Original Article
The lncRNA-DLEU2/miR-186-5p/PDK3 axis promotes the progress of glioma cells

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Abstract: Long non-coding RNAs (lncRNAs) have great value in research on tumour targeted therapy, including for glioma. In the present study, we investigated the role of the lncRNA deleted in lymphocytic leukaemia 2 (lncRNA-DLEU2) in glioma. First, we found that lncRNA-DLEU2 is highly expressed in glioma tissues and cell lines. Next, experiments in cells showed that lncRNA-DLEU2 knockdown inhibited, whereas lncRNA-DLEU2 overexpression promoted, the clone formation, migration and invasion of glioma cells. A luciferase reporter assay and an RNA immunoprecipitation assay demonstrated that lncRNA-DLEU2 acts as a sponge for miR-186-5p in glioma cells. Further, studies suggested that miR-186-5p inhibits the expression of PDK3, which is an oncogene in glioma. Moreover, with rescue experiments, we demonstrated that lncRNA-DLEU2 regulates the expression of PDK3 and the progression of glioma in a miR-186-5p-dependent manner. Finally, we also showed that lncRNA-DLEU2 promotes glioma growth in a manner that is related to miR-186-5p and PDK3 *in vivo*. In conclusion, our study reported for the first time that lncRNA-DLEU2 promotes glioma progression by targeting the miR-186-5p/PDK3 axis. These findings provide novel strategies for the gene therapy treatment of glioma.

Keywords: Deleted in lymphocytic leukaemia 2, miR-186-5p, PDK3, glioma

Introduction

Glioma is the most common intracranial malignancy, originates from glial cells and is one of the leading causes of neoplastic death in humans [1, 2]. Current research indicates that the incidence of brain tumours is 21/100,000, accounting for approximately 2% of all human cancers, and the incidence of gliomas accounts for approximately 60% of brain tumours [3]. Despite the rapid development of therapy, the prognosis and survival of gliomas have not improved as much as those of other human tumours [4, 5]. It is undeniable that genetic mutations are one of the important causes of glioma onset. Therefore, the molecular mechanism that underlies the pathogenesis and progression of glioma is of great significance for the diagnosis and treatment of glioma.

Long non-coding RNAs (lncRNA) were originally thought to be the "noise" of genomic transcription, as by-products of RNA polymerase II transcription that had no biological function [6, 7]. However, recent studies have shown that lncRNA is involved in many important regulatory processes in mammals and may be able to serve as therapeutic targets for a variety of human diseases, including central nervous system diseases. Numerous studies have indicated that lncRNAs are widely involved in the progression of central nervous system diseases, including brain tumours [8, 9], ischaemic stroke [10, 11], Parkinson's disease [12, 13] and Alzheimer's disease [14, 15]. Existing studies have shown that lncRNAs can affect glioma proliferation, migration, drug resistance and many other biological behaviours. For instance, miR155 host gene (miR155HG) promotes glioma growth by sponging miR-185-5p [16]. Gastric cancer-associated transcript 3 (GACAT3) promotes glioma migration and invasion by regulating nicotinamide phosphoribosyl transferase (NAMPT) [17]. The lncRNA HOX transcript antisense in-

Table 1. The primers used in this study

Gene	Primer Sequence
DLEU2	forward primer: 5'-TCTGGAGAACAGCCTCACTTC-3'
	reverse primer: 5'-TGCTGAGCTAAGTAGAGGTCTC-3'
miR-186-5p	forward primer: 5'-ACACTCCAGCTGGGCAGCAGCACACT-3'
	reverse primer: 5'-CTCAACTGGTGTCGTGGA-3'
PDK3	forward primer: 5'-AGATCTAGATCTCGTCCACTGAAGGCACAAAAGC-3'
	reverse primer: 5'-CCCAAGCTTAAGCTTGGCGCACAGACCCGCCTA-3'
β-actin	forward primer: 5'-CTCCATCCTGGCCTCGCTGT-3'
	reverse primer: 5'-GCTGTCACCTTCACCGTTCC-3'
U6	forward primer: 5'-CTCGCTTCGGCAGCACA-3'
	reverse primer: 5'-AACGCTTCACGAATTTGCGT-3'

tergenic RNA (HOTAIR) regulates the malignant progression of glioma by affecting glutamine metabolism and drug resistance [18]. Small nucleolar RNA host gene 20 (SNHG20) regulates the expression of p21 by recruiting RNA-binding proteins (RBP), thereby affecting the cell cycle of glioma [19]. These studies demonstrated that lncRNAs play an important role in the progression of glioma and thus further reveal the molecular mechanisms that are relevant for the treatment of gliomas.

LncRNA deleted in lymphocytic leukaemia 2 (DLEU2) is located on chromosome 13 and has been reported to act as an oncogene in many human cancers [20-22]. However, there has been no research on lncRNA-DLEU2 in glioma until now. In the present study, we demonstrated that compared with normal brain tissues (NBTs), lncRNA-DLEU2 is upregulated in glioma tissues. Next, we confirmed that lncRNA-DLEU2 can promote the clone formation, migration and invasion of glioma through a series of experiments in cells. Then, with the help of bioinformatics analysis, we identified the relevant molecular mechanisms of lncRNA-DLEU2 in glioma. These findings provide novel strategies for the gene therapy treatment of glioma.

Materials and methods

Clinical samples

Clinical samples (including 5 normal brain tissues and 15 glioma tissues) were obtained from the department of Neurosurgery, Nanjing First Hospital, Nanjing Medical University. All the tissues were frozen in liquid nitrogen immediately after resection. The pathological diagnosis of these tissues was independently confirmed by two experienced pathologists. Written

informed consent was obtained from the patients. This study was approved by the Ethics Committee of the Nanjing First Hospital, Nanjing Medical University.

Cell culture

All the glioma cell lines were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). The normal human astrocyte (NHA) cell line was obtained from JENNIO Biological Technology (Guangzhou, China). The cell lines used in this study were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, NY, USA) that was supplemented with 10% foetal bovine serum (FBS, Gibco, NY, USA) and were maintained in a humidified incubator (5% CO_2, 37°C).

