Silencing of lncRNA CCDC26 Restrains the Growth and Migration of Glioma Cells In Vitro and In Vivo via Targeting miR-203

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Gliomas are the most common primary brain tumors with high mortality. The treatment for gliomas is largely limited due to its uncomprehending pathological mechanism. Here we aimed to investigate the effect of long noncoding RNA (lncRNA) coiled-coil domain-containing 26 (CCDC26) in glioma progression. In our study, the expression of CCDC26 was found upregulated in glioma tissues and cell lines compared with normal tissues and cell lines. Further exploration detected decreased cell proliferation and increased cell apoptosis in U-251 and M059J cells transfected with CCDC26-siRNA. In addition, the silencing of CCDC26 strongly reduced the wound closing rate and the number of invasive cells compared with the scramble group. Simultaneously, the expression of miR-203 was found suppressed in glioma tissues and cells lines. Suppressed level of miR-203 was then elevated in U-251 and M059J cells transfected with CCDC26-siRNA. The result of the luciferase activity assay also showed that the luciferase activity was strongly strengthened by adding the miR-203 inhibitor into the CCDC26 WT group. Moreover, CDCC26-siRNA counteracted the effect of the miR-203 inhibitor in facilitating cell viability and mobility in U-251 cells. The in vivo experiment also revealed that CCDC26-siRNA inhibited glioma growth and metastasis. Taken together, our research indicated a CCDC26/miR-203 pathway in regulating the growth and metastasis of gliomas, providing new viewpoints and promising targets for glioma therapy.

Key words: Coiled-coil domain-containing 26 (CCDC26); miR-203; Growth; Migration; Glioma

INTRODUCTION

Gliomas are the most common primary brain tumors, and the grade IV gliomas [glioblastoma multiforme (GBM)] are highly aggressive and lethal malignant neoplasms[1,2]. Although many efforts have been made in surgical and medical therapy, the treatment for gliomas is still largely limited due to the highly metastatic characteristics of gliomas and their ability to resist current therapies[3,4]. The median survival of patients with GBM is merely 14.6 months, and the 5-year survival is 9.8%, so it is quite urgent to get a more thorough understanding of the molecular mechanisms on the growth and migration of gliomas[5,6].

Recently, more and more attention has been paid to the regulating role of long noncoding RNAs (lncRNAs) in cancer progression. For example, lncRNA antisense noncoding RNA in the INK4 locus (ANRIL) is overexpressed in bladder cancer and regulates cell proliferation and apoptosis through the intrinsic pathway[7]. Others reported that the overexpressed lncRNA maternally expressed gene 3 (MEG3) suppressed the proliferation, migration, and invasion capacities of breast cancer cells[8]. Coiled-coil domain-containing 26 (CCDC26; gene ID: 137196) is an lncRNA located on chromosome 8q24 and is conserved in primates[9]. Hirano et al. reported that overexpressed lncRNA CCDC26 promoted the growth of myeloid leukemia cells by regulating the expression of KIT (a tyrosine kinase receptor)[10]. The link between CCDC26 with low-grade glioma was also suggested by genome-wide association studies[11]. Moreover, CCDC26 was used as a diagnostic and prognostic marker for glioma patients in clinical study[12]. However, to date, the specific regulatory mechanism of CCDC26 in gliomas is still elusive.

miRNAs mediate the role of various target genes by base pairing with the 3′-untranslated region (3′-UTR) of target mRNA sequences[13]. According to a previous report, overexpressed miR-137 suppressed proliferation, inhibited cell cycle arrest, and induced cell apoptosis in human glioma cells by directly targeting Rac1[14]. miR-203,

a putative tumor suppressor gene, has been identified to be involved in the progression of many cancers. For example, miR-203 was verified to suppress cell proliferation, adhesion, and invasion in prostate cancer[15]. Zhao et al. revealed that miR-203 functioned as a tumor suppressor in ovarian cancer[16]. Additionally, He et al. demonstrated that the expression of miR-203 was downregulated in gliomas, and miR-203 might be an intrinsic regulator of tumor progression[17]. However, the related regulation mechanism of miR-203 in gliomas still needs to be investigated.

In this study, we aimed to investigate the role of CCDC26 in the growth and migration of gliomas. CCDC26 was found upregulated in glioma tissues and cell lines. In addition, the inhibition of CCDC26 suppressed the viability and motility of glioma cells by targeting miR-203. Moreover, CCDC26 small interfering RNA (siRNA) controlled the growth and metastasis of gliomas in vivo. Our study revealed the regulatory mechanism of the CCDC26/miR-203 pathway in pathogenesis of gliomas and provided new targets for glioma therapy.

MATERIALS AND METHODS

Sample Collection

Forty pairs of human gliomas and their adjacent noncancerous tissues were collected from patients who underwent surgical resection in Liaocheng People's Hospital. Before the experiment, prior consent from the patients and approval from the Institutional Ethics Committee of Liaocheng People's Hospital were obtained. Tissue samples were collected and immediately frozen in liquid nitrogen and preserved at −80°C before further use.

