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Research Progress on the Effect of Sphingosine Kinase 2 on Glioma

Chunpeng Wang[1†], Xiaohui Liu[2†], Shiwen Guo[1*]

[1]Department of Neurosurgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710000, Shaanxi Province, China

[2]Department of Internal Medicine-Oncology, Anyang Cancer Hospital, Anyang 455000, Henan Province, China

[†]These authors contributed equally to this work

***Corresponding author:** Shiwen Guo, gsw1325302@163.com

Abstract: ***Objective:*** The purpose of this study is to explore the role of sphingosine kinase 2 (SphK2) in the treatment of glioma, which is the most common primary tumor in the central nervous system. ***Methods:*** A total of 82 patients were included in this study, with 27 cases in the control group and 55 cases in the glioma group; the expressions of SphK2 and gp130 in the two groups were compared by immunohistochemical method, and the correlation between the two factors was analyzed. ***Results:*** Both SphK2 and gp130 were upregulated in the glioma group, and the two factors were significantly correlated. ***Conclusion:*** The high expression of SphK2 may play an important role in the occurrence, development, and diagnosis of glioma.

Keywords: Glioma; SphK2; Diagnosis; Biomarker

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1. Introduction

Infiltration and growth are the significant features of diffuse glioma in the brain parenchyma; hence, complete surgical resection of the lesion is impossible [1]. Diffuse gliomas infiltrate and grow anywhere in the host brain. Some tend to infiltrate to the edge of white matter and vascular walls [2]. The infiltration of peripheral brain tissue is determined by the complex interaction between glioma cells and the cell microenvironment [3]. Glioma is highly invasive and lethal [4]. In most cases, patients die within two years of diagnosis despite active treatment. Finding a scientific treatment for malignant glioma is the focus at present.

Sphingosine 1-phosphate (S1P) is an important bioactive lipid messenger [5], which can regulate cell proliferation and inhibit apoptosis. Sphingosine kinase (SphK) is a rate-limiting enzyme [6] involved in the formation of S1P, which can regulate the normal proliferation of cells. SphK can be divided into SphK1 and SphK2. SphK1 is expressed in many tumors, including glioma, and its expression level is related to the prognosis of patients [7]; however, there are only a few studies on the expression of SphK2. Therefore, the purpose of this study is to explore the relationship between SphK2 and glioma.

2. Material and methods

2.1. Sample collection

This study was carried out from March 15, 2020, to September 15, 2021. A total of 82 patients, with age ranging from 20 to 80 years old, participated in the study. The patients were divided into the control group

(decompression for brain trauma, n = 27) and the glioma group (diagnosed as having glioma, n = 55). The number of tumor specimens in each group and the specific histological types are shown in **Table 1** and **Table 2**, respectively.

Table 1. The demographic and clinical characteristics of the patients

Character	Control group	Glioma group
Number of cases	27	55
Male	10	26
Female	17	29
Age (years)	42.16 ± 11.10	39.49 ± 11.60

Table 2. Histological classification of glioma tissue specimens

Pathological type	n
Diffuse astrocytoma	12
Oligodendroglioma	11
Anaplastic astrocytoma	11
Anaplastic oligodendroglioma	9
Glioblastoma	12

2.2. Materials

Rabbit anti human immunoglobulin G antibody (AB_2868363) and gp130 monoclonal antibody (8D4D2) were purchased from Invitrogen, USA. Horseradish peroxidase (HRP)-conjugated sheep anti-rabbit immunoglobulin G and 3,3'-diaminobenzidine-tetrahydrochloride (DAB) were purchased from TaKaRa, Japan. Other commonly used chemical reagents were purchased from TransGen Biotech, China.

