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Effect of TAGLN2 in the regulation of meningioma tumorigenesis and development

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Abstract. – OBJECTIVE: To explore the role of transgelin-2 TAGLN2 in the development and progression of meningioma and the potential regulatory.

MATERIALS AND METHODS: TAGLN2 knockdown expression and overexpression *in vitro* models were constructed using lentivirus in meningioma cell line CH157; their corresponding transfection efficiencies were verified by qRT-PCR and Western Blot. Actions of TAGLN2 on the proliferation of meningioma cells were explored by CCK8 and colony formation assays. The effect of TAGLN2 on invasion of meningioma cells was analyzed by transwell cell invasion assay. Biological function of TAGLN2 on apoptosis of meningioma cells was determined by flow cytometry. Finally, Western Blot was used to investigate the detailed mechanism of TAGLN2 on regulating the biological functions of meningioma cells.

RESULTS: After down-regulating the expression of TAGLN2, there were significantly decreased capacities of cells proliferation and colony formation of meningioma cells, meanwhile, cell invasion was significantly decreased but the apoptosis rate was increased. On the contrary, up-regulation of TAGLN2 expression, the proliferation, colony formation ability were significantly increased as well as the invasion capacity, whilst apoptosis rate was decreased. Western Blot showed that expressions of p-PI3K and p-AKT were inhibited after knockdown of TAGLN2, which were significantly increased after TAGLN2 was overexpressed.

CONCLUSIONS: TAGLN2 can affect the proliferation, invasion and apoptosis of meningioma cells and may participate in the development of meningioma through regulating the PI3K/AKT signaling pathway.

Key Words:
TAGLN2, Meningioma, Proliferation, Apoptosis.

Introduction

Meningioma is a common central nervous system tumor, accounting for 20% of all intracranial tumors[1,2]. According to the pathological classification of CNS tumors by WHO, meningioma is divided into three grades (WHO I-III, with increased malignant degree)[3]. Currently, the ratio of WHO grade III meningioma (malignant meningioma) was significantly higher than before, accounting for 15-20% of all meningioma[4,5]. Pathological grade is the most important cause that affects tumor prognosis. To date, surgical-based comprehensive treatment is the main treatment[6,7]. However, overall recurrence rate of malignant meningioma was up to 80% and the 5-year survival rate was almost zero after comprehensive treatment[8,9]. The high recurrence rate and mortality make it one of the most intracranial tumors that is needed to be overcame[10]. Although we already had a certain understanding of meningioma molecular genetics, the potential development of meningioma mechanism still remains to be further explored. TAGLN2 is an important member of a family of actin cytoskeletal binding proteins that are widely expressed in normal tissue cells. Due to the role of the TAGLN protein family in cytoskeletal remodeling and cell morphological transformation, its potential function in the malignant phenotype of tumor cells has gained a lot of attention[11,12]. Meanwhile, it has been reported that TAGLN was not only closely linked to the actin family members to regulate cell motility, but also expressed in the nucleus and may affect cell phenotype by directly regulating expressions of related genes[13]. Therefore, the effects of TAGLN

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family on various types of tumors are needed to be further elucidated. At present, the role of TAGLN2 in the tumor is unclear. For example, studies in breast cancer have shown that TAGLN2 was down-regulated in invasive tumor cells[14]; however, it served as a promotion regulator in bladder cancer and cervical cancer to promote the invasion of tumor cells[15, 16]. Few studies have been carried out on the effect and clinical significance of TAGLN2 in meningioma, as well as the role in the regulation of meningioma development. Therefore, in this study, we aimed to explore the role of TAGLN2 in the development of meningioma and its potential regulatory mechanism.

Materials and Methods

Cell Lines and Reagents

Meningioma cell line CH157 was purchased from American Type Culture Collection (ATCC) company (Manassas, VA, USA), Dulbecco's Modified Eagle Medium (DMEM) medium and fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA); Cells were maintained in an incubator with 37°C and 5% CO_2 atmosphere and cultured in the DMEM medium supplemented with 10% FBS.

