Original Article
Correlation between VEGFR2 rs2071559 polymorphism and glioma risk among Chinese population

Guo-Zheng Xu[1], Ying Liu[2], Yi Zhang[3], Jing Yu[3], Bo Diao[1]

[1]Department of Neurosurgery, Wuhan General Hospital of Guangzhou Command, Hubei Key Laboratory of Central Nervous System Tumor and Intervention, Wuhan 430070, China; [2]Department of Clinical Laboratory, Wuhan General Hospital of Guangzhou Command, Wuhan 430070, China; [3]Department of Clinical Experiment, Wuhan General Hospital of Guangzhou Command, Wuhan 430070, China

Abstract: Objective: We investigated the correlation between vascular endothelial growth factor 2 (VEGFR2) polymorphism and glioma risk among Chinese population. Method: Case-control study design was adopted, and blood samples and clinical data of 250 glioma cases and 260 control subjects were collected. Epidemiological questionnaire survey was performed, and DNA extraction, concentration normalization and packaging were carried out using Qiagen Blood Kit. TaqMan method was performed for VEGFR2 rs2071559 genotyping. Results: C allele of VEGFR2 rs2071559 genotype was the susceptibility allele contributing to the risk of glioma (OR=1.813, 95% CI: 1.393-2.359, P=0.014). CC genotypes of VEGFR2 rs2071559 were associated with increased risk of glioma (OR=2.068, 95% CI: 1.164-3.674, P=0.014; Adjusted OR=1.883, 95% CI: 1.430~3.013, P=0.018). Conclusion: CC genotypes of VEGFR2 rs2071559 were associated with glioma risk among Chinese population. However, the role of VEGFR2 rs2071559 polymorphism in glioma susceptibility remains to be further clarified.

Keywords: Glioma, vascular endothelial growth factor 2 (VEGFR2), gene, polymorphism, genetics

Introduction

Glioma is a neuroepithelial-derived tumor with the highest incidence of all malignant tumors of the central nerve system. Accounting for over 80% of all malignant intracranial tumors, glioma features a higher malignancy than other tumors [1, 2]. Glioma is rich in blood vessels and has a fast growth and short course of disease. Invading the perivascular spaces of brain tissue and leading to indistinct border, glioma is hard to be completely removed by surgery [3]. Therefore, postoperative recurrence is very high and the patients usually have a short survival. Glioma represents one of the refractory tumors in the field of neurosurgery due to fast tumor cell proliferation, slow rate of apoptosis, high invasiveness and neovascularization. According to the latest statistics, the 2-year survival of patients with low-grade astrocytoma, degenerative astrocytoma and glioblastoma multiforme was respectively 66%, 45% and 9% even after surgical treatment, radiotherapy or chemotherapy [4]. So far we have very limited knowledge on pathogenesis, molecular biology changes, individual variation, diagnostic

and prognostic indicators and specific treatment measures relating to glioma. Most tumors are the result of combined action of environmental and genetic factors. One established external cause of glioma is exposure to therapeutic or high-dose radiation, especially ionizing radiation. However the risk of exposure may be augmented by genetic factors. The Human Genome Project (HGP) has revealed the stability of human genome, but gene mutation and polymorphism are not fully known. According to the human genome landscape, about 99.9% of human genome sequences are identical between the individuals, and the difference accounts for only 0.1%. Gene mutation and polymorphism result in interindividual differences, susceptibility to diseases and varying response to drugs and environmental factors. Human genome contains various forms of gene polymorphism, among which single nucleotide polymorphism (SNP) is the most simple and common. SNP refers to the single base change with frequency>1%, sometimes accompanied by the insertion or deletion of one or several nucleotides.

