



*Research article*

## **Kinesin family member 15 can promote the proliferation of glioblastoma**

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**Abstract:** Glioblastoma is one of the most dangerous tumors for patients in clinical practice at present, and since glioblastoma originates from the brain, it will have a serious impact on patients. Therefore, more effective clinical therapeutic targets are still needed at this stage. Kinesin family member 15 (KIF15) promotes proliferation in several cancers, but its effect on glioblastoma is unclear. In this study, differentially expressed gene analysis and network analysis were performed to identify critical genes affecting glioma progression. The samples were divided into a KIF15 high-expression group and KIF15 low-expression group, and the association between KIF15 expression level and clinical characteristics was summarized and analyzed by performing medical data analysis; the effect of KIF15 on glioblastoma cell proliferation was detected by employing colony formation and MTT assays. The effect of KIF15 on tumor growth in mice was determined. It was found that KIF15 was a potential gene affecting the progression of glioblastoma. In addition, KIF15 was highly expressed in glioblastoma tumor tissues, and KIF15 was correlated with tumor size, clinical stage and other clinical characteristics. After the KIF15 gene was knocked out, the proliferation ability of glioblastoma was significantly inhibited. KIF15 also contributed to the growth of glioblastoma tumors in mice. Therefore, we found KIF15 to be a promising clinical therapeutic target.

**Keywords:** glioblastoma; kinesin family member 15 (KIF15); proliferation; clinical characteristics; therapeutic target

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### **1. Introduction**

Glioblastoma originating in the brain has been found to be one of the most dangerous types of tumors in clinical studies for many years [1,2]. However, glioblastoma has a very strong metastatic

capacity, and distant metastasis will occur before radiotherapy or chemotherapy, so the effect of chemotherapy or radiotherapy is limited. According to the latest data, the prognosis of glioblastoma remained very poor from 2000 to 2022, with little change. When patients are diagnosed with glioblastoma, the 1-year survival rate is less than 65%, and the 5-year survival rate is nearly 5% [3–5]. In addition, glioblastoma also continuously infiltrates the surrounding normal brain tissue with a high degree of infiltration, and the high heterogeneity of glioblastoma cells brings great resistance to treatment; so, up to now, there is still a lack of a feasible treatment [6] in clinical practice. Over these years, glioblastoma has shown unprecedented strength in response to the application of targeted approaches [7]. More and more undiscovered therapeutic targets have been successfully identified, such as KPNB1 and EGFR [8,9]. However, we still need more favorable molecular therapeutic targets to fight the disease.

There are many proteins in the driver protein superfamily, among which there is a relatively conservative motor protein [10]. Many kinin proteins exhibit ATP-dependent activity, and these proteins can travel along microtubule orbits [11,12]. Up to 45 kinase peptides have now been identified in normal mammalian cells [13]. These drivers play different but important roles in different cellular processes in various cells in the human body, including mitosis, cytokinesis and bipolar spindle assembly [14–16]. KIF15, also known as kinesin-12, is a tetramer spindle motor protein of the kinesin superfamily, which is involved in a variety of microtubule functions [17]. Previous studies have shown that KIF15 is expressed in neurons during nerve development in zebrafish, thereby affecting the growth of nerve cell axons. In addition, the KIF15 protein is also closely related to the migration of cortical astrocytes [18,19].

In the process of tumor genesis, KIF15 has been considered to have some influence on the generation and proliferation of some tumors; for example, KIF15 is related to the proliferation and metastasis of breast cancer cells [20] and tamoxifen resistance. It was also found that KIF15 is highly expressed in pancreatic cancer tissues and can promote the proliferation of cancer cells through the MEK-ERK signaling pathway [21]. Moreover, KIF15 is associated with the generation and metastasis of lung adenocarcinoma and can lead to a poor prognosis of the tumor. KIF15 has been found to play an important role in the proliferation and metastasis of many types of tumor cells, but the role of KIF15 in glioblastoma remains unclear.

After this study and discussion, we found that KIF15 is an important gene affecting the development of glioblastoma. Interestingly, we found that KIF15 is highly expressed in human glioblastoma tissues, and we explored the correlation between KIF15 expression level and clinicopathological features of glioblastoma patients. We have also shown that downregulation of KIF15 expression can reduce the proliferation of mouse glioblastoma cells and inhibit tumor growth. Therefore, we provide a new therapeutic target for the treatment of glioblastoma—KIF15.

## 2. Materials and methods

### 2.1. Bioinformation analysis

DEG (Differential Expression Analysis) analysis of young and old rhesus macaques: The differential expression of differentially expressed genes (DEGs) in Grades 2 and 3 which were studied by using the edgeR software package. The nominal significance threshold  $P < 0.05$  and fold change (FC)  $> 1$  were used to identify the DEGs. Multiple test adjustments were made to the P values by using the Benjamini-Hochberg method to estimate the false discovery rate (FDR). Using two online

resources, namely, DAVID (<https://david.ncifcrf.gov/>) and g: Profiler (<https://biit.cs.ut.ee/gprofiler/>), evaluation of the DEG functional category (GO and KEGG) enrichment degrees was performed. Multiple test adjustments were made to the P values by using the Benjamini-Hochberg method to estimate the error detection rate (FDR).

