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miRNA-451 regulates the NF-κB signaling pathway by targeting IKKβ to inhibit glioma cell growth

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ABSTRACT

Glioblastoma multiforme (GBM) is associated with a poor prognosis, and effective treatments are lacking. Our previous studies have shown that miRNA-451 is closely related to the development and progression of glioma. miRNA-451 is a tumor suppressor whose expression is negatively correlated with the WHO grades of gliomas, but its specific mechanism is still unclear. Research shows that NF-κB is highly expressed in early malignant glioma, and thus, the NF-κB signaling pathway has become an important target for the treatment of malignant glioma. Activation of IKK is a critical step in the activation of the classical NF-κB pathway. By performing a bioinformatics analysis, we found that IKKβ is a potential direct target of miRNA-451 in glioma. In this study, we transfected lentivirus expressing miRNA-451 to test the effect of miRNA-451 overexpression on malignant glioma cell lines and confirmed that IKKβ is a target gene of miRNA-451 by luciferase assay. By targeting IKKβ, MTT, cell invasion and wound-healing assays showed that cell proliferation, cell invasion and migration were significantly suppressed in the LV-miRNA-451 group. Western blotting results showed that the expression levels of IKKβ, p-p65, MMP-2, MMP-9, Cyclin D1, p16 and PCNA were significantly decreased in the LV-miRNA-451 group. In vivo, miRNA-451 significantly decreased glioma cell growth, and the survival of BALB/c-A nude mice was significantly prolonged. Immunohistochemistry showed that p-p65, Cyclin D1 and Ki67 expression was significantly reduced in the LV-miRNA-451 group. Taken together, these results suggest that miRNA-451 could regulate the NF-κB signaling pathway by targeting IKKβ, which inhibits glioma cell growth in vitro and in vivo. Therefore, this study may provide novel insight into miRNA-451-targeted therapy for glioma.

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1. Introduction

Glioblastoma multiforme (GBM) is the most common primary malignant brain cancer. The poor prognosis and high lethality of this disease are largely attributed to the highly invasive and migratory nature of glioma cells, which are capable of wide migration and diffuse infiltration into the surrounding brain tissue [1,2]. In addition, various genetic mutations occur during the formation and development of GBM, which is why GBM treatment is very challenging [3,4,5]. In the clinic, it is urgent to further understand the molecular mechanisms by which glioma cells infiltrate the surrounding brain tissue to identify new targets for the treatment of GBM [1,6,7].

Nuclear factor-κB (NF-κB)-mediated signaling pathways are often activated in glioma cell lines and in most cases of high-grade GBM [6,8]. NF-κB is often considered to be one of the main factors that regulates tumor cell resistance to treatment including in glioma cells [9]. IκBs are controlled by the kinase IKK, which phosphorylates IκB in response to cellular stimuli and leads to ubiquitin-mediated degradation of IκB proteins [10,11]. When NF-κB is released and transferred to the nucleus, NF-κB can induce the expression of its target genes, induce cell proliferation and invasion and inhibit cell apoptosis [10,11,12]. Studies have confirmed that NF-κB can induce the proliferation and invasiveness of glioma cells [9,10]. However, currently, few studies have reported on inhibition of the IKK/NF-κB signaling pathway for the treatment of glioma.

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microRNAs (miRNAs), a class of noncoding RNAs, negatively regulate gene expression at the post-transcriptional level by binding to the 3′-untranslated regions (3′UTRs) of the messenger RNAs (mRNAs) of their target genes. miRNAs can act as both tumor suppressors and oncogenes by negatively regulating their mRNA targets through degradation or translational repression [5,13]. Liang *et al.* [14] revealed that downregulation of miRNA-137 and miRNA–6500–3p promotes cell proliferation in pediatric high-grade gliomas. Wang *et al.* [15] reported that miRNA–139–5p inhibits glioma cell proliferation and progression by targeting GABRA1. Cui *et al.* [16] found that miRNA-193a-3p regulates the AKT2 pathway to inhibit glioma cell growth and promote apoptosis of glioma cells by targeting ALKBH5. In recent years, other studies have reported that miRNA-451 expression is downregulated in glioma [5,13,17]. miRNA-451 expression is negatively correlated with the WHO grades of gliomas and suppresses EMT and metastasis in glioma cells [18,19]. However, the specific regulatory mechanism of miRNA-451 in glioma is not clear. Using a bioinformatics analysis, we identified IKKβ as a potential direct target of miRNA-451 in glioma. Thus, we investigated whether miRNA-451 can regulate the NF-κB signaling pathway by targeting IKKβ to inhibit glioma cell growth.

