Investigation of ICAM-1 and β3 Integrin Gene Variations in Patients with Brain Tumors

Umit Yilmaz¹, Umit Zeybek¹*, Ozlem Timirci Kahraman¹, Ali Metin Kafadar², Bahar Toptas¹, Nesibe Yamak¹, Faruk Celik¹, Ilhan Yaylim¹

Abstract

Background: Primary brain tumors constitute a small percent of all malignant cancers, but their etiology remains poorly understood. β3 integrin (ITGB3) has been recognized to play influential roles in angiogenesis, tumor growth and metastasis. Intercellular adhesion molecule-1 (ICAM-1) is a surface glycoprotein important for tumor invasion and angiogenesis. The aim of this study was to investigate whether specific genetic polymorphisms of ICAM-1 and ITGB3 could be associated with brain cancer development and progression in a Turkish population. Our study is the first to our knowledge to investigate the relationship between brain tumor risk and ICAM-1 and β3 integrin gene polymorphisms.

Materials and Methods: The study covered 92 patients with primary brain tumors and 92 age-matched healthy control subjects. Evaluation of β3 integrin (Leu33Pro (rs5918)) and ICAM-1 (R241G (rs1799969) and K469E (rs5498)) gene polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: According to results of our research, the A allele of the ICAM-1 R241G gene polymorphism appeared to be a risk factor for primary brain tumors (p<0.001). Similarly, the frequency of the A mutant allele of ICAM-1 R241G was statistically significant in patients with brain tumors classified as glioma (p<0.001). When allele and genotype distributions of ICAM-1 K469E, ICAM-1 R241G and β3 integrin Leu33Pro gene polymorphisms were evaluated with age, sex, and smoking, there were no statistically significant differences. Haplotype analysis revealed that the frequencies of GAC (rs1799969-rs5498-rs5918) and GAT (rs1799969-rs5498-rs5918) haplotypes were significantly lower in patients as compared with controls (p=0.001; p=0.036 respectively).

Conclusions: This study provides the first evidence that ICAM-1 R241G SNP significantly contributes to the risk of primary brain tumors in a Turkish population. In addition, our results suggest that ICAM-1 R241G in combination ICAM-1 K469E may have protective effects against the development of brain cancer.

Keywords: Cancer - brain tumor - polymorphism - ICAM-1 - β3-integrin

Asian Pac J Cancer Prev, 14 (10), 5929-5934

Introduction

Brain tumors are composed of a lot of different kinds of cells that constitute the brain tissue and membrane (Stenberg, 1997). Brain tumors are about 2% of all malignant tumors in humans (Fisher et al., 2007). Approximately 75% of all primary brain tumors are classified as glioma or meningioma (Stenberg, 1997). Meningioma generates approximately 25% of all primary intracranial neoplasms and is more commonly seen in middle-aged and elderly patients (Giles et al., 2002). Glioma is one of the most common form of brain tumor that is approximately 12-15% of all intracranial tumors and 50-60% of astrocytic tumors, accounting for creates (Parkin et al., 1998; D’Abaco and Kaye, 2007). Glioma can be seen at any age but is more common, especially in adults (Louis et al., 2000). The incidence of brain tumors shows differences according to gender, age, race, ethnic origin, and geography (Stenberg, 1997). The etiology of brain tumors is still poorly understood. Some studies have reported that environmental exposures and genetic alterations may lead to primary brain tumors development (Wrensch et al., 2005; Fischer et al., 2007; Gu et al., 2009).

Integrins are transmembrane adhesion molecules that are composed of non-covalently linked heterodimeric α and β chains (Sims and Dustin, 2002). For functional activity of the molecule are required both subunits, but the molecule binding specificity is associated with β subunit. By the possession of β subunit structures of β1 integrins, β2 and β3 integrins are called (Sims and Dustin, 2002). β3 Integrins are probably required in tumor growth and metastasis (Felding-Habermann et al., 2002; Trikha et al., 2002). The increased expression of cell surface integrins on cancer cells is related to the development of invasive properties, i.e., altered migration on and adhesion to the extracellular matrix (Hood and Cheresh,
we hypothesized that the ICAM-1 (K469E), (R241G) and β3 integrin subunit Leu33Pro polymorphism could influence cancer risk.