Transfection

Knockdown (shTAGLN2) and overexpression (TAGLN2) and their respective control sequences (shNC and NC) were designed and synthesized by Shanghai Gene Pharma Co., Ltd. (Shanghai, China) according to the TAGLN2 gene sequence to construct a corresponding lentiviral vector. Cells in logarithmic growth phase were seeded into a 6-well plate, the appropriate density was 50-70% the next day. The medium was replaced before transfection, 1 ml of medium without antibodies was added into each well. 1 μL of polybrene was added to each well and a suitable amount of virus solution (based on viral MOI). After incubation for 24 h, 1 mL of medium without antibodies was again added the next day. After transfection for another 24 h, fluorescence intensity was observed under a microscope to assess transfection efficiency. Finally, puromycin was used to screen stable transfected cells.

Cell Proliferation Assays

Stably expressed cells in logarithmic growth phase were harvested and plated in a 96-well plate at 2000 cells per well. After cells were cultured for 1, 2, 3 and 4 days respectively, cell counting kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) was replenished and continued culturing for 2 h. Absorbance values at 490 nm were detected by the microplate reader and then analyzed.

Cell Clone Formation Assay

Stably expressed cells in logarithmic growth phase were digested by trypsin and resuspended, then they were plated into a 6-well plate with a density of 1000 cells per well, maintained in an incubator with 37°C and 5% CO_2. The medium was replaced a week later. When colonies were grown into the appropriate size, 1 mL of paraformaldehyde was used to fix cells for 30 min, crystal violet was used to stain for 30 min, washed with PBS and dried to count the number of colonies.

Cell Apoptosis Assay

Cells were collected by EDTA-free trypsinization, phosphate-buffered saline (PBS) was used for washing twice, and 1 x 10^5 cells were collected. 5 μL of 7-AAD dye were added to 50 μL of Binding Buffer, cells were collected and the above 7-AAD dye was added and mixed at room temperature without light to react for 5-15 min. Then, 450 μL of Binding Buffer and 1 μL of Annexin V-PE were added and mixed at room temperature without light to react another 5-15 min; cells were then analyzed with a flow cytometry within 1 h.

Transwell Cell Invasion Assay

Stably expressed cells in logarithmic growth phase were digested with trypsin and resuspended in serum-free medium. Cell density was adjusted to 2.0 × 10^5 / mL. The transwell chamber containing matrigel was placed in a 24-well plate. In brief, there were 200 μL of cell suspension in the upper chamber and 500 μL medium containing 10% fetal bovine serum (FBS) in the lower chamber. Cells were maintained in a 37ºC incubator. The chamber was removed 48 h later, then fixed with 4% paraformaldehyde for 30 min, and washed with PBS after crystal violet staining for 15 min. The inner surface of the cell basement membrane was carefully cleaned and the inner cells were removed. Outer base of the basement membrane stained cells were observed under a microscope, randomly selected five visual fields for counting.

Quantitative Real-time PCR (qRT-PCR)

Total RNA was extracted from meningioma cells CH157 using TRIzol reagent (Invitrogen,

Carlsbad, CA, USA) and PrimeScript™ RT Master Mix (Perfect Real Time) from TaKaRa Inc. (Otsu, Shiga, Japan) was utilized for reverse transcription of RNA to cDNA. QRT-PCR reactions were performed using TaKaRa SYBR Green Master (ROX) kit and StepOne Plus Real-time PCR system. Primers used for the qRT-PCR reaction were as the following: TAGLN2: forward ATGGCACGGTGCTATGTGAG, reverse: CCCACCCAGATTCATCAGCG; GAPDH: forward: GGAGCGAGATCCCTCCAAAAT, reverse: GGCTGTTGTCATACTTCTCATGG. Data were analyzed using ABI Step One software and relative mRNA levels were calculated by the $2^{-\Delta\Delta Ct}$ method.

Western Blot

In short, transfected cells were lysed with lysis buffer on ice for 30 min, and then centrifuged at 14,000 ×g for 15 min at 4ºC. Total protein concentration was measured by bicinchoninic acid (BCA) protein assay reagent (Pierce, Rockford, IL, USA). Extracted proteins were separated by electrophoresis on a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Primary and secondary antibodies were incubated respectively. Western blot analysis was performed according to standard procedures. Primary antibodies used in this study were p-PI3K, PI3K, p-AKT, AKT and GAPDH, the secondary antibodies were anti-mouse and anti-rabbit, all purchased from Cell Signaling Technology (CST, Danvers, MA, USA).