In the present study, we transfected lentivirus expressing miRNA-451 to test its effect on malignant glioma cell lines and confirmed IKKβ as a target gene of miRNA-451. In vivo and in vitro experiments confirmed that miRNA-451 can significantly suppress glioma cell proliferation, invasion and migration by regulating the NF-κB signaling pathway through targeting IKKβ. Our results suggest that downregulation of miRNA-451 may be important for the development and progression of glioma, thus highlighting this miRNA as a potential target for glioma therapy.

2. Materials and methods

2.1 Cell culture

The human GBM cell lines U251 and U87 were purchased from the Chinese Academy of Sciences (Shanghai, China). Cells were cultured at 37°C in a humidified 5% CO_2 atmosphere with Dulbecco's Modified Eagle's Medium (DMEM, Gibco, NY, USA) supplemented with 10% fetal bovine serum (FBS, Gibco).

2.2 Lentiviral infection and cell transfection

Lentiviruses expressing hsa-miRNA-negative control (miRNA-NC) or hsa-miRNA-451 (miRNA-451) were obtained from GenePharma (Shanghai, China). The coding sequences were miRNA-NC, 5′-UUCUCCG AACGUGUCACGUTT-3′ and miRNA-451, 5′-AA ACCGUUACCAUUACUGAGUU-3′. For experiments, cells were divided into two treatment groups: the lentivirus-miRNA-negative control group (LV-miRNA-NC) and the lentivirus-hsa-miRNA-451-overexpressing group (LV-miRNA-451). U251 and U87 glioma cells were transfected with LV-miRNA-NC/LV-miRNA-451 according to the manufacturer's protocol.

2.3 RNA extraction and real-time PCR

After transfection for 48 h, total RNA was extracted from U251 and U87 glioma cells using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was prepared using 1 μg of total RNA from each sample. For quantitative PCR, oligonucleotide primers (GenePharma, China) were used as follows: U6 forward, 5′-ATTGGAACGATACAGAGAAGA TT-3′ and reverse, 5′-GGAACGCTTCACGAATT TG-3′; miRNA-451 forward, 5′-GCGGCGCAAAG AATTCTCCT-3′ and reverse, 5′-GTGCAGGGTC CGAGGT-3′. To compare relative RNA expression levels between samples, GAPDH and U6 served as controls. A Bio-Rad PCR instrument (Hercules, CA, USA) was used to analyze and process the data. All reactions were performed in triplicate. miRNA levels were quantified using the 2-ΔΔCT method. Real-time PCR was performed according to the manufacturer's protocol (Promega, USA).

2.4 Luciferase activity assay

The pGL3-IKKβ-3′UTR-Mut and pGL3-IKKβ -3′UTR-Wt plasmids were purchased from GenScript (Nanjing, China). The cDNA was cloned into the XbaI/XbaI site of the pGL3 control vector

downstream of the luciferase gene to generate pGL3-IKKβ vectors using the following oligonucleotide sequences: IKKβ-3′UTR-Wt, 5′-GCCCCGCGGTC TCACATGGTGGTTCCTG-3′ and IKKβ-3′UTR-Mut, 5′-GAAAAGTGCTTGGAGTACGGAATGCC ACACACGTGACTGG-3′. U251 and U87 cells were cultured in 96-well plates and transfected with 5 pmol of the miRNA-451 mimic oligonucleotide using Lipofectamine 3000 (2×10^3 cells per well). The cells were transfected again with 0.2 μg of either the pGL3-IKKβ-3-UTR-Wt plasmid or the pGL3-IKKβ-3′UTR-Mut plasmid using Lipofectamine 3000 after 24 h of transfection. A luciferase assay system (Promega, USA) was used to measure the luciferase activity after 48 h of transfection.

2.5 MTT assay

U251 and U87 glioma cells in the logarithmic growth phase were seeded into 96-well plates at a density of 2×10^3 cells per well. Five wells from each group were subjected to an MTT assay on days 1, 2, 3, 4, 5, and 6 after transfection. The optical density (OD) was measured at 570 nm in an automated microplate reader, and cell proliferation in each group was analyzed using GraphPad software. Each assay was performed in triplicate.

