IncRNA F11-AS1 Suppresses Glioma by Regulation miRNA-3146/PTEN

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Research

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Abstract

BACKGROUND: LncRNAs play key roles in glioma development, however, IncRNA F11-AS1’s effects has been unclearly in glioma.

OBJECTIVE: F11-AS1 is an anti-tumor factor in glioma has been clearly until now.

METHODS: Evaluating F11-AS1 expression by ISH in difference tissues and analysis the correlation with glioma prognosis in clinical. Using U87 and U251 cell to discuss IncRNA F11-AS1’s effect in glioma cancer cell biological activities by MTT, flow cytometry, transwell and wound healing assay. And measuring the relative miRNA and gene expressions by RT-qPCR and relative proteins expressions by WB assay. In next step, discuss the miR-3146 in F11-AS1 depressing cancer cell biological activities.

RESULTS: The authors observed in this study that F11-AS1 was down-regulation in glioma and negatively correlated with miRNA-3146. Servival analysis showed that low level of F11-AS1 predicted poor survival. In glioma cells, F11-AS1 knockdown led to up-regulate, whereas F11-AS1 overexpression led to down-regulated miRNA-3146. Analysis of cell biological activities showed that F11-AS1 overexpression had effects to suppress glioma cell lines biological activities.

CONCLUSION: Therefore, F11-AS1 down-regulates miRNA-3146 to depress cell biological activities in glioma.

1. Background

Glioma is a malignant tumor that occurs in the neuroderm. According to the latest statistics, the incidence of glioma in China increased from the 5.9/100,000 in 1973 to the 6.61/100,000 in 2016 [1]. Studies have shown that the pathogenesis of glioma is related to the inactivation of tumor suppressor genes, oxidative stress damage, genetic mutations and inhibition of autophagy. However, the specific pathogenesis and its mechanism remain unclear [2]. Therefore, it is urgent to clarify the pathogenesis and possible mechanisms of glioma and find more effective diagnosis and treatment strategies. In recent years, genome-wide transcription studies have shown that only about 1% of human genome is used as a blueprint for proteins, while a higher percentage of genome is transcribed into non-coding RNA [3]. Long non-coding RNA (lncRNA) means that non-protein-coding transcripts longer than 200 nucleotides (nt). It has been proven that LncRNA acts as a key molecule in the progression of various human tumors. Some researchers originally held that it is the by-product of the transcription of RNA polymerase E and has no biological function. However, several studies have shown that IncRNA can regulate gene expression at epigenetic level and transcriptional and post-transcriptional levels and participate in the regulation of different biological processes of cells, including cell cycle, gene stability and chromatin structure, and plays an important role in a series of different types of cells and diseases. This indicates that the regulation of cellular function by IncRNA is almost ubiquitous [4]. The results of the study carried out by Yu at al. showed that the abnormally decreased expression of F11-AS1 may be the main cause of pancreatic ductal adenocarcinoma (PDAC) [5]; the subsequent studies on F11-AS1 showed that the
expression of F11-AS1 is closely correlated to the poor prognosis of ovarian cancer [6]; recent relevant studies showed that F11-AS1 may inhibit the occurrence and development of liver cancer by regulating the corresponding miRNA [7]. Nevertheless, the clinical significance and acting mechanism of F11-AS1 in glioma are still unclear. In this study, in-situ hybridization (ISH) and RT-qPCR were first taken to detect the expression of F11-AS1 at different stages and in adjacent normal tissues and analyze the correlation between F11-AS1 and the prognosis and pathology of patients with glioma; afterwards, F11-AS1 was transfected into cell lines to observe its impacts on the bioactivity of glioma and analyze the relevant acting mechanism.