Cell Lines

The human glioma cells U-251 and M059J and the normal human astrocyte cell line were purchased from the American Type Culture Collection (Manassas, VA, USA). All the cell lines were cultured in RPMI-1640 (Cat. No. 11875-093; Gibco) supplemented with 10% fetal bovine serum (FBS; Life Technologies Inc., Grand Island, NY, USA) and were grown at 37°C in a humidified 5% CO_2 atmosphere.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was harvested from glioma tissues and cell lines using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and was reverse transcribed into cDNAs using the RT-PCR kit purchased from TaKaRa (Dalian, P.R. China) following the manufacturer's instructions. Quantitative real-time PCR was performed with SYBR Green PCR Master Mix reagents in the 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). The RT-PCR primers for CCDC26 and miR-203 were purchased from GeneCopoeia (San Diego, CA, USA). The raw data were presented as the relative quantity of target mRNA, normalized with GAPDH. Fold changes of CCDC26 and miR-203 were calculated by the equation $2^{-\Delta\Delta Ct}$.

Northern Blot Assays

Northern blot analysis was performed as previously described[18]. The expression levels of CCDC26 and miR-203 in glioma samples, adjacent normal tissues, glioma cell lines (U-251 and M059J), and normal human astrocyte cell line were determined by Northern blot assay.

Cell Transfection

The siRNA fragments targeting CCDC26 and inhibitors specific for miR-203 were designed and purchased from Invitrogen. The control and scramble fragments were designed as the negative control of CCDC26 and miR-203. The U-251 and M059J cells were seeded in 24-well plates (1×10^5 cells per well), followed by transfection with CCDC26-siRNA, miR-203 inhibitors, or control/scramble fragments, respectively, using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol.

Cell Proliferation Assay

Cell proliferation was assayed using the cell counting kit-8 (CCK-8; Dojindo Laboratories, Tokyo, Japan) according to the manufacturer's protocol. The U-251 and M059J cells were transfected with CCDC26-siRNA or siRNA scramble. Two days later, the cells were seeded onto 96-well plates at a density of 5,000 cells per well and were incubated for 24, 48, 72, and 96 h. Cells were then incubated with the CCK-8 solution for 2 h at 37°C according to the manufacturer's protocol. The absorbance was read at 450 nm via a microplate system. All experiments were repeated at least three times.

Evaluation of Cell Apoptosis by Flow Cytometry

Pretreated cells were seeded in six-well plates. Forty-eight hours later, cells were washed with cold PBS and were double-labeled with annexin V and propidium iodide (PI) apoptosis detection kits (Annexin-V-FITC Apoptosis Detection Kit; eBioscience) according to the manufacturer's protocol, and analyzed by a flow cytometer (Beckman Coulter, Brea, CA, USA). The apoptosis rate was evaluated for further analysis. All experiments were performed in triplicate.

Cell Migration and Invasion Analyses

Cell migration and invasion abilities were investigated through wound healing and Transwell invasion assay as previously described[19]. For the wound healing assay, the U-251 and M059J cells transfected with CCDC26-siRNA or siRNA scramble were seeded in a six-well

plate (1.5×10^6 cells/well) and cultured overnight until the cells reached 90% confluence. Then a straight scratch was created by a sterile pipette tip. Destroyed cells were rinsed off the with PBS, and the plate was cultured in medium for another 24 h. Cell migration was observed and imaged at 0 and 24 h with a digital camera (Leica DFC300FX).

For the Transwell invasion assay, pretreated U-251 and M059J cells with HAS1-siRNA or siRNA scramble (2×10^4 cells/well) were placed in a Transwell chamber with 8-μm pores coated with Matrigel (Becton-Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Cell suspension was placed in the upper chamber of the insert, and the lower chamber was filled with medium containing 10% FBS. Twenty-four hours later, noninvading cells were mechanically removed with cotton swabs, and the invasive cells on the lower chamber were stained with hematoxylin. The invading cells were quantified under a light microscope at 100× in five random fields per membrane. Each sample was assayed in triplicate.

Luciferase Activity Assay

Luciferase reporter assay was performed as described previously[20]. The 3′-UTR of the CCDC26 gene containing the putative binding sites of miR-203 was amplified by chemical synthesis and inserted into the luciferase reporter vector (pGL4.74) and named CCDC26 wild type (WT). The mutation of CCDC26 in the seed sequence was synthesized using a site-directed mutagenesis kit (Stratagene, San Diego, CA, USA). The U-251 cells were seeded onto six-well culture plates in DMEM containing 10% FBS and incubated overnight. Cells were cotransfected with 0.1 μg of Luc-CCDC26 WT or Luc-CCDC26 mutant (MUT), together with 40 nM miR-203 inhibitor or 40 nM negative control for 24 h. Luciferase activity assays were detected by a dual-luciferase reporter system according to the manufacturer's instructions (E2920; Promega, Madison, WI, USA).