Oligonucleotides, plasmid construction, and cell transfection

The short-hairpin RNAs targeting lncRNA-DLEU2 (sh-DLEU2-1 and sh-DLEU2-2), small interfering RNA targeting PDK3 (si-PDK3), miR-186-5p mimics, miR-186-5p inhibitors and corresponding negative controls (NCs) were purchased from GenePharma (Shanghai, China). Lipofectamine 2000 (Life Technology, Thermo Fisher Scientific, DE, USA) was used to conduct the cell transfection according to the manufacturer's protocol.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissues or cells by using TRIzol (Invitrogen, CA, USA). cDNAs were synthesized using M-MLV (Promega, WI, USA). qPCR was performed with TaqMan Noncoding RNA Assays and TaqMan miRNA Assays on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, MA, USA). The relative expression was normalized to that of U6. The $2^{-\Delta\Delta Ct}$ method was used to analyse the data. The primers used in this study are shown in **Table 1**.

Immunoprecipitation

The RNA immunoprecipitation assay was performed with an EZ-Magna RIP kit (Millipore, MA,

USA) according to the manufacturer's protocols. A human anti-Ago2 antibody and the IgG that was used as a control were also obtained from Millipore. A NanoDrop spectrophotometer was used to measure the RNA concentration, and the purified RNA was subjected to qRT-PCR analysis.

Luciferase reporter assay

The region that contained the miR-186-5p binding site on lncRNA-DLEU2 or PDK3 was inserted into the luciferase reporter vector pmirGLO (Promega, WI, USA). The cells were cultured in a 96-well plate and co-transfected with luciferase plasmids and with miR-186-5p or with the negative control miRNA. Forty-eight hours later, the luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega, WI, USA).

Clone formation assay

Cells in the logarithmic growth phase were collected and resuspended after digestion with trypsin. Then, the appropriate amount of cell suspension was added to 6.0 cm culture dishes. The cells were maintained in a humidified incubator (5% CO_2, 37°C). After 14 d, the colonies were washed with phosphate-buffered saline (PBS), fixed with 10% formaldehyde and stained with 1% crystal violet for 30 min.

Transwell migration and invasion assays

For invasion assays, transwell chambers were coated with 50 μl Matrigel (1:8 dilution, BD, NJ, USA) in a 24-well plate and maintained in a humidified incubator (5% CO_2, 37°C). Twenty-four hours after cell transfection, the cells were washed, digested, centrifuged and counted. Then, 20,000 cells were added into every upper chamber, and 500 μl of medium (supplemented with 10% FBS) was added to the lower chamber. Then, the plate was placed in a humidified incubator (5% CO_2, 37°C) for 48 h. The cells were wiped off of the upper chamber with a cotton swab, and the cells in the lower chamber were stained with 1% crystal violet for 30 min. For the migration experiments, except for coating the chamber with 50 μl Matrigel, the other steps were the same.

Western blot assay

Proteins were extracted with RIPA buffer (KeyGEN, Shanghai, China), and the protein con-

centrations were measured with a BCA Protein Assay Kit (Beyotime, Jinagsu, China). Protein samples were separated by SDS-PAGE (Beyotime, Jinagsu, China) and transferred onto PVDF membranes (Millipore, MA, USA). Then, the membranes were blocked with 5% non-fat milk and incubated with primary antibodies at 4°C overnight. Next, the membranes were incubated with secondary antibodies for 2 h. Finally, the proteins were visualized using an Image Quant LAS 4000 mini system.

Tumour xenograft model

U87 cells (1×10^7) that had been transfected with sh-DLEU2 or the corresponding negative control (NC) were harvested and subcutaneously injected into each flank of nude female mice. After 30 d, these mice were euthanized. The weight and volume of the tumours that had grown under the skin of the nude mice were measured. Tumour volume was calculated using the equation: volume = 0.5 × length × width^2.

Statistical analysis

SPSS 20.0 was used to analyse the data. The results are shown as the mean ± standard error. A t test (two groups) or one-way ANOVA (no less than three groups) was used to analyse the statistical significance. A *p*-value of less than 0.05 was considered statistically significant. A *p*-value of less than 0.01 was considered statistically very significant. All experiments were independently performed in triplicate.

Results

DLEU2 is highly expressed in glioma

To investigate the role of lncRNA-DLEU2 in glioma, we initially analysed the expression pattern of DLEU2 in TCGA (The Cancer Genome Atlas). The data showed that the expression of lncRNA-DLEU2 is higher in glioma tissues than in normal brain tissues (**Figure 1A**). Next, we validated the result that we obtained from TCGA using clinical tissue samples, and we obtained a similar result (**Figure 1B**). Furthermore, we also measured the expression of lncRNA-DLEU2 in glioma cell lines and found that lncRNA-DLEU2 has the highest expression in U87 and U251 cells and the lowest in U343 cells, but all five cell lines express higher levels of
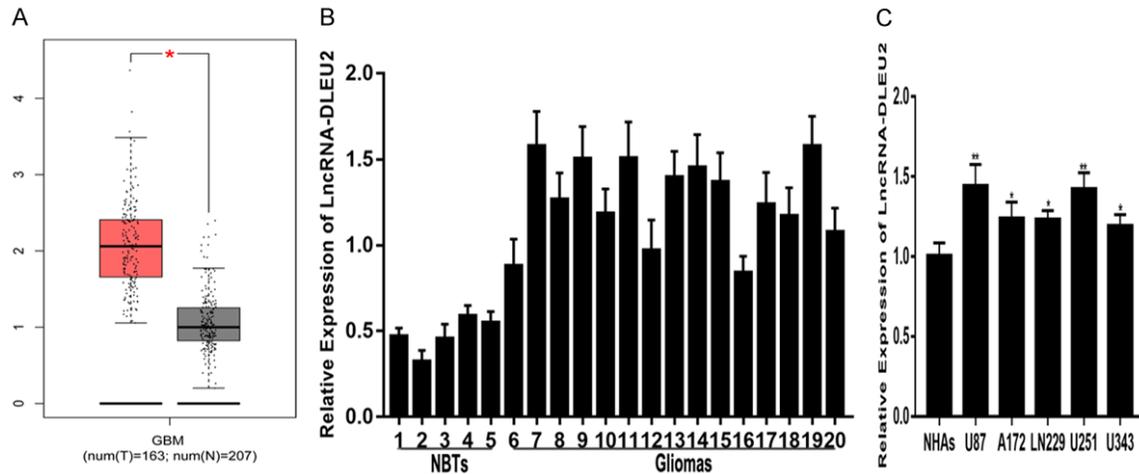
Figure 1. LncRNA-DLEU2 is highly expressed in glioma. A. TCGA data from GEPIA indicated that lncRNA-DLEU2 was upregulated in glioma tissues compared to the levels in normal brain tissues. B. The expression of lncRNA-DLEU2 in glioma tissues (n = 15) is higher than in normal brain tissues (n = 5) based on qRT-PCR. C. The expression of lncRNA-DLEU2 in glioma cell lines is higher than in normal human astrocytes (NHAs) based on qRT-PCR. $^*P<0.05$, $^{**}P<0.01$.

