RESEARCH COMMUNICATION

Polymorphisms in DNA Repair Gene XRCC1 and Increased Genetic Susceptibility to Glioma

Xue-Bin Hu[1], Zhe Feng[2]*, Yu-Cong Fan[1], Zhi-Yong Xiong[1], Qi-Wei Huang[1]

Abstract

Background: The *XRCC1* gene encodes the XRCC1 protein, which complexes with three other DNA repair enzymes involved in the base-excision repair (BER) pathways. Different XRCC1 polymorphisms may increase the risk of cancers by impairing interaction with other enzymatic proteins and consequently altering DNA repair activity, and result in carcinogenesis. Our study aimed to investigate any association between three polymorphisms of the XRCC1 gene at codon 194, 280 and 399 and potential glioma risk. Methods: We collected 127 patients with primary glioma and 249 controls who requested general health examinations from Union Hospital of Tongji Medical College hospital from March 2007 to September 2010. A total of 5 ml venous blood was drawn from each subject. The polymorphisms of XRCC1 gene at codons 194, 280 and 399 were analyzed based on duplex polymerase-chain-reactions with the confronting-two-pair primer (PCR-CTPP) method. Results: The homozygous Trp/Trp and heterozygotes Arg/Trp variants of codon 194 had a 2.12 fold and 1.46 fold increased risk of glioma compared to the homozygous Arg/Arg wide genotypes. The same effect was found in codon 399, the codon 399 Gln/Gln and Arg/Gln genotypes being associated with a 2.24 fold and 1.67 fold increased risk in glioma. When comparing the codon 194 Arg/Arg and 399 Arg/Arg genotypes, the combination of codon 194 Trp allele and 399 Gln allele had a heavy increase in glioma risk (OR=2.87, 95%CI=1.56-6.73). Conclusion: The present study provided evidence of a potential role for XRCC1 codon 194 and 399 polymorphisms in genetic predisposition to glioma among the Chinese population. This analysis of correlation of DNA repair genes and glioma may provide a deeper insight into the genetic and environment factors for cancer risk.

Keywords: DNA repair gene XRCC1 - polymorphism - glioma

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Introduction

The repair of DNA damage is a ubiquity process and has a key role in protecting the genome from assaults of various oncogenic mutations due to premutational DNA damage (Rajewsky et al., 2000). A potentially important source of inherent genetic susceptibility in relation to development of cancer is the interindividual variability in the DNA repair capacity within human populations. Common genetic polymorphisms in DNA repair genes may alter protein function and an individual's capacity to repair damaged DNA. Such deficits in DNA repair capacity may lead to genetic instability and carcinogenesis.

The X-ray cross-complementing group 1 gene (XRCC1) is a major DNA repair gene involved in BER (Vidal et al., 2001). It is located on chromosome 19q13.2, spans a genetic distance of 32 kb, comprises of 17 exons, and encodes a 70-kDa protein consisting of 633 amino acids (Lamerdin et al., 1995; Lindahl and Wood, 1999). XRCC1 has no catalytic activity of its own, but it acts both as a scaffold and a modulator by interacting with and bringing together BER components (Whitehouse et al., 2001). Furthermore, mutations and polymorphisms in DNA repair genes are associated with variations in repair efficiency of DNA damage, and this repair deficit may eventually predispose an individual to cancer risk, birth defects and a reduced life span. It reported that a total of eight nonsynonymous single nucleotide polymorphisms (SNPs) have been reported in XRCC1 gene (Han et al., 2003; Hu et al., 2005), and the three common polymorphisms in XRCC1 are located 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).