2.6 Transwell invasion and wound-healing assays

A 24-well BD Matrigel invasion chamber (BD, USA) was used for the Transwell invasion assay according to the manufacturer's instructions. Transfected U251 and U87 glioma cells were seeded in Transwell cell culture inserts at a concentration of 5×10^4 cells/well. After incubation for 24 h, the remaining glioma cells were removed from the top well with a cotton swab, whereas the invading cells in the bottom well were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet. Finally, the number of cells was counted under a light microscope, and the results were analyzed. Each test was repeated in triplicate. Cell motility was determined by wound-healing assays. Glioma cells were seeded in 6-well plates and cultured for 48 h after transfection. The cell layers from each group were scratched using a 200-μL pipette tip to form wound gaps. After gentle washing in PBS three times, the wounded cell monolayer was allowed to heal in DMEM containing 5% FBS. Images of three randomly selected fields at the lesion border were acquired using an inverted microscope at 0 and 24 h. ImageJ (version 1.45 software) was used to analyze the extent of wound closure. The experiments were performed in triplicate.

2.7 Western blot analysis

RIPA lysis buffer (Solarbio, R0010, China) containing protease inhibitors (Solarbio, A8260, China) was used to isolate proteins from U251 and U87 glioma cells, and the protein concentrations were determined using a BCA kit (Solarbio, PC0020, China). Then, the denatured proteins were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, USA). The membranes were blocked in TBST with 5% skim milk for 1 h and were then incubated with mouse antibodies against GAPDH, IKKβ, p-p65, MMP-2, MMP-9, p16, and PCNA (1:1000; CST, USA) and a rabbit antibody against Cyclin D1 (1:1000; CST, USA) at 4°C overnight. The next day, the membranes were washed with PBS/Tween three times for 5 min each time and were then incubated with goat anti-rabbit or anti-mouse HRP-conjugated secondary antibodies (1:2000; CST, USA) for 1 h. The protein blots were developed with an ECL Protein Detection Kit (Pierce, USA) and analyzed in a gel imaging analyzer (Syngene, UK). The relative protein level was determined by normalizing the densitometry value of related proteins to that of GAPDH.

2.8 Animal experiments

Four-week-old BALB/c-A nude mice were purchased from the Animal Center of the Cancer Institute of the Chinese Academy of Medical Sciences. The mice were randomly assigned to two groups and were intracranially implanted

with 5×10^5 U87 cells (pretreated with lentivirus expressing the miRNA-451 or negative control sequences) using a stereotactic instrument. Intracranial tumor growth was detected by bioluminescence imaging. After the mice were anesthetized, they were intraperitoneally injected with 50 mg/mL D-luciferin (Promega, Wisconsin). An IVIS imaging system (Caliper Life Sciences) was used to image the animals for 10 120 s. The overall survival of the mice was monitored throughout the experimental period. All animal experiments were performed in accordance with the Animal Welfare Act and were approved by the Institutional Committee for Animal Research of Tianjin Medical University.

2.9 Immunohistochemistry

Paraffin-embedded sections (5 μm thickness) of brain specimens were stained. Brain tissue sections embedded in paraffin blocks were deparaffinized in xylene, after which antigen retrieval was performed by heating the sections in citrate buffer for 20 min. After blocking, the samples were incubated with primary antibodies (1:100 dilution) against p-p65, Cyclin D1 and Ki67 overnight at 4°C. The samples were equilibrated to room temperature for 1 h, washed with PBS three times, and incubated with a biotinylated secondary antibody (1:200 dilution) at room temperature for 2 h. After washing with PBS, the sections were incubated with ABC-peroxidase reagent for 40 min. The sections were then stained with diaminobenzidine (DAB) (Carpinteria, USA) for 5 min, washed in water, and counterstained in hematoxylin. The expression of p-p65, Cyclin D1 and Ki67 in the tumor tissue samples was compared between the LV-miRNA-NC and LV-miRNA-451 groups.

2.10 Statistical analyses

Student's t test was performed to compare differences among the groups with respect to parametric variables. Overall survival was evaluated using the Kaplan-Meier method and log-rank test. All the data are presented as the mean ± standard error. *P* values <0.05 were considered significant.

3. Results

3.1 miRNA-451 expression is upregulated after transfection, and IKKβ is a target gene of miRNA-451 in glioma

To verify the transfection efficiency of the lentivirus expressing miRNA-451 in glioma cells, we performed RT-PCR and found that miRNA-451 expression was significantly increased in the LV-miRNA-451 group (Figure 1(a)). The transfected cells were used in subsequent Western blot assays, and IKKβ expression was found to be significantly downregulated in cells transfected with LV-miRNA-451 (Figure 1(b)). By performing a bioinformatics analysis (http://starbase.sysu.edu.cn/), we found that the 3′UTR of IKKβ contains a complementary base sequence for miRNA-451 binding and that IKKβ is a possible target gene of miRNA-451 in glioma (Figure 1(c)). To determine whether miRNA-451 directly interacts with the IKKβ mRNA 3′UTR, we used a luciferase reporter system. Luciferase activity was significantly decreased in cells cotransfected with LV-miRNA -451 and the pGL3-IKKβ-3′UTR-Wt plasmid compared with cells cotransfected with LV-miRNA-NC and the pGL3-IKKβ-3′UTR-Mut plasmid (Figure 1(d)). Together, these data demonstrate that IKKβ is a target gene of miRNA-451 in glioma.