lncRNA-DLEU2 than those in normal human astrocytes (**Figure 1C**). All of these data show that lncRNA-DLEU2 is highly expressed in glioma tissues and cell lines.

DLEU2 promotes the clone formation, migration and invasion of glioma

Since lncRNA-DLEU2 is upregulated in glioma tissues and cell lines, we speculated that it may be related to the progression of glioma. To investigate the biological function of lncRNA-DLEU2 in glioma, two shRNAs (short hairpin RNAs, shDLEU2-1 and sh-DLEU2-2) and the overexpression vector pcDNA (DLEU2) were constructed. Then, we transfected the shRNA or pcDNA into U87 and U251 cells. The transfection efficiency was verified by qRT-PCR (**Figure 2A**, **2B**). Through a clone formation assay, we found that compared to the controls, upregulated lncRNA-DLEU2 promoted the clone formation of U87 and U251 cells, whereas downregulated lncRNA-DLEU2 had the opposite effect on the clone formation of U87 and U251 cells (**Figure 2C**, **2D**). To assess the effect of lncRNA-DLEU2 on migration and invasion, we conducted transwell assays. The results showed that compared to the controls, upregulated lncRNA-DLEU2 promoted the migration and invasion of U87 and U251 cells, and downregulated lncRNA-DLEU2 suppressed the migration and invasion of U87 and U251 cells (**Figure 2E-H**). These findings suggest that lncRNA-DLEU2

promotes the clone formation, migration and invasion of glioma in vitro.