Previous established etiologic factors for glioma included ionizing radiation (IR), heterocyclic aromatic amines, alcohol, diet generate reactive oxygen radicals, bulky DNA adducts, oxidized DNA bases, and DNA strand breaks (Audebert et al., 2004). Therefore, deficient DNA repair might contribute to genomic instability and glima. Genetic variability in DNA repair may contribute to hypersensitivity to ionizing radiation and susceptibility to glioma. Previous study pointed out the DNA repair genes

*[1]Department of Neurosurgery, Union Hospital, Tongji Medical College, [2]School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China *For correspondence: Fengzhou_2020@yahoo.com.cn, hxb7276@163.com*

may greatly contribute to brain tumor susceptibility (Anne et al., 2008). However, there is no one study regarding on the relationship between polymorphism of XRCC1 gene and glioma in Chinese population. Therefore, the current study is to investigate the hypothesis that the genetic polymorphisms of DNA repair gene XRCC1, resulting in the three non-conservative amino acid substitutions at codons 194 (Arg→Trp), 280 ((Arg→His) and 299 ((Arg→Gln) may increase the susceptibility to glioma by modifying individual DNA repair capability.

Materials and Methods

Study population

This study was a hospital based case-control study composing of 127 patients with primary glioma in Union Hospital of Tongji Medical College hospital from March 2007 to September 2010. The patients were identified from oncology, neurosurgery and neurology departments. Their blood samples and clinic-pathological information from patients were obtained with informed consent and ethical review board approval from Union Hospital of Tongji Medical College. 249 controls were randomly selected from people who requested general health examinations in the same hospital during the same period and were confirmed to have no malignancy, digestive diseases, chronic diseases and also no prior history of malignancy. The controls were frequency matched to cases by 5 year age group and sex.

DNA extraction and PCR analysis

A total of 5 ml venous blood was drawn from each subject. DNA was extracted from the whole blood by using the methods of Daly et al. (1996). Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pairprimer (PCR-CTPP) method (Priya et al., 2005). Briefly, the sequences of primers used for polymorphism of XRCC1 codons 194, 280 and 399 were amplified by using the following primers. XRCC1 codon 194: 5'-GTT CCG TGT GAA GGA GGA GGA-3' and 5'-CGA GTC TAG GTC TCA ACC CTA CTC ACT-3'; XRCC1 codon 280: 5'-TTG ACC CCC AGT GGT GCT AA-3'; and 5'-AGT CTG CTG GCT CTG GGC TGG-3'. Each 25 μL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L Mg^{2+}, 0.24 mmol/L 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 4 min, followed by 35 cycles at 94°C for 40 s, at 55°C for 30 s, at 72°C for 40 s, and a final extension at 72°C for 10 min. After transient centrifugation, agarose electrophoresis was conducted. The PCR products included 138 bp for Arg allele of codon 194, and 75 bp for Trp allele; For codon 280, 63 bp and 205 were in Arg allele, and 65 and 205 bps were in His allele. For the codon 399, the Arg allele gave the products of 285 and 461 bps, and the Gln allele gave the products of 285 and 276 bps.

Data analysis

All analysis was performed by using the STATA statistical package (version 9, STATA, College Station, TX). Demographic data, ionizing radiation exposure, smoking and drinking status were collected. The ionizing radiation (IR) exposure history included medical and occupational exposure. The medical ionizing radiation exposure included diagnostic and radiotherapy for medical problem. Occupational exposure included work as a pilot, flight attendant, astronaut, uranium miner, workers in the nuclear power industries, radiologists or X-ray medical worker, dentist or dental hygienist, and other participants who self-reported ionizing radiation exposure in the workplace. The association between XRCC1 codon 194, 280 and 399 genotypes and glioma were analyzed by calculating the crude odds ratios (OR) and 95% confidence Intervals (95% CI) using the χ^2-test. The OR adjusted for sex, age, smoking and drinking were calculated using unconditional logistic regression analysis with the low risk genotype as the referent category to evaluate potential effect modification for these variables. The effects of combined genotypes on glioma were also estimated by using unconditional logistic regression analysis, and the low risk genotype also used as the reference group. Two sided probability test with a significance level of $p<0.05$ was used in our test. Chi-square test was used to check the Hardy-Weinberg equilibrium (HWE) in controls for the assessment of discrepancies between genotype and allele frequencies.

