotyping

Blood samples were obtained from each patient before chemotherapy for DNA isolation and determination of genotypes. And DNA was extracted from these samples using Blood Genomic DNA Isolation Kit (ShangHai,Watson). XRCC1 Arg399Gln polymorphism was assessed by nuclease allelic discrimination assay (TaqMan-MGB) using a fluorescent temperature cycler (ABI 7500 Real Time PCR System, USA). Briefly, the 25 ul PCR mixture contained DNA 20 ng, 12.5 ul Taqman Universal PCR Master Mix (Applied

Biosystems, Foster City, CA, USA), 1.25 ul 20× Taqman SNP Genotyping Assay Mix (Applied Biosystems, Foster City, CA, USA), 9.25 ul ultrapure water. The PCR conditions were for 95 °C 10 min, followed by 40 cycles at 92 °C for 15 s and at 60 °C for 1 min. Sequences of primers and probes were designed by Applied Biosystems (Lot Number 480524 Assay ID c-622564-10). A minimum of 32 randomly selected DNA samples were genotyped at least twice to confirm the results.

Statistical Analysis

Distribution difference of genotypes between good and poor responders was calculated using generalized Fisher's exact test (the P values for the two-sided exact significance are presented) and Chi-square test, to show the relation of each genotype with the sensibility to chemotherapy. Logistic regression model was used to calculate Odds ratios (OR) and 95 % confidence interval (CI). The Kaplan-Meier method was adopted to estimate TTP, and the log-rank test was used to compare patients' TTP between genotype groups. Cox proportional hazard model was used to obtain the P-value for genotype corrected by the other clinical parameters, such as ECOG PS (Eastern Cooperative Oncology Group performance status), tumor stage or grade. All P-values were two-sided. Statistical significance was defined as $P<0.05$. All analyses were performed with the SPSS Version 13.0 software (SPSS Inc., Chicago, IL, USA) .

Results

Genotype Frequencies

All patients were assessable to test the possible association between the XRCC1 Arg399Gln polymorphism and the response to chemotherapy using the above-cited criteria. Polymorphism of XRCC1 Arg399Gln Amplification was successful for all DNA prepared from the samples. The analysis of the polymorphism located at XRCC1 Arg399Gln showed that 52 patients (52.53 %) had the homozygous for the codon (G/G genotype), 38 (38.38 %) were heterozygous (G/A genotype), and 9 (9.09 %) (A/A genotype). The genotype frequencies of the XRCC1 399 G→A variation was in Hardy-Weinberg equilibrium.

Response to Chemotherapy in Relation to Genetic Polymorphism

A statistically significant association was found between polymorphisms of XRCC1 and successful treatment with oxaliplatin-based chemotherapy in stage IV patients ($P=0.029$, Fisher's exact test). Nineteen of 31 cases with response were homozygous for 399Gln allele compared with 12 of the 31 cases with a nonresponse. Logistic regression analysis showed a significant increased chance of treatment response in patients with the G/G genotype versus the A/A+A/G genotype (odds ratio 3.845; 95 % CI 1.231–12.01; $P=0.018$). The gender, age, number of metastasis, differentiation and PS of patients were not correlated with the response to chemotherapy after calculated by Logistic regression model (Table 2).

XRCC1 Arg399Gln Polymorphism and Time to Progress

The median TTP of all patients was 7 (5.57–8.43)months. Individuals with the G/G genotype had an 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. The log-rank test was marginally significant ($x^2=29.20$, $p<0.01$). The Cox proportional hazards model, adjusted for stage, performance status, and chemotherapy regimen, showed that only XRCC1 G/G genotypes increases the OR significantly (OR=3.555; 95 % CI, 2.119~5.963; $P<0.01$) (Table 3; Fig. 1).