Glioma Xenografts

For exploring the effect of CCDC26 on tumor growth and metastasis in vivo, a glioma xenograft mouse model was created by subcutaneous injection of 1×10^7 U-251 cells transfected with CCDC26-siRNA or siRNA scramble to SPF nude mice. After development of a palpable tumor, the tumor volume was monitored every 6 days for 30 days and assessed by measuring the two perpendicular dimensions using a caliper and the formula $(a \times b^2)/2$, where a and b are the largest and the perpendicular diameters, respectively. Then the mice were put to death, and tumor weights were assessed and photographed. Tumors from each mouse were randomly selected for immunohistochemical (IHC) analysis. All the animal experiments were performed according to relevant national and international guidelines and were approved by the animal experimental ethical committee.

Immunohistochemistry Analysis

Formalin-fixed paraffin-embedded glioma tumors were cut into 5-μm-thick paraffin sections with a microtome. The sections were deparaffinized and rehydrated according to the previous protocols[21]. Antigen retrieval was carried out in heated 10 mM citrate buffer (pH 6.0) for 10 min at 96–98°C. Slides were incubated with primary antibodies against vascular endothelial cell growth factor (VEGF; Boster Bioengineering, Wuhan, P.R. China). Corresponding mouse horseradish peroxidase (HRP)-conjugated secondary antibody was added for 1 h at room temperature. Sections were subsequently incubated with the cell and tissue staining kit HRP-DAB system (R&D Systems, Minneapolis, MN, USA) and then viewed under a bright-field microscope.

Statistical Analysis

The significance of differences between two groups was estimated using Student's *t*-test. Data are shown as mean ± standard deviation (SD) of at least three independent experiments performed in triplicate. All of the *p* values were two sided, and differences were considered statistically significant with a value of $p < 0.05$.

