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Variation in DNA repair gene XRCC3 affects susceptibility to astrocytomas and glioblastomas

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ABSTRACT. The gene XRCC3 (X-ray cross complementing group 3) has the task of repairing damage that occurs when there is recombination between homologous chromosomes. Repair of recombination between homologous chromosomes plays an important role in maintaining genome integrity, although it is known that double-strand breaks are the main inducers of chromosomal aberrations. Changes in the XRCC3 protein lead to an increase in errors in chromosome segregation due to defects in centrosomes, resulting in aneuploidy and other chromosomal aberrations, such as small increases in telomeres. We examined XRCC3 Thr241Met polymorphism using PCR-RFLP in 80 astrocytoma and glioblastoma samples. The individuals of the control group (N = 100) were selected from the general population of the São Paulo State. Odds ratio and 95%CI were calculated using a logistic regression

model. Patients who had the allele Met of the XRCC3 Thr241Met polymorphism had a significantly increased risk of tumor development (odds ratio = 3.13; 95% confidence interval = 1.50-6.50). There were no significant differences in overall survival of patients. We suggest that XRCC3 Thr241Met polymorphism is involved in susceptibility for developing astrocytomas and glioblastomas.

Key words: Polymorphism; XRCC3; Astrocytoma; Glioblastoma

INTRODUCTION

Tumors of the central nervous system (CNS) represent approximately 2% of all cancers, with an estimated 4.2 to 5.4 per 100,000 individuals per year (Ohgaki and Kleihues, 2005). Although the incidence of CNS tumors is small compared with other cancers, these are among the most serious human malignancies, since they affect coordination and integration of all organic activities. Moreover, as each region of the brain has a vital function, the therapy used in other cancers (total surgical removal of the organ or tumor with a generous margin of normal tissue) cannot be applied to cure brain tumors (Louis et al., 2002; Ohgaki and Kleihues, 2005). Gliomas are the most common tumors of the CNS. Despite the remarkable progress in the characterization of the molecular pathogenesis of gliomas, these tumors remain incurable and, in most cases, refractory to treatment due to their molecular heterogeneity (Kleihues et al., 2002).

Astrocytomas account for the large majority of gliomas, making up 70% of the total, and can be divided into: pilocytic astrocytomas (grade I), including low-grade astrocytomas (grade II), anaplastic (grade III) and glioblastoma (grade IV) (Kleihues et al., 2002). The relevance of the graduated scheme of malignancy based on histopathology is indicated by patient survival. Patients with low-grade astrocytomas (grade II) have a median survival of about seven years, patients with anaplastic astrocytomas (grade III) have a mean survival of half of that time (McCormack et al., 1992), while patients with glioblastoma have in average 9 to 11 months (Simpson et al., 1993). Unlike grade I astrocytomas, the progression of tumors of grades II and III for most malignant tumors is well documented (Ino et al., 2001; Collins, 2004; Hartmann et al., 2004; Ichimura et al., 2004; Ohgaki, 2005). Ng and Lam (1998) suggested that glioblastomas should be divided into two clinically and molecularly distinct entities: primary glioblastomas or new ones, that occur in elderly patients and are clinically very aggressive, and secondary glioblastomas, which develop from preexisting low-degree astrocytomas and have a more prolonged clinical course.

The XRCC3 (X-ray cross complementing group 3) gene is needed to repair damage caused when recombination occurs between homologous chromosomes (Loizidou et al., 2008; Andreassi et al., 2009). The repair of recombination between homologous chromosomes is a mechanism that fixes various types of DNA damage. The large number of repeated sequences can potentially lead to a large number of undesirable interactions between chromosomes. The repair of recombination between homologues plays an important role in maintaining genome integrity. Although it is known that double-strand breaks are the main inducers of chromosomal aberrations, the mechanisms by which these are formed are still unresolved (Griffin and Tracker, 2004).

Deficiencies in the XRCC3 protein lead to an increase in errors in chromosome segregation due to defects in centrosomes, resulting in aneuploidy and other chromosomal aberrations such as small increases in telomeres (Thacher, 2005). XRCC3 is an important component in the repair machinery via homologous recombination. Changes in this machine due to the presence of polymorphisms lead to damage that results in tumor development (Jiao et al., 2008).

The XRCC3 gene is mapped to 14q32.2 (Tebbs et al., 1995; Zou et al., 2009). The XRCC3 Thr241Met polymorphism (rs861539) located in exon 7 is characterized by the substitution of thymine (T) to cytosine (C) at codon 241, leading to a change of threonine (Thr) to methionine (Met), and could affect enzyme function and/or its interaction with other proteins involved in repair of DNA damage (Matullo et al., 2001; Jiao et al., 2008; Andreassi et al., 2009).

In the present research, a case-control study was conducted to examine the genotype distribution of Thr241Met SNP and to search for an association between astrocytomas and glioblastomas and XRCC3 SNP, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach.