Materials and Methods

Subjects

We investigated the β3 integrin Leu33Pro, ICAM-1 K469E and ICAM-1 R242G gene polymorphisms in 92 brain cancer patients (including 54 cases with glioma and 38 cases with non-glioma brain tumors) and 92 healthy control matched subjects who were in the follow-up Cerrahpasa Faculty of Medicine, Department of Neurosurgery in Istanbul University. The mean ages of brain cancer patients and control group were 47.37±14.67 and 48.12±14.79 years, respectively.

The specimens were taken after obtaining informed consent and the study was conducted prospectively. Medical Ethics Committee of Istanbul Medical Faculty approval was obtained for the study. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

Genotyping

DNA was extracted from whole blood using Pure Link DNA extraction kit (Invitrogen, USA). The DNA concentration was determined by measuring the OD at 260 nm. Genotyping was performed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). For β3 Integrin Leu33Pro polymorphism, the following primers were used to amplify the β3 Integrin gene; sense primer 5'-GCT CCA TGG TGA CTT GAG TGA CCT-3'; antisense primer 5'-GGT GAG GAT TGC CCG AGC-3'. For detection of the β3 Integrin Leu33Pro RFLP, 20 ng genomic DNA was amplified with 1 X PCR buffer, 3 mM MgCl₂, 0.2 mM of each dNTP, 0.2 mM of each primer and Taq polymerase in a 25 µl final volume. The PCR conditions involved an initial denaturation of 5 min at 95°C, followed by 35 cycle of 94°C 45 sec, annealing at 59°C for 45 sec and extension 72°C for 45 sec. A final extension step at 72°C for 5 min was also studied. PCR products were digested with Mspl (Fermentas, Lithuania) restriction enzyme at 37°C for 16 h and electrophoresised on 3% agarose gels and stained with ethidium bromide. Genotypes were determined as TT (23 pairs), TC (212,175,125 bp) or CC (157,125 bp) for β3 Integrin Leu33Pro polymorphism. The primers for ICAM-1 K469E polymorphism were sense 5'-GG-TCA TAC GGG GTA AAC-3'; antisense primer 5'-GGG GAC TGA CCT TTT CCT GAT GTA-3'. The DNA template was amplified by PCR using 3 mM MgCl₂, 0.2 mM of each dNTP, 0.2 mM of each primer and Taq polymerase (Fermentas, Lithuania) in a 25 µl reaction volume. The PCR conditions involved an initial denaturation of 5 min at 95°C, followed by 35 cycle of 94°C 45 sec, annealing at 59°C for 45 sec and extension 72°C for 45 sec. A final extension step at 72°C for 5 min was also studied. PCR products were digested with BstUI (Fermentas, Lithuania) restriction enzyme at 37°C for 2 h followed by electrophoresis in a
3% agarose gel. Genotypes were determined as AA (223 bp), AG (223,137,87 bp) or GG (136,87 bp) for ICAM-1 K469E polymorphism. The oligonucleotide primers to amplify region of ICAM-1 R241G were: 5’-CCG TGG TCT GTT CCC ‘TGT AC-3’ (sense), 5’-GAA GGA GTC GTT GGC ATA GG-3’ (antisense) respectively. PCR was performed in a total volume of 25 µl with 0.3 µl Taq DNA polymerase (Fermentas, Lithuania). The PCR mix was incubated for 5 min at 95°C followed by 35 cycles of 45 sec at 94°C, 45 sec at 57°C for ICAM-1 R241G polymorphism. PCR products were digested with BsrGI (Fermentas, Lithuania) restriction enzyme at 37°C for 16 h followed by electrophoresis in a 3% agarose gel. Genotypes were determined as GG (110 bp), AA (90, 20 bp) or GA (110, 90, 20 bp) for ICAM-1 R241G polymorphism.

### Statistical Analysis

Statistical analysis were performed using SPSS version 11.5 (SPSS Inc, Chicago, USA) including the Chi-square ($\chi^2$) test, Fischer’s exact test and the Pearson correlation test, odds ratio (OR) and 95% confidence intervals (CI) were calculated. Mean values were compared among subjects by the unpaired Student’s t-test. Haplotype analysis was undertaken using Haploview 4.2 software program. Values of p<0.05 were considered as statistically significant.

### Results

#### Subject characteristics

The analysis included 92 brain cancer (including 54 cases with glioma and 38 cases with non-glioma brain tumors) and 92 healthy controls. Information on study groups are shown in Table 1. There was no significant difference statistically between brain tumor cases and controls in terms of age and gender parameters (p=0.848; p=0.184, respectively).