RESULTS

lncRNA-CCDC26 Is Overexpressed in Gliomas

In order to investigate the regulating role of CCDC26 in the pathogenesis of gliomas, the expression of CCDC26 in glioma tissues and cell lines was first detected through qPCR and Northern blotting. As shown in Figure 1A, the expression level of CCDC26 in glioma tissues was obviously higher than that of the normal tissues ($p < 0.05$). Similarly, the level of CCDC26 in glioma cell lines (U-251 and M059J) was largely upregulated compared with the astrocyte group ($p < 0.01$) (Fig. 1B). Then the change in the expression of CCDC26 was further verified at the protein level. Compared with normal tissues, the expression of CCDC26 in glioma tissues was significantly increased, detected through Northern blotting (Fig. 1C). Simultaneously, elevation of CCDC26 expression was also detected in related glioma cells compared with control cells (Fig. 1D). Statistical analysis of relative expression of CCDC26 in related tissues and cell lines was presented in the form of histograms ($p < 0.01$, $p < 0.001$) (Fig. 1E and F). The results revealed that the expression of CCDC26 in glioma tissues and cell lines is largely increased compared with normal tissues and cells lines, indicating a certain relationship between CCDC26 and the progression of gliomas.
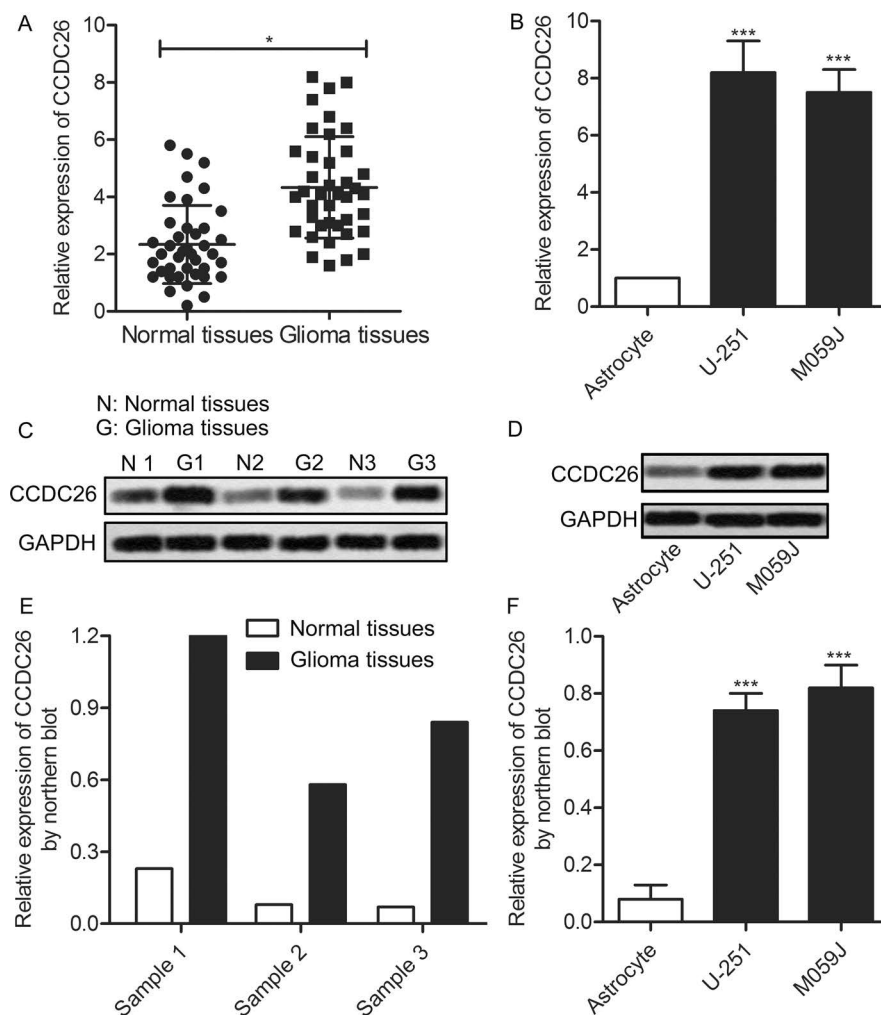
Figure 1. Long noncoding RNA-coiled-coil domain-containing 26 (lncRNA CCDC26) is overexpressed in gliomas. (A) Relative expression of CCDC26 in glioma tissues and adjacent histologically normal tissues was detected by quantitative polymerase chain reaction (qPCR) (*$p<0.05$ vs. normal tissues). (B) Relative expression of CCDC26 in glioma cell lines (U-251 and M059J), and normal human astrocytes was detected through quantitative reverse transcription (qRT)-PCR. (C) Expression of CCDC26 in glioma tissues and adjacent histologically normal tissues was detected through Northern blotting. (D) Expression of CCDC26 in above-related glioma cell lines and astrocytes was valued through Northern blotting. GAPDH was used as an endogenous reference. (E) Histogram represents the statistical analysis of relative expression of CCDC26 in glioma tissues and normal tissues. (F) Histogram represents the statistical analysis of the relative expression of CCDC26 in glioma cell lines and astrocytes. ***$p<0.001$ versus astrocytes. The bars show means±SD of three independent experiments.
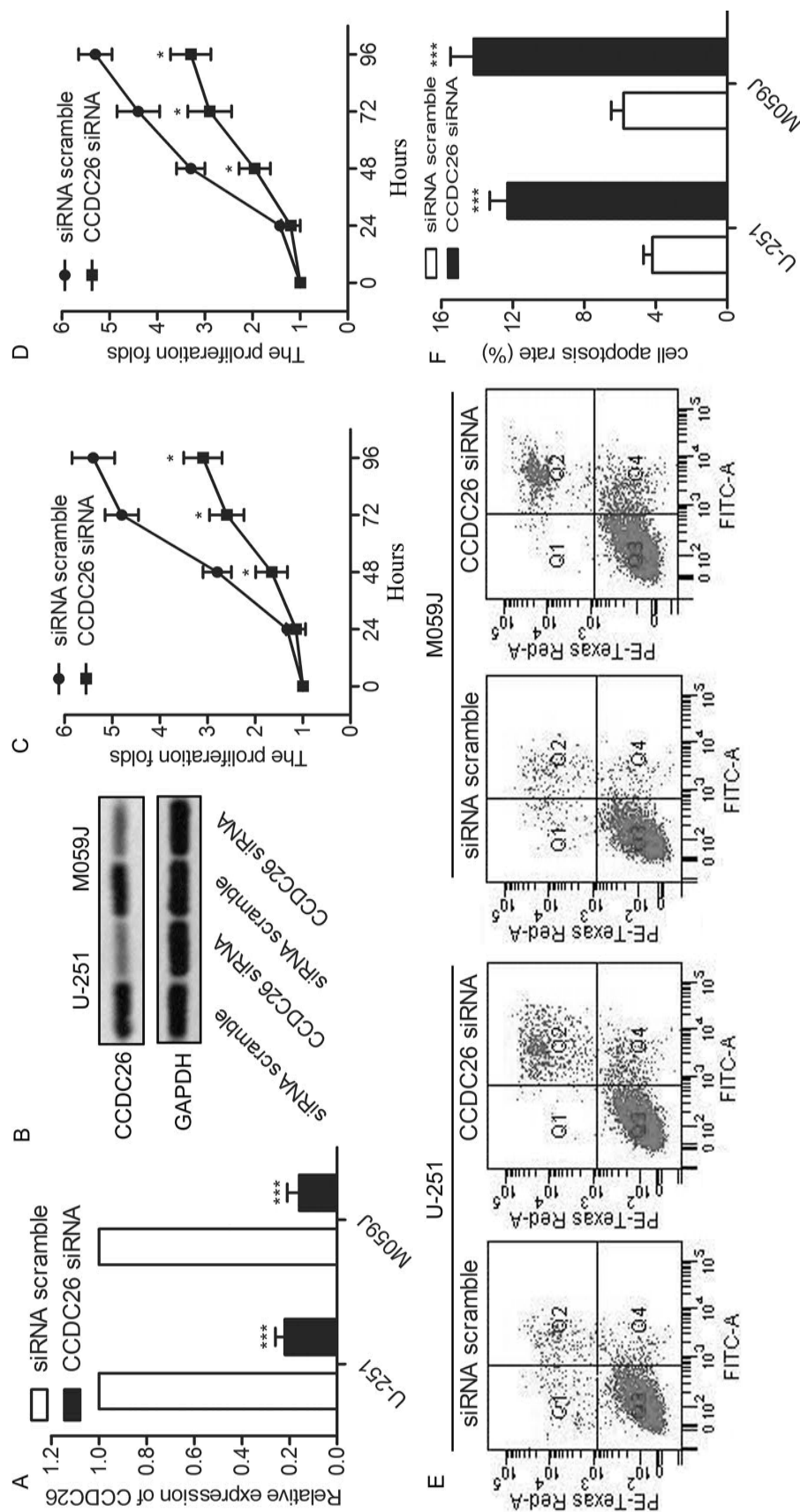
Inhibition of CCDC26 Suppresses Cell Viability in Gliomas