Results

The characteristics of subjects are listed in Table 1. The mean ages of 127 cases and 249 controls were 49.5±3.6 and 48.9±4.1, respectively. The ESCC patients had more cancer history in first relatives than controls ($p<0.05$). In terms of ionizing radiation exposure, 14 glioma cases (2 medical exposure, 6 medical and occupational exposure, 6 occupational exposure) and 11 controls (1 medical exposure, 2 medical and occupational exposure, 8 occupational exposure) reported a history of ionizing radiation exposure, and there was significant difference between them ($p<0.05$). No significant difference was found in cases and controls in terms of sex, age, drinking and smoking.

The frequencies of XRCC1 codon 194 Trp/Trp and Arg/Trp, and coden 399 Gln/Gln and Arg/Gln in the cases were significantly more frequent than those in controls ($p<0.05$). However, the XRCC1 codon 280 His/His did not found more in cases than that in controls ($p>0.05$) (Table 2). χ^2 analysis of the distribution of XRCC1 genotypes in controls were in according to Hardy-Weinberg equilibrium. The association between polymorphisms of XRCC1 codon 194, 280 and 399 gene variants and glioma was also showed in Table 3. The homozygous Trp/Trp and heterozygotes Arg/Trp variants of codon 194 had a 1.44(1.01-2.56) fold and 2.12(1.32-4.45) fold increased risk of glioma compared to the homozygous Arg/Arg wide genotypes. The same effect was found in codon 399, the codon 399 Gln/Gln and Arg/Gln genotypes were associated with a 1.69 (1.13-3.23) fold and 2.24 (1.32-3.76) fold increased risk in glioma.

A combined genotype analysis was performed to evaluate the potential combined effects of codon 194 and 399 genotypes on the risk of glioma. When comparing

Table 1. Characteristics of Study Subjects by the Case-control Status

Characteristics	Cases n=127(%)	Controls n=249(%)	Test statistics	P
Sex				
Male	87(69)	166(67)	χ^2=0.13	0.72
Female	40(31)	83(33)		
Age (mean±SD)	49.5±3.6	48.9±4.1	t=1.39	0.08
Smoking				
Yes	47(37)	82(33)	χ^2=0.62	0.43
No	80(63)	167(67)		
Drinking				
Yes	59(54)	98(35)	χ^2=1.74	0.19
No	68(46)	151(65)		
Cancer history of first relatives				
Yes	8(5)	4(2)	χ^2=6.0	0.014
No	119(95)	245(98)		
IR exposure				
Yes	14(11)	11(4)	χ^2=5.91	0.015
No	113(89)	238(96)		

Table 2. Genotype Frequencies of XRCC1 Codon 194, 280 and 399 Polymorphisms and Their Associations with Risk of Glioma

XRCC1 genotype	Cases n=127(%)	Controls n=249(%)	OR[1](95% CI)	OR[2](95% CI)
Codon 194				
Arg/Arg	71(56)	163(65)	1.0 (Reference)	1.0 (Reference)
Arg/Trp	38(30)	64(26)	1.36(0.81-2.28)	1.44(1.01-2.56)
Trp/Trp	18(14)	22(9)	1.88(0.89-3.92)	2.12(1.32-4.45)
Codon 280				
Arg/Arg	72(57)	153(61)	1.0 (Reference)	1.0 (Reference)
Arg/His	28(22)	58(23)	1.03(0.58-1.80)	1.27(0.67-1.94)
His/His	27(21)	38(15)	1.51(0.82-2.76)	1.54(0.88-2.85)
Codon 399				
Arg/Arg	58(46)	145(58)	1.0 (Reference)	1.0 (Reference)
Arg/Gln	48(38)	75(30)	1.6(0.97-2.64)	1.69(1.13-3.23)
Gln/Gln	21(17)	29(12)	1.81(0.90-3.58)	2.24(1.32-3.76)

[1]Unadjusted odds ratio; [2]Odds ratio after adjusted for age, sex, smoking, drinking, cancer history of first relatives and IR exposure

Table 3. Combined Genotype Analysis of Codon 194 and 399 Genotypes on the Risk of Glioma

