RESEARCH COMMUNICATION

DNA Repair Gene ERCC1 and XPD Polymorphisms Predict Glioma Susceptibility and Prognosis

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Abstract

Aims: We conducted a case-control study in a Chinese population to clarify the association between polymorphisms in ERCC1 and XPD and susceptibility and survival of glioma. Methods: A total of 393 cases and 410 controls were selected from March 2007 to December 2011. Genotyping of ERCC1 and XPD was conducted by TaqMan assays using the ABI Prism 7911HT Sequence Detection System. All analyses were performed using the STATA statistical package. Results: Polymorphisms in ERCC1 118C/T, ERCC1 8092C/A and XPD Asp312Asn showed no statistically significant difference between glioma cases and controls. However, individuals with the XPD 751Gln/Gln genotype had an increased risk of developing glioma compared with those with the Lys/Lys genotype (adjusted OR=1.64, 95% CI: 1.06-2.89). The ERCC1 118T/T genotype was associated with significantly higher median survival than the ERCC1 C/C genotype (HR=0.67, 95%CI=0.35-0.96). In addition, individuals with XPD 751Gln/Gln had a lower median survival time than XPD Lys/Lys carriers (HR=0.54, 95%CI=0.37-0.93). Conclusion: In conclusion, we observed that the XPD 751Gln/Gln genotype is associated with glioma susceptibility, and ERCC1 118 T/T and XPD 751Gln/Gln genotypes confer a significantly better prognosis.

Keywords: - ERCC1 - XPD - polymorphisms - glioma - susceptibility - prognosis

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Introduction

Glioma is the most common type of primary brain malignancy in adults. Currently, the etiology of glioma has not been completely understood. Previous studies have reported that a number of single nucleotide polymorphisms (SNPs) in DNA repair gene may modify glioma risk (Wang et al., 2004; Wrensch et al., 2005; Kiuru et al., 2008; Liu et al., 2009; Zhou et al., 2009; Rajaraman et al., 2010; Hu et al., 2011; Yosunkaya et al., 2010; Zhou et al., 2011). However, the clinical value of most glioma-associated molecular aberrations in terms of their significance as diagnostic, prognostic or predictive molecular markers has remained unclear (Batchelor et al., 2004; Ohgaki et al., 2004; Houillier et al., 2006). To date, the polymorphisms of DNA repair genes are plausible candidates that can modify the risk of human cancer, and are associated with the prognosis in cancer patients with radiotherapy or radiotherapy plus chemotherapy (Hayes et al., 2011; Kuwabara et al., 2011; Liao et al., 2012; Milovic-Kovacevic et al., 2011; Yan et al., 2012).

The excision repair cross-complementing rodent repair deficiency complementation group 1 (ERCC1) gene and xeroderma pigmentosum group D (XPD) are two essential subunit of the nucleotide excision repair (NER) system, and are responsible for effecting repairs to bulky adducts and UV-induced DNA damage (Sung et al., 1993; Weeda and Hoeijmakers, 1993). The DNA repaired mechanisms are hypothesized to be play an important role in the treatment of patients with chemotherapy and radiotherapy (Leichman et al., 2006; Goyal et al., 2010; Kuwabara et al., 2011; Milovic-Kovacevic et al., 2011; Liao et al., 2012; Yan et al., 2012). However, the studies pertaining to these DNA-repair genes focusing on prognosis of glioma would appear to be limied and controversial. Our previous study failed to find the association between ERCC1 gene polymorphism and glioma (Zhang et al., 2012). In present paper, we conducted a perspective case-control and case cohort study to examine the role of genetic polymorphisms of XRCC1 and XPD in the risk of glioma and association with prognosis in Chinese population.

