

RESEARCH COMMUNICATION

Evaluation of DNA Repair Gene XRCC1 Polymorphism in Prediction and Prognosis of Hepatocellular Carcinoma Risk

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Abstract

We conducted a case-control study in China to clarify the association between XRCC1-Arg399Gln polymorphism and HCC risk. A total of 150 cases and 158 controls were selected from the the Affiliated Hospital of Qingdao University from May 2008 to May 2010. XRCC1-Arg399Gln polymorphism was based upon duplex polymerase-chain-reaction with the confronting-two-pair primer (PCR-CTPP) method. All analyses were performed using the STATA statistical package. A significantly increased risk was associated with the Arg/Gln genotype (adjusted OR 1.78, 95% CI=1.13-2.79) compared with genotype Arg/Arg. In contrast, the Gln/Gln genotype had non-significant increased risk of HCC with adjusted OR (95% CI) of 1.69 (0.93-2.66). A significant association was found between positive HBsAg and Arg/Gln, with an OR of 3.43 (95% CI=1.45-8.13). Patients carrying Gln/Gln genotypes showed significantly lower median survival than Arg/Arg genotypes (HR=1.38, 95% CI=1.04-1.84). Further Kaplan-Meier analysis showed decreased median survival in Arg/Gln+Gln/Gln genotype carriers in comparison to Arg/Arg carriers (HR=1.33, 95% CI=1.02-1.76). In conclusion, we observed that XRCC1-Arg399Gln polymorphism is associated with susceptibility to HCC, and XRCC1 Gln allele genotype showed significant prognostic associations.

Keywords: Polymorphism - XRCC1-Arg399Gln - hepatocellular carcinoma risk - prognosis - China

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men (523,000 cases, 7.9% of the total) and the seventh in women (226,000 cases, 6.5% of the total), and most of the burden is in developing countries, where almost 85% of the cases occur, and particularly in men: the overall sex ratio male: female is 2.4. The regions of high incidence are Eastern and South-Eastern Asia, Middle and Western Africa, but also Melanesia and Micronesia/Polynesia (particularly in men). Low rates are estimated in developed regions, with the exception of Southern Europe where the incidence in men (ASR 10.5 per 100,000) is significantly higher than in other developed regions (IARC, 2008). The wide geographic variation at an international levels of EC in terms of incidence and mortality suggested the role of genetic and environmental factors in the pathogenesis of this cancer.

In recent years, it has been shown that variability in DNA repair capacity plays a role as a modifier of cancer risk. The XRCC1 gene (located at chromosome 19q13.2) produces an enzyme involved in the base excision repair (BER) pathway, amending small lesions such as singlestrand breaks (SSBs), non-bulky adducts, oxidative damage, alkylation, and methylation. Recently, the XRCC1 complex has also been described as part of

an alternative route of DNA double-strand break (DSB) nonhomologous end-rejoining, i.e., PARP1-dependent end-joining of DSBs (Audebert et al., 2004). The XRCC1 protein is essential for mammalian viability and XRCC1-deficient cells are genetically unstable and sensitive to DNA damaging agents. Three common SNPs lead to amino acid substitutions in XRCC1 at codons 194 (exon 6, C-T, Arg-Trp), 280 (exon 9, G-A, Arg-His), and 399 (exon 10, G-A, Arg-Gln) (Shen et al., 1998). These mutations could alter XRCC1 function, diminish repair kinetics, influence susceptibility to adverse health effect, such as cancer.

Several studies have explored the impact of XRCC1-Arg399Gln polymorphism in various cancers including lung, gastric, esophageal, breast, prostate and glioma cancers (Geng et al., 2008; Yin et al., 2009; Kiyohara et al., 2010; Rajaraman et al., 2010; Zipprich et al., 2010). However, association studies of XRCC1-Arg399Gln polymorphism in HCC risk have been conflicting (Kiran et al., 2009; Liu et al., 2011). Our study has showed the XRCC1-Arg399Gln has been associated with HCC (Liang et al., 2011), but did not explore the gene interaction with environment.

The role of XRCC1-Arg399Gln polymorphism on the survival of patients has also been reported in different types of malignancies (Grimminger et al., 2010; Ott et

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al., 2011; Rybarova et al., 2011). Furthermore, there are population-specific differences in cancer susceptibility as well as allele frequency of gene polymorphism. Moreover, it has not been explored in a Chinese population.