Network analysis: Using string analysis (String: Functional Protein Association Networks ([string-db.org](http://string-db.org))), we have constructed 429 DEG networks that have changed at Levels 3 and 4 compared to Level 2. The network is represented by the cell wall. CytoHubba software was used to analyze the hub genes in the network.

## 2.2. Antibodies, primers and shRNA plasmids

Rabbit anti-KIF15 (for immunohistochemistry (IHC) assays, 1 : 50 dilution; for immunoblot assays, 1 : 500 dilution; PA5-57305, Invitrogen, USA) and mouse anti- $\beta$ -actin (1 : 1000 dilution; ab8226, abcam, Cambridge, UK) were employed. Rabbit anti-Ki67 (for IHC assays, 1 : 50 dilution; for immunoblot assays, 1 : 1000 dilution; ab16667, abcam, Cambridge, UK) and rabbit anti-proliferating cell nuclear antigen (PCNA) (for IHC assays, 1 : 50 dilution; for immunoblot assays, 1 : 500 dilution; ab92552, abcam, Cambridge, UK) were also employed.

The quantitative PCR primer sequences of KIF15 are as follows: forward, 5'-AAGCAGGTAACATAAATCG-3', and reverse, 5'-AAATCCCGTACTAAGAAGGT-3'. The qRT-PCR primer sequences of GAPDH are as follows: 5'-CGACCACTTTGTCAAGCTCA-3' and 5'-GGTTGAGCACAGGGTACTTTATT-3'.

Ready-to-package AAV shRNA plasmids targeted by KIF15 (pAV-KIF15-shRNA, Catalog number: SH813982) were bought from the Addgene plc. The shRNA sequences targeted by KIF15 were as follows: sense and 5'-AACCAACCAAGTAATGAAGGTGA-3'.

## 2.3. Human tissue samples and analysis

Sixty-two human glioblastoma tissue samples and corresponding adjacent tissue samples were collected from patients receiving routine treatment in Tianjin Lake Hospital. Tumor cells were then isolated from the tumor. The clinicopathological characteristics of the patients, including age and clinical stage, were collected as shown in Table 1. The study has been approved by the Ethics Committee of Hubin Hospital in Tianjin.

IHC was performed to observe the possible relationship between KIF15 presentation level and glioblastoma development. That is, the resulting sample was fixed in 4% PFA (Polyfluoroalkoxy), for up to 30 minutes, after which we sealed it with 2% BSA (Bovine Serum Albumin Solution) for up to 20 minutes. KIF15, Ki67 and PCNA antibodies were cultured at room temperature for nearly 2 hours. Diamobenzidine was used as a color substrate and cultured for 1.5 hours.

KIF15 mainly exists in the cytoplasm of glioblastoma cells. The scoring method was as follows: 1 = 1–25% staining cells; 2 = stained cells 26–50%; 3 = 51–100% color cells. Color intensity has been divided as follows: 0 (no color), 1 (low color), 2 (medium color) and 3 (strong color). The expression level of KIF15 was determined by using a staining index: staining intensity score  $\times$  staining cell percentage score. A color index  $< 4$  indicated low expression, and a color index of 4 or  $> 4$  indicated high expression. The experimental results were analyzed by Double-blind method.

**Table 1.** Relationship between MUC21 expression and clinicopathological characteristics of LUAD (adenocarcinoma of lung) patients (N = 47).

Feature	All n = 62	KIF15 expression		$\chi^2$	P
		Low n = 20	High n = 42		
Age (year)				1.586	0.208
< 55	38	10	28		
≥ 55	24	10	14		
Gender				1.168	0.280
Male	40	11	29		
Female	22	9	13		
Tumor lateralization				0.712	0.399
Subtentorial	42	15	27		
Supratentorial	20	5	15		
Recurrence				8.062	0.005*
Yes	50	12	38		
No	12	8	4		
IDH1 mutations				0.102	0.750
No	42	13	29		
Yes	20	7	13		

#### 2.4. Cell culture and transfection

Human glioblastoma cell lines U87 and U251 were purchased from ATCC in 2018 and frozen and thawed at the Tianjin Institute of Neurosurgery. U87 and U251 were grown in humid air in a 5% carbon dioxide incubator in Dulbecco's Modified Eagle's Medium, which was supplemented with 10% FBS, 100 µg/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL dimycin B.