DLEU2 acts as a ceRNA to sponge miR-186 in glioma

In recent years, the mechanism of action of lncRNAs in tumours has mainly been shown to occur by acting as competing endogenous RNA (ceRNA). That is, lncRNAs could sponge microRNAs (miRNAs), thereby regulating the expression of particular targeted messenger RNAs (mRNAs) [23, 24]. Inspired by excellent previous studies, we speculated that lncRNA-DLEU2 may also influence the progression of glioma by acting as a ceRNA. To verify our hypothesis, we performed a series of experiments. First, we used the website starbase V3.0 to identify that miR-186-5p may be a functional target miRNA of lncRNA-DLEU2 (**Figure 3A**). Then, we measured the expression level of miR-186-5p in the clinical samples and found that the expression of miR-186-5p is lower in glioma tissues than in normal brain tissues (**Figure 3B**). Simultaneously, we measured the expression of miR-186-5p in NHAs and glioma cell lines, and the results showed that, compared with NHAs, miR-186-5p was present at much lower levels in glioma cell lines (**Figure 3C**). Furthermore, in an in vitro model, we found that the downregulation of lncRNA-DLEU2 led to the upregulation of miR-186-5p, whereas the upregulation of lncRNA-DLEU2 led to the downregulation of miR-186-
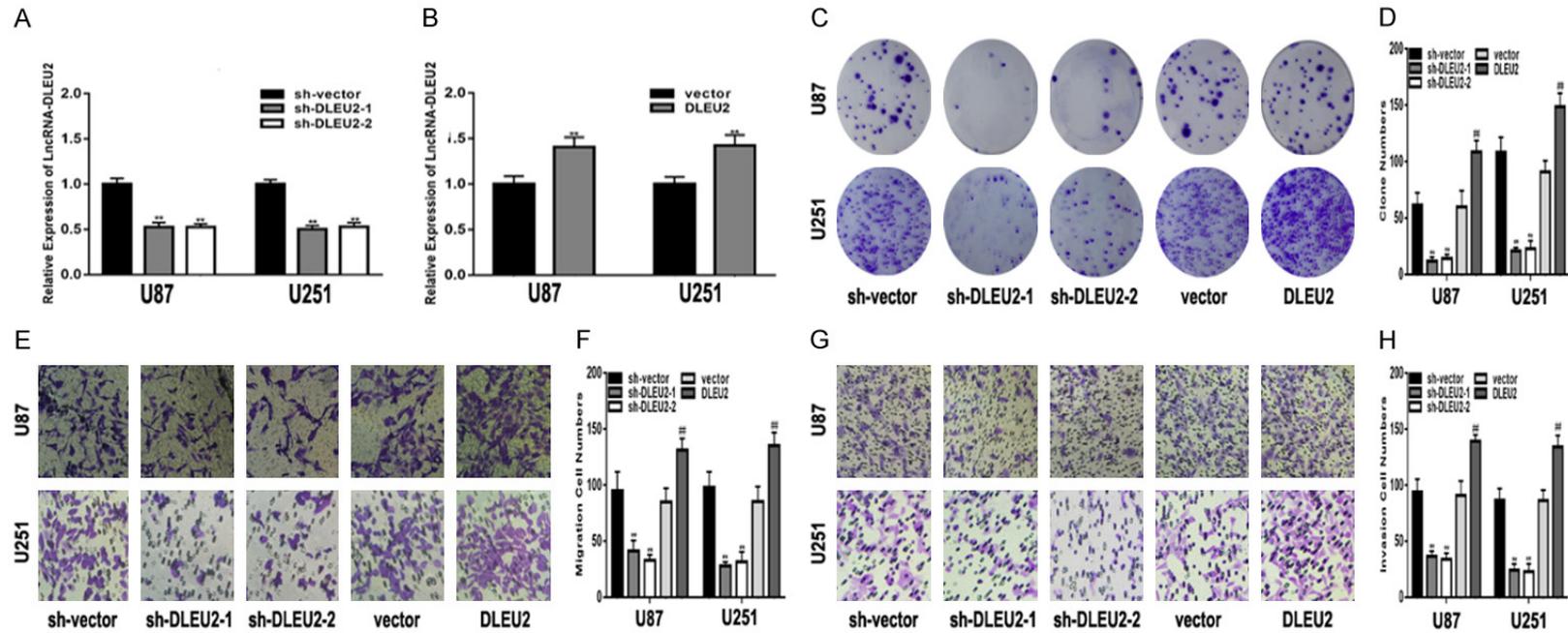
Figure 2. LncRNA-DLEU2 promotes the clone formation, migration and invasion of glioma. A, B. The expression of lncRNA-DLEU2 in U87 and U251 cells transfected with sh-vector, sh-DLEU2-1, sh-DLEU2-2, vector and DLEU2. C, D. The clone formation assay revealed that the clone formation ability was impaired by lncRNA-DLEU2 downregulation, whereas it was enhanced by lncRNA-DLEU2 upregulation. E, F. The migration assay revealed that the migration capacity was impaired by lncRNA-DLEU2 downregulation, whereas it was enhanced by lncRNA-DLEU2 upregulation. G, H. The transwell assay revealed the invasion capacity was impaired by lncRNA-DLEU2 downregulation, whereas it was enhanced by lncRNA-DLEU2 upregulation. $^{*}P<0.05$ and $^{**}P<0.01$ compared to cells treated with sh-vector, $^{#}P<0.05$ and $^{##}P<0.01$ compared to cells treated with vector.
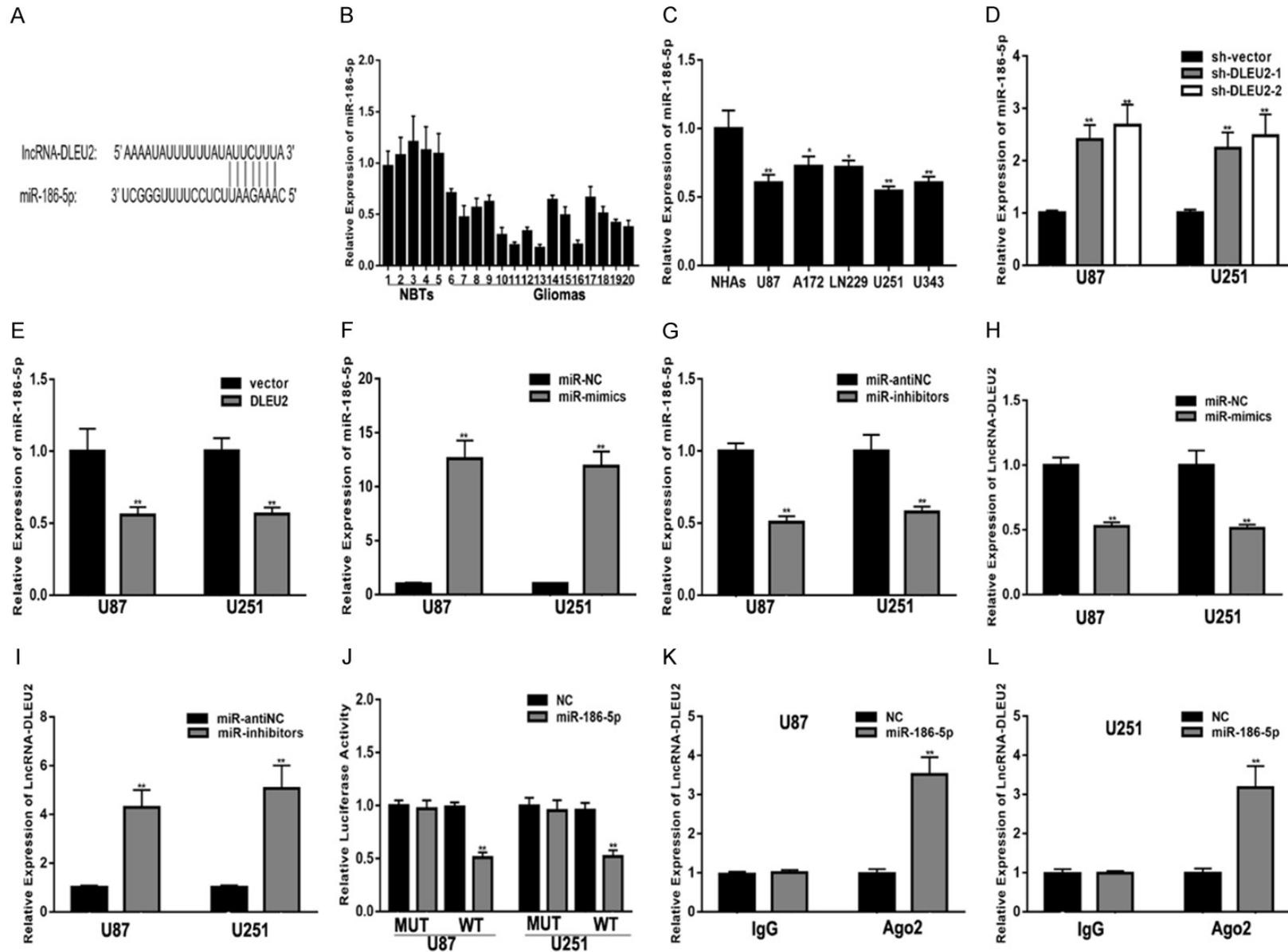
Figure 3. LncRNA-DLEU2 acts as a ceRNA by sponging miR-186 in glioma. A. The putative binding sites of miR-186-5p on lncRNA-DLEU2 as predicted by starbase V3.0 (http://starbase.sysu.edu.cn/). B. The expression of miR-186-5p is lower in glioma tissues than in normal brain tissues measured by qRT-PCR. C. Expression

