Original Investigation

The Evaluation of Killer Cell Immunoglobulin-Like Receptor Gene Polymorphism in Glioblastoma Patients

Mustafa Emre SARAC[1], Kadir OKTAY[2], Semih Kivanc OLGUNER[3], Ozlem GORUROGLU OZTURK[4],
Alp Iskender GOCER[5]

[1]Private South Adana Hospital, Department of Neurosurgery, Adana, Turkey
[2]Gaziantep Medical Park Hospital, Department of Neurosurgery, Gaziantep, Turkey
[3]Adana City Training and Research Hospital, Department of Neurosurgery, Adana, Turkey
[4]Cukurova University School of Medicine, Department of Clinical Biochemistry, Adana, Turkey
[5]Cukurova University School of Medicine, Department of Neurosurgery, Adana, Turkey

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Corresponding author: Kadir OKTAY ✉ drkadiroktay@hotmail.com

ABSTRACT

AIM: To assess the distribution of genetic polymorphisms of killer cell immunoglobulin-like receptors (KIRs) to predict the clinical course of glioblastoma, report on the genetic mechanisms, and provide guidance on potential therapeutic methods.

MATERIAL and METHODS: Our study included 31 adult patients who were admitted to the Department of Neurosurgery at our institution and diagnosed with glioblastoma between October 2013 and January 2014 together with 50 control subjects.

RESULTS: The mean age of the patients was 53.5 vs. 53.9 years, respectively, and the gender distribution (male/female: 64.5/35.5% vs. 64/36%, respectively) was comparable among patients and controls (p>0.05). Sixteen different KIR genes including inhibitory, activating, and pseudogenes were investigated for each sample, and the framework genes including KIR2DL4, 3DL2, 3DL3, and 3DP1 were present in all patients and controls. In addition, the inhibitory KIR genes and the 2DL3 gene were significantly more common in patients compared to controls (p<0.05).

CONCLUSION: This study demonstrated that the inhibitory KIR gene 2DL3 has a predisposition for glioblastoma. Identifying the potential link between glioblastoma cells and immune system genetics is critical in predicting familial predisposition and early diagnosis. In addition, this clue may be a key factor in developing post-surgery individual immunotherapy models in the future.

KEYWORDS: Glioblastoma, Killer cell immunoglobulin-like receptor gene, Natural killer cells, Spesific oligonucleotid probes method

■ INTRODUCTION

Glioblastoma is the most common and the most deadly primary brain tumor (29). The primary course of treatment includes maximal surgical resection, followed by radiotherapy with concurrent temozolomide therapy (29). In recurring tumors, salvage therapy options include repeat surgical resection, antiangiogenic therapy (bevacizumab), and various investigational therapies including immunotherapy and other chemotherapeutic agents (7,21). Despite aggressive treatment, the median survival time is only 18–24 months. The limited treatment success is partially due to intrinsically aggressive tumor behavior but also to the heterogeneity of the disease (2,15).

The most important cells of the immune system are natural killer cells (NK), which are large granular lymphocytes originating from the bone marrow. The cytotoxic response

Mustafa Emre SARAC	: 0000-0003-2420-3428	Ozlem GORUROGLU OZTURK	: 0000-0002-8657-2964
Kadir OKTAY	: 0000-0003-2420-2734	Alp Iskender GOCER	: 0000-0002-4148-3852
Semih Kivanc OLGUNER	: 0000-0006-2830-3442		

of NK cells is controlled by the balance between signals from the activating or inhibitory receptors on the surface. One such group of receptors is the NK cell immunoglobulin-like receptors (KIR). KIR receptors distinguish the cells in an organism by their human leukocyte antigen (HLA) class I molecules, while foreign cells are distinguished by the absence of these molecules (31). Since the characterization of KIR genes is very recent, few studies have reported on their relationship to specific diseases (8,25,28,31).

Positive results from genetic studies are leading to the development of possible gene therapies for diseases with high mortality and morbidity and no definitive treatment. Based on our current knowledge, no studies have reported on the polymorphism of KIR genes in glioblastoma. In this study, we aimed to compare the KIR gene polymorphism in cases of glioblastoma with healthy controls to predict the possible genetic predisposition and clinical course of the disease. It is also possible that the obtained results could be used in the future in individual gene therapy for this disease.