3.2. miRNA-451 inhibits glioma cell proliferation, invasion, and migration by targeting IKKβ

To confirm whether inhibition of the NF-κB pathway via the targeting of IKKβ by miRNA-451 could suppress tumorigenesis, cell growth and survival were evaluated. The MTT assay showed that the viability and proliferation of cells in the LV-miRNA-451 group were inhibited by miRNA-451 (Figure 2(a)). Cell invasion and wound-healing assays indicated that invasion and migration of cells in the LV-miRNA-451 group were suppressed by miRNA-451 (Figure 2(b-c)). Furthermore, Western blotting results showed that the expression levels of proteins related to the malignant behavior of glioma cells, IKKβ, p-p65, MMP-2,

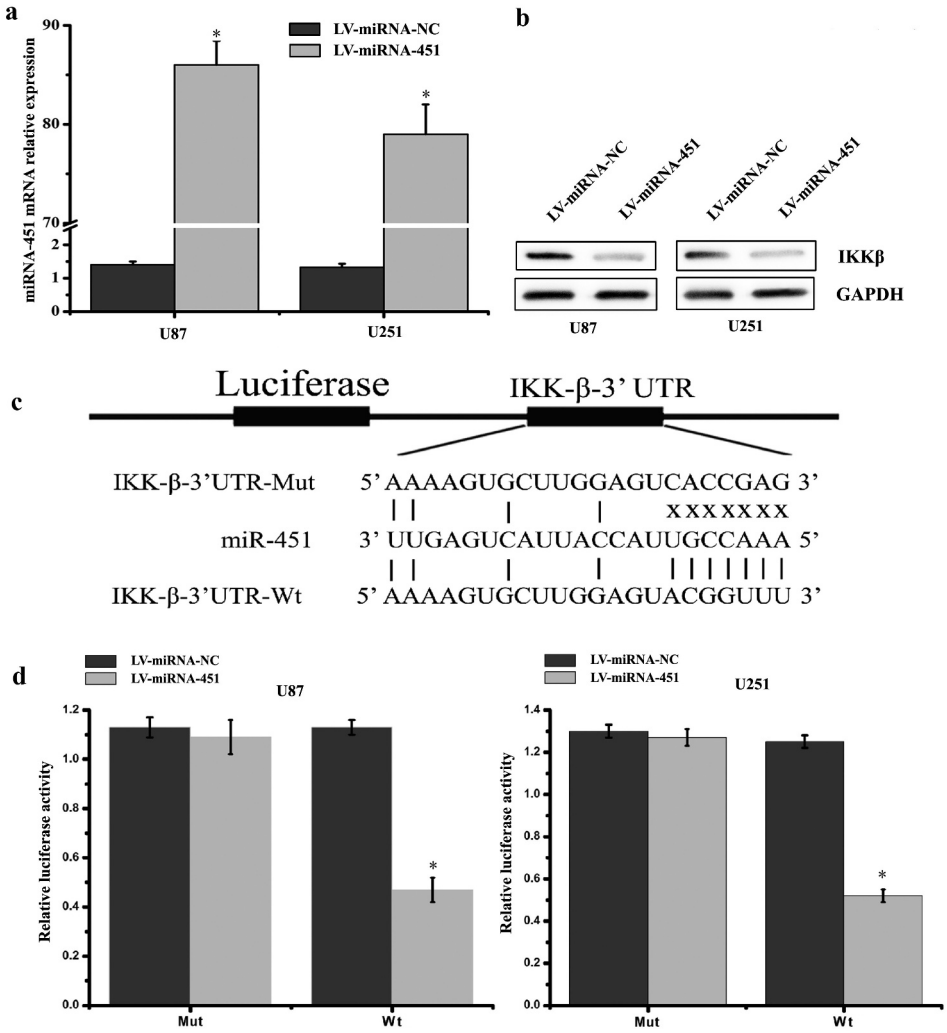

Figure 1. Upregulated miRNA-451 decreases the expression of IKKβ, which is a target gene of miRNA-451, in glioma cells. (a) miRNA-451 expression level in U251 and U87 glioma cells transfected with LV-miRNA-NC or LV-miRNA-451. (b) protein band of IKKβ in U251 and U87 glioma cells. (c) the binding site of miRNA-451 on the 3′UTR of IKKβ. (d) verification results of the luciferase activity assay. the data are shown as the mean ± standard deviation (SD) of three independent experiments. *P< 0.05 between the two groups.