### Association between ICAM-1 and β3 integrin polymorphisms and risk of brain tumors

Genotype and allele frequencies of brain tumor cases and controls are shown in Table 2. Firstly, we evaluated association between ICAM-1 R241G polymorphism and study groups. The frequency of the A mutant allele was significantly higher patients compared to controls ($\chi^2=15.311$, p<0.001, respectively). When ICAM-1 K469E and β3 Integrin Leu33Pro polymorphisms genotype and allele frequencies compared in study groups, no statistically significant difference was detected (p>0.05).

In the present prospective case-control study, the primary brain tumors were divided into two groups, namely glioma with a malignant tumors and namely meningioma with benign tumors. Genotype and allele distributions of ICAM-1 K469E and R241G SNPs were statistically significant in patients with brain tumors classified as glioma. Frequency of A wild type allele for ICAM-1 K469E was significantly lower in glioma patients compared to the control group ($\chi^2=4.089$, p=0.043), (Table 3). However, frequency of A mutant allele for ICAM-1 R241G was significantly higher in glioma patients compared to the control group ($\chi^2=16.341$, p<0.001), (Table 3). The observed genotype frequencies of ICAM-1 and β3 Integrin polymorphisms between meningioma and control group were in agreement with Hardy-Weinberg equilibrium (p>0.05). When allele frequencies of these polymorphisms compared between meningioma and control groups, no statistically significant difference was detected (p>0.05).

According to the grade of tumor patients with brain tumors are grouped as grade I-II and grade III-IV, genotype GG for ICAM K469E were significantly higher than AA+AG genotype in patients with high grade (III+IV)

Chi-square test was used to compare genotypes and alleles characteristics in the study group. *p values less than 0.05 denoted statistical significance; **Haplotype consist of rs1799969 (ICAM-1 R241G), rs5498 (ICAM-1 K469E) and rs5918 (β3Integrin Leu33Pro). p values of haplotype association were calculated using Haploview 4.2.

Table 4. According to the Grade of Tumor Patients with Brain Tumors are Grouped as Grade I-II and Grade III-IV

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients with grade I-II tumors n (%)</th>
<th>Patients with grade III-IV tumors n (%)</th>
<th>OR 95% (CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 K469E</td>
<td>AA+AG 15 (93.8)</td>
<td>24 (63.2)</td>
<td>0.674 (0.512-0.886)</td>
<td>*0.022</td>
</tr>
<tr>
<td></td>
<td>GG 1 (6.3)</td>
<td>14 (36.8)</td>
<td>5.895 (0.845-41.137)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG+AG 13 (81.3)</td>
<td>31 (81.6)</td>
<td>1.004 (0.759-1.328)</td>
<td>0.977</td>
</tr>
<tr>
<td></td>
<td>AA 3 (18.8)</td>
<td>7 (18.4)</td>
<td>0.982 (0.290-3.327)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA+GG 4 (15)</td>
<td>21 (51.3)</td>
<td>2.211 (0.903-5.413)</td>
<td>**0.042</td>
</tr>
<tr>
<td></td>
<td>AG 12 (75)</td>
<td>17 (44.7)</td>
<td>0.596 (0.379-0.938)</td>
<td></td>
</tr>
<tr>
<td>ICAM-1 R241G</td>
<td>GG+GA 16 (100)</td>
<td>33 (86.8)</td>
<td>0.868 (0.767-0.983)</td>
<td>0.128</td>
</tr>
<tr>
<td>β3Integrin Leu33Pro</td>
<td>AA 0 (0)</td>
<td>5 (13.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG+AA 2 (12.5)</td>
<td>7 (18.4)</td>
<td>1.474 (0.343-6.339)</td>
<td>0.584</td>
</tr>
<tr>
<td></td>
<td>GG 14 (87.5)</td>
<td>31 (81.6)</td>
<td>0.932 (0.734-1.184)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA 2 (12.5)</td>
<td>2 (5.3)</td>
<td>0.421 (0.065-2.734)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG+GA 14 (87.5)</td>
<td>36 (94.7)</td>
<td>1.083 (0.887-1.322)</td>
<td></td>
</tr>
</tbody>
</table>
| Allele and genotype distributions of ICAM-1 K469E, ICAM-1 R241G and β3 Integrin Leu33Pro polymorphisms were evaluated with age, sex, and smoking, there was no statistically significant difference (p>0.05). Genotype combinations of ICAM-1 K469E, ICAM-1 R241G and β3 Integrin Leu33Pro polymorphisms were examined, a statistically significant difference was not found between the carrying combined genotype in patients with brain tumors and the control group (p>0.05).