■ **MATERIAL and METHODS**

Our research was performed according to the principles of the Declaration of Helsinki, and the Institutional Ethical Review Board approval was obtained (6 December 2013, no: 17). In total, we included 31 adult patients who were diagnosed and treated for glioblastoma in the department of neurosurgery at our institution between October 2013 and January 2014. The control group comprised 50 individuals without any nervous system trauma, vascular or inflammatory diseases, or neoplasia. After obtaining informed consent from each patient or the patient's relatives, we gathered patient data including age, sex, place of birth, chronic viral diseases, autoimmune diseases (familial mediterranean fever [FMF], rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus [SLE]), cancer history, family history, and pregnancy. After isolating the DNA, we performed KIR genotyping using the sequence-specific oligonucleotide probes method.

KIR Genotyping

We extracted DNA from venous blood samples of each subject using a DNA isolation kit (QIAamp DNA Blood Mini Kit, Cat No. 51104; QIAGEN Vertriebs GmbH, Vienna, Austria). Genotyping of the KIR genes was performed using the multiplex KIR-SSO typing kit (Tepnel Lifecodes Corp., Ref: 545110; Connecticut, USA), which consists of a mixture of locus-specific oligonucleotide probes coupled to color-coded microspheres (Luminex Corp.) and two polymerase chain reaction (PCR) tests to amplify KIR exons 4, 5, 7, 8, and 9. PCR was performed on each sample, and the product was hybridized with the SSO-probe mixture according to the manufacturer's instructions. After hybridization, the sample plate was placed in a Luminex instrument for analysis (24).

Prediction of Group A/B KIR Haplotype

The frequencies of groups A and B KIR haplotypes were deduced from the genotyping data (9,12). Individuals carrying only KIR3DL3, 2DL3, 2DL1, 2DP1, 3DP1, 2DL4, 3DL1, 2DS4 and 3DL2, a fixed gene content characteristic of group A

haplotypes, were considered to carry two copies of group A KIR haplotypes (AA genotypes), whereas the those carrying KIR2DL2, 2DL5, 3DS1, 2DS1, 2DS2, 2DS3, and/or 2DS5 were considered to carry a B haplotype (Bx).

Statistical Analyses

The percentage of each KIR gene in the two groups was calculated by direct counting (individuals positive for the gene/individuals tested per population × 100). Data analysis was performed using the statistical software Minitab, version 17. Differences in the distribution of each KIR gene between the two groups were estimated using a two-tailed Fisher's exact test, and $p < 0.05$ was considered statistically significant.

■ **RESULTS**

In the present study, we evaluated a total of 81 subjects, including 31 with glioblastoma who were referred to neurosurgical wards and outpatient services together with 50 individuals in the control group. Overall, 20 males (64.5%) and 11 females (35.5%) comprised the patient group. Of controls, 32 were male (64%) and 18 were female (36%). Glioblastoma patients had a mean age of 53.5 years compared to a mean age of 53.9 years in the control group.

Figure 1 shows the genotype distribution in our 31 glioblastoma patients. In the study, a total of 16 different KIR genes, inhibitory, activating, and pseudogene, were studied, and the framework genes, including KIR2DL4, 3DL2, 3DL3, and 3DP1, were present in all patients in both groups (Table I). The 2DL3 gene, one of the inhibitory genes, was more common in patients than controls, and the difference was statistically significant ($p < 0.05$) (Figure 2).

■ **DISCUSSION**

Glioblastomas are classified as glioblastoma, IDH-wild-type; glioblastoma, IDH-mutant; and glioblastoma, NOS according to the 2016 World Health Organization Classification of Tumors of the Central Nervous System. Glioblastoma, IDH-wild-type constitutes approximately 90% of cases and corresponds most frequently with clinically defined primary or de novo glioblastoma. It is mainly found in patients aged >55 years. Glioblastoma, IDH-mutant, which usually affects younger patients, comprises only 10% of cases and corresponds to secondary glioblastoma with a history of prior lower-grade diffuse glioma. Glioblastoma, NOS is a diagnosis reserved for those tumors in which full IDH evaluation cannot be performed (16,23). Epithelioid glioblastoma is a new variant of glioblastoma that joins giant cell glioblastoma and gliosarcoma under the umbrella of IDH-wild type glioblastoma (7). This tumor is usually identified in children and younger adults, typically presents as superficial cerebral or diencephalic masses, and often harbors a BRAF V600E mutation (which can be detected immunohistochemically) (5,10,11).