2. Materials And Methods

2.1 Clinical Data

The clinical data and samples of tumor and adjacent normal tissue of 70 patients with glioma admitted to Department of Neurosurgery Zaozhuang municipal hospital from June 2009 to June 2014 were collected. The acquisition of all samples conformed to the agreement of the Ethics Committee of our hospital. All patients enrolled in this study had signed an informed consent. All patients enrolled had been confirmed by surgical pathology and had not received radiotherapy, chemotherapy or other anticancer therapies before histopathological collection. The age of patients enrolled was 25–75, with an average of 50. 30 patients were < 50 and 40 patients ≥ 50; there were 32 male and 38 female patients; 27 patients had a KPS score ≥ 80 and 43 patients < 80; 26 patients had a tumor diameter < 4 cm and 44 patients ≥ 4 cm; 33 patients had single tumor and 37 patients had multiple tumors; 34 had postoperative recurrence and 36 had no recurrence. According to the classification criteria for nervous system tumors of WHO (2007), 24 patients were classified into Grade I-II and 46 into Grade III-IV.

2.2 Quantitative real-time polymerase chain reaction PCR (RT-qPCR)

Trizol method (Invitrogen, CA, USA) was adopted to extract the total RNA from tissues and cells. Prime Script T Reagent Kit (Takara, Dalian, Liaoning, China) or One-step miRNA Reverse Transcription Kit (Haigene, Harbin, China) were used for reverse transcription. Under the conditions of Roche LightCycler 480 system (Roche, Switzerland), SYBR Select Master Mix (Thermo Fisher Scientific, Waltham, MA) was adopted for RT-qPCR. U6 and GAPDH were used as internal reference. $2^{-\Delta\Delta Ct}$ method was adopted to calculate the expression of relevant mRNA.

2.3 Follow-up visit

After the operation, patients were followed up once every 3 months. Short-term follow-up visits were mainly in the form of outpatient visits; and long-term follow-up visits were mainly conducted by telephone. The deadline of follow-up visits was June 2019. The contents of follow-up visits were general data, KPS score and postoperative recurrence.

2.4 In situ hybridization (ISH)
After the tissue chip was dewaxed and hydrated, it was incubated in protease K (15 mg/L) at 37°C for 15 min. After dewatering with gradient ethanol, the tissue chip was prehybridized at 55°C for 30 min; and then 90°C denatured digoxin labeled probe F11-AS1 (Denmark Exiqon, YD00610917-BCG, 1:500) hybrid working fluid was added for incubation at 55°C overnight. On the next day, after the gradient washing and sealing, anti-DIG-AP (1:800) was added for incubation at room temperature for 1 h. Finally, NBT/BCIP staining and nuclear solid red counterstaining were adopted for sealing and microscopic examination. The known positive section was used as a positive control; PBS was used to replace the probe as a negative control. The film was taken under a 200-fold optical microscope. Image J image analysis software was used to analyze the difference in the expression of F11-AS1 in tissues among groups.

2.5 Cell culture

Normal human glial cells HEB and human glioma cell lines U87, U251, T98G, HS693 and SW1783 were purchased from the cell bank of Shanghai Institute of Life Sciences, Chinese Academy of Sciences. The cell culture fluid for HEB, U251, T98G, HS693 and SW1783 was DMEM high glucose medium containing 10% fetal bovine serum (FBS, Hyclone); the culture fluid for U87 cell was EME high glucose medium with 10% FBS. All cells were cultured in 5% CO₂ incubator (Thermo) at 37°C.

2.6 Main reagents and instruments

DMEM purchased from Shanghai Gino Biotech Company; Opti-MEM optimized medium purchased from American Invitrogen; M-MLV reverse transcriptase purchased from Promega; INTERFERin™ purchased from Shenzhen Dakewe Biotech Co., Ltd.; Annexin V-FITC cell death detection kit purchased from American Sigma; Transwell chamber purchased from American BD; and CCK-8 cell counting kit purchased from Japan DOJINDO Institute of Chemistry. Main instruments include PCR instrument (Eppendorf), real-time quantitative PCR instrument (MJ research), DK-8D electric constant temperature water bath, Eppendorf-centrifuge 5810R high speed freezing centrifuge and Western blot fluorescence imaging analyzer (FUJIFILM: flow cytometry (American BD)).