MMP-9, Cyclin D1, p16 and PCNA, were decreased in the LV-miRNA-451 group (Figure 3). These results suggest that miRNA-451 significantly inhibits glioma cell proliferation, invasion, and migration.

3.3. miRNA-451 regulates the NF-κB signaling pathway by targeting IKKβ in glioma in vitro

The NF-κB pathway plays a critical role in controlling various cellular functions, such as protein synthesis, cell cycle progression, cell survival, and apoptosis. Therefore, we investigated NF-κB-related pathways. Consistent with the results above, miRNA-451 overexpression led to a marked down-regulation of IKKβ, p-p65, MMP-2, MMP-9, Cyclin D1, p16 and PCNA expression. The results of RT-PCR, MTT, Transwell invasion, wound-healing, and Western blot assays suggest that the effect of miRNA-451 on glioblastoma tumor cell growth activity is likely mediated through its regulation of the NF-κB pathway via IKKβ.

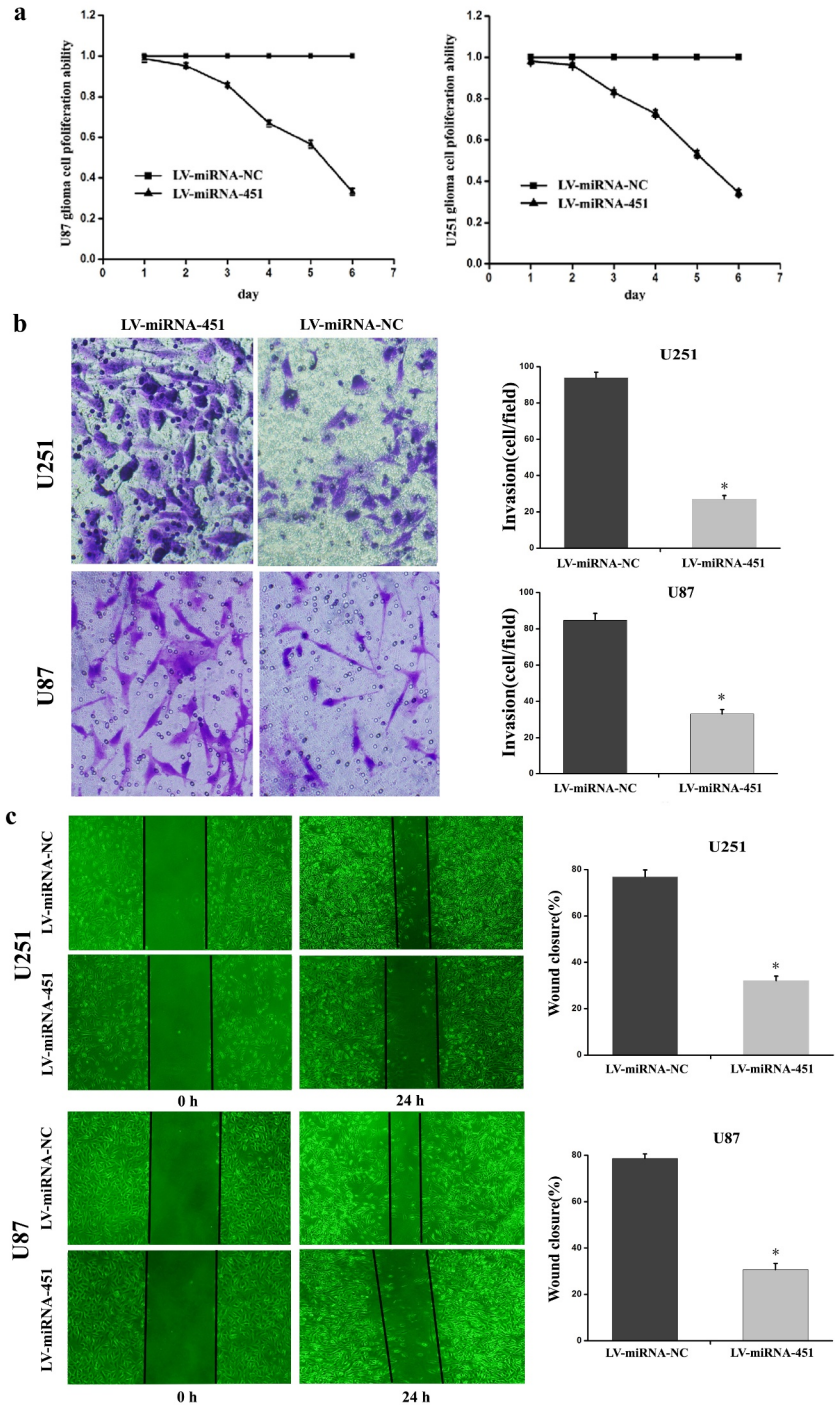

Figure 2. Upregulated miRNA-451 inhibits the proliferation, invasion and migration of glioma cells. (a) the proliferation ability of the abovementioned cells was determined by MTT assay. (b, c) the invasion and migration abilities of U251 and U87 glioma cells treated as described above were evaluated by invasion and wound-healing assays. three independent experiments with biological repeats. *P< 0.05 between the two groups.

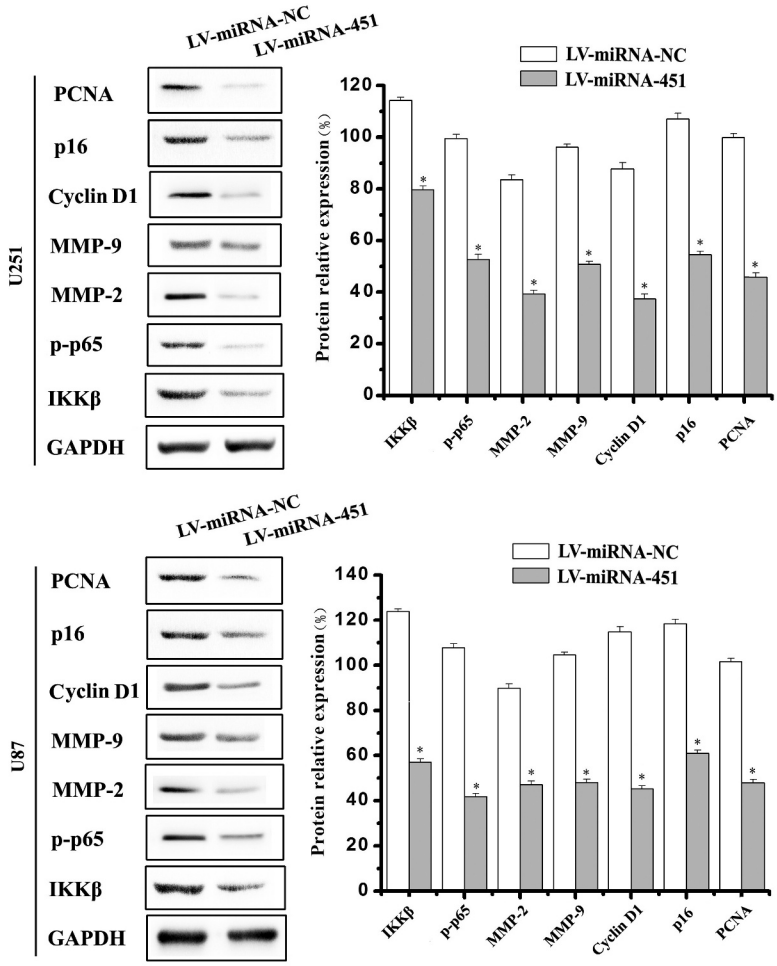