Glioblastoma with primitive neuronal component was added to the 2016 WHO Classification as a pattern in glioblastoma. This pattern, previously known in the literature as glioblastoma with PNET-like component, usually consists of a diffuse

astrocytoma of any grade (or oligodendroglioma in rare cases) with well-demarcated nodules containing primitive cells that display neuronal differentiation (e.g., Homer-Wright rosettes, gain of synaptophysin positivity, and loss of GFAP expression) and that sometimes have MYC or MYCN amplification; these tumors have a tendency for craniospinal fluid dissemination (26).
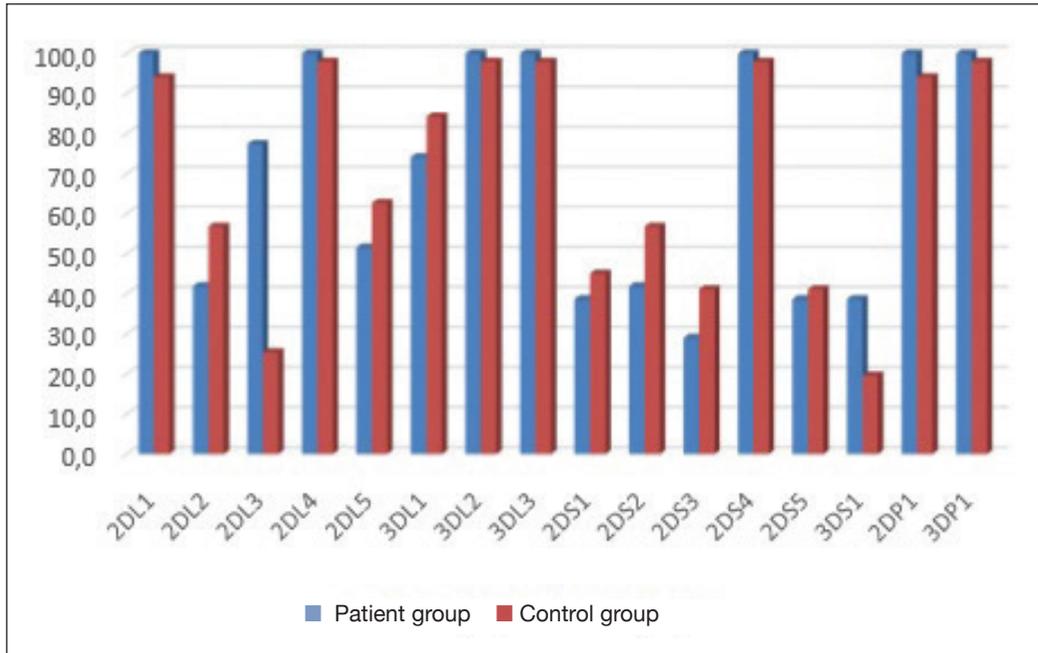
Table I: Frequency of Inhibitor and Activator Genes

	Gene	Patient Group		Control Group		
		n	%	n	%	p
Inhibitor KIR	2DL1	31	100.0	48	94.1	0.522
	2DL2	13	41.9	29	56.9	0.177
	2DL3*	24	77.4	13	25.5	**0.002**
	2DL4	31	100.0	50	98.0	1.0
	2DL5	16	51.6	32	62.7	0.353
	3DL1	23	74.2	43	84.3	0.242
	3DL2	31	100.0	50	98.0	1.0
	3DL3	31	100.0	50	98.0	1.0
Activator KIR	2DS1	12	38.7	23	45.1	0.645
	2DS2	13	41.9	29	56.9	0.177
	2DS3	9	29.0	21	41.2	0.344
	2DS4	31	100.0	50	98.0	1.0
	2DS5	12	38.7	21	41.2	0.819
	3DS1	12	38.7	10	19.6	0.077
Pseudogene	2DP1	31	100.0	48	94.1	0.522
	3DP1	31	100.0	50	98.0	1.0