Having known that the expression of CCDC26 was upregulated in gliomas, to examine the function of the CCDC26 gene, knockdown was performed using specific siRNAs. U-251 and M059J cells were transfected with CCDC26-siRNA and siRNA scramble, respectively. The expression of CCDC26 was successfully suppressed by CCDC26-siRNA, as shown in Figure 2A and B ($p<0.001$). Then cell proliferation in U-251 and M059J cells was examined through the CCK-8 assay. Compared with the scramble group, cell proliferation

rate was largely suppressed by CCDC26-siRNA ($p<0.05$) (Fig. 2C and D). Moreover, the cell apoptotic rate was markedly increased in the CCDC26-siRNA group compared with the scramble group ($p<0.001$) (Fig. 2E and F). The results above indicate that inhibition of CCDC26 suppresses cell viability in gliomas.

Inhibition of CCDC26 Suppresses Cell Motility in Gliomas

After the inhibiting role of CCDC26-siRNA on cell proliferation in gliomas was verified, we explored the effect of CCDC26-siRNA on cell motility. U-251 and

Figure 2. The inhibition of CCDC26 suppresses cell viability in gliomas. U-251 and M059J cell lines were transfected with the CCDC26-siRNA or siRNA scramble, respectively. (A) Relative expression of CCDC26 in U-251 and M059J cells was detected through qRT-PCR. (B) Expression of CCDC26 in U-251 and M059J cells was detected through Northern blotting. GAPDH was used as an endogenous reference. (C, D) The numbers of cells per well in U-251 and M059J cells were measured every 24 h (0–96 h) through the cell counting kit-8 (CCK-8) assay at 570 nm. Statistical analysis of the proliferation folds was presented in the form of a line chart (*$p < 0.05$ vs. scramble group). (E) Cell apoptosis in U-251 and M059J cells was examined through flow cytometry. (F) Histogram represents the statistical analysis of cell apoptosis rate. ***$p < 0.01$ versus the scramble group. The bars show means±SD of three independent experiments.

M059J cells were transfected with the CCDC26-siRNA and siRNA scramble, respectively. Wound healing assay exhibited that the closing rate of scratch wounds was significantly decreased in the CCDC26-siRNA group compared with the scramble group. The wound closing rate in every group was aggregated and shown in the form of histograms ($p < 0.01$) (Fig. 3A and B). Similarly, the number of invasive cells in the CCDC26-siRNA group was largely decreased compared with the scramble group detected through the Transwell invasion assay ($p < 0.001$) (Fig. 3C and D). We therefore suggest that the inhibition of CCDC26 suppresses cell motility in gliomas.

