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High expression of GSKIP is associated with poor prognosis in meningioma

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Abstract

Meningiomas are the most common extra-axial primary central nervous system tumors. There is no effective treatment or targeted therapy for meningioma except excision and radiotherapy. glycogen synthesis kinase 3β interaction protein (GSKIP) is an A-kinase anchor protein that has cytosolic scaffolding function and binds to a protein kinase A and glycogen synthesis kinase 3β to modulate different biological processes and malignant tumorigenesis through the Wnt pathway. The purpose of this study was to investigate the relationship between GSKIP expression and the clinico-pathological parameters in meningioma using immunohistochemical staining. We collected samples from 74 patients, from 2008 to 2012, in the Kaohsiung Medical University Hospital that had data on the staging and prognosis of the meningioma pathological section. Chi-square, Kaplan-Meier method, and cox regression were used to analyze the correlation between clinical parameters and immunohistochemistry staining for GSKIP. Following our immunohistochemical score, we found that higher expression of GSKIP was associated with high World Health Organization grading, recurrence, malignant transformation, and reduced overall survival time and recurrence-free survival time in meningioma. GSKIP may be a biomarker of poor prognosis and a target protein for therapy in meningioma.

Abbreviations: GSK3β = glycogen synthesis kinase 3β, GSKIP = GSK3β interaction protein, IHC = immunohistochemical, OS = overall survival, WHO = World Health Organization.

Keywords: GSK3β interaction protein, meningioma, prognosis

1. Introduction

Meningiomas are the most common extra-axial primary central nervous system tumors, accounting for approximately 30% of all primary central nervous system tumors. Over 90% of meningiomas are benign.[1,2] Previous research predicted that an estimated 34,210 people in the United States of America would be diagnosed with meningioma in 2020. Incidence rates increase with age, with a large increase in diagnosis rates in adults above the age of 65. Diagnoses have been found to have a peak at 40 years of age and a female-to-male ratio of approximately 2:1.[2] Meningiomas are characterized by growing neoplasms that are thought to arise from meningothelial (arachnoid) cells. The World Health Organization (WHO) grading system classified meningiomas into 15 subtypes across 3 grades based on histopathological criteria. Grade I presents benign performance and includes 3 common types: transitional, fibrous, and meningiothelial type. Grade II and grade III represent 17.7% and 1.7% of meningiomas, respectively,[3] and have atypical to malignant histology with a more aggressive clinical course. The prognosis of atypical meningiomas is worse, with a 10-year progression-free survival and overall survival (OS) rate from 23% to 78% and 50% to 79%, respectively. Treatment for meningiomas is highly individualized and includes a combination of observation, surgical resection, radiotherapy, and, rarely, chemotherapy. Surgical resection is the primary choice for symptomatic, observation failure meningiomas, or large tumors that are anticipated to quickly become symptomatic. The goal of surgery is to achieve gross total resection (Simpson I, GTR); however, meningiomas of grades II and III show a higher recurrence rate. Biological behavior and risk of recurrence can range from indolent to highly aggressive despite being phenotypically identical, and optimal management has not been defined and remains controversial.

Glycogen synthesis kinase 3β (GSK3β) GSK3β interaction protein (GSKIP) is an A-kinase anchor protein with cytosolic scaffolding function.[4] GSKIP binds to a protein kinase A and GSK3β, and blocks the phosphorylation of β-catenin protein at ser-33/

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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ser-37/thr-4, negatively regulating the Wnt signaling pathway of GSK3β.[5,6] Wnt signaling modulates different biological processes and its deregulation is linked to malignant tumor neogenesis.[7,8]

This was the first study to assess the role of GSKIP as a potential target in meningioma. We investigated the relationship between GSKIP and clinicopathological characteristics in a cohort of patients with meningioma.

2. Materials and Methods

2.1. Patients

We used clinico-pathological parameters and paraffin-embedded section slides. In this study, a total of 74 patients with meningioma that had had surgical resection at the Department of Neurosurgery, Kaohsiung Medical University Hospital, were included. Specifically, the exclusion criteria were diagnosis only via biopsy, incomplete medical records, lack of follow-up data, and poor quality pathological and immunohistochemical (IHC) staining results. Subgroup analysis was performed by categorizing the study population according to 6 different criteria: presence or absence of malignant tumor, tumor recurrence, brain invasion, brain edema, hemorrhage, and intratumoral necrosis. All the data in this study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-E(I)-20180277).