of miR-186-5p in glioma cell lines is much lower than in NHAs measured by qRT-PCR. D, E. qRT-PCR analysis showing that miR-186-5p was negatively regulated by lncRNA-DLEU2. F, G. The interfering efficiency of miR-186-5p mimics and inhibitors in U87 and U251 cells measured by qRT-PCR. H, I. qRT-PCR analysis showed that lncRNA-DLEU2 was negatively regulated by miR-186-5p. J. Luciferase reporter assays showed that miR-186-5p reduced the luciferase activity of lncRNA-DLEU2-WT but not of lncRNA-DLEU2-MUT. K, L. An RNA immunoprecipitation assay showed that both lncRNA-DLEU2 and miR-186-5p were enriched in the Ago-2 precipitate compared with the levels in the IgG precipitate. *P<0.05 and **P<0.01.
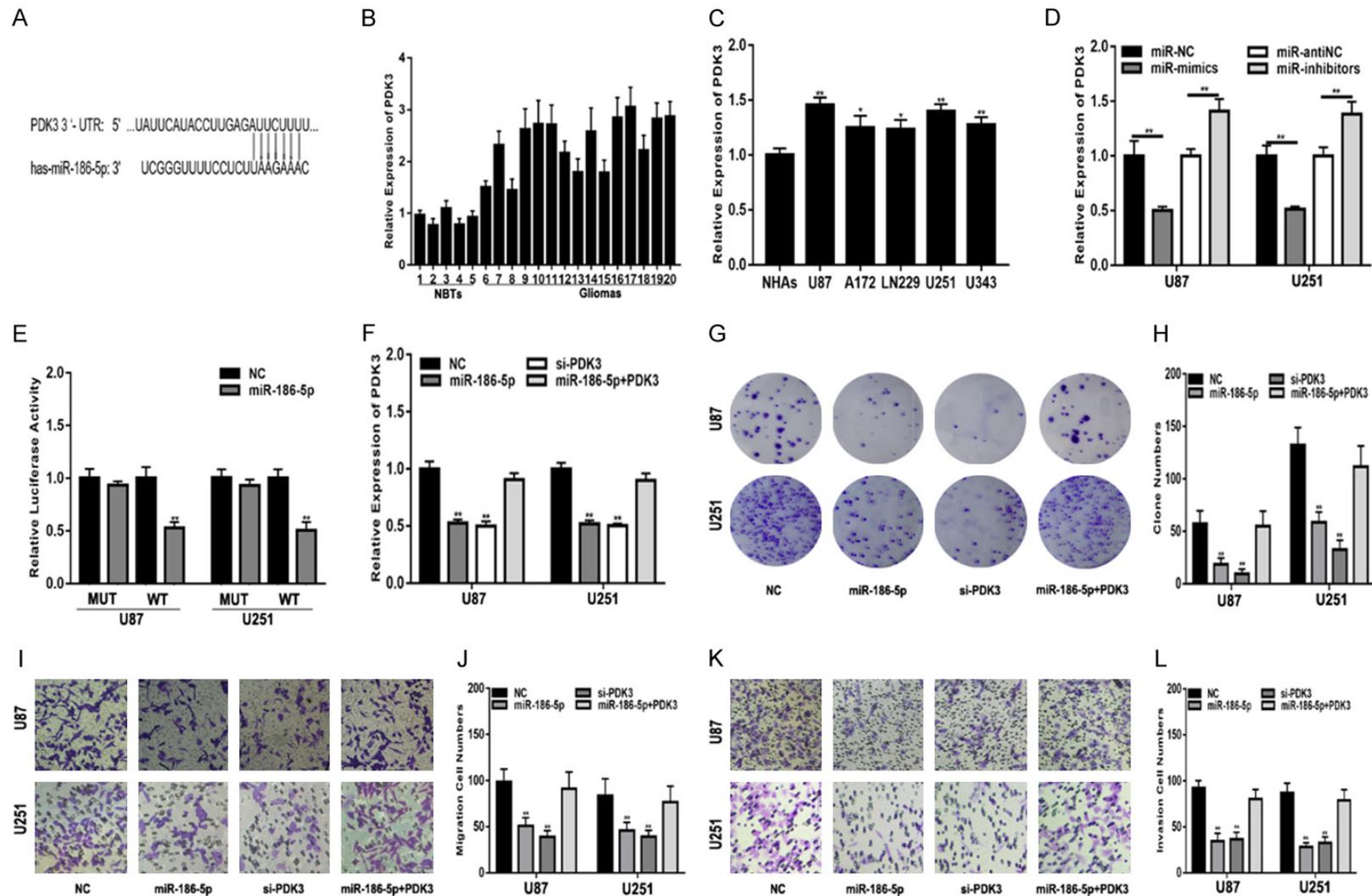
Figure 4. miR-186-5p functions as a suppressor by targeting PDK3. A. The binding sites of miR-186-5p on PDK3 were predicted by starbase. B. Expression of PDK3 in glioma tissues is much higher than in normal brain tissues measured by qRT-PCR. C. Expression of PDK3 in glioma cell lines is much higher than in NHAs measured by qRT-PCR. D. qRT-PCR analysis showed that PDK3 was negatively regulated by miR-186-5p. E. Luciferase reporter assays showed that miR-186-5p reduced the luciferase activity of PDK3-WT but not of PDK3-MUT. F. qRT-PCR showing the expression of PDK3 in glioma cell lines transfected with NC, miR-186-5p, si-PDK3 