In glioma, VEGF and VEGF receptor 2 (VEGRF2) are closely related to blood vessels and angiogenesis [5]. VEGFR2 shows a strong ligand-dependent tyrosine phosphorylation in normal cells, which mediates the proliferation and differentiation of vascular endothelial cells. VEGFR2 plays a role in increasing VEGF expression and inducing tumor angiogenesis [6]. By binding to VEGFR2, VEGF2 can promote the division of vascular endothelial cells and angiogenesis and enhance the invasiveness of glioma. Liu et al. [7] showed that the VEGF2 level of patients with glioma was significantly higher than that of the controls. Hence VEGFR2, when abnormally expressed, has an adverse impact on the activity of neural cells, thereby leading to glioma. At present, there are very few studies on the correlation of SNP in glioma-related genes and VEGFR2 to the susceptibility to glioma. We adopted case-control study to analyze the correlation between VEGFR2 rs2071559 polymorphism and glioma risk among 250 glioma cases and 260 normal cases. The purpose of the present study was to find molecular-level evidence for the occurrence of glioma.

Subjects and methods

Subjects

For glioma group 250 glioma patients hospitalized at Wuhan General Hospital of Guangzhou Commandy from October 2011 to February 2015 were recruited and received surgical treatment. The inclusion criteria were as follows: 1) Pathologically diagnosed as glioma by referring to the 2007 WHO Classification of Tumors of the Central Nervous System; 2) Having no past history of malignant tumors of other organs; 3) Having received no radiotherapy or chemotherapy; 4) No limitation by age, gender and pathological type. For control group 260 patients hospitalized for traumatic brain injury at Huashan Hospital Affiliated to Fudan University in the same period were recruited. They satisfied the following inclusion criteria: 1) No past history of tumors; 2) No other diseases of the central nervous system; 3) Having received no radiotherapy or chemotherapy for unknown reasons; 4) Matched for gender and age with glioma patients. All subjects were from the Han Nationality and randomly chosen without blood relationship between them.

The protocol was approved by College of Life Sciences Ethics Committee of Fudan University.

Informed consent was obtained from the subjects before epidemiological survey and blood sample collection.

Methods

Epidemiological questionnaire was designed based on the questionnaire for epidemiological survey of central nervous system tumors by MD Anderson Cancer Center, University of Texas. Investigators received strict, unified professional training before face-to-face interview with the subjects. The information inquired was as follows: general demographic characteristics (age, gender, place of birth), occupation, history of diseases, history of tumors in first-degree relatives, smoking and diet, clinical data (diagnostic and treatment). The subjects were stratified by smoking status, namely, non-smoker, former smoker and current smoker. Current smokers were defined as those who smoked at least 1 cigarette/d for at least 1 year; former smokers were those who had stopped smoking for over 1 year, and smokers not satisfying this definition were all considered as current smokers. Gliomas were pathologically classified into three categories: glioblastoma, astrocytoma other than glioblastoma (mainly diffuse and anaplastic astrocytoma) and other types of glioma (oligodendroglioma, anaplastic oligodendroglioma, ependymocytoma, choroid plexus papilloma and mixed glioma). All contents of questionnaire were stored in the computer, and checking, revision and conversion of assigned values were performed to establish the database of glioma cases and controls.

Blood sample collection

From each subject 5 ml of peripheral venous blood sample was collected and placed into a tube containing anticoagulant citrate dextrose solution (citric acid, sodium citrate, glucose). All samples were stored at 20°C prior to use.

DNA extraction and genotyping

2 ml venous blood of subjects was extracted with EDTA anticoagulant tube. Qiagen reagent kit (Germany) was used to extract the DNA according to the instructions.

The TaqMan genotyping technique was used to genotype VEGFR2 rs2071559 polymorphism. The PCR reaction system was 5 ul, which contained 5 ng DNA template, and it was placed in

Table 1. The characteristics of participants

Item	Control group (N=260)	Glioma group (N=250)	P
Sex (M/F)	141/119	135/125	0.108
Age (year)	40.6±11.4	40.4±11.8	0.452
Smoking (n, %)	84 (32.3)	77 (30.8)	0.446
Family cancer history (n, %)	38 (14.6)	31 (12.4)	0.193

a 96-well plate for reaction. The reaction conditions of PCR were as follows: pre degeneration at 95°C for 10 min, denaturation at 92°C for 15 s, annealing at 60°C for 1 min, 45 cycles in total.