Thus, based on the literature and current understanding, this study was designed to investigate the association of XRCC1-Arg399Gln polymorphism with HCC, to analyze its interaction with environment factors, and explore the role of this polymorphism in determining survival outcome in Chinese patients.

Materials and Methods

HCC patients were consecutively collected from May 2008 to May 2010. All HCC patients with newly diagnosed primary HCC in the hospital were invited for face-to-face interviews within one month after diagnosis. All cases recruited in our study were histologically confirmed. Among a total of 161 eligible cases, 150 were interviewed with a participation rate of 93.16%. 169 controls were randomly selected from people who requested general health examinations in the same hospital during the same period, and 158 controls approved to participate our study with a participation rate of 93.49%. The controls were confirmed to have no malignancy, digestive diseases, chronic diseases and also no prior history of malignancy. Controls were matched with cases by age within five years

Genotyping of XRCC1

Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pair-primer (PCR-CTPP) method. Briefly, the sequences of primers used for XRCC1 polymorphisms are: 5'-GAAGTCCCTGAAAAGCTAAAGC-3' and 5'-GTTGGGCTCAAATATACGGTGG-3'. Each 30 µL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L Mg²⁺, 2.4 µL dNTPs, 8 primers, 15 pmol of each primer and 5-8 µL template. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, at 62°C for 30 s, at 72°C for 30 s, and a final extension at 72°C for 5 min. After transient centrifugation, agarose electrophoresis was conducted.

Statistical analysis

All analysis was performed by using the STATA statistical package (version 9, STATA, College Station, TX). Hardy-Weinberg equilibrium of alleles at controls was assessed by using exact tests. Categorical variables were compared with use of the chi-square test or Fisher's exact test (when one expected value was <5). Unconditional logistic regression was undertaken to estimate odds ratios (ORs) and their 95% confidence intervals (95% CIs) after controlling for potential confounding factors, including age, sex, cigarette smoking (yes or no), alcohol consumption (yes or no), first degree family history of HCC. The outcome for the study was overall survival, which was estimated using the Kaplan-Meier method. A univariate Cox's regression analysis was used to assess the association between XRCC1-Arg399Gln gene polymorphism and survival. The relative risk [hazard ratio(HR)] and 95% CI were calculated from

Table 1. Demographic Characteristics of the Study Population

Parameter	Cases N=150(%)	Controls N=158(%)	P
Sex			
Male	95(63.33)	98(62.03)	0.81
Female	55(36.67)	60(37.97)	
Age (mean±SD)	51.3±8.9	50.8±8.5	0.31
Smoking			
Ever	58(38.67)	50(31.65)	0.159
No	92(61.33)	108(68.35)	
Drinking status			
Ever	89(59.33)	62(39.24)	<0.001
No	61(40.67)	96(60.76)	
HBsAg			
+	85(56.67)	74(46.84)	0.08
-	65(43.33)	84(53.16)	
Anti-HCV			
+	17(11.33)	13(8.23)	0.36
-	133(88.67)	145(91.77)	

Table 2. The Gene Frequencies of XRCC1-Arg399Cln in Cases and Controls

XRCC1-Arg399Cln	Cases N=150(%)	Controls N=158(%)	OR	OR ¹
Arg/Arg	32(21.33)	46(29.11)	1.0 (reference)	1.0 (reference)
Arg/Gln	78(52.0)	73(46.20)	1.53 (0.85-2.77)	1.78 (1.13-2.79)
Gln/Gln	40(26.67)	39(24.68)	1.47 (0.75-2.91)	1.69 (0.93-2.66)

¹Adjusted for sex, age, smoking, drinking and HBsAg status

the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). All statistical tests were two sided, and differences were taken as significant when the P value was less than 0.05.

Results

The mean age at enrollment of this case-control study was 51.3±8.9 years for cases and 50.8±8.5 years for controls. There were no significant differences for sex, age, smoking, HBsAg and anti-HCV status (P>0.05, Table 1). The HCC patients had significant higher consumption of drinking than controls (P<0.05, Table 1).