MATERIAL AND METHODS

Study population

Eighty gliomas were analyzed, which had been surgically resected from previously untreated patients under the care of the Neurosurgery Department of Fundação Pio XII, Cancer Hospital of Barretos (Barretos, SP, Brazil). The samples, classified according to WHO criteria, were: 43 astrocytomas and 37 glioblastomas. The clinical outcome, including length of survival, was obtained from patient records. For SNP studies, blood samples from 100 healthy individuals were collected as control. Because of the highly heterogeneous ethnic composition of the Brazilian population, the individuals of the control group were selected from the general population of São Paulo State, with no family history of cancer in first-degree relatives.

DNA extraction and primer construction

DNA extraction was performed using proteinase K and phenol-chloroform according to routine molecular biology protocols. Primers were constructed using the Gene Runner 3.05 program (Hasting Software, Inc.) from the gene sequence of the XRCC3 Thr241Met polymorphism, obtained in the dbSNP of NCBI (accession No. rs861539). Table 1 shows the primers and PCR product sizes.

Table 1. Polymerase chain reaction primers.

SNP XRCC3 Thr241Met	Primers	Sequence (5'-3')	Length (bp)	PCR product (bp)
	Thr241MetF	GCT GTC TCG GGG CAT GGC TC	20	235
	Thr241MetR	GCT TCC GCA TCC TGG CTA AA	20	

PCR = polymerase chain reaction; SNP = single nucleotide polymorphism.

PCR was carried out in a final volume of 25 µL containing 50 ng genomic DNA template, 1X PCR buffer with 2 mM $MgCl_2$, 0.4 µM of each primer (Invitrogen), 50 µM dNTPs (Amersham Biosciences) and 0.5 U DNA polymerase (Biotools). The PCR cycling conditions were: 94°C for 5 min, followed by 35 denaturation cycles of 30 s at 94°C, 30 s of annealing at 56°C, and 30 s of extension at 72°C, and a final elongation cycle at 72°C for 5 min. For RFLP, the PCR products were digested by *Hsp*92II (1 U at 37°C for 16 h - Thr241Met). *Hsp*92II recognizes a restriction site at Met241 (CATG/NN) and generates two fragments of different sizes (125 and 110 bp), while Thr241 allele generates only one fragment of 235 bp. DNA fragments were electrophoresed through a 10% acrylamide:bisacrylamide gel (19:1) and stained with silver nitrate.

Statistical analysis

PCR-RFLP - The independence of alleles (Hardy-Weinberg equilibrium) was ensured using the chi-square test. The distribution of genotype and allele frequencies among patients and controls was compared using chi-square and Fisher exact tests. Overall survival curves were obtained using the Kaplan-Meier method and compared with the log-rank test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using a logistic regression model. Statistical significance was set at $P < 0.05$. Statistical analyses were performed with GraphPad InStat 4.0 and GraphPad Prism 5.0 software programs (GraphPad Software, Inc.).

RESULTS

Analysis of tumors and control populations according to XRCC3 Thr241Met

Eighty patients and 100 control subjects were included in this study. The patient sample comprised 28 females and 52 males (M/F ratio = 0.65) and the control sample consisted of 63 males and 37 females (M/F ratio = 1.7). Average age in the patient group was 45 years (range = 1-75) and in the control group it was 45 years (range = 18-72). Genotype frequencies in controls and patients were in Hardy-Weinberg equilibrium. Allele and genotype frequencies of XRCC3 Thr241Met in controls and patients are shown in Table 2. The frequencies of Thr/Thr, Thr/Met and Met/Met among patients were 66.3, 22.5 and 11.3%, while for the controls the frequencies of Thr/Thr, Thr/Met and Met/Me were 86.0, 9.0 and 5.0%, respectively ($P = 0.007$). The Met241 allele frequency was statistically significant between cases and controls (0.22 and 0.09, respectively; $P = 0.001$).

Table 2. Allele and genotype frequencies in case and control groups.

SNP XRCC3 Thr241Met	Genotype	Case group	Control group	P
	Thr/Thr	53 (66.3%)	86 (86.0%)	0.007
	Thr/Met	18 (22.5%)	9 (9.0%)	
	Met/Met	9 (11.3%)	5 (5.0%)	
	Met 241 allele frequency	0.22	0.09	0.001

Data are reported as number with percent in parentheses. SNP = single nucleotide polymorphism.

Logistic regression analysis for the investigation of polymorphism association with

risk of astrocytomas and glioblastomas is presented in Table 3. Compared to Thr/Thr, the most common genotype of the polymorphism XRCC3 Thr241Met in the study population, the genotypes with the presence of the allele Met revealed an increased risk of tumor development (OR = 3.13; 95% CI = 1.50-6.50; P = 0.002).

Table 3. Association of XRCC3 Thr241Met single nucleotide polymorphisms with risk of cancer.

SNP XRCC3 Thr241Met	Genotype	Case/Control	OR (95%CI)	P
	Thr/Thr	53/86	1	-
	Thr/Met	18/9	3.24 (1.36-7.75)	0.006
	Met/Met	9/5	2.92 (0.93-9.18)	0.057
	Thr/Met; Met/Met	27/14	3.13 (1.50-6.50)	0.002

SNP = single nucleotide polymorphism; OR = odds ratio; 95%CI = 95% confidence interval.