Statistical analysis

Data analyses were done using SPSS 17.0 software. The differences in gender, age, smoking status and family history of tumors between the two groups were analyzed using χ^2 test. The goodness-of-fit was subject to χ^2 test using HaploView 4.2 to determine whether the genotype frequencies for SNPs were in Hardy-Weinberg equilibrium. Multifactor logistic regression analysis was adopted to calculate odds ratio (OR) and the correlation between VEGFR2 rs2071559 polymorphism and glioma risk was evaluated at 95% CI. The results corrected for gender and age, for gender, age and family history of tumors, or for gender, age, smoking status and family history of tumors were analyzed, respectively. $P \le 0.05$ was considered as statistically significant.

Results

General information

Gender, age, smoking status, family history of tumors, pathological classification and WHO classification of the two groups were compared. The average age of glioma group and the control group was 40.4±11.8 and 40.6±11.4 years old, respectively. The two groups were matched for gender and age without significant differences in smoking status and family history of tumors (**Table 1**).

Genotype analysis

The success rate of rs2071559 genotyping in glioma group and control group was 100%. Genotype frequencies for SNPs of rs2071559

were in Hardy-Weinberg equilibrium among the controls (P>0.05).

The two groups showed significant differences in genotype frequencies of rs2071559 (P<0.05, **Table 2**). Compared with T allele, C allele was associated with increased glioma risk (OR=1.813, 95% CI: 1.393-2.359, P= 0.014). CC genotypes of VEGFR2 rs2071559 were associated with increased risk of glioma (OR=2.068, 95% CI: 1.164-3.674, P=0.014).

Logistic regression analysis of genotypes indicated that CC genotypes were associated with increased glioma risk compared with TT genotype (Adjusted OR=1.883, 95% CI: 1.430~3.013, P=0.018, **Table 3**).

Discussion

The occurrence of glioma is controlled by multiple factors. Many studies focus on environmental factors, but explicit environmental factors other than therapeutic ionizing radiation have been identified so far. Compared with other tumors of the central nervous system, we have very limited knowledge on SNPs in glioma. New-born capillary network is crucial for the growth of solid tumors such as glioma. Tumor angiogenesis is regulated by soluble factors released by tumor cells, and the latter acts on vascular endothelial cells via paracrine action. VEGF is the primary regulator of normal blood vessels and tumor angiogenesis. Both VEGF and VEGFR2 are expressed by the same glioma cells. A tyrosine kinase (TK), VEGFR2 has an extracellular domain (ECD) that consists of 7 Ig-like loops and 1 split TK domain, and acts as the receptor for different VEGF subtypes and VEGFC. VEGF2 expression is usually elevated in vascular tissues in glioblastoma multiforme, anaplastic oligodendroglioma and ependymocytoma complicate by necrosis. In contrast, VEGF2 is only weakly expressed in WHO grade-II astrocytoma, anaplastic astrocytoma and oligodendroglioma and even to an undetectable extent. In addition, VEGF mRNA expression is usually elevated in most low-grade glioma, especially in malignant glioma. Glioma and the endothelial cells from adjacent tissues may also have a significantly elevated VEGFR2 mRNA expression. This implies that VEGF over-expression and VEGFR2 can influence neural cell activities and lead to glioma.