Discussion

The aim of this study was to determine whether the polymorphisms of XRCC1 Arg399Gln could predict the chemosensitivity and clinical outcome of patients with advanced colorectal cancers treated with oxaliplantin-based regimen. We anticipated that polymorphisms of DNA repair genes and genes related to metabolism may influence tumor response to oxaliplantin-based chemotherapy. In the current study, patients carrying XRCC1 G/G genotype

Table 2 Response to chemotherapy according to XRCC1 genetic polymorphism

Arg399Gln genotype	Case[n(%)]		Chi-square test		Logistic regression analysis		
	Responder	Nonresponder	x^2	p	OR	95 %CI	p
G/G	19(61.29 %)	12 (38.71 %)					
G/A+A/A	7(29.17 %)	17(70.83 %)	5.6	0.029	3.845	1.231~12.01	0.018
Total	26(47.27 %)	29(52.73 %)					

Table 3 The relationship between XRCC1 genotypes and time to progression of colorectal cancer

Arg399Gln genotype	Median TTP months (95 %CI)	Log-rank test		Cox regression analysis		
		x^2	*P*	OR	95 %CI	*P*
G/G	10.0(8.88,11.12)					
G/A+A/A	5.0(4.26,5.74)	29.20	<0.01	3.555	2.119~5.963	<0.01
Total	7.0(5.57,8.43)					

showed enhanced sensitivity to chemotherapy, and the results corresponded with the hypothesis that XRCC1 are strongly associated with tumor response in oxaliplantin-based chemotherapy in patients with advanced colorectal cancers.

The self-repairing capability of DNA is one of the most important factors which affect genome stability. And DNA repairing syetem includes base excision repair (BER), nucleotide excision repair(NER), mismatch repair (MMR) pathways [6]. There has been increasing evidence that reduced DNA repair capacity resulting from genetic polymorphisms of DNA repair genes is associated with improved survival with oxaliplantin-based chemotherapy in cancers [7–9]. One of the remaining challenges is to identify markers that dramatically influence clinical outcome to specific chemotherapeutic agents. In this respect, we selected XRCC1, one of key DNA repair genes in the BER pathway as the focus of this study because of its pivotal role in carcinogenesis and in function of oxaliplantin. Several studies have reported the association of XRCC1 with the risk in non-small-cell lung cancer, breast cancer, gastric cancer, and colorectal cancer [10–13]. All the results suggest that XRCC1 polymorphism may affect the repairing ability of DNA.

XRCC1 encodes a protein that complexes with DNA ligase to repair DNA gaps. The polymorphism of XRCC1 was thought to reduce DNA repair activity and hence lead to increased DNA damage, mutation induction and increased chemosensitivity. However, scientific publications are although available but with diverging results in the XRCC1 Arg399Gln polymorphism and cancer risk or treatment efficiency. Suh KW et al. [14] showed that XRCC1 G/G genotype predicted superior survial and better prognosis in