KIF15 shRNA plasmid was transfected into U87 or U251 cells by using LiPO-2000 (#11668019, Invitrogen, Carlsbad, CA, USA). In animal experiments, KIF15 stabilized knockout U87 cells by screening for shRNA lentivirus infection.

#### 2.5. Quantitative PCR assay

Total mRNA was extracted from human glioblastoma cells by using the TRIzol reagent (#15596026, Invitrogen, Carlsbad, CA, USA). RNA was then reversed by using m-MLV reverse transcriptase (#M1701, Promega, Madison, Wisconsin, USA).

cDNA was synthesized via the reverse transcription of total mRNA by using a cDNA synthesis system. Quantitative PCR was performed by using a SYBR Ex Taq kit (#638319, Takara, Japan) to normalize the KIF15 expression level to the GAPDH expression level.

#### 2.6. Immunoblot assays

Patients' glioblastoma tumor cells or tissues were decomposed by using a RIPA buffer (#9800, Cell signaling pathway, Danfoss, MA). SDS-PAGE was used to analyze the total protein. And then, we used 5%

milk, which was used as a buffer; then, we encapsulated it and incubated it with primary antibodies. KIF15, Ki67, PCNA and  $\beta$ -actin were detected at room temperature for 2 hours. The PVDF (polyvinylidene fluoride) membrane was then correlated with HRP (Horseradish Peroxidase) and incubated again for up to 45 minutes. Finally, an ECL (Enhanced chemiluminescence) kit was used for Western blotting detection. ImagePro software was used for the experimental calculations.

### 2.7. Colony formation assay

We then used about 500 U87 or U251 cells, which were successively added to 6-well culture plates and transfected with KIF15 shRNA plasmid; then, these transferred cells were cultured at 37 °C. The medium was replaced every 2 days and kept fresh. After 14 days, the cells were fixed with PFA for 30 minutes, stained with 0.1% crystal violet at room temperature for 30 minutes and then washed with PBS (Phosphate Buffered Saline) reagent. We then counted and analyzed the colony count manually.

### 2.8. MTT assay

The cells were placed into 96-well plates with 1000 grains per well, transfected with the control or KIF15shRNA plasmid and cultured for 1 day. These cells were then incubated with MTT for up to 4 hours, after which the medium was removed. The tumor cells were then washed with PBS reagent. These stained cells were extracted by adding 150  $\mu$ l of dimethyl sulphoxide to each well; the OD (optical density) values were measured by using a 570-nm microplate analyzer.

### 2.9. Tumor growth assays

Naked ball/C mice (6–8 weeks, female, 18–22 g, n = 10) were purchased from Beijing Weisheng He Experimental Animal Science and Technology Co., Ltd. (Beijing, China). All animal testing procedures were approved by our facility's Animal Care and Use Committee. Regarding the animal experiments, our hospital has carried out animal work, and the approval number obtained from the relevant institutional review board is LLSP2019-016. Briefly, U87 cells were stably transfected with a control or KIF15 shRNA lentivirus.  $2 \times 10^6$  control cells or KIF15 ablated cells were implanted subcutaneously in nude mice. The tumor was isolated 2 weeks later and photographed; its volume was measured every 3 days.

### 2.10. Statistical analysis

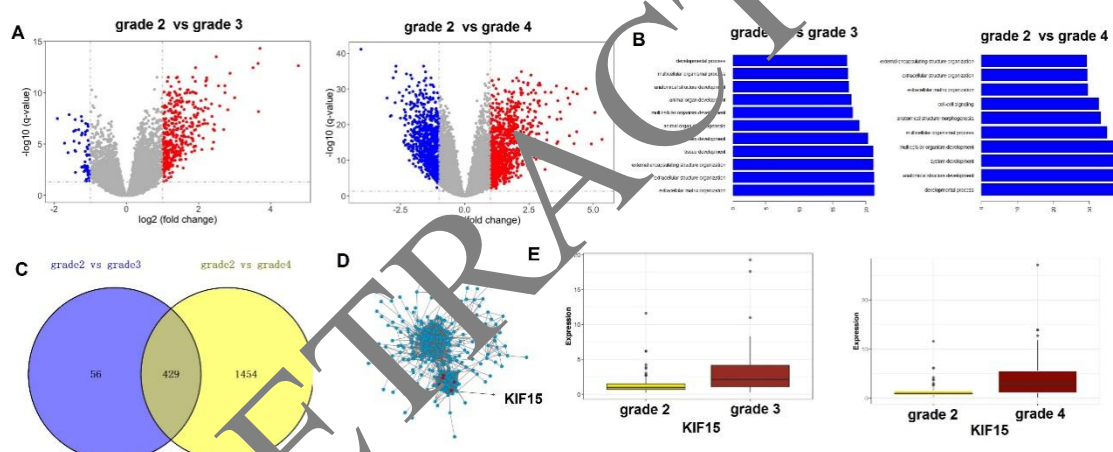
Graphpad software was used for statistical analysis in this study. All results of this study are presented as mean  $\pm$  standard deviation. The correlation between clinical features and protein levels was calculated by conducting  $\chi^2$  analysis and Fisher's exact testing. Statistical comparisons were made by using the student T-test. An asterisk indicates that  $P < 0.05$ .  $P < 0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Identification of *KIF15* as a potential gene to affect the progression of glioma