**Discussion**

Cancer is the abnormal cell growth that causes cell proliferation and morbidity due to multiple gene expressions, and if not treated, it may result in tissue invasion and metastasis leading to death (Ruddon, 2007). Despite primary brain tumors are less than 2% of all malignancies, primary brain tumors have been reported which are second only to stroke as a cause of death from neurologic disorders (Burgess, 2010). Brain tumors are graded on the basis of histopathology into the following main histologic groupings: neuroepithelial tissue tumors such as glioma, including astrocytoma (grade II), anaplastic astrocytoma (grade III), glioblastoma (grade IV), oligodendroglioma, and ependymoma, meninges tumors, germ cell tumors, and sellar region tumors including pituitary tumors and craniopharyngioma (Brooks et al., 1994).

β3 Integrin play a role on the pathophysiology of malignant tumors. It is implicated on endothelial cells, where β3 Integrin is involved in tumor angiogenesis (Tian et al., 2012). The tumor cells express β3 Integrin and this expression has been shown in several malignancies. β3 Integrin expression is associated with tumor progression in melanoma, glioma, and ovarian and breast cancer (Kammerer et al., 2004). ICAM-1 has a key role in cell-to-cell, cell-to-extracellular matrix interaction and cell signaling. Some studies indicate which ICAM-1 has an important role in the development and progression of human cancers. Overexpression of ICAM-1 in melanoma, gastric breast, renal cell carcinoma and colorectal cancer...
has been shown (Arandi et al., 2008). The SNP in ICAM1 gene has been also reported which is correlated with the risk of cancers, such as breast cancer, colorectal cancer and melanoma (Howell et al., 2005; Cox et al., 2006; Vinceti et al., 2006; Wang et al., 2009).

In our study, genotype and allele distribution of ICAM K469E were not observed statistically significant difference between patients with brain tumors and control group. Similarly, Vigano et al. (2003) have not found a significant relationship for ICAM K469E polymorphism allele and genotype frequencies between endometriodm patients and the control group. At the same time, Arandi et al. (2008) have reported that distribution of ICAM K469E polymorphism allele and genotype was not statistically significant difference between breast cancer and control. However, AA genotype for ICAM-1 K469E has been shown that increased cancer risk in colorectal cancer (Wang et al., 2009), stomach cancer (Tian et al., 2012) and the colon (Theodoropoulos et al., 2006) cancer studies. Our results also demonstrated that genotype and allele distributions of ICAM R241G were found statistically significant differences between the control and patient groups.

In the present prospective case-control study, the primary brain tumors were divided into two groups, namely gliomas with a malignant tumors and namely meningiomas with benign tumors. Frequency of A mutant allele of ICAM-1 R241G were statistically significant in patients with brain tumors classified as glioma. Gliomas are common tumors and account for almost 80% of primary malignant brain tumors, usually resulting in poor survival compared to other types of brain tumors.

In addition to SNP analyses, haplotypes were evaluated for association with primary brain tumors. Haplotypic analysis suggested that primary brain tumor risk was highly reduced among individuals with specific haplotypes. Haplotype association analysis showed that GAC (ICAM-1 K469E G allele: ICAM-1 R241 A allele: β3 Integrin Leu33Pro C allele) and GAT (ICAM-1 K469E G allele: ICAM-1 R241 A allele: β3 Integrin Leu33Pro T allele) haplotype was associated with the risk of primary brain tumors in our study. The combination of ICAM-1’s SNPs may have protective effect on the risk of brain tumors.

In conclusion, our findings provides new evidence for the association between ICAM-1 R241G SNP and the risk of primary brain tumors in a Turkish population. These findings also suggest that ICAM-1 R241G gene polymorphism might affect the development of glioma tumors. At the same time, we identified a previously undescribed ICAM-1 R241G and ICAM-1 K469E haplotype in association with the primary brain tumor in our population. The haplotype of ICAM-1 K469E mutant G: ICAM-1 R241 mutant A may be a protective marker for genetic susceptibility to primary brain tumors. Our results needs to be confirmed in larger cohorts in order to better understand their role in development of brain cancer.

References


DOI:http://dx.doi.org/10.7314/APJCP.2013.14.10.5929

Polymorphisms in ICAM-1 and β3 Integrin and Brain Tumors in Turkey