Figure 3. miRNA-451 regulates the NF-κB signaling pathway by targeting IKKβ in glioma. the protein levels of IKKβ, p-p65, MMP-2, MMP-9, cyclin D1, p16 and PCNA were detected using western blot assays in U251 and U87 glioma cells. the data are shown as the mean ± standard deviation (SD) of three independent experiments. three independent experiments with biological repeats. *P< 0.05 between the two groups.

3.4. miRNA-451 inhibits the growth of U87 glioma cells through regulation of the NF-κB pathway by targeting IKKβ in xenograft models

Due to the large difference between the growth environments of glioma cells in vitro and in vivo, it is particularly important to design in vivo models to verify the results of in vitro experiments. We established tumors as intracranial xenografts in nude mice to evaluate the effects of miRNA-451 inhibition on NF-κB signaling through its targeting of IKKβ and tumor growth in vivo. U87 cells were pretreated with lentiviruses containing a luciferase reporter. Compared with LV-miRNA-NC, LV-miRNA-451 significantly decreased tumor growth (Figure 4(a)). We generated Kaplan-Meier survival curves to analyze the survival of the different treatment groups and found that the survival of BALB/c-A nude mice was significantly prolonged (Figure 4(b-c)). Compared with that in the LV-miRNA-NC group, immunohistochemistry showed that p-p65, Cyclin D1 and Ki67 expression was significantly reduced in tumor sections from the LV-miRNA-451 group (Figure 4(d)). Therefore, miRNA-451 significantly inhibits tumor growth, prolongs the survival time of xenogeneic tumor-bearing mice and inhibits the expression of p-p65, CyclinD1 and Ki67.