To explore the pathology of glioma, we first searched the potential genes abnormally expressed in glioma tissues by conducting bioinformatics analysis. We first used the dataset GLIOMASdb (<http://cgga.org.cn:9091/gliomasdb/>) for different stages of glioma transcriptome analysis. Analysis of the gene expression differences between Grades 2 and 3 glioma and Grades 2 and 4 glioma was performed. Interestingly, we found that 485 DEGs were specifically altered between Grades 2 and 3 gliomas (Figure 1A), and that 1883 genes were specifically altered between Grades 2 and 4 gliomas (Figure 1B).

Furthermore, by analyzing the functions of these DEGs, we found that the biological processes of these DEGs are abundant during development and in extracellular tissue (Figure 1C). We found that there were 429 gene changes in Grades 3 and 4 (Figure 1D). Following the network analysis of 429 genes, we found a *KIF15* central gene network (Figure 1E) and that the information on the expression level analysis of *KIF15* was upregulated in Grade 4 compared with Grades 2 and 3 (Figure 1E). So, we hypothesized that *KIF15* is a candidate gene for glioma.



**Figure 1.** New DEGs in glioma tissues were identified based on bioinformatics analysis, and *KIF15* was identified as a potential gene affecting the progression of glioma. (A) Volcanic map showing genetic changes in Grade 2 gliomas compared to Grade 3 gliomas. The red and blue dots indicate the upregulation and downregulation of genes in Grade 3 glioma ( $P < 0.05$ , fold change  $> 2$ ). (B) Volcanic map showing genetic changes in Grade 2 gliomas and Grade 4 gliomas. The red and blue dots indicate the upregulation and downregulation of gene expression in Grade 4 glioma ( $P < 0.05$ , fold change  $> 2$ ). (C) Venn diagram showing the overlapping DEG for Grades 2 and 3 and for Grades 2 and 4. There were 429 DEGs in Grades 2 and 3 and Grades 2 and 4. (D) Network analysis showing the interaction of 429 DEGs co-existing at Level 2 to Level 3 and Level 2 to Level 4. The red circle shows the top 5 HUB genes in the network, including *KIF15*. (E) Box plots showing *KIF15* expression levels in Grades 2 and 3 gliomas (left) and *KIF15* expression levels in Grades 2 and 4 gliomas (right).

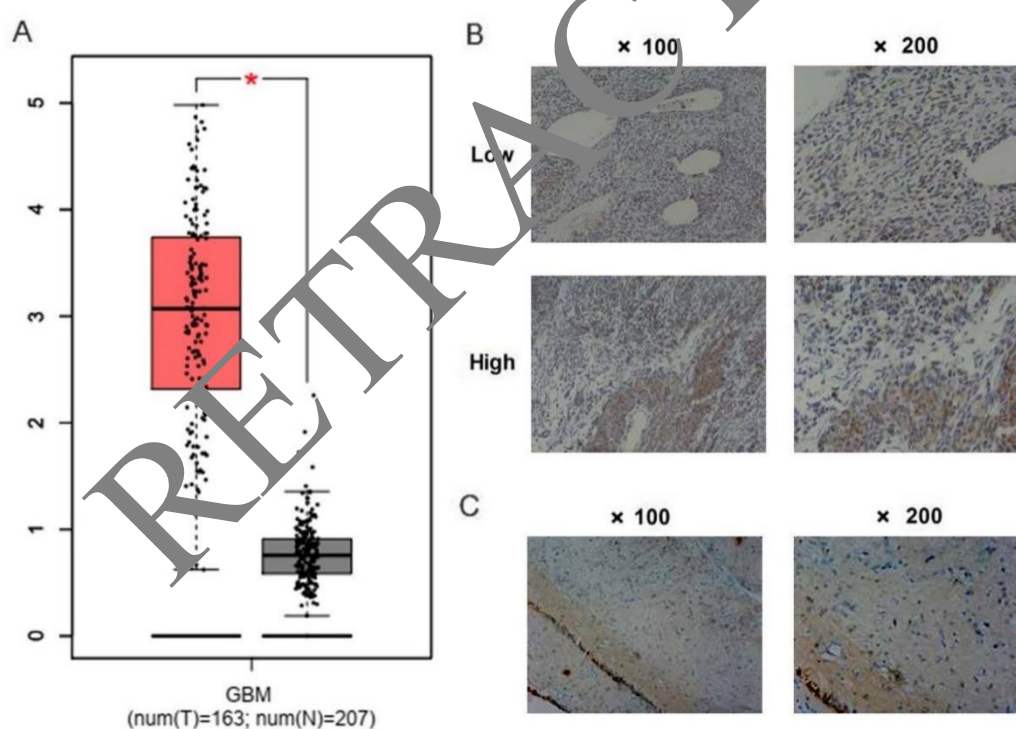
### 3.2 KIF15 expression is largely upregulated in human glioblastoma tissues

To investigate the possible role of KIF15 in the development and progression of glioblastoma, an immunohistochemical method was used to detect the expression level of KIF15 in glioblastoma tissues of patients undergoing surgical resection. We found that KIF15 is mainly located in the cytoplasm of glioblastoma cells (Figure 2B).