2.7 Cell transfection

F11-AS1 was transfected into glioma cells via pcDNA3.1 vector (Invirogen, Carlsbad, CA). miR-NC and miR-3146 were purchased from GenePharma (Shanghai, China). All plasmids were transfected into U87 and U251 via Lipofectamine 2000 (Invitrogen). Cells in all groups received the corresponding treatment for 48 h for subsequent experiments.

2.8 Detection of cell proliferation activity

Cells were taken from all groups and inoculated into 96-hole plate by the density of 1 x 10⁴ cells/hole. Each group had 3 multiple holes. All cells were further cultured for 48 h, after which 20 µl MTT solution (5 g/L) was added. After 4 h of culture, 100 µl dimethyl sulfoxide was added to terminate the reaction. The absorbance (A) of each hole at 492 nm wavelength was detected. The survival rate was calculated according to the formula: survival rate (%) = (control group A - experiment group A/control group A) *100%.
2.9 Detection by flow cytometry

The cells after 48 h of transfection were taken from each group and washed twice with pre-cooled PBS. The cell concentration was adjusted to $1 \times 10^6$ cells/ml by buffer suspension. 100 ml was taken and placed in 5 ml culture tube, to which 5 µl of Annexin V-FITC and PI was added, respectively. After incubation at 37°C for 15 min, the mixture was placed on the machine to detect the apoptosis rate.

2.10 Detection by Transwell test

The cells after 48 h of transfection were collected from each group for Transwell test. In the migration test, $5 \times 10^4$ cells were added to the upper chamber without serum medium. However, in the invasion assay, Matrigel should be laid in advance, before inoculation. $1 \times 10^4$ cells were added to the upper chamber with serum medium. Lower chambers contained medium with 10% FBS. After 24 h, chambers were taken out and fixed by formaldehyde and stained by crystal violet successively. Inverted microscope was used to calculate the number of invading or migrating cells. This experiment was repeated three times.

2.11 Wound healing detection

$1 \times 10^6$ cells were incubated into six-hole plate. When the degree of cell fusion reached 90%, a sterilized 200 µl pipette tip was used to make a straight scratch. The floating cells were washed off with PBS. DMEM was used for further culture. Photos were taken 0 h, 24 h and 48 h after making the scratch. The migration ability of cells was shown by the healing rate of the scratch.

2.12 Western blot assay

Cells were collected 48 h after the transfection. The protein concentration was determined by BCA method. After electrophoresis in 10% sodium dodecyl sulfate - polyacrylamide gel (SDS-PAGE), cells were transferred to PVDF membrane, sealed with TBST containing 5% defatted milk at room temperature for 1 h, incubated in primary antibodies at 4°C overnight. The membrane was washed with PBST for 3 times. Cells were incubated in secondary antibodies at room temperature for 1 h; the membrane was washed with PBST for 3 times. ECL chemiluminescence detection (CLD) was used for detection. PTEN, PI3K, AKT, P53 and MMP-9 were purchased from ABcam (Cambridge, UK).

2.13 Luciferase report analysis

Luciferase report method was adopted to detect the ability of miR-3146 to combine with F11-AS1 or PTEN and clone
wild or mutant F11-AS1 and PTEN into an empty pGL3 carrier (Ambion, Inc, Austin, TX). According to experimental needs, 48 h after the transfection of the LIHC cells cotransfected with negative control (NC) mimics or miR-3146 mimics with the above carrier, the Luciferase activity of different carriers were determined via the dual-luciferase reporter system (Promega, Madison, WI).