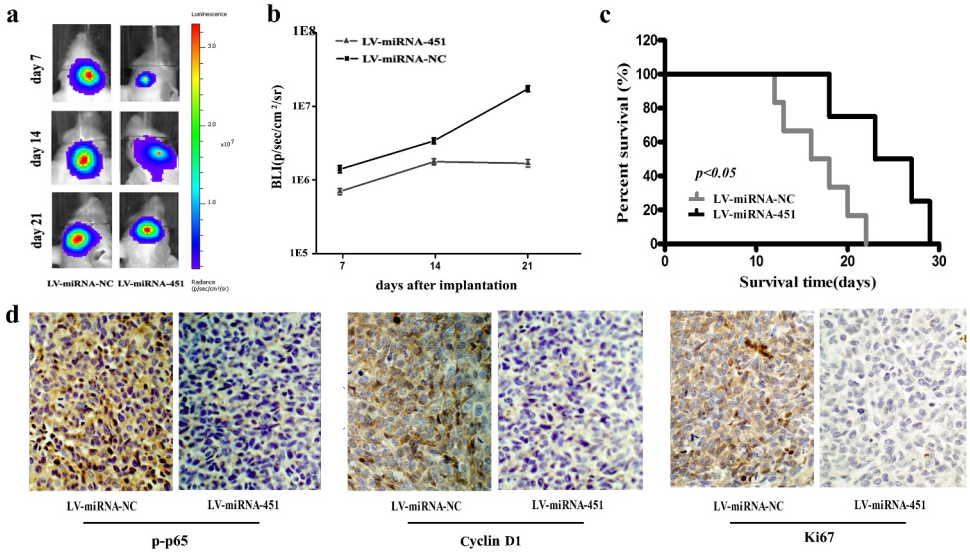

Figure 4. Antitumor effect of miRNA-451 on the growth of human U87 glioma cell-derived tumors in xenograft models. (a) bioluminescence images from treated animals at 7, 14, and 21 days after tumor implantation. (b) tumor growth curves were evaluated. (c) the survival time of the treated mice was determined. (d) the expression levels of p-p65, cyclin D1 and Ki67 were detected using immunohistochemistry (200x). the data are shown as the mean ± standard deviation (SD) of three independent experiments. *P< 0.05 between the two groups.

4. Discussion

GBM is the most common fatal brain cancer in adults [1,4,7]. At present, the median survival time of highly malignant glioblastoma after surgical treatment, concurrent postoperative radiotherapy and chemotherapy, and late adjuvant chemotherapy is only 14–16 months [9,20,21]. The clinical treatment of glioma is still suboptimal, and thus, it is urgent to further understand the molecular mechanisms by which glioma cells infiltrate surrounding brain tissue so that new targets for the treatment of malignant gliomas can be identified [1,2,35,19].

miRNAs play important roles in various biological processes, such as cell differentiation, proliferation, metastasis, and apoptosis [5,8,13,16]. Many recent studies have demonstrated that miRNAs play an important role in the occurrence and development of tumors [8,10,12,13,14,15]. For example, Zhong *et al.* [22] demonstrated that miRNA-1225 inhibits apoptosis of pancreatic cancer cells by targeting JAK1. Yang *et al.* [23] showed that miRNA-20b-5p functions as a tumor suppressor microRNA by targeting cyclin D1 in colon cancer. Other research has reported that miRNAs are associated with IKKβ in cancer. For example, Song *et al.* [24] showed that miRNA-218 inhibits the invasive ability of glioma cells by direct downregulation of IKKβ. Kong *et al.* [25] reported that the tumor-suppressive miRNA-497 targets IKKβ to regulate the NF-κB signaling pathway in human prostate cancer cells. Okada *et al.* [26] found that miRNA–139–5p and miRNA–139–3p act as tumor-suppressive miRNAs in renal cell carcinoma and that the expression levels of the PXN, ARHGEF19, ELK1, and IKKβ genes are independent prognostic factors for patient survival. Another study showed that miRNA-199a-5p functions as a tumor suppressor in oral squamous cell carcinoma by targeting the IKKβ/NF-κB signaling pathway [12]. Recent advances in cancer biology have shown that miRNA-451 is closely related to the occurrence and development of tumors. Wang *et al.* [27] found that miRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). Liu *et al.* [28] reported that miRNA-451 inhibits cell growth and invasion by targeting MIF and is associated with survival in nasopharyngeal carcinoma. Additionally, another study revealed that miRNA-451 can not only serve as an indicator for poor prognosis in postoperative gastric cancer patients but that it is also related to increased Th17 distribution in gastric cancer [29]. Moreover, Li *et al.* [30] showed that miRNA-451 expression is markedly downregulated in hepatocellular carcinoma cells and