### 3.3 KIF15 expression is associated with the prognosis of patients with glioblastoma

According to our staining conclusions, the histopathological specimens of 62 surgically resected patients with glioblastoma were categorized into the low-expression group of KIF15 and high-expression group of KIF15 according to staining intensity (Figure 2B and Table 1). There were 20 cases of low KIF15 expression and 42 cases of high KIF15 expression (Table 1).

We then analyzed the significance of KIF15 in patients with glioblastoma. Each patient's age, gender, tumor size and other characteristics were analyzed. Among them, we found that age and other characteristics of patients with low KIF15 expression and high KIF15 expression were not clinically significant (Table 1). Interestingly, our results showed that KIF15 expression levels were significantly associated with recurrence in patients with glioblastoma ( $P < 0.05$ ) (Table 1).

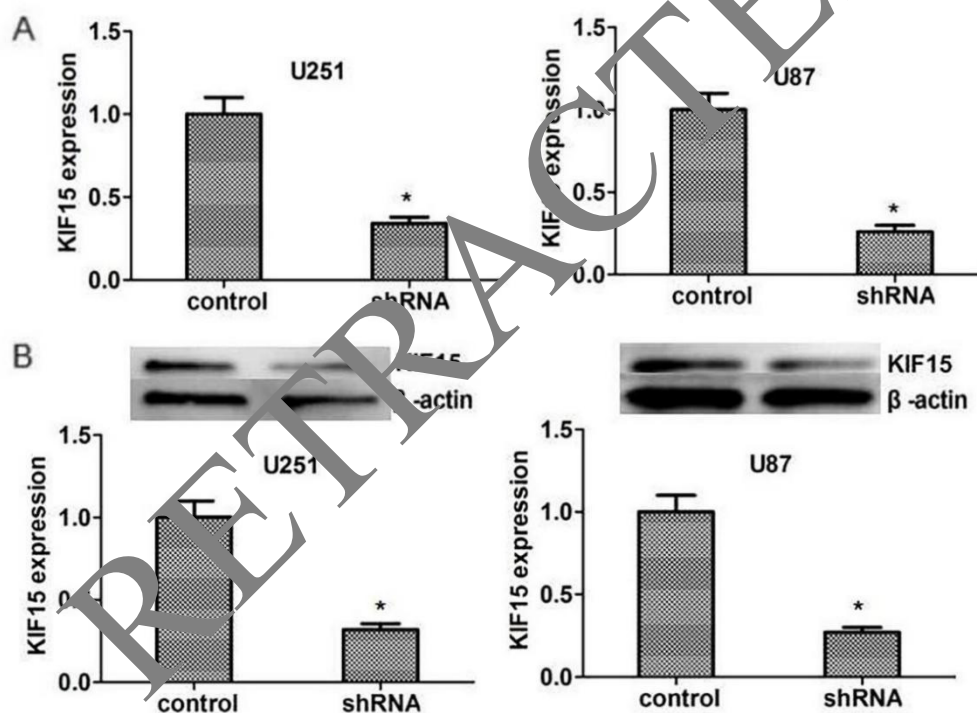


**Figure 2.** KIF15 is highly expressed in human glioblastoma tissues. (A) KIF15 expression levels in cancerous and normal tissues are shown (GBM: glioblastoma,  $*P < 0.05$ ). (B) Immunohistochemical tests were performed to show representative images of low and high KIF15 expression in glioblastoma tissues (enlarged  $\times 100$  and  $\times 200$ , respectively). (C) Immunohistochemical staining showed negative KIF15 expression level in non-adjacent tissues (magnified  $\times 100$  and  $\times 200$ , respectively).