Statistical Analysis

Statistic package for social science (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis. Measurement data were compared with *t*-test and presented as mean ± standard deviation ($\bar{x} \pm s$), categorical data were analyzed by x^2 test or Fisher exact probability. $p<0.05$ indicated significantly difference; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

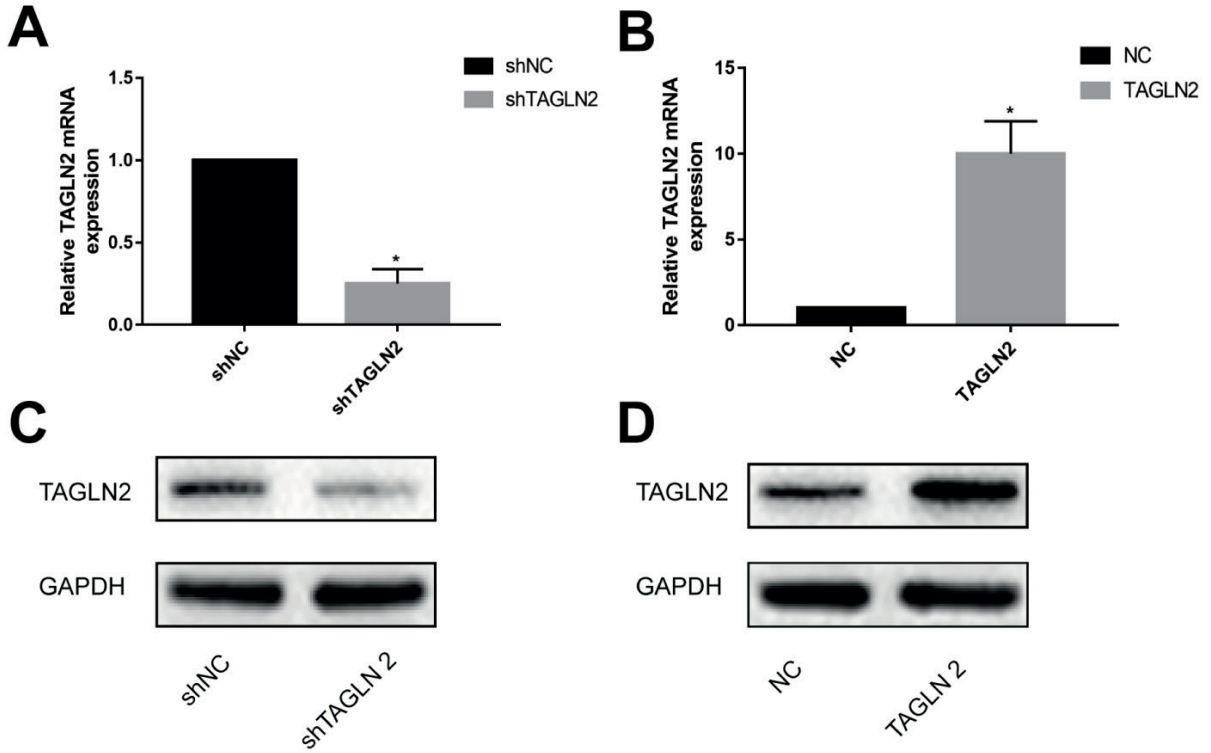

Figure 1. ***(A-C)*** Western blot and qRT-PCR were used to verify the efficiency of TAGLN2 knockdown. shTAGLN2 indicates TAGLN2 knockdown; shNC indicates cells transfected with an empty vector; ***(B-D)*** Western blot and qRT-PCR were used to verify the efficiency of TAGLN2 overexpression (named as TAGLN2).