2.14 Statistical analysis

The software SPSS22.0 (SPSS Inc, Chicago, IL, USA) was applied for statistical analysis of experimental data. The measurement data were expressed as Mean ± SD. T test was adopted for comparison among groups; / test was adopted for parameter data analysis; Kaplan-Meier method was taken to draw a survival curve; log-rank test was adopted for comparison among groups; univariate and multivariate Cox proportional hazard regression models were used to evaluate the correlation between different clinicopathologic features and clinical prognosis of patients with glioma. The difference was statistically significant for P < 0.05.

3. Results

3.1 The expression level of F11-AS1 in glioma tissues was lower than that in adjacent normal tissues and was correlated to the postoperative prognosis of patients with glioma.

Figure 1A showed that the cell boundaries in adjacent tissues were obvious. With the increase of stage, the cell boundaries in I-II stage and III-IV stage tissues were more ambiguous. ISH staining showed that, compared with adjacent tissues, the expression of F11-AS1 decreased significantly with the increase of stage (P < 0.01, Fig. 1B); the results of RT-qPCR verification were consistent with those of ISH detection (Fig. 1C); the median 0.23 of the expression of F11-AS1 was taken to divide 70 patients with glioma into a low F11-AS1 expression group and a high F11-AS1 expression group; Kaplan-Meier was used to draw a survival curve and log-rank for survival rate test. The results showed that the postoperative prognosis for the high F11-AS1 expression group was significantly better and the 5-year survival rate significantly higher than those in the low expression group (P = 0.0072, Fig. 1D).

3.2 Correlation between F11-AS1 expression and clinicopathological markers of glioma
The analysis results showed that the low expression level in glioma tissues is not correlated to patients’ age (P = 0.514) and gender (P = 0.517), but is partly correlated to tumor number (P = 0.048), tumor size (P = 0.015), KPS score (P = 0.006) and patients’ postoperative recurrence (P = 0.011), see Table 1 for details.

3.3 Gene expression level of F11-AS1 in various cell strains and groups

Figure 2A showed that, compared with normal glial cell HEB, the expression level of F11-AS1 mRNA in glioma cell lines U87, U251, T98G, HS683 and SW1783 was significantly lower (P < 0.001, respectively), and the gene expression of F11-AS1 in U87 and U251 cells were the lowest; compared with the NC group, after F11-AS1 was transfected into U87 and U251 cells, the expression level of F11-AS1 mRNA in F11-AS1 group was significantly higher (P < 0.0001, respectively, Fig. 2B & 2C).

3.4 Impacts of F11-AS1 on proliferation, apoptosis, and cycle of glioma cells

Compared with the NC group, the proliferation rate of U87 and U251 cells in the F11-AS1 group was significantly lower (P < 0.001, respectively, Fig. 3A); the apoptosis rate and G1 cell retention rate increased significantly (P < 0.001, respectively, Fig. 3B-3E).

3.5 Impacts of F11-AS1 on invasion and migration of glioma cells

Compared with the NC group, the number of invading U87 and U251 cells in the F11-AS1 group after transfection into F11-AS1 was significantly lower (P < 0.001, respectively, Fig. 4A & 4B); the observation of the impacts of F11-AS1 on the migration ability of glioma cells by wound healing showed that, compared with the NC group, the 24 h and 48 h wound healing rate of U87 and U251 cells in F11-AS1 group were significantly lower (P < 0.001, respectively, Fig. 4C & 4D).

3.6 Impacts of F11-AS1 on relevant mRNA

The results of RT-qPCR detection showed that, compared with the NC group, in U87 and U251 cell lines, the expression level of miRNA-3146, PI3K, AKT and MMP-9 mRNA in the F11-AS1 group was significantly lower, but the expression level of PTEN and P53 mRNA was significantly higher (P < 0.001, respectively, Fig. 5A & 5B).

3.7 Impacts of F11-AS1 on relevant proteins

The results of WB detection showed that, compared with the NC group, in U87 and U251 cell lines, the expression level of PI3K, AKT and MMP-9 proteins in the F11-AS1 group was significantly lower, but the expression level of PTEN and P53 proteins was significantly higher (P < 0.001, respectively, Fig. 6A & 6B).