Distribution of XRCC1-Arg399Cln Genotypes

The genotype distribution of XRCC1-Arg399Cln for both HCC cases and controls were shown in Table 2. The genotype frequency distribution in controls fit well to Hardy-Weinberg equilibrium (p=0.07). The frequencies of Arg/Arg, Arg/Gln and Gln/Gln in cases were 21.33%, 52.0% and 26.67%, respectively, which was significant difference with those in controls (29.11%, 46.20% and 24.68%, respectively). A significant increased risk was associated with the Arg/Gln genotype (adjusted OR 1.78, 95%CI=1.13-2.79) compared with genotype Arg/Arg. But Gln/Gln genotype had non-significant increased risk of HCC with adjusted OR (95%CI) of 1.69 (0.93-2.66).

Interaction of XRCC1-Arg399Cln genotypes with alcohol drinking status and HBsAg

In case-only analysis, for the interaction of XRCC1-Arg399Cln polymorphism with environmental risk factors

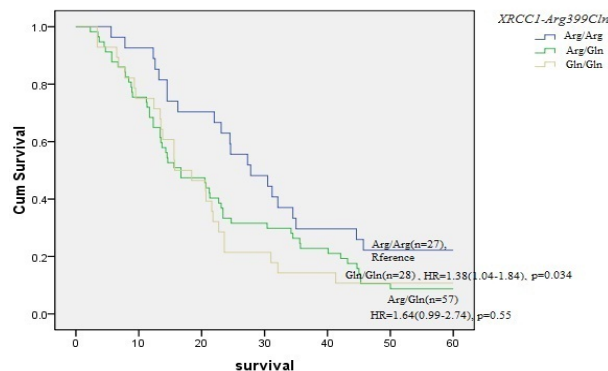
Table 3. Interaction of XRCC1-Arg399Cln Genotypes with Smoking, HBsAg in liver Cancer Patients

XRCC1-Arg399Cln	Drinkers(n=89, %)	Nondrinkers (n=61, %)	OR(95%CI), P	HBsAg(+)(n=85, %)	HBsAg(-)(n=65, %)	OR(95%CI), P
Arg/Arg	17(11.33)	15(10.0)	1.0(Reference)	14(9.33)	28(18.67)	1.0(Reference)
Arg/Gln	45(30.0)	33(22.0)	1.28(0.52-3.15)	49(32.67)	29(19.33)	3.43(1.45-8.13)
Gln/Gln	28(18.67)	12(8.0)	2.16(0.84-6.39)	22(14.67)	18(12.00)	2.59(0.96-6.78)

¹Adjusted for age, sex and smoking status

Table 4. Kaplan-meier Survival Estimation of Median Survival and HRs with Gene Polymorphism

XRCC1-Arg399Cln	n(%)	Median Survival (in month)	HR (95% CI), P value
Arg/Arg	27(24.1)	31.84	1.0(Reference)
Arg/Gln	57(50.9)	23.36	1.64(0.99-2.74)
Gln/Gln	28(25.0)	21.58	1.38(1.04-1.84)
Gln allele	85(75.9)	22.77	1.33(1.02-1.76)

**Figure 1. Overall Survival (in months) among Different Genotypes of XRCC1-Arg399Cln Polymorphism**

such as alcohol intake and HBsAg status, we found that patients with Arg/Gln and Gln/Gln genotypes had a positive association with alcohol intake and positive HBsAg (Table 3). A significant association was found between positive HBsAg and Arg/Gln, and a significant increased OR was showed (OR=3.43, 95% CI=1.45-8.13).

Kaplan-Meier survival analysis of HCC patients

We could follow up those patients (112) who continued their treatment in the same hospital and received chemotherapy after surgery or radiotherapy with or without chemotherapy. Median survival of patients was 23.2 mo (SD=±8.22). A five-year survival rate was found 12.5% (14/112) cases. When the survival time of the patients was compared among XRCC1-Arg399Cln genotypes (Figure 1), a difference in median survival of patients carrying the Arg/Arg and Arg/Gln genotypes (31.84 vs 23.36 months, respectively) were obtained (Table 4), but it was statistically nonsignificant. Patients carrying Gln/Gln genotypes showed significantly lower median survival than Arg/Arg genotypes (HR=1.38, 95% CI=1.04-1.84). Further Kaplan-Meier analysis showed decreased median survival in Arg/Gln+Gln/Gln genotype carrier in comparison to Arg/Arg carriers (HR=1.33, 95% CI=1.02-1.76).