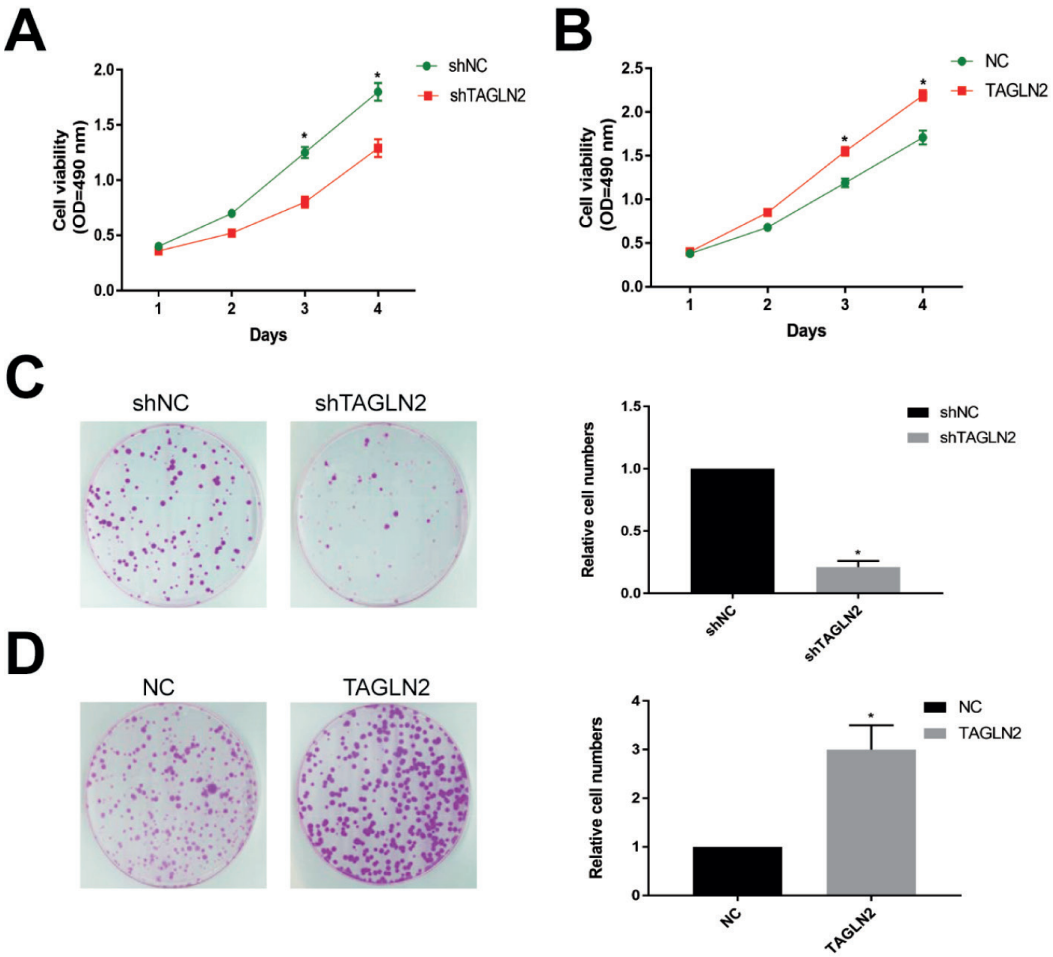

Figure 2. ***(A-B)*** Growth curve analysis showing the cell growth of CH157 cells with TAGLN2 knockdown or overexpression. ***(C-D)*** The efficiency of cell colony formation in CH157 cells with TAGLN2 knockdown or overexpression.

Results

Knockdown of TAGLN2 Inhibited Cell Proliferation

First, we explored the effect of TAGLN2 on the proliferation of meningioma cells, we successfully constructed a TAGLN2 knockdown expression and overexpression model. Western blot and PCR results showed that there was a significant decrease of TAGLN2 expression in the knockdown group (shTAGLN2), while an increase was observed in the overexpression group (TAGLN2) (Figure 1). CCK8 assay revealed that the proliferation rate in TAGLN2 knockdown group (TAGLN2) was significantly decreased in comparison with the control group (shNC). Also, there was a significant increase on the cell proliferation in the TAGLN2 over-expression group (TAGLN2) compared with that of control group (NC) (Figure 2A-B). Similarly, colony formation assay also showed a decreased capacity after knockdown of TAGLN2 expression, and an increased capacity after overexpressing TAGLN2.

Effect of TAGLN2 on Cell Apoptosis

Next, we constructed flow cytometry to investigate the effect of TAGLN2 on apoptosis of meningioma cells. Results suggest that, after TAGLN2 downregulation, as can be seen, the apoptosis rate of CH157 cells was notably higher than that of the negative control group, while the apoptosis rate decreased after overexpressing TAGLN2 (Figure 3A). It suggested that TAGLN2 can inhibit the apoptosis and promote the proliferation of meningioma cells.