3.8 Difference in expression of F11-AS1 and miRNA-3146 among groups
The results of RT-qPCR detection showed that, compared with the NC group, the expression level of F11-AS1 mRNA in U87 and U251 cell lines in F11-AS1, F11-AS1 + miR-NC and F11-AS1 + miR-3146 groups was significantly higher (P < 0.001, respectively, Fig. 7A); the expression level of mRNA-3146mRNA in U87 and U251 cell lines in F11-AS1 and F11-AS1 + miR-NC groups was significantly lower (P < 0.001, respectively, Fig. 7B); compared with the F11 + AS1 + miR-NC group, the expression level of miRNA-3146mRNA was significantly higher (P < 0.001, Fig. 7B).

3.9 Role of miRNA-3146 in F11-AS1’s inhibiting proliferation of glioma cells and promoting apoptosis and cell cycle

The results of MTT detection showed that, compared with the NC group, the cell proliferation rate in U87 and U251 cell lines in F11-AS1 and F11-AS1 + miR-NC groups was significantly lower (P < 0.001, respectively, Fig. 8A); compared with the F11-AS1 + miR-NC group, the cell proliferation rate in the F11-AS1 + miRNA-3146 group was significantly higher (P < 0.001, respectively, Fig. 8A). Flow cytometry was used to detect the apoptosis and cycle of cells in each group. The results showed that, compared with the NC group, the apoptosis rate of U87 and U251 cells in F11-AS1 and F11-AS1 + miR-NC groups without intervention of miRNA-3146 significantly increased (P < 0.001, respectively, Fig. 8B & 8C), accompanied by a significant increase in G1 phase cells and a significant decrease in G2 phase cells (P < 0.001, respectively, Fig. 8D & 8E). After miRNA-3146 was transfected into U87 and U251 cells, compared with the F11-AS1 + miR-NC group, the apoptosis and cycle of cells in the F11-AS1 + miR-3146 group significantly reversed (P < 0.001, respectively, Fig. 8B-8E).

3.10 Role of miRNA-3146 in F11-AS1’s inhibiting invasion and migration of glioma cells

Transwell experiment proved that, compared with the NC group, the number of invading U87 and U251 cells in F11-AS1 and F11-AS1 + miR-NC group was significantly inhibited (P < 0.001, respectively, Fig. 9A & 9B). After the cotransfection of miRNA-3146, compared with the F11-AS1 + miR-NC group, the number of invading U87 and U251 cells in the F11-AS1 + miR-3146 increased significantly (P < 0.001, respectively, Fig. 9A & 9B). Wound healing experiment proved that, compared with the NC group, the 24 h and 48 h wound healing rate in U87 and U251 cell lines in F11-AS1 and F11-AS1 + miR-NC groups was significantly inhibited (P < 0.001, respectively, Fig. 9C & 9D). However, after the transfection of miRNA-3146 into cells, compared with the F11-AS1 + miR-NC group, the 24 h and 48 h wound healing rate in U87 and U251 cell lines in the F11-AS1 + miR-3146 increased significantly (P < 0.001, respectively, Fig. 9C & 9D).

3.11 Expression of relevant genes in groups in RT-qPCR detection
Compared with the NC group, the expression of PTEN and P53mRNA in F11-AS1 and F11-AS1 + miR-NC groups increased significantly, but the expression of PI3K, AKT and MMP-9 genes was significantly inhibited (P < 0.001, respectively, Fig. 10A & 10B); however, after the transfection of miRNA-3146 into cells, compared with the F11-AS1 + miR-NC group, the expression of PTEN and P53mRNA in the F11-AS1 + miR-3146 was significantly inhibited, but the expression of PI3K, AKT and MMP-9 genes increased significantly (P < 0.001, respectively, Fig. 10A & 10B).