*miR-203 Is a Direct Target of CCDC26*

Recent studies have suggested that lncRNAs participate in molecular regulation pathways through interacting with miRNA[22]. Therefore, the related regulation mechanism of CCDC26 in gliomas was investigated. Interestingly, miR-203 was found suppressed in glioma tissues and cell lines at the mRNA level compared with normal tissues and cell lines ($p < 0.05$, $p < 0.01$, $p < 0.001$) (Fig. 4A and B). The expression of miR-203 in related tissues and cell lines was also valued through Northern blotting. As shown in Figure 4C, the level of CCDC26 was obviously downregulated in glioma tissues and cells
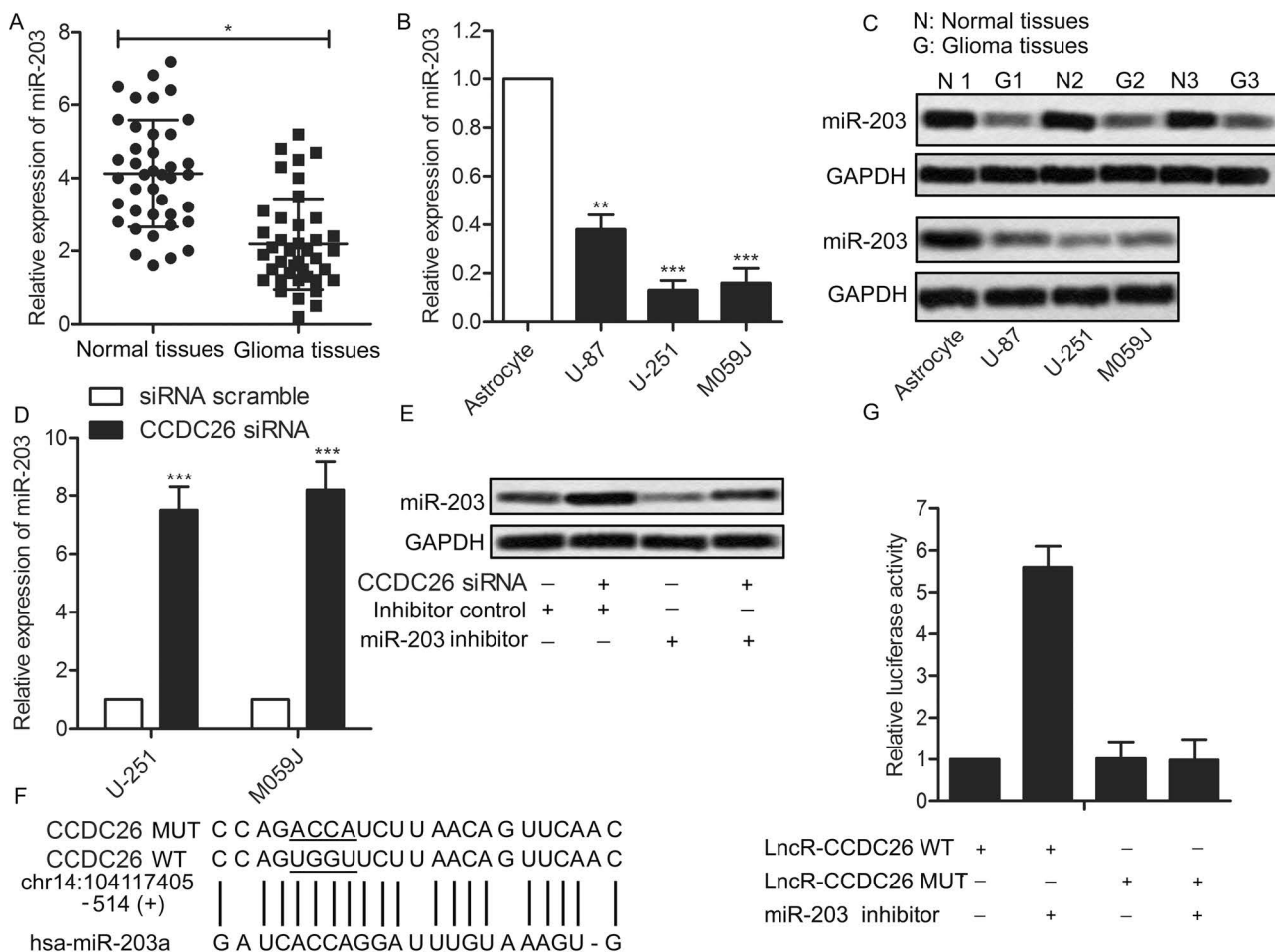
compared with the control. Then U-251 and M059J cells were transfected with CCDC26-siRNA and siRNA scramble, respectively. The expression of miR-203 in U-251 and M059J cells was strongly elevated by CCDC26-siRNA compared with the scramble group ($p < 0.001$) (Fig. 4D). Moreover, the elevated expression of miR-203 was suppressed by adding the miR-203 inhibitor into U-251 cells transfected with CCDC26-siRNA (Fig. 4E). These results indicated a targeting relationship between CCDC26 and miR-203. Subsequently, the complementary site of miR-203 in CCDC26 RNA was predicted through bioinformatics analysis (Fig. 4F). Luciferase activity assay was conducted to further verify the targeting relationship. The result showed that the relative luciferase activity was largely strengthened by cotransfecting the miR-203 inhibitor in the CCDC26 WT group compared with the control group ($p < 0.01$) (Fig. 4G). These results revealed that miR-203 is a direct target of CCDC26.