or miR-186-5p together with PDK3. G, H. The clone formation assay showing the clone formation capacity of glioma cell lines transfected with NC, miR-186-5p, si-PDK3 or miR-186-5p together with PDK3. I, J. Migration assay showing the migration capacity of glioma cell lines transfected with NC, miR-186-5p, si-PDK3 or miR-186-5p together with PDK3. K, L. Transwell assay showing the invasion capacity of glioma cell lines transfected with NC, miR-186-5p, si-PDK3 or miR-186-5p together with PDK3. *P<0.05, **P<0.01.
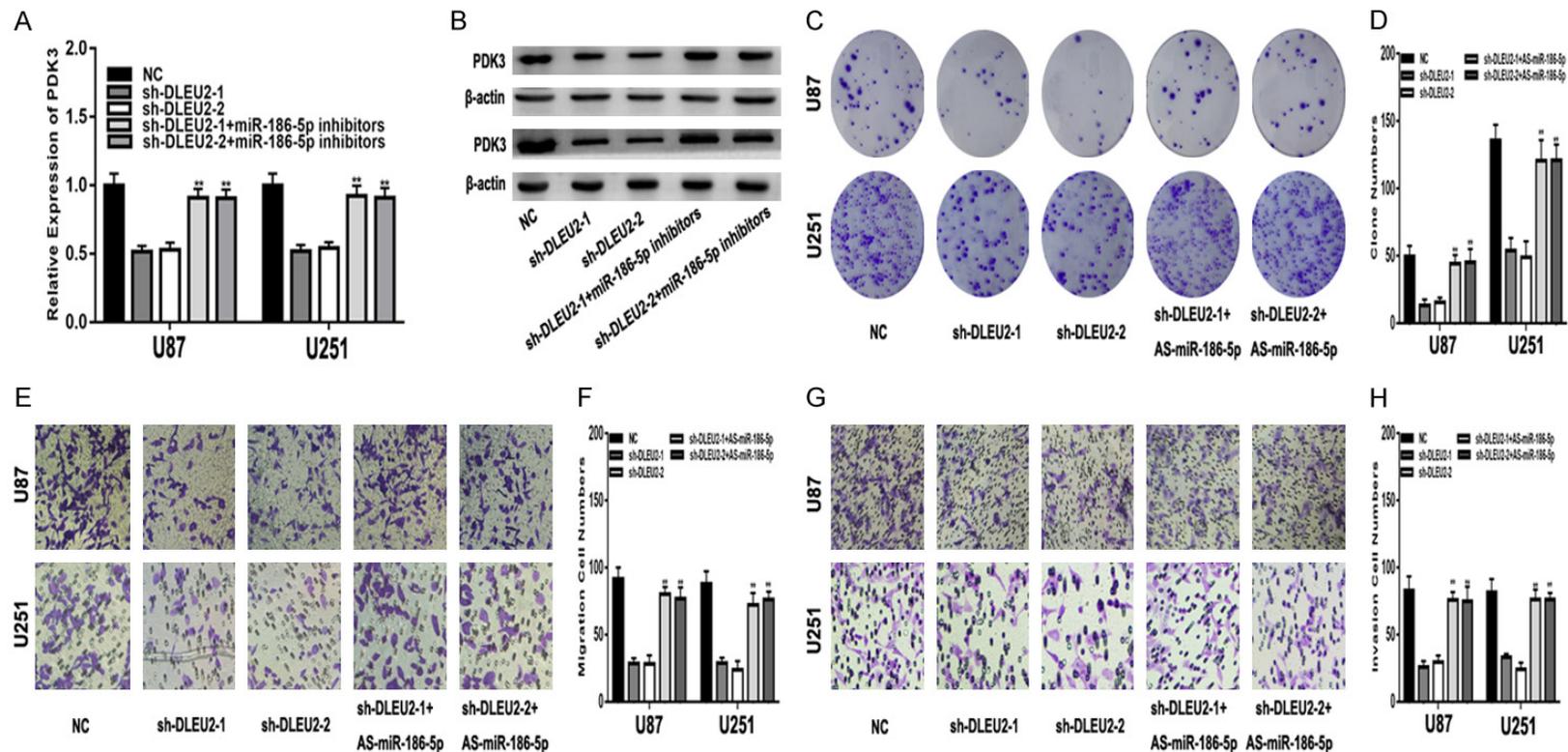
Figure 5. LncRNA-DLEU2 regulates the clone formation, migration and invasion of glioma in a miR-186-5p-independent manner. A, B. The expression of PDK3 in glioma cell lines that were transfected with NC, sh-DLEU2 or sh-DLEU2 together with miR-186-5p inhibitors, as measured by qRT-PCR and western blotting. C, D. Clone formation assay showing the clone formation ability of glioma cell lines was impaired by sh-DLEU2, whereas it was restored by miR-186-5p inhibitors. E, F. Migration assay showing the migration capacity of glioma cell lines was impaired by sh-DLEU2, whereas it was restored by miR-186-5p inhibitors. G, H. Transwell assay showing the invasion capacity of glioma cell lines was impaired by sh-DLEU2, whereas it was restored by miR-186-5p inhibitors as well. *P<0.05, **P<0.01.