3.12 Expression of relevant proteins in groups in WB detection

Compared with the NC group, the expression of PTEN and P53 proteins in F11-AS1 and F11-AS1 + miR-NC groups increased significantly, but the expression of PI3K, AKT and MMP-9 proteins was significantly inhibited (P < 0.001, respectively, Fig. 11A & 11B); however, after the transfection of miRNA-3146 into cells, compared with the F11-AS1 + miR-NC group, the expression of PTEN and P53 proteins in the F11-AS1 + miR-3146 was significantly inhibited, but the expression of PI3K, AKT and MMP-9 proteins increased significantly (P < 0.001, respectively, Fig. 11A & 11B).

3.13 Analysis of correlation among F11-AS1, miRNA-3146 and PTEN

Luciferase report analysis was used to analyze the correlation among F11-AS1, miRNA-3146 and PTEN. The results showed that, F11-AS1 can realize the targeted regulation of miRNA-3146 (P < 0.001, Fig. 12A); the further analysis of the correlation between miRNA-3146 and PTEN showed that miRNA-3146 can realized the targeted regulation of PTEN (P < 0.001, Fig. 12B).

4. Discussion

Since the tumor shows invasive growth, the recurrence rate of glioma is high and the prognosis is extremely poor [8]. At present, radiotherapy, chemotherapy and other adjuvant therapies play a certain role in the treatment of glioma, but the effect is not significant and the 5-year survival rate is still below 5%, with an average survival time is only 14 months [9]. Therefore, a new therapy for glioma is urgently needed. This study showed that except for mutations or abnormal expression of protein-coding genes, the mutation and regulation of non-coding RNA, especially LncRNA, play a leading role in cancer [10]. Under normal circumstances, LncRNA may show tumor inhibition and carcinogenesis; its abnormal expression may directly affect the malignant biological behavior of tumor cells; and LncRNA is significantly correlated to the drug resistance of tumor cells and diagnosis and prognosis of tumor [11]. The study of Nie et al. [12] showed that LncRNA-UCA1 can up-regulate the expression of ERBB4 protein and affect the proliferation and metastasis of non-small cell lung cancer (NSCL) by acting on downstream microRNA (miR-193a-3p). After the silence of the expression of LncRNA-UCA1, the proliferation and metastasis ability of NSCL increased significantly. Meanwhile, multiple LncRNAs,
including CRNDE, MEG3 and HOTAIR, have been identified as new regulators in the development of glioma and can be used as potential therapeutic targets [13]. Therefore, LncRNA plays an exact role in tumor pathogenesis. However, whether it has specificity and sensitivity and its correlation with the diagnosis and prognosis of in vivo glioma are still unclear. IncRNA F11-AS1 is a kind of IncRNA discovered in recent years. The findings in recent years have shown that F11-AS1 shows low expression in tumor tissues [6, 7]. This study proved that the expression level of F11-AS1 in glioma tissue decreased significantly and that the F11-AS1 with low expression level is an important cause for the poor prognosis of glioma patients. In order to explore the specific acting mechanism of F11-AS1 in glioma, the over-expressed F11-AS1 was transfected into glioma cell lines to observe and analyze the acting mechanism of F11-AS1 therein.

miRNA is a short-chain RNA composed of 20 ~ 22 nucleotides and regulates the expression of target gene by combining the post-transcriptional level with 3′-UTR [14, 15]. More and more studies have indicated that, miRNA plays an important role in tumorigenesis and development. It forms RNA induction or silencing complexes by combining with the 3′-UTR of target gene and up-regulates or down-regulates target gene expression to affect the biological behavior of tumor cells [16]. Previous studies have shown that F11-AS1 can inhibit the bioactivity of liver cancer by regulating miR-3146/PTEN [7]. The results of this study showed that after the transfection of F11-AS1 into neuroblastoma cells, the bioactivity of such cells was significantly inhibited. Besides, the expression of miRNA-3146 decreased significantly. The targeted regulation of PTEN by miRNA-3146 was proven by dual-luciferase reporter.