Materials and Methods

This case-control study was conducted in the largest teaching hospital in Wenzhou, a city in Eastern China. All Chinese cases with newly diagnosed primary glioma between March 2007 and December 2011 in this hospital were invited to participate within 2 months after diagnosis. All cases recruited in this study were histologically confirmed. Among a total of 429 eligible cases, 393 were successfully interviewed and donated blood samples with a participation rate of 91.6%. Controls were randomly selected from people who requested general health

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examinations in the same hospital during the same period. Controls were required to be without any history of any type of cancer and frequency matched by sex and 5-year age groups. A total of 457 eligible controls, 410 were successfully interviewed and donated blood samples with a participation rate of 89.7%. Informed consent was obtained before each interview and blood taking. Trained interviewers conducted face-to-face interviews using a structured questionnaire to collect information on sociodemographic characteristics, family history of cancer, occupational radiation exposure and other potential confounders, which were introduced in our pervious study (Zhang et al., 2012). All the interviews were finished within two weeks after their diagnosis and each interview took around 10-15 mins. Approval to conduct this study was granted by the Ethics Committee of Wenzhou Medical College and the Ethics Committee for Clinical Research of Wenzhou Medical College. All interviews and blood samples collection were conducted after obtaining signed informed consent from participants.

A total of 393 patients who continued their treatment in the same hospital and received chemotherapy after surgery or radiotherapy with or without chemotherapy were followed-up. All 393 patients were followed up and 22 patients were loss to follow-up due to immigrant, psychological factor or cognitive disability. A total of 371 patients were followed-up till March 2012.

Genotyping

Genomic DNA was extracted from whole-blood samples by using the Qiagen Blood Kit (Qiagen, Chatsworth, CA). Genotyping of ERCC1 and XPD was conducted by TaqMan assays using the ABI Prism 7911HT Sequence Detection System (Applied Biosystems, Foster City, CA). Primer, probes, and reaction conditions were available upon request. Genotyping was done by laboratory personnel blinded to case-control status. 10% of the samples were used for quality control in our study.

Statistical analysis

All statistical analysis were performed by using the Statistical Package for the Social Sciences (SPSS) software 13.0 for windows. Chi-square or t-tests were used to test differences of potential confounders between cases and controls. The Hardy-Weinberg equilibrium (HWE) was used to assess the gene frequency distribution in controls. Unconditional logistic regression was used to estimate the odds ratios (ORs) and corresponding 95% confidence interval (CIs) for each polymorphism. The association between each gene polymorphism and risk of glioma were further analyzed by adjusting the potential confounders including sociodemographic characteristics, family history of cancer and occupational radiation exposure. 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 and XRCC3 gene polymorphism and survival. The relative risk (hazard ratio (HR)) and 95% CI were calculated from 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 distribution of basic characteristics among cases and controls are showed in Table 1. The successfully genotypes mean age at enrollment of this case-control study was 50.4±7.9 years for cases and 49.6±8.5 years for controls. There was no significant differences for gender, age and family history of glioma cancer. More cases than controls had occupational ionizing radiation histories (p=0.001). The 393 glioma cases consisted of 122 (31%) glioblastoma, 136 (34.6%) anaplastic astrocytoma or diffuse astrocytoma or other astrocytoma, and 135 (34.4%) other gliomas. The distributions of the four genotype frequencies were in agreement with those expected from the HWE model at the 0.05 level for controls (p=0.14, 0.32, 0.18 and 0.36 for 118C/T, 8092C/A, Lys751Gln and Asp312Asn, respectively).

Table 2 shows the genotype frequencies of the four polymorphisms in cases and controls and the corresponding ORs with CIs. Polymorphisms in ERCC1 118C/T, ERCC1 8092C/A and XPD Asp312Asn showed

Table 1. Demographic Characteristics of the Study Population

Variable		Cases N=393	Controls N=410	P value
Age		50.4±7.9	49.6±8.5	
	<30	45(11.4)	50(12.3)	0.93
	30-50	126(32.1)	133(32.4)	
	>50	222(56.5)	227(55.3)	
Sex				
	Male	242(61.5)	254(62.0)	0.92
	Female	151(38.5)	156(38.0)	
Occupational IR exposure history				
	Yes	19 (4.9)	4 (1.0)	0.001
	No	374 (95.1)	406 (99.0)	
History of glioma cancer in first relatives				
	Yes	5(1.3)	1(0.2)	0.09
	No	388(95.4)	409(99.8)	