5p (**Figure 3D**, **3E**). To assess the effect of miR-186-5p on lncRNA-DLEU2 expression, miR-186-5p mimics and inhibitors were purchased. The interference efficiency of the miR-186-5p mimics and inhibitors was verified by qRT-PCR (**Figure 3F**, **3G**). With this in vitro model, we found that the downregulation of miR-186-5p led to the upregulation of lncRNA-DLEU2, whereas the upregulation of miR-186-5p led to the downregulation of lncRNA-DLEU2 (**Figure 3H**, **3I**). In addition, a luciferase reporter assay showed that compared to the controls, miR-186-5p decreased the fluorescence of lncRNA-DLEU2-WT but not of lncRNA-DLEU2-MUT (**Figure 3J**). Finally, an RNA immunoprecipitation assay was performed, and the results demonstrated that lncRNA-DLEU2 and miR-186-5p were both enriched in when the Ago 2 protein was pulled down (**Figure 3K**, **3L**). These data suggested that lncRNA-DLEU2 acts as a sponge for miR-186-5p in glioma cell lines.

miR-186 functions as a suppressor by targeting PDK3

To further reveal the ceRNA mechanism of lncRNA-DLEU2, we used the website starbase V3.0 to identify that PDK3 may be a functional target mRNA of miR-186-5p. We first verified the binding sequence of miR-186-5p and PDK3 (**Figure 4A**). Next, we examined the expression level of PDK3 in clinical samples, and the results showed that, compared with normal brain tissues, PDK3 was upregulated in glioma tissues (**Figure 4B**). Then, we measured PDK3 expression in glioma cell lines by qRT-PCR. The results showed that the expression of PDK3 in glioma cell lines was higher than in normal human astrocytes (**Figure 4C**). We also detected the expression of PDK3 in glioma cell lines that were interfered with miR-186-5p mimics or inhibitors. The results suggested that compared to the controls, miR-186-5p mimics acted as an inhibitor of PDK3, whereas miR-186-5p inhibitors promoted the expression of PDK3 (**Figure 4D**). Furthermore, a luciferase reporter assay showed that miR-186-5p decreased the fluorescence of PDK3-WT but not of PDK3-MUT (**Figure 4E**). To further confirm the regulatory relationship between miR-186-5p and PDK3, we transfected miR-186-5p mimics, PDK3 siRNA or miR-186-5p mimics together with a PDK3 plasmid into U87 and U251 cells. qRT-PCR indicated that compared to the controls, miR-186-5p mimics/DK3 siRNA could inhibit PDK3 ex-

pression and that the inhibitory effect of miR-186-5p mimics could be restored by the PDK3 plasmid (**Figure 4F**). Additionally, a series of cell experiments demonstrated that compared to the controls, miR-186-5p mimics/PDK3 siRNA could inhibit the clone formation, migration and invasion of glioma cell lines, and the inhibitory effect could be reversed by the PDK3 plasmid (**Figure 4G-L**). Taken together, we concluded that miR-186-5p functions as a suppressor by targeting PDK3 in glioma.

DLEU2 regulates the expression of PDK3 in a miR-186-5p-dependent manner

To verify the validity of the ceRNA mechanism of lncRNA-DLEU2 in glioma, we performed a series of assays to explore whether lncRNA-DLEU2 regulates the clone formation, migration and invasion of glioma by targeting miR-186-5p. First, we transfected sh-DLEU2-1, sh-DLEU2-2, sh-DLEU2-1 together with miR-186-5p inhibitors or sh-DLEU2-2 together with miR-186-5p inhibitors into U87 and U251 cells. qRT-PCR and western blot assays indicated that compared to the controls, sh-DLEU2 (including sh-DLEU2-1 and sh-DLEU2-2) inhibited PDK3 expression and that the inhibitory effect could be partly reversed by miR-186-5p inhibitors (**Figure 5A**, **5B**). Moreover, the clone formation assay and transwell assay indicated that the effect of sh-DLEU2 on clone formation, migration and invasion were restored by miR-186-5p inhibitors (**Figure 5C-H**). Thus, we concluded that lncRNA-DLEU2 regulates the clone formation, migration and invasion of glioma in a miR-186-5p-dependent manner.

DLEU2 promotes glioma growth in vivo

To explore the role of lncRNA-DLEU2 *in vivo*, U87 cells that had been transfected with a negative control or sh-DLEU2 (including sh-DLEU2-1 and sh-DLEU2-2) were subcutaneously injected into nude mice. 30 days later, the tumours were recovered and measured. The data indicated that the average weight and volume of the tumours in the sh-DLEU2 group was much lower than those in the negative control group (**Figure 6A**, **6B**). Furthermore, we detected the expression of lncRNA-DLEU2, miR-186-5p and PDK3 in the two groups. Compared with the negative control, in the sh-DLEU2 group, lncRNA-DLEU2 and PDK3 were downregulated, whereas miR-186-5p was upregulated (**Figure**
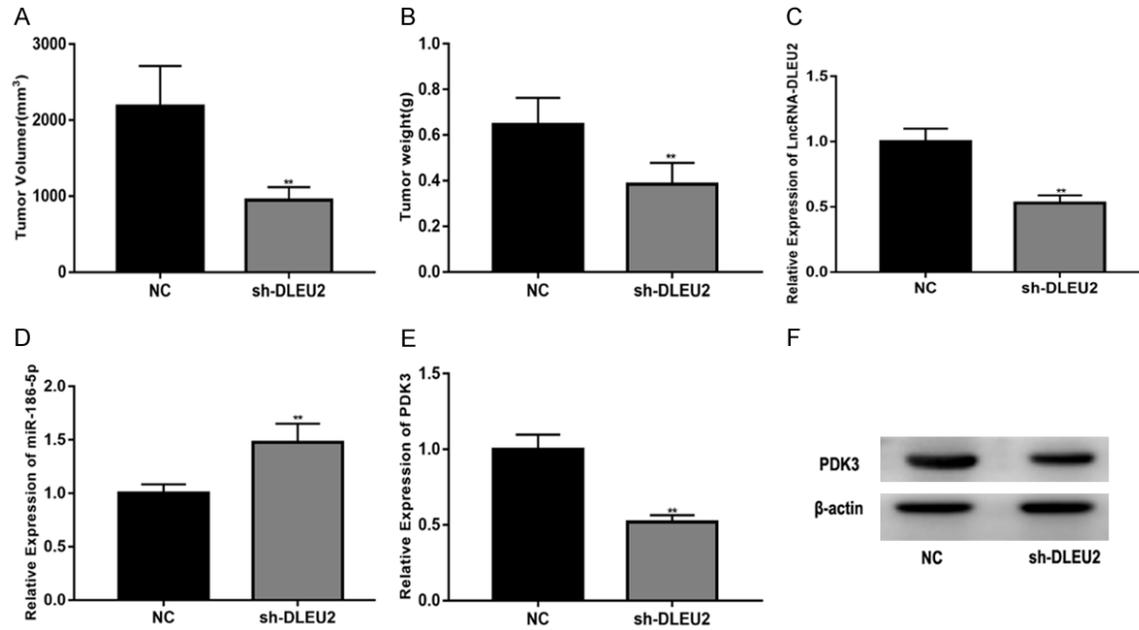
Figure 6. LncRNA-DLEU2 promotes glioma growth *in vivo*. A, B. LncRNA-DLEU2 knockdown led to a reduction in tumour growth (including volume and weight) in nude mice compared to those in the control group. C. LncRNA-DLEU2 knockdown led to a decrease in lncRNA-DLEU2 in the tumour compared to that in the controls. D. LncRNA-DLEU2 knockdown led to an increase in miR-186-5p in the tumour compared to that in the controls. E, F. LncRNA-DLEU2 knockdown led to a decrease in PDK3 in the tumour compared to that in the controls, as measured by qRT-PCR and western blot. *P<0.05, **P<0.01.