This study showed that PTEN is an important tumor suppressor gene in the body. It is closely correlated to the genesis and development of multiple tumors by allele deletions, gene mutations and methylation patterns. PTEN is a negative regulator of P13K depending on AKT signal. PTEN protein has lipid phosphatase activity and can antagonize the action of P13K to allow PIP3 to dephosphorylate and generate PIP2 and maintain the low level of PIP3, so as to down-regulate the expression of protein in P13K/AKT pathway, accelerate cell apoptosis and inhibit cell survival [17]. PTEN is intervened by gene or small-molecule inhibitor. Some good laboratory results on the inhibition of the proliferation of tumor cells by P13K/AKT pathway have been obtained [18–23]. The results of this study proved that the over-expression of F11-AS1 causes the decrease of the expression of miRNA-3146, so as to inhibit the PI3K/AKT signaling pathway after the activation of PTEN and inhibit the bioactivity of glioma cells. p53 gene is an important tumor suppressor gene. The normal p53 gene is divided into mutant and wild genes, both of which participate in the regulation of cell apoptosis [24]. The results of this study indicated that the increase of the expression of p53 protein and gene caused a large number of glioma cells to remain in G1 phase and die. The high expression of MMP-9 was closely correlated to the invasion and migration of tumor cells [25, 26], accompanied by the inhibition of the activity of PI3K/AKT signaling pathway; the expression level of the downstream MMP-9 protein and gene expression was also significantly inhibited, which might be the acting mechanism of F11-AS1’s inhibition of the invasion and migration of glioma cells.
Conclusions

Thus it can be seen that F11-AS1 has the ability to be used as the potential indicator for the diagnosis and analysis of the prognosis effect of patients with glioma. Meanwhile, the over-expression of F11-AS1 can effectively regulate the proliferation, migration, invasion and migration of glioma cells via miRNA-3146/PTEN.

Declarations

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Authors’ contributions

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Availability of data and materials

The authors’ confirm that the data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

This experimental procedure and animal use protocol have been approved by the Animal Ethics Committee of Zaozhuang municipal hospital.

Consent for publication

Not applicable

Competing interests

The authors declare no conflicts of interest.
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Tables

Table 1. Correlation between IncRNA F11-AS1 expression level and clinicopathological indicators in glioma patients (n, %)
| Characteristics                  | F11-AS1 expression (Fold) | P     |
|---------------------------------|--------------------------|-------|
|                                 | Low (n=43)               | High (n=27) |
| Age                             |                          | 0.541 |
| 50 yrs (n=30)                   | 18 (60.0)                | 12 (40.0) |
| ≥ 50 yrs (n=40)                 | 25 (62.5)                | 15 (37.5) |
| Gender                          |                          | 0.511 |
| Male (n=32)                     | 19 (59.4)                | 13 (40.6) |
| Female (n=38)                   | 24 (63.2)                | 14 (36.8) |
| Histological grade              |                          | 0.002 |
| I-II stage (n=24)               | 9 (37.5)                 | 15 (62.5) |
| III-IV stage (n=46)             | 34 (73.9)                | 12 (26.1) |
| Tumor number                    |                          | 0.048 |
| Single (n=33)                   | 10 (30.3)                | 23 (69.7) |
| Multiple (n=37)                 | 33 (89.2)                | 4 (10.8)  |
| Tumor size                      |                          | 0.015 |
| ≤ 4 cm (26)                     | 16 (61.5)                | 10 (38.5) |
| ≥ 4 cm (44)                     | 27 (61.4)                | 17 (38.6) |
| KPS score                       |                          | 0.006 |
| ≥ 80 (n=27)                     | 11 (40.7)                | 16 (59.3) |
| ≤ 80 (n=43)                     | 32 (74.4)                | 11 (25.6) |
| Postoperative recurrence        |                          | 0.011 |
| Yes (n=34)                      | 24 (70.6)                | 10 (29.4) |
| No (n=36)                       | 19 (52.8)                | 17 (47.2) |