2.2. IHC staining

Based on immunohistochemical staining following previously described in HP Tsai,[9] 3-micrometer thick sections were cut from formalin-fixed paraffin-embedded tissue samples collected from each patient. After deparaffinizing and rehydrating, the sections were autoclaved at 121°C for 10 minutes in 200 µL of Target Retrieval solution (pH 6.0) (S2369; Dako, Glostrup, Denmark) for antigen retrieval. Then, 100 µL of 3% H_2O_2 was added and the sample was incubated for 5 minutes at 25°C to block endogenous peroxidase activity. After 2 washes with TBS including 10% Tween 20, the sections were incubated with 100 µL anti-GSKIP antibody (1:50 dilution; PA5–29165, Thermo Fischer Scientific, Waltham, MA) for 1 h at room temperature, followed by incubation with secondary horseradish peroxidase-conjugated antibody for 30 minutes at room temperature. Finally, the slides were incubated with 3,3-diaminobenzidine (K5007; Dako) for 5 minutes, counterstained with 200 µL Mayer's hematoxylin for 90 s, and then mounted with malinol mounting medium. Based on IHC staining, expressions were classified as low and high levels. Staining intensity was classified as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. Scores 2 and 3 were considered as high GSKIP expression and scores 0 and 1 were considered as low GSKIP expression (Fig. 1).

2.3. Revise-transcription (RT) and quantitative polymerase chain reaction (Qpcr)

4 × 10 µm Formalin-Fixed Paraffin-Embedded (FFPE) meningioma sections was cut, and mRNA from these sections were extract following RNeasy FFPE kit. For reverse transcription, 1 µg total RNA are reverse transcribed into cDNA in 20 µL of TOOLS Easy Fast RT Kit and the conditions are 42°C for 15 minutes and 95°C for 3 minutes. GSKIP (Forward: 5'-ATGCGCTAGCTAT GGAAACAGACTG TAATCCCATG-3' and Revise: 5'-GCATACCGG TCCTGACTGTCCATCT CTTTTCAAAG-3') and GAPDH (Forward: 5'-CATGAGAA GTATGACAA CAGCCT-3' and Revise: 5'-AGTCCTTCC ACGATACCAAAGT-3') gene are amplified by polymerase chain reaction using TOOLS 2xSYBR qPCR Mix. The PCR procedures are 94°C for 15 minutes followed by 40 cycles of 95°C for 10 seconds, and extension at 60°C for 32 seconds.

2.4. Data analysis

Statistical Package for Social Sciences 24.0 software (SPSS, IBM, Armonk, NY) was used for statistical analysis. The Chi-squared test was used to assess the correlation between GSKIP protein expression and specific clinicopathological characteristics. The Kaplan-Meier method and log-rank test were used for survival analysis. P values < 0.05 were considered statistically significant for all analyses.

3. Results

3.1. Clinical-pathological parameters of patients with meningioma

In this study, we collected data of 74 patients with meningioma (42 females and 32 males); of these, 41 were aged ≥ 60 years and 33 were < 60 years old (mean age: 58.05 years). Based on the WHO classification, 58 and 16 patients with meningioma were classified as grade I and II/III, respectively. Furthermore, 7, 26, 13, 58, 5, and 10 patients had malignant transformation, recurrence, brain invasion, brain edema, tumor bleeding, and necrosis, respectively (Table 1).

3.2. Correlation between clinical-pathological parameters and GSKIP

Figure 1 shows that the IHC staining for the low expression and high expression of GSKIP; GSKIP is shown in the nucleus (Fig. 1). We analyzed the GSKIP staining to assess the correlation between the GSKIP expression and clinical parameters of patients with meningioma. Chi-square analysis showed that GSKIP was significantly associated with WHO grade (P = .029),

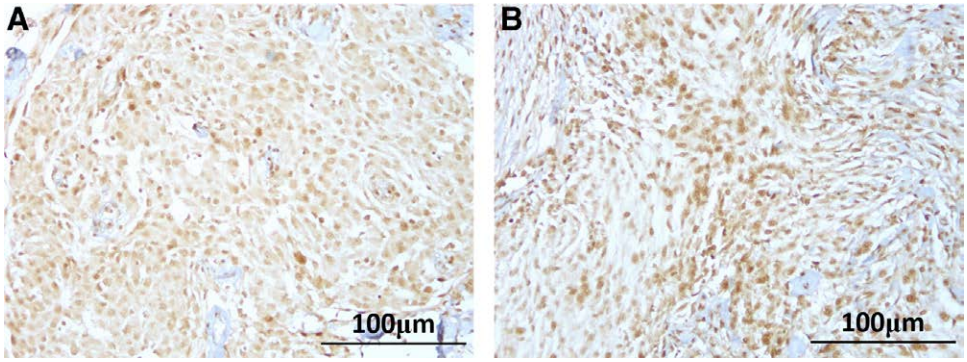

Figure 1. Immunohistochemistry staining for GSKIP in meningioma. (A) Low expression of GSKIP in grade I meningioma. (B) High expression of GSKIP in grade II/III meningioma. GSKIP = GSK3 β interaction protein.

malignant transformation (P = .047), and recurrence (P = .022). However, the GSKIP protein expression exhibited no significant correlation with age (P = .350), gender (P = 1), brain invasion (P = .761), brain edema (P = .052), tumor bleeding (P = .662), and necrosis (P = .740) (Table 1).