### 3.4. Loss of KIF15 reduced the ability of cell proliferation *in vitro*

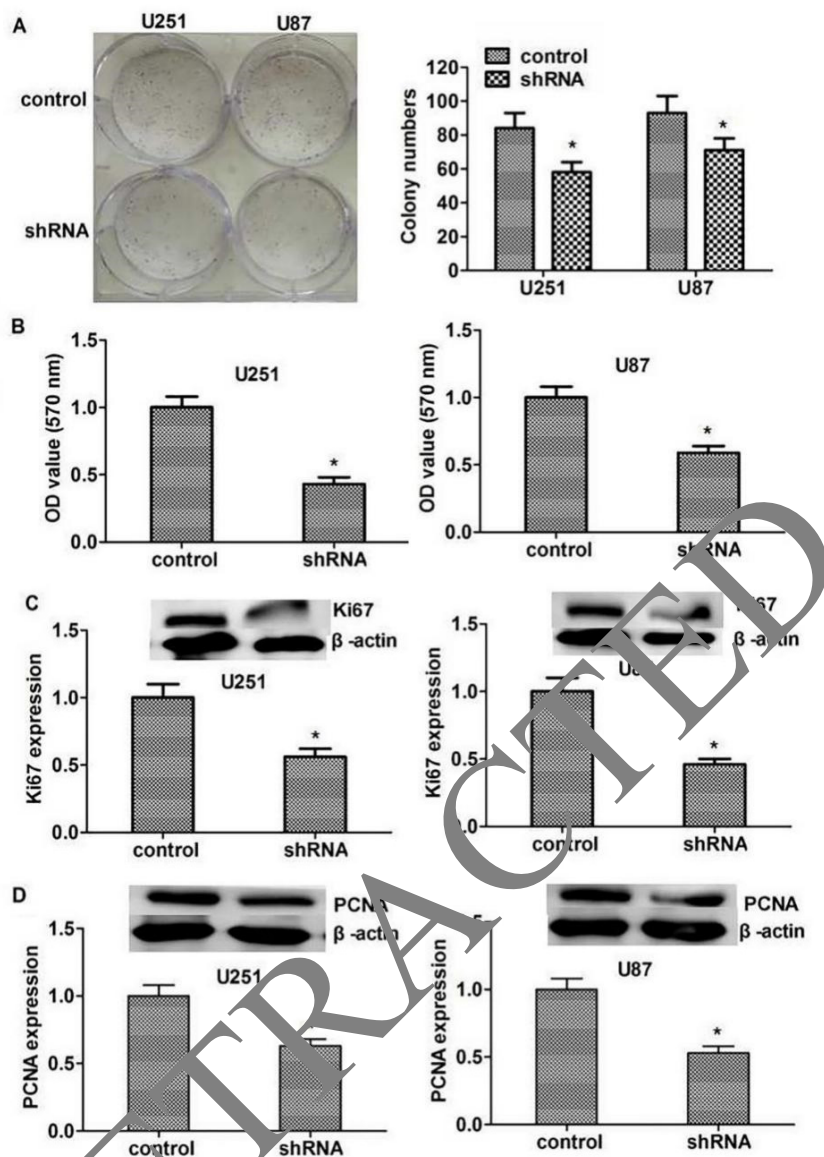
To investigate the molecular mechanism of KIF15 in glioblastoma, we transfected KIF15 shRNA plasmid into two human glioblastoma cell lines, U87 and U251, to reduce KIF15 expression. By performing quantitative PCR (Figure 3A), we found that the transfection of KIF15 shRNA plasmid effectively inhibited its expression in U87 and U251 cells. Similarly, Western blot analysis confirmed that the KIF15 expression levels were significantly reduced in both the U87 and U251 cells transfected with KIF15 shRNA plasmid (Figure 3B).

Next, colony formation experiments were performed to determine cell proliferation in the glioblastoma. We found that the ablation of KIF15 significantly reduced the number of colonies, as confirmed by colony formation experiments (Figure 4A). Similarly, the MTT assay results showed that the OD values of U87 and U251 cells at 570 nm were significantly reduced (Figure 4B). Then, Western blotting was used to detect the expression levels of Ki67 and PCNA, two markers reflecting proliferation ability. Consistent with previous studies, KIF15 downregulation significantly reduced the expression levels of Ki67 and PCNA in the U87 and U251 cells (Figure 4C, D).



**Figure 3.** After KIF15 deletion, the expression level of KIF15 in human glioblastoma cells U87 and U251 was significantly decreased. (A) Quantitative PCR showed that the expression level of KIF15 shRNA was significantly decreased after transfection in U87 and U251 cells. (B) Western blot analysis showed that KIF15 shRNA could effectively inhibit KIF15 expression in both the U87 and U251 cells after transfection. The results are expressed as mean  $\pm$  standard deviation, and all results were obtained from three independent experiments. \* $P < 0.05$  (A:  $P = 0.032, 0.026$ , B:  $P = 0.029, 0.027$ ).