2.3. Immunohistochemistry

The tissue sections were successively placed in xylene for 15 minutes, 100% ethanol for 15 minutes, then methanol hydrogen peroxide for 30 minutes, 95% and 80% ethanol for 10 minutes were necessary. Then, all the sections were removed from 80% ethanol, fully rinsed with tap water, and fully rinsed again with distilled water. After that, the tissue sections were stored in the antigen repair solution at 96~100ºC for 20 minutes. Upon returning to room temperature, they were washed with triethanolamine buffered saline (TBS) for 2 times (5 minutes each time). The samples were blocked at room temperature for 30 minutes by dripping about 100 μL sheep serum, shaking off the sheep serum on the slices, and gently wiping off a small amount of liquid left on the blank slide next to the tissue. The samples were covered with 100 μL diluted rabbit anti human SphK2 antibody (1:100 dilution) and rabbit anti human gp130 antibody (1:100 dilution). They were then incubated in a wet box at 4ºC for 12 hours. The samples were rewarmed and washed with TBS vibration twice for 5 minutes each time on the second day and incubated with 100 μL sheep anti rabbit IgG antibody (1:1000 dilution) for 1 hour. After TBS washing, ABC complex was added and incubated for 1 hour; then, 10 ml of DAB color developing solution was added after TBS washing at room temperature; following that, 0.1 ml of 3% hydrogen peroxide solution was added. The discoloration of the slices were observed carefully, and the slices were washed using tap water after a satisfactory color was observed using the microscope. Then, hematoxylin counterstaining, dehydration, transparency, and sealing were carried out. The positive cells were counted after the sections were completely dried. After the above steps, the percentage of SphK2 and gp130 positive cells were calculated in 8 grids under the

microscope field of view at 400X.

2.4. Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, New York, USA). The measurement data were expressed in M ± SD by t-test, and the count data were expressed as percentage by chi-square (χ^2) test. $P < 0.05$ indicates a statistically significant difference.

3. Results

3.1. Expression of SphK2 in glioma tissues and in the brain tissues of the control group

SphK2 positive cells were occasionally seen in the brain tissue of the control group, but SphK2 positive cells were significantly higher in the glioma group. The difference of SphK2 positive labeling index (LI) between the glioma group and the control group was statistically significant ($P < 0.05$) (**Table 3**).

Table 3. Analysis of SphK2 in the control group and glioma group

Group	SphK2 (LI) (M ± SD)
Control group	0.6±0.23
Glioma group	0.9±0.31*

Note: *$P < 0.05$

3.2. gp130 in glioma tissues and the brain tissues of the control group

This study found that gp130 LI in glioma tissue was significantly higher than that in the control group ($P < 0.05$). The difference was statistically significant (**Table 4**).

Table 4. Analysis of gp130 LI in the control group and glioma group

Group	gp130 (LI) (M ± SD)
Control group	0.1 ± 0.09
Glioma tissue group	0.6 ± 0.21*

Note: *$P < 0.05$

3.3. Relationship between SphK2 expression and tumor cell proliferation in glioma

In glioma, SphK2 was found to be positively correlated with gp130 (as shown in **Table 5**), suggesting that there may be a certain correlation between SphK2 and glioma cell proliferation.

Table 5. Correlation between gp130 and SphK2 expression in glioma

	Control group	Glioma group
Control group	-	0.28*
Glioma group	0.28*	-

Note: *Significant correlation at 0.01 level

4. Discussion

The results showed that the expression of SphK2 in glioma was higher than that in the control group, indicating that SphK2 is a potential molecular marker to assist the diagnosis of glioma [8]. This study did not determine the difference of SphK2 expression between different grades of gliomas, which may be related to the limited number of samples in this study. Malignant glioma cells have the characteristics of rapid proliferation [9]. In the glioma tissue samples used in this study, the gp130 positive labeling index was significantly higher than that of the control group, and the gp130 positive labeling index was found to be positively correlated with SphK2 positive labeling index, suggesting that the overexpression of SphK2 may be an important component that drives the proliferation of glioma cells.

This study studied the expression of SphK2 and gp130 in gliomas. The data showed that the high expression of SphK2 in gliomas may play an important role in the occurrence and development of tumors. The relationship between the expression of SphK2 and gp130 index suggests that SphK2 may promote the proliferation of glioma cells by regulating the secretion of exosomes, but the specific relationship between them requires further verification.

Disclosure statement

The authors declare that there is no conflict of interest.

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