CCDC26-siRNA Neutralizes the Facilitating Role of the miR-203 Inhibitor on Cell Viability and Motility in Gliomas

Considering that miR-203 is a direct target of CCDC26 in gliomas, the regulating role of the CCDC26/miR-203 pathway on cell viability and motility was further explored.
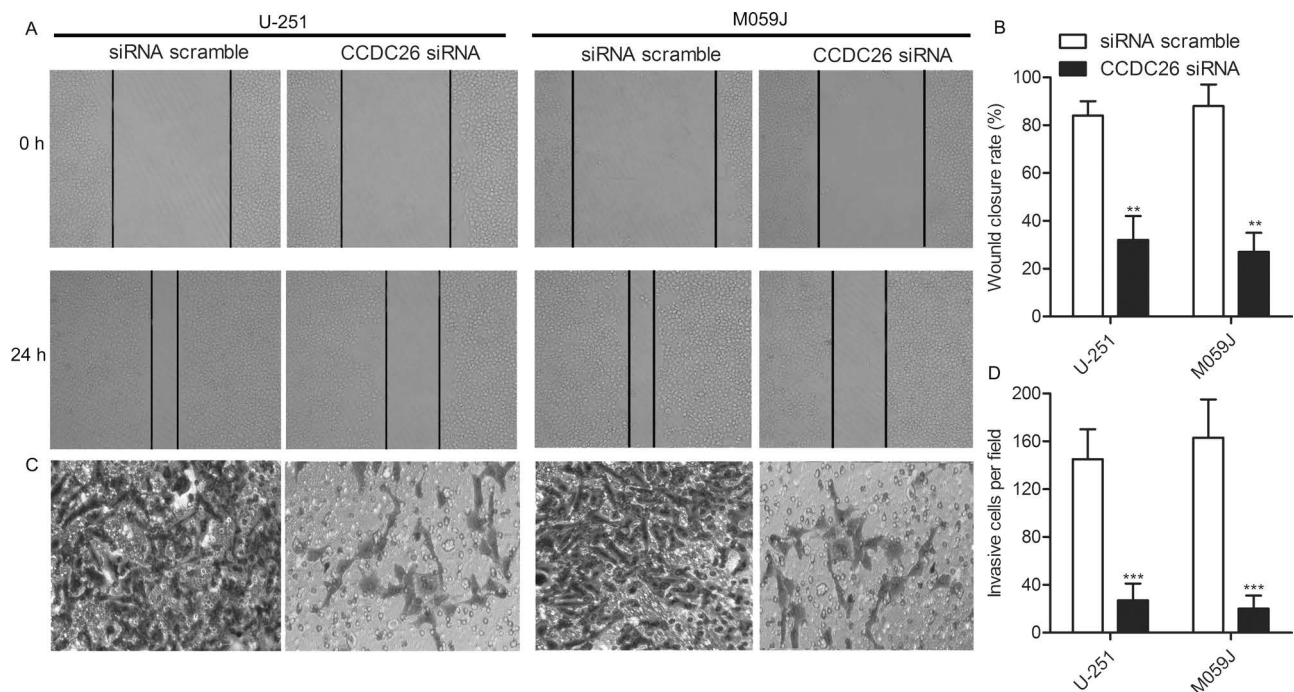
Figure 3. The inhibition of CCDC26 suppresses cell motility in gliomas. U-251 and M059J cell lines were transfected with CCDC26-siRNA or siRNA scramble, respectively. (A) The migration rate of U-251 and M059J cell was detected through wound healing assays. Cells were photographed at 0 and 24 h after transfection. (B) Histogram represents the statistical analysis of wound healing assays. (C) Transwell invasion assay was conducted to observe the invasive cells in U-251 and M059J cells. (D) Histogram represents the statistical analysis of Transwell invasion assay. **$p < 0.01$, ***$p < 0.001$ versus the scramble group. The bars show means ± SD of three independent experiments.

Figure 4. miR-203 is a direct target of CCDC26. (A) Relative expression of miR-203 in glioma tissues and adjacent histologically normal tissues was detected by qPCR (*$p < 0.05$ vs. normal tissues). (B) Relative expression of miR-203 in glioma cell lines (U-251 and M059J) and normal human astrocytes was detected through qRT-PCR (**$p < 0.01$, ***$p < 0.001$ vs. astrocyte). (C) Expression of miR-203 in above-related tissues and cell lines was detected through Northern blotting. GAPDH was used as an endogenous reference. (D) U-251 and M059J cell lines were transfected with CCDC26-siRNA or siRNA scramble, respectively. Relative expression of miR-203 in U-251 and M059J cells was detected through qRT-PCR (***$p < 0.001$ vs. scramble group). (E) U-251 cells were transfected with CCDC26-siRNA and/or miR-203 inhibitor or inhibitor control. Twenty-four hours posttransfection, the expression of miR-203 in U-251 cells was valued through Northern blotting. GAPDH was used as an endogenous reference. (F) Complementary sites of miR-203 in CCDC26 mRNA were predicted through bioinformatics analysis. (G) U-251 cells were transfected with CCDC26 WT/MUT luciferase reporter and/or miR-203 inhibitor or inhibitor control. Luciferase reporter assay was conducted to detect the luciferase activity in U-251 cells [**$p < 0.01$ vs. the CCEC26 wild-type (WT) group]. The bars show means±SD of three independent experiments.