tissues. miRNA-451 upregulation leads to cyclin D1 and c-Myc downregulation through NF-κB pathway inhibition, which is initiated by direct targeting of the IKBKB 3′-UTR. Other studies have shown that miRNA-451 targets TSC1 to regulate PI3K/Akt/mTOR signaling, which plays an essential role in myeloma stem cell biology [31]. miRNA-451 overexpression inhibits proliferation, migration, invasion, and adhesion and induces apoptosis of bladder cancer cells [32,33]. Gal *et al.* [34] showed that miRNA-451 and imatinib mesylate inhibit tumor growth of glioblastoma stem cells. Godlewski *et al.* [35,36] found that miRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells and that miRNA-451 is a conditional switch that controls glioma cell proliferation and migration. In addition, another study showed that knockdown of lncRNA LSINCT5 suppresses the growth and metastasis of human glioma cells by upregulating miRNA-451 [18]. We also found that miRNA-451 acts as a tumor suppressor in human glioma cells [5,13,17]. miRNA-451 downregulates the PI3K/AKT pathway through CAB39 in human glioma and suppresses EMT and metastasis in glioma cells [18,19]. These published data are consistent with our results, which indicates that miRNA-451 exerts a tumor suppressor effect in cancer cells, especially in gliomas. However, the specific mechanisms require further investigation.

NF-κB signaling orchestrates several key biological processes during the development and progression of cancer by inducing the transcription of a variety of target genes that regulate cell apoptosis, the cell cycle, and invasion [37,38]. NF-κB-regulated target genes include chemokines, cytokines, adhesion molecules and their own inhibitors, IκBα and IKKβ [10,11,38]. IKKβ is a critical kinase required for cytokine-induced phosphorylation of IκB proteins, which leads to their ubiquitination and subsequent degradation by the proteasome [6,38,39]. By performing a bioinformatics analysis, we found that the 3′UTR of IKKβ contains a complementary base sequence for miRNA-451 binding and that IKKβ is a possible target gene of miRNA-451 in glioma. However, it was unclear whether miRNA-451 could affect glioma cell growth and invasion by targeting IKKβ via modulation of the NF-κB pathway. Therefore, we evaluated the effects of miRNA-451 on glioma cell growth by targeting IKKβ and tested whether this effect was linked with the NF-κB pathway.

In this study, we introduced a lentivirus expressing miRNA-451 into glioma cells and found that miRNA-451 expression was significantly increased in the LV-miRNA-451 group. Luciferase activity experiments showed that IKKβ is a target gene of miRNA-451 in glioma. The MTT, cell invasion and wound-healing assays showed that cell viability, proliferation, invasion and migration were significantly suppressed by miRNA-451. In addition, Western blotting results revealed that the expression levels of IKKβ, p-p65, MMP-2, MMP-9, Cyclin D1, p16 and PCNA, which are related to the malignant behavior of glioma, were significantly suppressed by miRNA-451. In vivo, in a nude mouse intracranial xenograft model, miRNA-451 significantly decreased the growth of glioma cells by targeting IKKβ-mediated suppression of the NF-κB pathway, and the survival of mice was significantly prolonged. Immunohistochemistry showed that p-p65, Cyclin D1 and Ki67 expression was significantly inhibited by miRNA-451. The in vitro and in vivo results indicate that miRNA-451 inhibits glioma cell growth by targeting IKKβ-mediated suppression of the NF-κB pathway.

In conclusion, our results revealed that miRNA-451 expression is markedly downregulated in glioma. miRNA-451 can regulate the NF-κB signaling pathway by targeting IKKβ to inhibit glioma cell growth in vitro and in vivo. Our results help increase our understanding of the complex molecular regulation in glioma cells and demonstrate that targeting miRNA-451 may be a useful therapeutic strategy for glioma treatment in the future.

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Disclosure statement

The authors declare that they have no competing interests.

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