Knockdown of TAGLN2 Inhibited Cell Invasion

Transwell invasion assay was used to explore the role of TAGLN2 on invasion of meningioma cells. It pointed out that when comparing with the control group, the number of transmembrane cells in transwell chamber decreased significantly after knockdown of TAGLN2, suggesting that the invasion ability was inhibited. However, the number of transmembrane cells in transwell chamber increased significantly after overexpressing TAGLN2, which promoted the invasion of meningioma cells (Figure 3B).

Effect of TAGLN2 on Protein Expressions of PI3K and AKT

In order to analyze that whether TAGLN2 may participate in the development of meningioma through regulating PI3K/AKT pathway, we selected PI3K/AKT pathway key proteins, including p-PI3K, PI3K, p-AKT and AKT as research molecules. Western blot showed that the expressions of p-PI3K and p-AKT decreased significantly after knockdown of TAGLN2, while they were notably increased after overexpressing TAGLN2, the expressions of total PI3K and AKT did not change significantly (Figure 4).

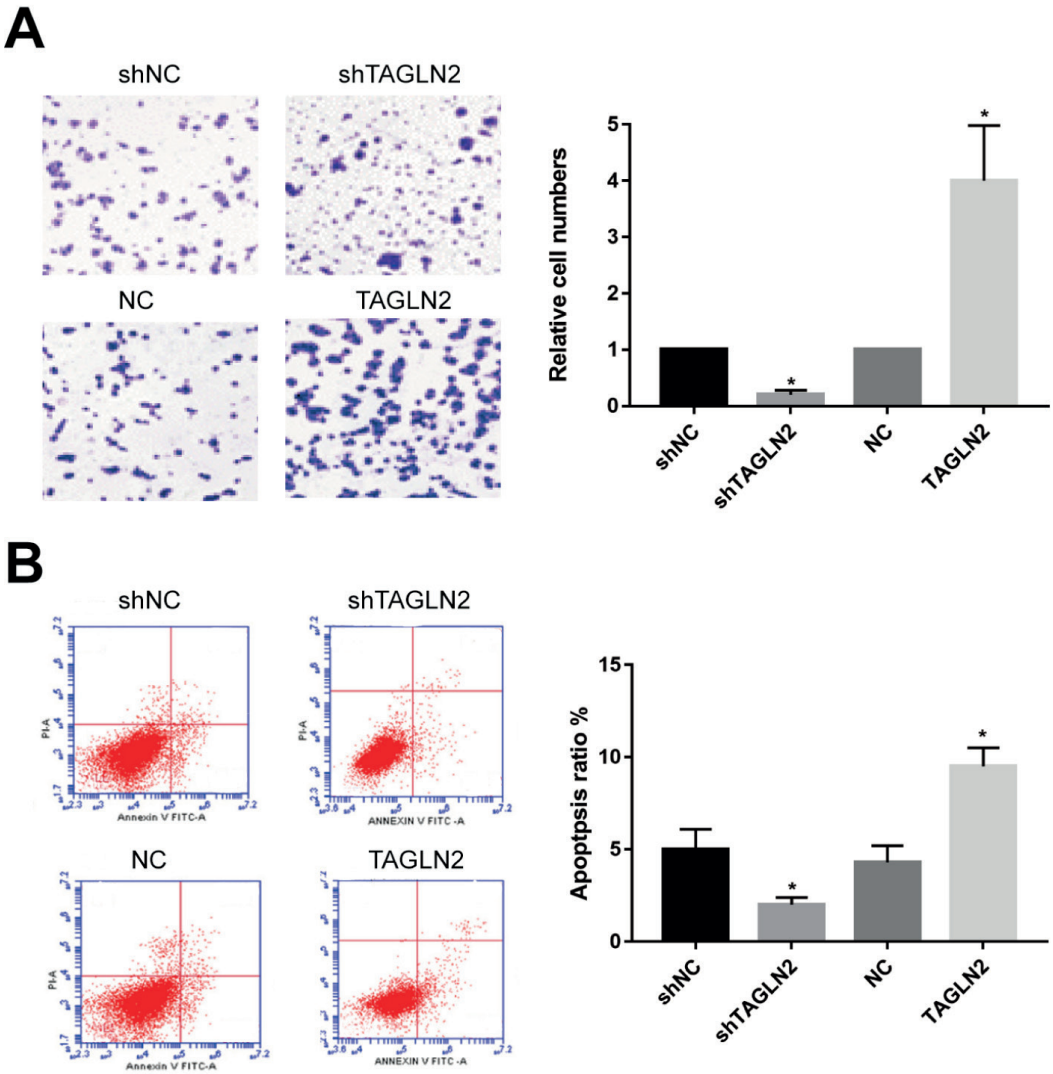

Figure 3. ***(A-B)*** Transwell Matrigel invasion assay in CH157 cells with TAGLN2 knockdown or overexpression; ***(C-D)*** Effect of TAGLN2 on cell apoptosis in CH157 cells with TAGLN2 knockdown or overexpression.

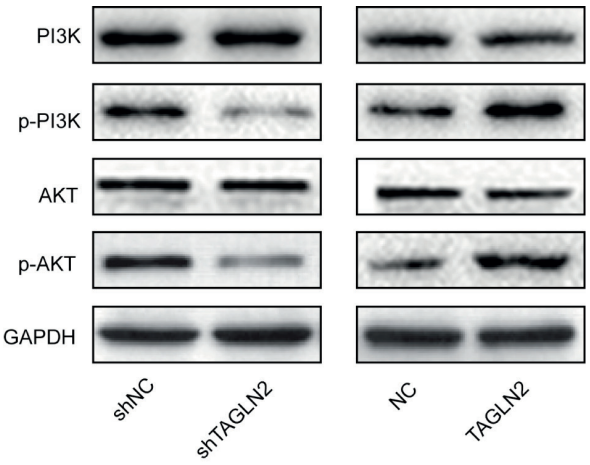

Figure 4. TAGLN2 affects the protein expression levels of p-PI3K and p-AKT in CH157 cells.

Discussion

At present, the main treatment of meningioma are surgical treatment, stereotactic radiotherapy, etc., but there are about 50% of cases which cannot be cured due to tumor location, high pathological grade and surgical complications[17,18]. Therefore, studies on molecular mechanism of meningioma are required, which is of great significance in looking for new treatment for recurrent, atypical and malignant meningioma besides surgery and radiotherapy.