The relation of 2DL3 gene to Susceptibility to disease is statistically significant (p<0.05).
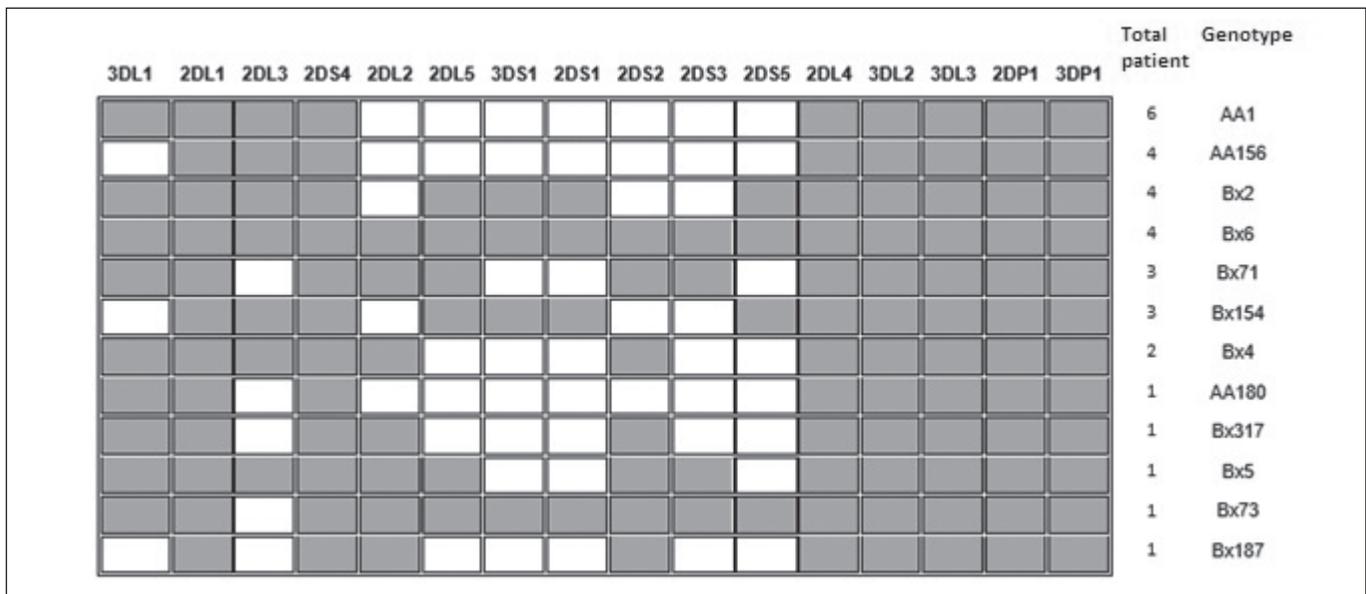
Figure 1: Genotype distributions of patients.

Figure 2: The frequency of inhibitor and activator genes in the patient and control groups.

KIRs are a family of inhibitory and activating receptors produced by NK cells that regulate the activation of NK cells, and they are polymorphic markers. KIR genes are widely believed to co-develop with HLA ligands (28). In addition, genotypic KIR diversity is a result of allelic and haplotypic diversity. Thus, it is extremely difficult to detect the same KIR genotype in two unrelated individuals (25). Due to these characteristics, KIR genes are considered good markers of determining population genetics (8,20).

Independent studies on the KIR genes were combined and added to the knowledge database prepared for HLA in 2003. T-cell-mediated suppression of class I HLA surface molecules used by tumor cells as an escape mechanism makes NK cells more susceptible to cell-mediated lysis. In this case, individual changes in the KIR genes of NK cells only explain the ability of some NK cells to destroy tumors. NK cells may interfere with viral-infected or malignant transformed cells via KIR with lysis and cell-mediated cytotoxicity and antibody-mediated cellular cytotoxicity (8,20,25,28).

Studies have reported that specific KIR genes and KIR ligands may be associated with prophylaxis or susceptibility to leukemia and solid tumors (1,3,4,6,14,18,19,22,24,32). However, the role KIR gene polymorphism plays in the development of glioblastoma is not known. The purpose of our study was to analyze the possible roles of KIR gene polymorphisms in glioblastoma patients. We investigated a total of 81 subjects, with 31 having glioblastoma patients and 50 serving as the control group, in order to evaluate the relationship between KIR polymorphism and glioblastoma. KIR framework genes 2DL4, 3DL2, 3DL3, and 3DP1 were present in all individuals in both groups. 2DL3, an inhibitory KIR gene, was more common in the patient group (p<0.05). These results demonstrated a statistically significant relationship between the 2DL3 gene and glioblastoma development.