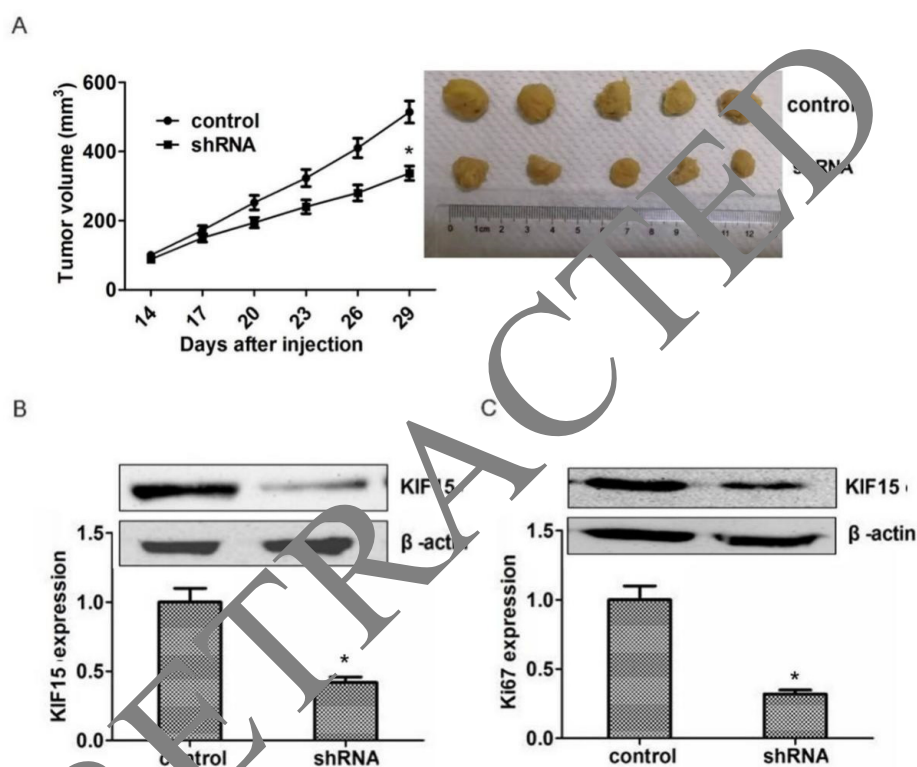
**Figure 4.** KIF15 promotes glioblastoma cell proliferation *in vitro*. (A) U87 and U251 cells were transfected with control or KIF15 shRNA plasmids and colony formation analysis was performed. Colony formation experiments showed that KIF15 ablation resulted in impaired cell proliferation. (B) MTT assay results showing that KIF15 ablation resulted in impaired cell proliferation. (C) Western blotting results showing the Ki67 expression levels in the control or KIF15-deficient U87 and U251 cells. (D) Western blotting results showing PCNA expression levels in the control or KIF15-knockout glioblastoma cells. Results are expressed as mean  $\pm$  standard deviation, and all results were obtained from three independent experiments. \* $P < 0.05$ . (A:  $P = 0.043, 0.045$ , B:  $P = 0.032, 0.037$ , C:  $P = 0.036, 0.034$ , D:  $P = 0.048, 0.043$ ).

### 3.5 KIF15 depletion blocked tumor growth of glioblastoma in mice

Subsequently, we investigated the correlation between KIF15 and glioblastoma tumor growth promotion *in vivo*. To test our hypothesis, we infected U87 cells with the control or KIF15 shRNA

lentivirus and injected them subcutaneously into nude mice. After 14 days, tumors were isolated from the mice every 72 hours, photographed and measured. See Figure 5A. Meanwhile, the tumor growth curves for the mice were calculated (Figure 5A). As expected, the volume of tumor isolated in the KIF15 knockout group was significantly smaller than that in the control group (Figure 5A).

In addition, immunohistochemical tests showed that KIF15 was effectively silenced in tumor tissues in the KIF15 knockout group (Figure 5B). Western blotting further detected Ki67 expression levels in tumor tissues of the control group and the KIF15-deficient group. Interestingly, a decrease in Ki67 expression was detected in the tumors of the KIF15 ablation group, suggesting that KIF15 deletion leads to a significant reduction in tumor cell proliferation (Figure 5C). Overall, all results suggest that KIF15 is involved in the development of glioblastoma *in vivo*.



**Figure 5.** KIF15 promotes the growth of glioblastoma in mice. (A) U87 cells infected with the control or KIF15 shRNA lentivirus were subcutaneously implanted into nude mice. The tumor was isolated 2 weeks later and the tumor volume was measured every 3 days ( $n = 5$  for each group). Tumor growth curves were calculated and analyzed based on the mean volume of five tumors in the KIF15 deletion group and control group. (B) Western blot analysis revealed the KIF15 expression levels in the control and KIF15-ablated tumors isolated from mice. (C) Western blot analysis revealed the Ki67 expression levels in the control and KIF15-knockout tumors isolated from mice. Results are expressed as mean  $\pm$  standard deviation, and all results were obtained from three independent experiments. \* $P < 0.05$ . (A:  $P = 0.033$ , B:  $P = 0.031$ , C:  $P = 0.028$ ).