Previous studies have shown that TAGLN2 played an important role in breast cancer, bladder cancer and cervical cancer cells[14,15]. In the present study, we successfully constructed the TAGLN2 overexpression and knockdown cell models by lentiviral transfection. The effect of TAGLN2 on the proliferation of meningioma cells was verified by CCK-8 and colony formation assay. Transwell invasion assay showed the effect of TAGLN2 on meningioma cells. It suggested that overexpression of TAGLN2 can enhance the proliferation and invasion abilities of meningioma cells, while knockdown of TAGLN2 presented opposite results. In addition, apoptosis assay showed that overexpression of TAGLN2 can reduce the apoptosis rate of cells, whilst knockdown of TAGLN2 promoted apoptosis rate.

The above results showed that TAGLN2 played an important role in developing and progressing meningioma. Then, what is the specific regulatory mechanism? Some studies have shown that the PI3K/AKT signaling pathway was related to different grades of benign and malignant meningioma, and activating PI3K/AKT signaling pathway helped contribute to the invasion of malignant meningioma[19,20]. The PI3K/AKT pathway is the most common aberrant activation pathway in human cancer responsible for promoting cell growth, migration, survival and proliferation[21,22]. PI3K inhibitors can improve tumor degeneration but reduce tumor recurrence[23]. However, whether TAGLN2 is involved in the proliferation and invasion of meningioma cells through the PI3K/AKT signaling pathway has not been reported yet.

To investigate whether TAGLN2 is involved in the development and progression of meningioma through the PI3K/AKT pathway, expression changes of p-PI3K, PI3K, p-AKT and AKT was performed by Western blot after knockdown and overexpression of TAGLN2. Western blot showed that there were decreased expressions of p-PI3K and p-AKT, which turned out to be significantly increased after overexpressing TAGLN2, while expressions of total PI3K and AKT did not change significantly. It suggested that TAGLN2 could promote the development and progression of meningioma by activating the phosphorylated PI3K/AKT pathway.

Conclusions

TAGLN2 can affect the proliferation, invasion and apoptosis of meningioma cells and may participate in the development of meningioma through regulating the PI3K/AKT signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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