3.3. Correlation between prognosis and GSKIP

The Kaplan-Meier analysis with the log-rank test was conducted to establish the correlation between the GSKIP expression and the survival time in patients with meningioma revealed that the high GSKIP expression level was significantly correlated with the poor outcome of the OS (P = .001) and recurrence-free survival (P < .001; Fig 2).

Table 1

GSKIP expression correlated with clinicopathologic parameters in meningioma.

	No. of patients	GSKIP expression (n, %)		*P* value
		Low	High	
Age (yr)				.350
≥60	41	24 (32.4%)	17 (23%)	
<60	33	15 (20.3%)	18 (24.3%)	
Gender				1
Male	32	17 (23%)	15 (20.3%)	
Female	42	22 (29.7%)	20 (27%)	
WHO grade				.022*
I	58	35 (47.3%)	23 (31.1%)	
II/III	16	4 (4.1%)	12 (16.2%)	
Malignant transformation				.047*
Yes	7	1 (1.4%)	6 (8.1%)	
No	67	38 (51.4%)	29 (39.2%)	
Recurrence				.029*
Yes	26	9 (12.2%)	17 (23%)	
No	48	30 (40.5%)	18 (24.3%)	
Brain invasion				.761
Yes	13	6 (8.1%)	7 (9.5%)	
No	61	31 (41.9%)	30 (40.3%)	
Brain edema				.052
Yes	58	27 (36.5%)	31 (41.9%)	
No	16	12 (16.2%)	4 (5.4%)	
Tumor bleeding				.662
Yes	5	2 (2.7%)	3 (4.1%)	
No	69	37 (50%)	32 (43.2%)	
Necrosis				.740
Yes	10	6 (8.1%)	4 (5.4%)	
No	64	33 (44.6%)	31 (41.9%)	

GSKIP = GSK3 β interaction protein.

3.4. Compared GSKIP mRNA between different grade meningioma

To compare GSKIP mRNA level in grade I, II, and III, mRNA from 30 meningioma patients were extracted, including 17 WHO grade I, 10 WHO grade II, and 3 WHO grade III patients. The results from real-time PCR showed that the GSKIP mRNA level of WHO grade II and III patients individually were 3.31 and 8.22 folds than that of WHO grade I patients. These data showed that the GSKIP mRNA level of low-grade patients were lower than high-grade patients (Fig. 3).

4. Discussion

To the best of our knowledge, this is the 1st report on the correlation between GSKIP and meningioma. GSKIP binds to glycogen synthesis kinase 3β (GSK3β) to regulate cell physiological reaction. Glycogen synthase kinase 3 (GSK3), a serine/threonine kinase, participates in several signaling pathways, including the protein kinase A, protein kinase B, protein kinase C, and Wnt pathways.[4] This protein plays a multifaceted physiological role in the regulation of cell fate, signal transduction, protein synthesis, glycogen metabolism, mitosis, and apoptosis.[10,11] The Wnt pathway includes a large family of signaling molecules that have well-established roles in regulating cell fate, differentiation, proliferation, and potential tumor formation in many cancers, including meningioma.[12–14] The pro-survival functions of GSKIP have been revealed in several cell types and its upregulation contributes to the growth of cancer cells.[15] Moreover, GSKIP overexpression increases the resistance of cancer cells to chemotherapeutic drug-induced apoptosis.[8] In our study, we found that the expression of GSKIP was associated with OS and recurrence-free survival. The Kapan-Meier analysis showed that the patients with high expression of GSKIP had reduced OS and recurrence-free survival times. The predisposing factors for the recurrence of meningiomas have been well documented in previous studies, and include histological grade, extent of resection, postoperative radiotherapy, presence of brain invasion, and proliferation index.[16–18] In addition, malignant transformation of intracranial meningioma is rare, occurring in only 0.16 to 2.0% of cases, and the time to malignant transformation ranges from 8 months to 26 years.[19–22] In our results, high expression