The 2012 study by Ozturk et al. showed that the framework genes KIR2DL4, 3DL2, 3DL3, and 3DP1 were seen in all patients and controls (24). The rate of activating KIR2DS1 was much higher in patients with breast cancer than in healthy controls, while the allelic types of activating 2DS4 (2DS4*003/4/6/7) were lower in patients with breast cancer than in healthy controls. In addition, a statistically significant negative correlation was seen between 2DL1 genes and breast cancer development. They concluded that activating KIR2DS1 may trigger breast cancer development, while 2DL1 gene and 2DS4*003/4/6/7 alleles may possibly protect against breast cancer (24).

Middleton et al. analyzed the frequency of KIR in cohorts of Turkish patients with acute lymphocytic leukemia, acute myeloid leukemia, and chronic myeloid leukemia and compared the results with those of 154 controls (18). They proposed a model of protection against chronic myeloid leukemia via KIR2DL2 and/or KIR2DS2 with the presence of the ligand HLA-C1 group and susceptibility via HLA-Bw4 homozygosity. Besson et al. indicated that KIR3DS1 and KIR2DS1 activating genes were associated with protection against Hodgkin lymphoma (4).

A study by Lopez-Vazquez et al. examined the HLA and KIR genotypes, showing that the interaction between KIR3DS1 and HLA-Bw4 allotypes was protective against the development of hepatocellular carcinoma (14). Abnormal KIR expression has been associated with numerous malignant conditions. An increasing number of activating KIR genes has been associated with nasopharyngeal carcinoma (6). KIR3DL2 is shown to synthesize in phenotypically abnormal T cells in patients with Sézary syndrome (3). Inhibitory KIR is synthesized in subtypes of T-cell large granular lymphocytic leukemia, and the absence of HLA ligands for synthesized KIRs increases disease severity (19,22).

Al Omar et al. found no statistically significant difference in terms of KIR genes when comparing healthy individuals with those patients who had solid tumors (non-small-cell lung cancer, small-cell lung cancer, colon cancer, kidney cancer) (1). Zhang et al. reported that the activating KIR2DS4 gene was associated with the development of chronic myeloid leukemia (32). Meanwhile, 2DS3, another activating gene, had a lower frequency in patients with acute lymphoblastic leukemia compared to healthy individuals.

In the present study, we demonstrated that patients with glioblastoma had increased levels of KIR2DL3. In the literature, this gene is associated with clinical conditions including HIV infection, trachoma, SLE, and hepatitis C virus infection (13,17,27,30). Ma et al. evaluated 24 patients diagnosed with SLE and found that the number of KIR2DL3 (+) NK cells was negatively correlated with SLE Disease Activity Index Scores at 4 weeks post-treatment (17). In another study, increased KIR2DL3 inhibitory receptor expression by NK and cytotoxic T cells in patients with chronic hepatitis C were associated with cellular immune defects (30). Roberts et al. indicated that high levels of KIR2DL2 and KIR2DL3 increased the risk of conjunctival scarring in patients with trachoma (27). However, we could not find any study reporting a relationship between the KIR2DL3 gene and brain tumors. According to our review of the literature, this is the first article to report an association between KIR2DL3 gene and brain tumors.

Our study has certain limitations. The study was performed between October 2013 and January 2014; therefore, due to the timing of the study, the patients' pathological assessments were not performed according to the new classification, and this is the main limitation of the study. In addition, the patient group was small. There are also no data regarding the impact of this finding on patient survival or prognosis. Further studies including large groups of patients and long follow-up would aid in making more definite conclusions.

We think that such complex information regarding the role of KIR genes in tumor development is due to the existence of many different potential pathological mechanisms, immuno-logical responses, and immunological escape mechanisms in differentiated cancers. These data suggest that the increase in activating KIR genotypes may have protective effects against various types of cancer, while the increase in inhibitory KIR may provide a basis for cancer development.

■ CONCLUSION

The results of the present study suggest that high levels of KIR2DL3, an inhibitory receptor of NK cells, in a glioblastoma patient population negatively impact the protective role of these cells in the immune system and, as a result, the necessary immune response was not achieved. These data provide stronger evidence that the immune system has the ability to recognize and exterminate glioblastoma cells. They also suggest that glioblastoma cells are able to escape from the immune system. Understanding the genetic structure of tumors encourages the development of new, individual-specific treatments for tumors such as glioblastoma that do not have a definitive treatment modality. The relationship of glioblastoma cells to immunosystemic genetics is important in predicting familial predispositions, early diagnosis, and the development of personalized therapy after surgery.

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