6C-F). These data demonstrated that lncRNA-DLEU2 might promote glioma growth *in vivo* via miR-186-5p and PDK3.

Discussion

Glioma is an important cause of cancer-related death [25-27]. Although basic research on glioma has made some progress in the past two decades, the prognosis of glioma has not improved significantly. With the improvement of next-generation sequencing technology, researchers have found that lncRNAs, which were previously considered to be non-functional in human physiological and pathological processes, can play an important role in the progression of tumours [28, 29]. As the research continues to progress, the role of a large number of lncRNAs in the progression of human diseases has been revealed. For example, in gliomas, lncRNAs can affect the metabolism, proliferation, and chemotherapy resistance of glioma cells [8, 30]. Since lncRNAs have an important function in the progression of glioma, uncovering their mechanism of action is of great significance for the early diagnosis and prognostic improvement of glioma. Through the analysis of

TCGA, we found that the expression of lncRNA-DLEU2 in glioma tissue was significantly higher than that in normal brain tissue. Next, we found similar expression differences in the lncRNA-DLEU2 expression in clinical specimens and glioma cell lines. Previous studies have shown that lncRNA-DLEU2 is transcribed from the LEU2 gene and can regulate the malignant progression of oesophageal adenocarcinoma [20], chronic lymphocytic leukaemia [31], gastric cancer [22] and laryngeal carcinoma [21]. Based on these findings, we believe that the function and mechanism of lncRNA-DLEU2 in the malignant progression of glioma is worthy of further study.

With the help of shRNA and the overexpression vector pcDNA, we constructed cell models with different expression levels of lncRNA-DLEU2. Through a series of cell experiments, we found that lncRNA-DLEU2 can promote the clone formation, migration and invasion of glioma cells in vitro. There are several mechanisms through which lncRNAs contribute to the progression of human disease, the most widely studied of which is as acting as a ceRNA; that is, by adsorbing specific miRNAs and then affecting

the transcription of the targeted mRNA. For instance, lncRNA-SNHG16 promotes osteosarcoma progression by sponging miR-1301 [32]. Linc00202 acts as a sponge for miR-3619-5p in retinoblastoma [33]. LncRNA-NEAT1 sponges miR-34a-5p to regulate nasopharyngeal carcinoma progression [34]. Inspired by these studies, we speculated that lncRNA-DLEU2 may affect the progression of glioma through a ceRNA mechanism. With bioinformatics analysis, we found that miR-186-5p may be a target miRNA of lncRNA-DLEU2. Experiments such as RNA immunoprecipitation confirmed that lncRNA-DLEU2 can sponge miR-186-5p. MiR-186-5p has a dual role in tumour progression. For instance, miR-186-5p suppresses the progression of osteosarcoma [35], cutaneous malignant melanoma [36] and oral squamous cell carcinoma [37], whereas promotes the progression of cutaneous squamous cell carcinoma [38] and prostate cancer [39]. Studies have also shown that miR-186-5p can inhibit the malignant progression of glioblastoma-initiating cells [40], glioma stem cells [41] and glioma cells [42]. In addition, in this study, we demonstrated that miR-186-5p is much lower in glioma tissues than in normal brain tissues. Combined with the results of luciferase reporter assays, an RNA immunoprecipitation assay and cell functional assays, we conclude that lncRNA-DLEU2 promotes glioma progression by sponging miR-186-5p.

Though bioinformatics analysis, we identified PDK3 as a candidate target gene of miR-186-5p. PDK3 acts as an oncogene in many tumours [43, 44]; however, its role in glioma has not been previously reported. In this study, we found that PDK3 is upregulated in glioma tissues and cell lines. Furthermore, the cell experiments indicated that PDK3 could promote the clone formation, migration, and invasion of glioma cells and is regulated by miR-186-5p. In addition, we performed a series of rescue experiments and showed that miR-186-5p inhibitors could partly restore the effect of lncRNA-DLEU2 on glioma, which suggested that lncRNA-DLEU2 regulates the progression of glioma in a miR-186-5p-dependent manner. Finally, in a nude mouse model, we found that lncRNA-DLEU2 can promote tumour progression, and this promotion is related to miR-186-5p and PDK3.

In summary, our study showed that lncRNA-DLEU2 is upregulated and acts as an oncogene in glioma. A series of experiments have strongly supported that lncRNA-DLEU2 can regulate the expression of PDK3 through miR-186-5p, thus affecting the progression of glioma. This finding will provide a new direction for targeted therapy in glioma.

Disclosure of conflict of interest

None.

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