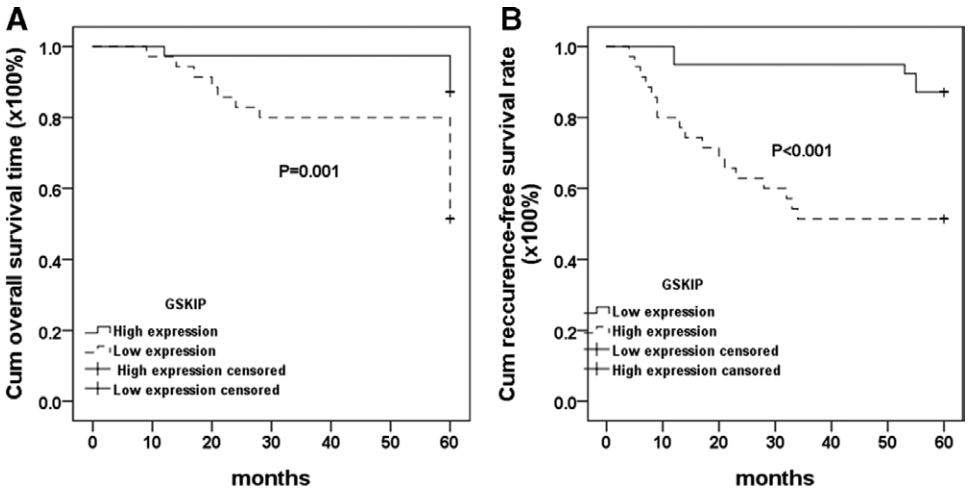

Figure 2. Kaplan-Meier survival analysis for (A) OS time and (B) recurrence-free survival time in patients with meningioma. SO = overall survival.

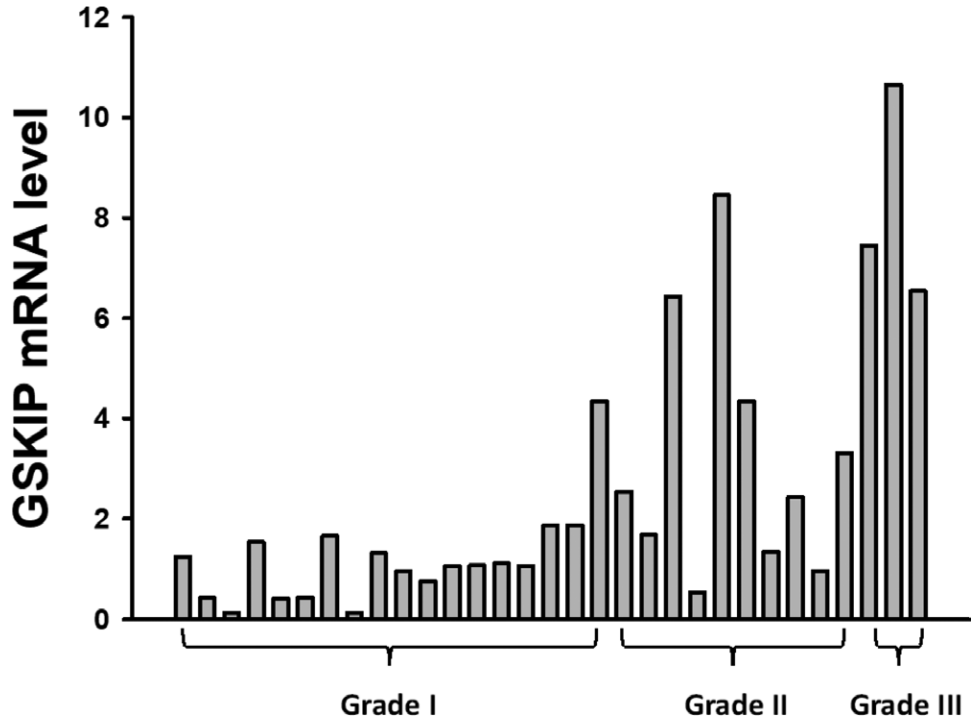

Figure 3. GSKIP mRNA levels in meningioma patients. 30 meningioma patients were 17 WHO grade I, 10 WHO grade II, and 3 WHO grade III patients. qPCR was analyzed GSKIP mRNA levels in different grading patients. Normalization with the average of GSKIP mRNA level in all grade I patients. GSKIP = GSK3 β interaction protein, WHO = World Health Organization.

of GSKIP in meningioma patients was associated with higher WHO grade, malignant transformation, and recurrence.

5. Conclusion

However, the limitation was too little to have malignancy meningioma. Further studies are thus required to provide clearer evidence of their association with GSKIP expression levels. In this study, high expression of GSKIP was associated with WHO high grading, recurrence, malignant transformation, and reduced OS and recurrence-free survival times in meningioma. Therefore, GSKIP may be a biomarker of poor prognosis and a target protein for therapy in meningioma.

Author contributions

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Writing – review & editing: Hung-Pei Tsai, Aij-Lie Kwan.

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