Table 2. The Gene Frequencies ERCC1 and XPD in Cases and Controls

Genotype	Case (%) N=393	Control (%) N=410	Crude OR (95% CI)	Adjusted OR (95% CI)
ERCC1 118C/T				
C/C	171(43.5)	196(47.7)	1.0(Ref.)	1.0(Ref.)
T/C	154(39.1)	152(37.1)	1.16(0.84-1.71)	1.23(0.88-1.89)
T/T	68(17.4)	62(15.2)	1.26(0.83-1.92)	1.58(0.91-2.04)
ERCC1 8092C/A				
C/C	202(51.5)	221(53.8)	1.0(Ref.)	1.0(Ref.)
A/C	141(35.9)	154(37.6)	1.01(0.74-1.36)	1.10(0.83-1.48)
A/A	50(12.6)	35(8.6)	1.51(0.92-2.50)	1.60(0.97-2.57)
XPD Lys751Gln				
Lys/Lys	139(35.3)	175(42.8)	1.0(Ref.)	1.0(Ref.)
Lys/Gln	198(50.4)	186(45.3)	1.34(0.94-1.83)	1.43(1.04-2.01)
Gln/ Gln	56(14.3)	49(11.9)	1.58(0.98-2.56)	1.64(1.06-2.89)
XPD Asp312Asn				
Asp/Asp	155(39.5)	177(43.2)	1.0(Ref.)	1.0(Ref.)
Asp/Asn	182(46.2)	186(45.4)	1.12(0.82-1.52)	1.20(0.89-1.67)
Asn/ Asn	56(14.3)	47(11.4)	1.36(0.85-2.18)	1.45(0.96-2.34)

[1]Adjusted for age, sex, smoking, and ionizing radiation exposure history

Table 3. Kaplan-Meier Estimation of median survival and HRs with gene polymorphism

Gene polymorphisms	N (%) N=371	Median survival(months)	HR(95% CI)
ERCC1 118C/T			
C/C	164(44.2)	29.5±7.7	1.0(Ref.)
T/C	144(38.7)	25.4±6.2	0.86(0.57-1.24)
T/T	63(17.1)	22.3±5.5	0.67(0.35-0.96)
XPD Lys751Gln			
Lys/Lys	132(35.6)	30.6±8.1	1.0(Ref.)
Lys/Gln	181(48.7)	25.1±5.9	0.83(0.62-1.45)
Gln/Gln	58(15.7)	23.9±4.7	0.54(0.37-0.93)

no statistically significant difference between glioma cases and controls. Individuals with the XPD 751 Lys/Gln and Gln/Gln genotypes had an increased risk of developing glioma compared with those with the Lys/Lys genotype (adjusted OR=1.43, 95% CI: 1.04-2.01 for Lys/Gln genotype and adjusted OR=1.64, 95% CI: 1.06-2.89 for Gln/Gln), and those with the TT genotype of ERCC1 118 TT genotype polymorphism had a non-significant increased risk compared with those carrying CC genotype (adjusted OR=1.58, 95% CI: 0.91-2.04).

A total of 371 patients who continued their treatment in the same hospital and received chemotherapy after surgery or radiotherapy with or without chemotherapy were followed-up. The median survival of patients was 28.1±6.3 months (SD, ±10.6). When the survival time of the patients was compared with ERCC1 118C/C genotype, a significant difference in median survival of patients carrying T/T and T/C genotype (25.4±6.2 and 22.3±5.5 months, respectively) was obtained (Table 3). Individuals with XPD Lys/Gln and Gln/ Gln genotypes had a significantly lower risk than Lys/Lys genotype (25.1±5.9 and 23.9±4.7 months vs 30.6±8.1 months). Individuals carrying ERCC1 118 T/T genotype showed significantly higher median survival than the ERCC1 C/C genotype and significant hazard ratio was found (HR=0.67, 95%CI=0.35-0.96). Meanwhile, further Kaplan Meier analysis showed individuals with XPD 751Gln/Gln had a lower median survival time in comparison to XPD Lys/Lys carriers, and we found a moderate HR for XPD 751 Gln/Gln (HR=0.54, 95%CI=0.37-0.93).