Table 2. Distribution of genotype and alleles

Genotype or Allele	Glioma group (N=250)	Control group (N=260)	OR (95% CI)	P
TT	98 (39.2)	128 (49.2)	1	
CT	114 (45.6)	108 (41.5)	1.378 (0.950-2.000)	0.095
CC	38 (15.2)	24 (9.3)	2.068 (1.164-3.674)	0.014
T	0.62	0.70	1	<0.01
C	0.38	0.30	1.813 (1.393-2.359)	<0.001

Table 3. Logistic regression of the relation between gene polymorphism and glioma risk

Parameters	OR	95% CI	P value
VEGFR2 polymorphism	1.883	1.430~3.013	0.018
Smoking	1.982	1.110~4.209	0.019
Age	1.527	0.786~3.011	0.124
History of cancer disease	1.557	0.775~3.101	0.281

We tested the correlation between VEGFR2 rs2071559 polymorphism and glioma risk and found that the rs2071559 polymorphism in the promoter (-604T>C) was associated with the risk of glioma. It has already been established that VEGFR2 rs2071559 polymorphism plays a crucial role in interindividual difference of VEGFR2 expression. Millauer et al. [8] found that VEGFR2 was associated with the growth of several solid tumors including breast cancer, ovarian cancer, lung cancer and glioblastoma. Wang et al. [9] indicated that rs2071559 genotype was associated with increased risk of coronary heart disease. Another correlation analysis suggested that rs2071559 polymorphism was connected with decreased susceptibility to atherosclerotic ischemic stroke, showing a negative correlation with the intima-media thickness of carotid artery. Galan et al. [10] found that VEGFR promoter encompassing SNP -604T>C had a high transcriptional activity. Compared with homozygous C allele, the carriers of homozygous T allele had a 3-fold increased risk of age-related macular degeneration (AMD). Another study [11] showed that AMD patients had a lower frequency of rs2071559 AA genotype. Dong et al. [12] observed 408 cases after surgical treatment for cancer of large intestine and demonstrated the critical association between rs2071559 and tumor recurrence and the significant correlation with the prognosis of glioblastoma.

In the present study, we identified the correlations of rs2071559 CC genotypes to glioma risk. According to haplotype analysis, the haplotype containing susceptibility allele (rs2071559 C allele) was associated with higher glioma risk. All above findings are evidences of the correlation between VEGFR2 rs2071559 polymorphism and glioma risk. According to our results, rs2071559 polymorphism (-604T>C) led to the downregulation of VEGFR2 and increased glioma risk. VEGF and high-affinity VEGFR are key regulators of tumor angiogenesis. Blocking VEGF/VEGFR2 signaling pathway can inhibit experimental tumor growth, implying that VEGFR2 is the main endothelial cell signaling molecule during tumor proliferation. Ebos et al. [13] reported the soluble form of VEGFR2, the expression of which was negatively correlated with tumor size. Of 7 ECDs of VEGFR2, domain 1-3 bind to VEGF and VEGFR2 is soluble. KDRn3 gene therapy for mouse model of glioma can reduce the average weight and volume of tumor and greatly decrease the microvessel density within the tumors. Silva et al. [14] recently reported that VEGF2 had an inhibitory effect on BON cell proliferation, invasion and migration. They assumed that part of the effect was contributed by the co-expression of soluble VEGFR2 (sVEGFR2) in cells. The above results further corroborated our finding that rs2071559 polymorphism was associated with glioma risk. Rs2071559 is localized to the VEGFR2 promoter domain that binds to nucleoprotein (presumably transcriptional factor). The rs2071559 (-604T>C) polymorphism can lead to a decline in the efficiency of the binding of VEGFR2 promoter to transcriptional factor, thereby downregulating VEGFR2 and its soluble form.

We found that VEGF2 polymorphism correlated to glioma risk among Chinese Han people, especially rs2071559 polymorphism. VEGFR2 may be inhibitory on tumor progression. We only studied one SNP in VEGFR2 gene, but other SNPs or SNPs in genes adjacent to VEGFR2 may also play a role. These domains require further study in terms of its function and correlation with glioma risk among Chinese people.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bo Diao, Department of Neurosurgery, Wuhan General Hospital of Guangzhou Command, Hubei Key Laboratory of Central Nervous System Tumor and Intervention, Wuhan 430070, China. Tel: +86-02750772489; Fax: +86-02750772489; Email: dpitao@163.com

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