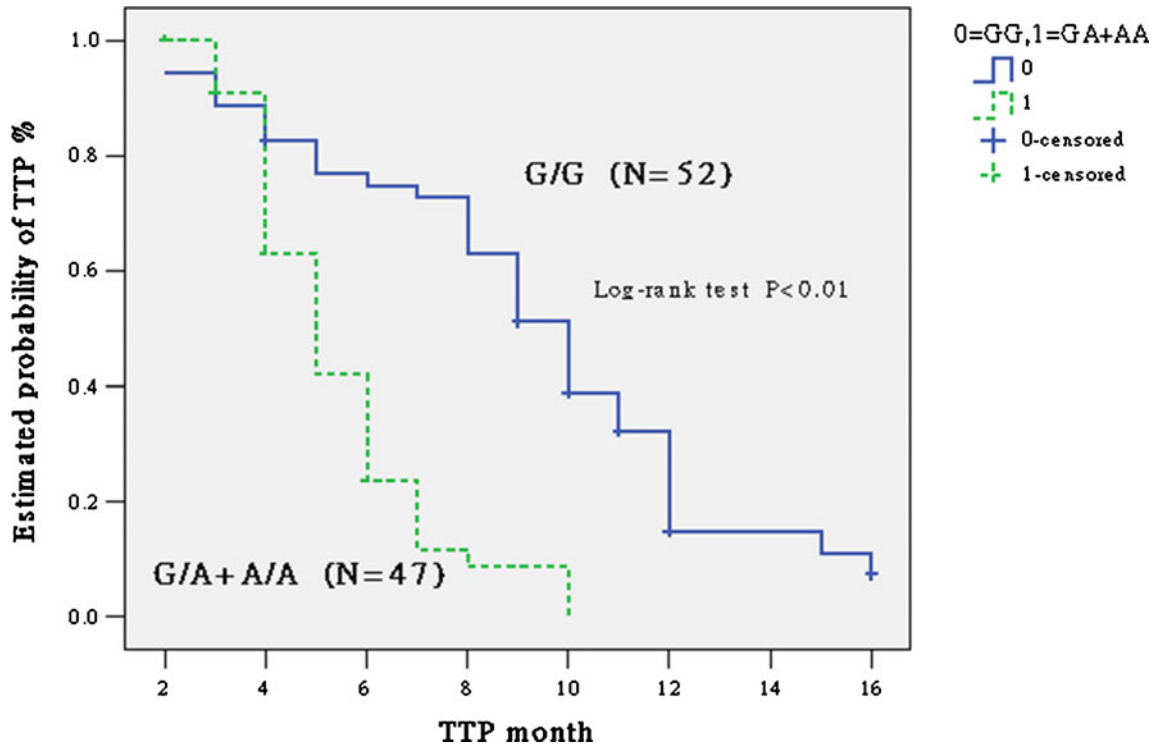

Fig. 1 Kaplan-Meier curves of XRCC1 Arg399Gln polymorphism (*P*<0.01, log-rank test) The median TTP of all patients was 7 (5.57–8.43)months. Individuals with the G/G genotype had an TTP of 10.0 (8.88–11.12)months, those with the G/A+A/A genotype had an TTP of 5.0(4.26–5.74) months. The log-rank test was marginally significant (x^2=29.20, p<0.01). The Cox proportional hazards model, adjusted for stage, performance status, and chemotherapy regimen, showed that only XRCC1 G/G genotypes increases the OR significantly (OR=3.555; 95 % CI, 2.119~5.963; *P*<0.01)

54 advanced colorectal cancer patients who received FOLFOX chemotherapy. Stoehlmacher et al. [15] analyzed the XRCC1 genetic polymorphism of 61 patients suffering advanced colorectal cancer who received oxaliplatin and 5-fluorouracil based treatment, and they found that XRCC1 Arg399Gln played a significant role in the prediction of clinical outcomes. 73 % responded to the chemotherapy was patients with G/G genotype, while 66 % responseless patients was A/A or G/A genotype. Baorui Liu et al. [16] reported that the median survival times for patients with Arg/Arg or Arg/Gln genotypes of XRCC1 gene were significantly longer than others in 58 gastric patients treated with FOLFOX chemotherapy. But Sarada Gurubhagavatula et al. [17] found that patients with XRCC1 A/A genotype had longer mean survival time (MST) than those of with XRCC1 G/G and G/A genotypes. Our results showed that XRCC1 G/G enhanced cancer chemosensitivity to oxaliplantin-based chemotherapy and prolonged TTP in advanced colorectal cancer patients.

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 to elucidate further the mechanism by which DNA repair affects outcome. These parameters can distinguish whether XRCC1 polymorphisms are predictive of treatment response or are prognostic by determining outcome. Ideally, prospective validation studies should be carried out to measure these additional end points. On the basis of the expected outcome of the patient, both predictive and prognostic factors may be important in the choice of chemotherapeutic regimens. Second, the number of the cases in this study was small, and it may have limitation to be generalized in advanced colorectal cancers. Third, chemotherapeutic regimens were not equal. If same chemotherapeutic regimen had been used in the treatment, the predictive role of XRCC1 polymorphism would have been generalized. Fourthermore repair of DNA damage is a complex process. Functional close proximity in the genome between XRCC1 and other polymorphic DNA repair genes such as the xeroderma pigmentosum group D(XPD) and excision repair cross complementing (ERCC1) may influence the interaction between them leading to different or synergic DNA repair capability. More genes and their interactions are needed for further study in this situation.

To our knowledge, this is the first time we demonstrated that the XRCC1 Arg399Gln polymorphisms is associated with the response to oxaliplantin-based chemotherapy in advanced colorectal cancer in Chinese population. A larger study will be necessary to validate our data that suggest that the XRCC1 Arg399Gln genotype may influence the response and clinical outcome of advanced colorectal cancer patients to oxaliplantin-based chemotherapy, together with functional studies to establish its mechanistic basis. We propose that the XRCC1 Arg399Gln polymorphism should be routinely done to select patients who are more likely to benefit from treatment with oxaliplantin-based chemotherapy in advanced colorectal cancer.