Discussion

In this study from the Chinese population, we observed XPD Lys751Gln polymorphism was associated with susceptibility to giloma. We did not observed any significant association between the three polymorphisms in ERCC1 118C/T, ERCC1 8092C/A and XPD Asp312Asn, and the risk of glioma in a Chinese population. Moreover, we found a significant direct correlation between polymorphisms of ERCC1 118C/T and XPD Lys751Gln and the risk of disease prognosis, especially for patients receiving chemotherapy. Genetic information on the polymorphisms and gene expression could play an important role in creating successful pharmacogenetic-guided chemotherapy. The use of rapid and sensitive PCR assays for diagnostic screening, coupled with ready accessibility to peripherad blood from patients with glioma, will help facilitate application of our study.

Our study did not found a significant association between polymorphism of ERCC1 118C/T and ERCC1 8092C/A and risk of glioma. Our finding were in line with previous studies that indicated no association between ERCC1 and risk of glioma (Wrensch et al., 2005; Yosunkaya et al., 2010). Both of these studies were from population based case-control series with a large sample size in the United States, about 500 to 1000 glioma cases. The two study found a non-significant increased risk for individual heterozygous ERCC1 genotype (Wrensch et al., 2005; Yosunkaya et al., 2010). Even in our previous study conducted in 2011, it showed no association between polymorphisms in ERCC1 gene and cancer risk (Zhang et al., 2011). However, another previous study found no significant association between the ERCC1 118C/T and 8092C/A and risk of glioma (Liu et al., 2009). This study was also a hospital-based study with 373 Caucasian glioma cases. The inconsistency of these study could be due to differences in population background, source of control subjects, sample size, and also by chance. Further confirmation is still needed in future large sample studies.

Our study indicate that the XPD Lys751Gln polymorphism may moderate increase the risk of glioma cancer. The reason might be explain that XPD protein is involved in the nucleotide excision repair (NER) pathway, which recognizes and repair a wide range of structurally unrelated lesions such as bulky adducts and thymidine dimmers (Flejter et al., 1992; Lindahl et al., 1997; Braithwaite et al., 1999; de Laat et al., 1999). The XPD gene encodes a helicase that is a component of the transcription factor TFIH. Mutations in the XPD gene can diminish the activity of TFIH complexes increasing the likelihood of repair defects, transcription defects and abnormal response to apoptosis (Sung et al., 1993; Coin et al., 1999). The low activity of repair defects may induce the progression of carcinogenesis. XPD polymorphisms have been analyzed particularly in epidemiological studies on skin and smoking-related cancers and no obvious relationship has been found for these types of cancer (Benhamou and Sarasin, 2002; Benhamou and Sarasin, 2005), however, this polymorphism with glioma has not established in previous study in Chinese population. Our study firstly investigated the association of this polymorphism and glioma and provide important information to investigate the biomarker for risk of glioma.

There are limited report on the role of ERCC1 118C/T and XPD Lys751Gln polymorphisms on the survival of cancer patients. The DNA repair systems are critical for repairing DNA damage induced by carcinogens. Moreover, they also play an important role in repairing the cross-linking and oxidative damage caused by chemotherapy drugs (Altaha et al., 2004). Therefore, the impaired DNA repair capacity may not only increase carcinogenesis and lead to more biologically aggressive tumors and decrease survival, but also contribute to the persistence of functional platinum-DNA adducts that confer anti-tumor activity and impart more favorable prognoses. To date, no previous study has examined the association between ERCC1 and XPD polymorphism and prognosis of glioma yet. Several studies indicated that

the wide type ERCC1 and XPD were important markers of improved patients survival (Milovic-Kovacevic et al., 2011; Leng et al., 2012; Yan et al., 2012). In the present study, we found a significantly slight decreased risk of death from glioma. A moderately strong correlation has been found between ERCC1 118T/T and XPD 751Gln/Gln gene expression levels and death risk from glioma, in agreement with a previous study (Chen et al., 2007).

In conclusion, our present data indicated that polymorphisms in ERCC1 and XPD have a role in the susceptibility and survival of glioma. The limitation of our study was a lower number of patients with survival data. In future, studies with a higher sample size are warranted.

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