Discussion

In this study from Chinese population, we observed that XRCC1-Arg399Cln polymorphism is associated with

susceptibility to HCC, and XRCC1 Gln allele genotype showed significant prognostic associations.

A wide variety of DNA damage may be induced by normal endogenous metabolic processes or by environmental carcinogens. Most of these alterations, if not repaired, may result in genetic instability, mutagenesis and cell death. DNA repair mechanisms are important for maintaining genome integrity and preventing carcinogenesis. BER is the predominant DNA damage repair pathway for the processing of small base lesions, derived from oxidation and alkylation's damage. XRCC1 gene is regarded an important proteins in the multistep BER pathway, and it is the first mammalian gene isolated that affects cellular sensitivity to ionizing radiation (Thompson et al., 1990). Mutations of XRCC1 may increase the risk of cancers by impairing the interaction of XRCC1 with other enzymatic proteins and consequently altering DNA repair activity (Basso et al., 2007; Tudek, 2007), and subsequently induce the carcinogenesis of head and neck and cancer of lung, esophagus, breast and many other malignancies (Yu et al., 2003; Han et al., 2004). As we known, HBV and HCV may promote chromosomal instability or insertional mutations, and thus to induce the carcinoma development risk. The XRCC1, DNA repair gene, play a protective way of genetic abnormalities, but the polymorphism of XRCC1 has been functional in altering the XRCC1 enzyme activity and DNA repair captivities, further leading to carcinoma development. There were three reported polymorphisms at codons 194, 280 and 399 of XRCC1, codon 194 and 280 do not locate in the important domain, but codon 399 locates in the BRCT1 domain. Previous experimental study showed the amino acid replacement of codon 399 could injury the DNA repair captivities and increase the susceptibility to ionizing radiation. Therefore, the polymorphism of XRCC1-Arg399Cln could increase the cancer risk of individuals. Our study also found the Arg/Gln genotype could increase the risk of HCC, which was in line of a previous study conducted in Taiwan (Yu et al., 2003).

Our study also found the polymorphism of XRCC1-Arg399Cln may increase the risk among drinkers. Chronic alcohol consumption is associated with the production of free radical intermediates, such as hydroxyethyl free radicals and reactive oxygen species, which are produced during ethanol metabolism (Clot et al., 1994). Several reports suggest that free radicals and oxidative stress play an important role in the pathogenesis of alcoholic and toxic liver diseases (Nordmann et al., 1992; Ishii et al., 1997; Navasumrit et al., 2000). The XRCC1-Arg399Cln may have functional significance in the repair of alcohol induced genetic lesions. However, when the amino acid replacement of codon 399, the DNA repair capacity may be reduced. Our study showed the Arg/Gln genotype may

increase the risk of HCC, but the p value of interaction analysis did not show significant, and the little sample size may limit the statistic power to find the difference. Additionally, we found the a significant association was found between positive HBsAg and Arg/Gln and a higher OR was found, which indicated XRCC1-Arg399Cln and hepatitis B virus may have a strong superimposed effect on carcinogenesis of HCC.

There are limited reports (Sakano et al., 2006; Ang et al., 2010; Long et al., 2010) on the role of XRCC1-Arg399Cln polymorphism on the survival of cancer patients. We found decreased median survival in variant allele carriers (Arg/Gln+Gln/Gln), which is obvious because Arg/Gln and Gln/Gln genotypes carriers have reduced XRCC1 enzyme activity and thus may have decrease DNA repair captivities. We observed Gln allele carriers had significant prognostic role on the survival of HCC patients in Chinese population. However, no significant decreased median survival was found in Arg/Gln genotype carriers, the reason might be the limited samples of HCC patients, which reduced the statistic power to find the difference.

Overall, our study demonstrates that polymorphisms in DNA repair genes have a role in the susceptibility and survival of HCC patients. The limitation of our study was a lower number of patients with survival data. In future, studies with a higher sample size are warranted. In conclusion, we found XRCC1-Arg399Cln play a significant role in determining susceptibility or prognosis of HCC in Chinese population.

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