Comparison of overall survival of patients according to the XRCC3 Thr241Met genotype did not show significant differences (P = 0.862). In the XRCC3 Thr241Metl genotype, the median survival of patients with Thr/Met and Met/Met was 61 weeks (Figure 1).

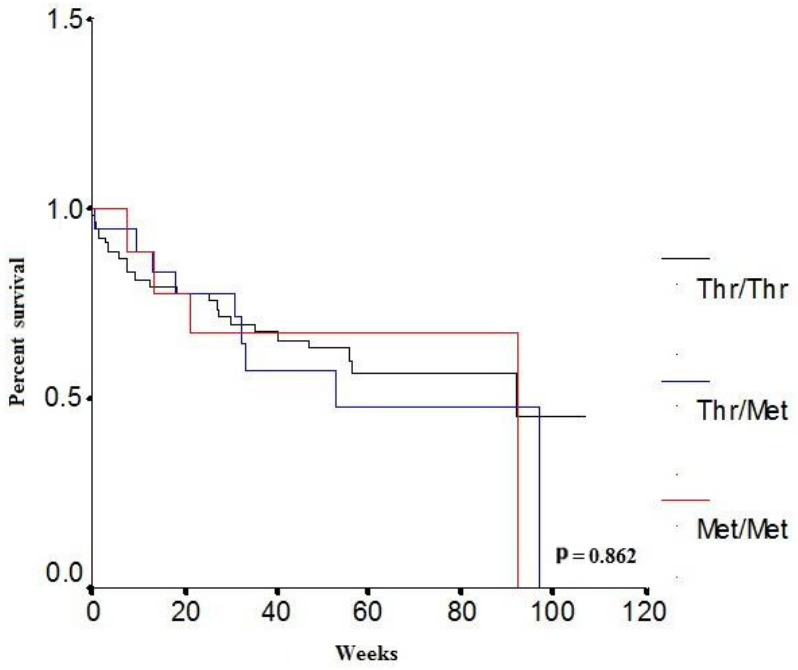

Figure 1. Overall survival in patients according to the XRCC3 Thr241Met single nucleotide polymorphism.

DISCUSSION

SNPs are recognized as important tools in human genetics and medicine and have been widely used in genetic association studies of various complex diseases, such as cardio-

vascular, psychiatric and autoimmune diseases, obesity, osteoporosis, diabetes, and cancer (Curran et al., 2001; Miller and Kwok, 2001; Lin et al., 2003; Tamura et al., 2003; Yamada et al., 2003; Hirai et al., 2005). In humans, several reviews of SNPs have also been conducted throughout the genome, with the intention of determining the patterns of the haplotypes in the populations (Daly et al., 2001; Jeffreys et al., 2001; Patil et al., 2001; Reich et al., 2001; Gabriel et al., 2002). Data from these tests are extremely useful for studying the genetic basis of common complex diseases (Phillips et al., 2003). Polymorphisms in DNA repair genes may be associated with differences in the efficient repair of DNA damage and may influence the risk for developing tumors. This can result in subtle changes in the structure of proteins from genes and alter their function (Sreeja et al., 2008).

In this study, we determined the relationship between XRCC3 Thr241Met SNPs and susceptibility to cancer and patient survival in 80 astrocytomas and glioblastomas. Some studies have linked this variant with some types of cancer, including breast cancer (Smith et al., 2003), lung (Jacobsen et al., 2004) and skin melanoma (Winsey et al., 2000).

The present case-control study showed that Met241 was more frequent in the cancer population than in non-cancer populations (0.22 and 0.09, respectively; $P = 0.001$), and that the presence of this genotype may increase the risk of developing astrocytomas and glioblastomas (OR = 3.13; 95%CI = 1.50-6.50; $P = 0.002$).

Lee et al. (2007) in a meta-analysis on Thr241Met reported that Met241 is associated with risk of developing breast cancer in Asian and Caucasian populations. The Thr241Met substitution is the most thoroughly investigated polymorphism in XRCC3 due to the C>T transition at exon 7. Functional data also suggest that the polymorphism may be associated with slightly decreased DNA repair capacity (Jara et al., 2010).

Kiuru et al. (2008) studied the XRCC3 Thr241Met polymorphism in samples of nervous system tumors and concluded that the genotype Thr/Met and homozygous Met/Met are associated with increased risk for developing this kind of tumors.

Zhou et al. (2009) conducted a case-control study in a Chinese population on the XRCC3 Thr241Met polymorphism in 771 samples of gliomas and 752 control samples, concluding that the presence of genotype Met/Met may contribute to the development of gliomas.

The XRCC3 Thr241Met polymorphism was analyzed in samples from breast cancer. The result demonstrated that the presence of the polymorphism does not interfere with disease development (Loizidou et al., 2008).

In summary, our study provides evidence that XRRC3 Thr241Met may contribute to the etiology of human astrocytomas and glioblastomas, since the allele Met241 was found more frequently in patients than controls, but there was no association between the genotypes and the patients' survival.

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