Genotype combinations	Cases n=127(%)	Controls n=249(%)	OR[1](95% CI)	OR[2](95% CI)
Codon 194 Arg/Arg and Codon 399 Arg/Arg	36	97	1.0 (Reference)	1.0 (Reference)
Codon 194 Arg/ Arg and Codon 399 Gln allele	35	66	1.43(0.78-2.60)	1.37(0.75-2.56)
Codon 194 Trp allele and Codon 399 Arg/Arg	29	48	1.63(0.85-3.09)	1.85(0.97-3.15)
Codon 194 Trp allele and Codon 399 Gln allele	27	38	1.91(0.97-3.74)	2.87(1.56-6.73)

[1]Unadjusted odds ratio; [2]Odds ratio after adjusted for age, sex, smoking, drinking, cancer history of first relatives and IR exposure

the codon 194 Arg/Arg and 399 Arg/Arg genotypes, the combination of codon 194 Trp allele and 399 Gln allele had a heavy increased in glioma risk (OR=2.87, 95%CI=1.56-6.73, p=0.04).

Discussion

This study first analyzed the association between polymorphism of DNA repair gene XRCC1 and glioma in Chinese population of our case-control study. Our study showed the XRCC1 codon 194 Trp allele plays a significant role as a risk modifier for glioma. Our study also showed an over representation of the codon 399 Gln allele in cases compared to controls, which suggested that this polymorphism may modify the risk for glioma. In our study, we fond a 2.24-fold increased risk of glioma with the codon 399 Gln polymorphism. This result is not consistent with study conducted in the United State, which did not found significant increased risk(Liu et al., 2009). However, the association between this particular polymorphism and cancer risk has been reported in previous epidemiologic studies, such as an association between this polymorphism and increase risk of breast cancer, hepatocellular carcinoma, colorectal carcinoma and carcinoma of head and neck as well as lung cancer (Sturgis et al., 1999; Abdel-Rahman et al., 2000; Divine et al., 2001 ; Chacko et al., 2005; Kiran et al., 2009).

The XRCC1 gene encodes the XRCC1 protein, which complexes with three other DNA repair enzymes involved in the BER pathways, including DNA ligase III, DNA polymerase and poly (ADP-ribose) polymerase PARP (Stern et al., 2001). The XRCC1 codon 194 and codon 280 polymorphisms are located in the linker region that separates the PARP interacting domain (Kubota et al., 1996). The codon 399 polymorphism resides on the COOH-terminal side of the PARP interacting domain, within the BRCT1 domain, that are thought to mediate several protein-protein interactions (Masson et al., 1998). Amino acid substitutions in the BRCT domina and in the DNA polymerase β interacting domain in hamster is reported to disrupt the functionality of XRCC1 (Shen et al., 1998). The mutations of XRCC1 polymorphisms 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 resulted in carcinogenesis development. Our study found significant an odds ratio of codon 194 Trp allele and codon 399 Gln allele for glioma, which strongly implicates that these polymorphisms may alter the normal protein function by encoding for a twisted protein (Tuimala et al., 2002), resulting in altered affinity to its interactive proteins suggesting an association with a deficiency in DNA repair capacity. This finding indicated the XRCC1 gene variants in the impairment of DNA repair mechanism and their consequent biological effect could induce the carcinogensis.

There are several strengthens in our study. Firstly, all the interviewers were trained and all of them used the same query mode for case and controls, which could avoid the measurement bias. Secondly, an extensive effort was made to collect information on related factors of XRCC1 and glioma, which was further considered and adjusted throughout the analysis. Thirdly, the controls in our study were collected from those who came to hospitals for routine health examination, which may be more representative than the hospitalized individuals with

a higher chance than the general population to share a common exposure with cases.

In conclusion, the present study suggested evidence of a potential role for XRCC1 codon 194 and 399 polymorphisms in the genetic predisposition to glioma among Chinese population. Further studies incorporating more DNA repair genes and their phenotypic correlation with biomarkers of DNA damage and other gene-environment interactions are in progress. This analysis of correlation of DNA repair genes and glioma may provide a deeper insight into the genetic and environment factors to cancer risk.

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