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Declaration of interest We have no conflicts of interest to report

References

1. Pisani P, Parkin DM, Bray F, Ferlay J (1999) Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer 83 (1):18–29. doi:10.1002/(SICI)1097-0215(19990924)83:1<18::AID-IJC5>3.0.CO;2-M
2. Faivre S, Chan D, Salinas R, Woynarowska B, Woynarowski JM (2003) DNA strand breaks and apoptosis induced by oxaliplatin in cancer cells. Biochem Pharmacol 66(2):225–237
3. Lentz F, Tran A, Rey E, Pons G, Treluyer JM (2005) Pharmacogenomics of fluorouracil, irinotecan, and oxaliplatin in hepatic metastases of colorectal cancer: clinical implications. Am J Pharmacogenomics 5(1):21–33
4. Kang D (2003) Genetic polymorphisms and cancer susceptibility of breast cancer in Korean women. J Biochem Mol Biol 36(1):28–34
5. Friedberg EC (2001) How nucleotide excision repair protects against cancer. Nat Rev Cancer 1(1):22–33. doi:10.1038/35094000
6. Tian M, Shinkura R, Shinkura N, Alt FW (2004) Growth retardation, early death, and DNA repair defects in mice deficient for the nucleotide excision repair enzyme XPF. Mol Cell Biol 24(3):1200–1205
7. Mort R, Mo L, McEwan C, Melton DW (2003) Lack of involvement of nucleotide excision repair gene polymorphisms in colorectal cancer. Br J Cancer 89(2):333–337. doi:10.1038/sj.bjc.6601061
8. Motycka TA, Bessho T, Post SM, Sung P, Tomkinson AE (2004) Physical and functional interaction between the XPF/ERCC1 endonuclease and hRad52. J Biol Chem 279(14):13634–13639. doi:10.1074/jbc.M313779200
9. Nagasubramanian R, Innocenti F, Ratain MJ (2003) Pharmacogenetics in cancer treatment. Annu Rev Med 54:437–452. doi:10.1146/annurev.med.54.101601.152352
10. Chacko P, Rajan B, Joseph T, Mathew BS, Pillai MR (2005) Polymorphisms in DNA repair gene XRCC1 and increased genetic susceptibility to breast cancer. Breast Cancer Res Treat 89(1):15–21. doi:10.1007/s10549-004-1004-x
11. Kiyohara C, Takayama K, Nakanishi Y (2006) Association of genetic polymorphisms in the base excision repair pathway with lung cancer risk: a meta-analysis. Lung Cancer 54(3):267–283. doi:10.1016/j.lungcan.2006.08.009
12. Stern MC, Siegmund KD, Conti DV, Corral R, Haile RW (2006) XRCC1, XRCC3, and XPD polymorphisms as modifiers of the effect of smoking and alcohol on colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 15(12):2384–2390. doi:10.1158/1055-9965.EPI-06-0381
13. Zhang Z, Miao XP, Tan W, Guo YL, Zhang XM, Lin DX (2006) Correlation of genetic polymorphisms in DNA repair genes ADPRT and XRCC1 to risk of gastric cancer. Ai Zheng 25(1):7–10
14. Kim DY, Kim YB, Choi HS, Kim JM, Suh K (2006) Which gene is a dominant predictor of response during FOLFOX chemotherapy for the treatment of metastatic colorectal cancer, MTHFR or XRCC1 gene? Ann Surg Oncol 13(2):7–7

15. Stoehlmacher J, Ghaderi V, Iobal S, Groshen S, Tsao-Wei D, Park D, Lenz HJ (2001) A polymorphism of the XRCC1 gene predicts for response to platinum based treatment in advanced colorectal cancer. Anticancer Res 21(4B):3075–3079
16. Liu B, Wei J, Zou Z, Qian X, Nakamura T, Zhang W, Ding Y, Feng J, Yu L (2007) Polymorphism of XRCC1 predicts overall survival of gastric cancer patients receiving oxaliplatin-based chemotherapy in Chinese population. Eur J Hum Genet 15(10):1049–1053. doi:10.1038/sj.ejhg.5201884
17. Gurubhagavatula S, Liu G, Park S, Zhou W, Su L, Wain JC, Lynch TJ, Neuberg DS, Christiani DC (2004) XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. J Clin Oncol 22(13):2594–2601. doi:10.1200/JCO.2004.08.067JCO.2004.08.067