#### 4. Discussion

The growth rate of glioblastoma is fast, as about 80% of patients have a course of disease that is less

than 6 months, and only 10% of patients have a course of disease that is more than 1 year [22,23]. In recent years, for glioblastoma, a highly malignant disease, conventional clinical treatment procedures such as surgical resection, radiotherapy and chemotherapy have not seen significant progress, so the 5-year overall survival of patients has remained at a low level [24]. In recent years, molecular targeted therapy has made great progress in various tumors. Based on current research results, most researchers believe that it can play a great role in the treatment of glioblastoma [24,25]. At present, several targeted therapy drugs, including EGFR and VEGF, have been proved to have positive treatment effects for glioblastoma in clinical therapeutic effect experiments [26]. In this study, we found that KIF15 was highly expressed in human glioblastoma tissues. Our data further demonstrate the association between KIF15 expression and clinical features.

In this study, through bioinformatics analysis, we found that KIF15 may have the ability to interfere with the progress of glioblastoma. We further investigated both the clinical features and biological function of KIF15 in glioma progression. According to the IHC assay-based analysis of KIF15 expression levels in human glioblastoma tissues and the non-tumor adjacent tissues, we noticed the high expression levels of KIF15 in tumor tissues. Moreover, the expression levels of KIF15 were obviously associated with clinical characteristics, including the tumor size and clinical stage. This clinical analysis further confirmed the important role of KIF15 in the progression of glioblastoma. Subsequent experiments confirmed the hypothesis that KIF15 acts as a contributor to glioblastoma proliferation. By including IHC assays, we next found a reduction in Ki67 and PCNA expression levels in the mouse tumor knockout group due to KIF15, confirming the involvement of KIF15 in the regulation of glioblastoma cell proliferation; this suggests that KIF15 will be a possible therapeutic target, and that KIF15 inhibitors are of great value for study.

KIF15 and Eg5 are the two end-added directional motors of spindle sliding [27]. Human Eg5 inhibitors such as Monastrol and Ispinesib can be used as potential antitumor agents [28]. In addition, HR22C16 can target ovarian cancer cells as an Eg5 inhibitor [29]. However, tumor cells can develop resistance to Eg5 by upregulating KIF15 as a substitute for Eg5 [27]. KIF15 is highly expressed in a variety of cancers, such as breast cancer, lung cancer and pancreatic cancer [21,22]. KIF15 has been reported to regulate mitosis and cytokinesis, which may further affect tumor cell proliferation [30]. It has been reported that KIF15 is uniformly expressed in glioblastoma stem cells, and even highly co-expressed in all glioblastoma subtypes. KIF15 may be worthy of further study as a therapeutic target for glioblastoma [31]. Interestingly, *in vitro* and in mice (*in vivo*), we found that KIF15 deletion leads to the impaired proliferation of glioblastoma cells, which may be caused by abnormal cell division. Based on our results, the development of KIF15 inhibitors may hold promise against glioblastoma. However, the precise molecular mechanism by which KIF15 promotes glioblastoma cell proliferation remains to be further studied [32].

In recent years, the driver protein motor has been shown to play an important role in cell division and become an important target for tumor therapy. Therefore, excitins may affect the proliferation of tumor cells and participate in tumorigenesis [33]. In this study, we found that a member of the driver superfamily, KIF15 [driver 12], is involved in the regulation of glioblastoma cell proliferation. Interestingly, various studies have shown that kinin is involved in the regulation of cancer cell proliferation. KIF14 promotes cell proliferation by activating the AKT signaling pathway in colorectal cancer [34]. Similarly, KIF20A promotes the malignant phenotype of lung cancer by promoting cell proliferation [35]. In addition, KIF26B deletion can inhibit the proliferation and migration of breast cancer cells [36]. More recently, in 2020, a new study showed KIF15 as a potential therapeutic target and prognostic factor for glioma [37], which is similar to our results. Then, we can announce

that we started our own study before this article was published, and that the glioblastoma mentioned in our study is different from the glioblastoma mentioned in the published article. Of course, in our study, we had *in vivo* results to back up our conclusions, which were not shown in the published article. Whether these proteins have a similar proliferation mechanism to KIF15, and whether they affect proliferation by regulating microtubules and spindles, remains to be further studied.

In conclusion, our results revealed that KIF15 could serve as a potential gene affecting glioblastoma progression and the high expression of KIF15 in human glioblastoma tissues. We also found the correlation between KIF15 expression levels and the clinical features of glioblastoma patients. Furthermore, KIF15 contributed to glioblastoma proliferation *in vitro* and promoted tumor growth in mice. Therefore, we preliminarily discussed the role of KIF15 in the development of glioblastoma and provided a new therapeutic target for the treatment of glioblastoma.

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### Conflict of interest

The authors declare that they have no conflict of interest to report regarding the present study.

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