U-251 cells were transfected with the CCDC26-siRNA and/or miR-203 inhibitor or inhibitor control. As shown in Figure 5A, elevated cell proliferation rate was suppressed by the addition of CCDC26-siRNA in U-251 cells transfected with the miR-203 inhibitor ($p < 0.05$). Similarly, suppressed cell apoptosis rate was elevated by adding CCDC26-siRNA in U-251 cells transfected with the miR-203 inhibitor ($p < 0.05$) (Fig. 5B). The high wound closing rate was reduced by CCDC26-siRNA in U-251 cells transfected with the miR-203 inhibitor ($p < 0.05$, $p < 0.01$) (Fig. 5C). The increased number of invasive cells observed through the Transwell invasion assay was

also decreased by the cotransfection of CCDC26-siRNA and miR-203 inhibitor in U-251 cells ($p < 0.001$) (Fig. 5D and E). The results revealed the tumor suppressor role of CCDC26-siRNA by targeting miR-203 in gliomas.
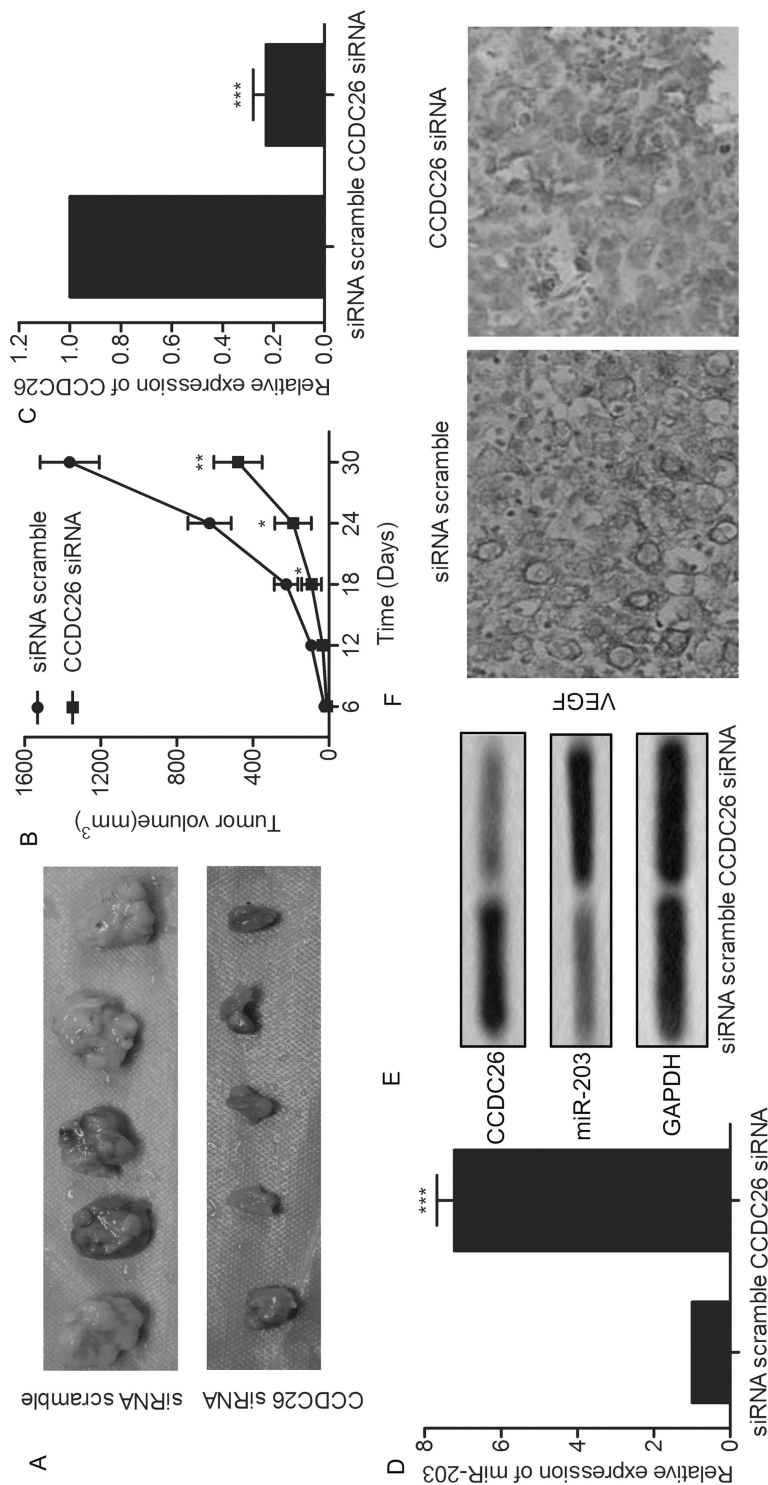
CCDC26-siRNA Depresses Tumor Growth and Metastasis In Vivo

To investigate the effect of CCDC26 on tumor growth and metastasis in vivo, U-251 cells lines were transfected with CCDC26-siRNA or siRNA scramble. A glioma xenograft mouse model was created by injecting recombinant U-251 cell lines to SPF nude mice subcutaneously.
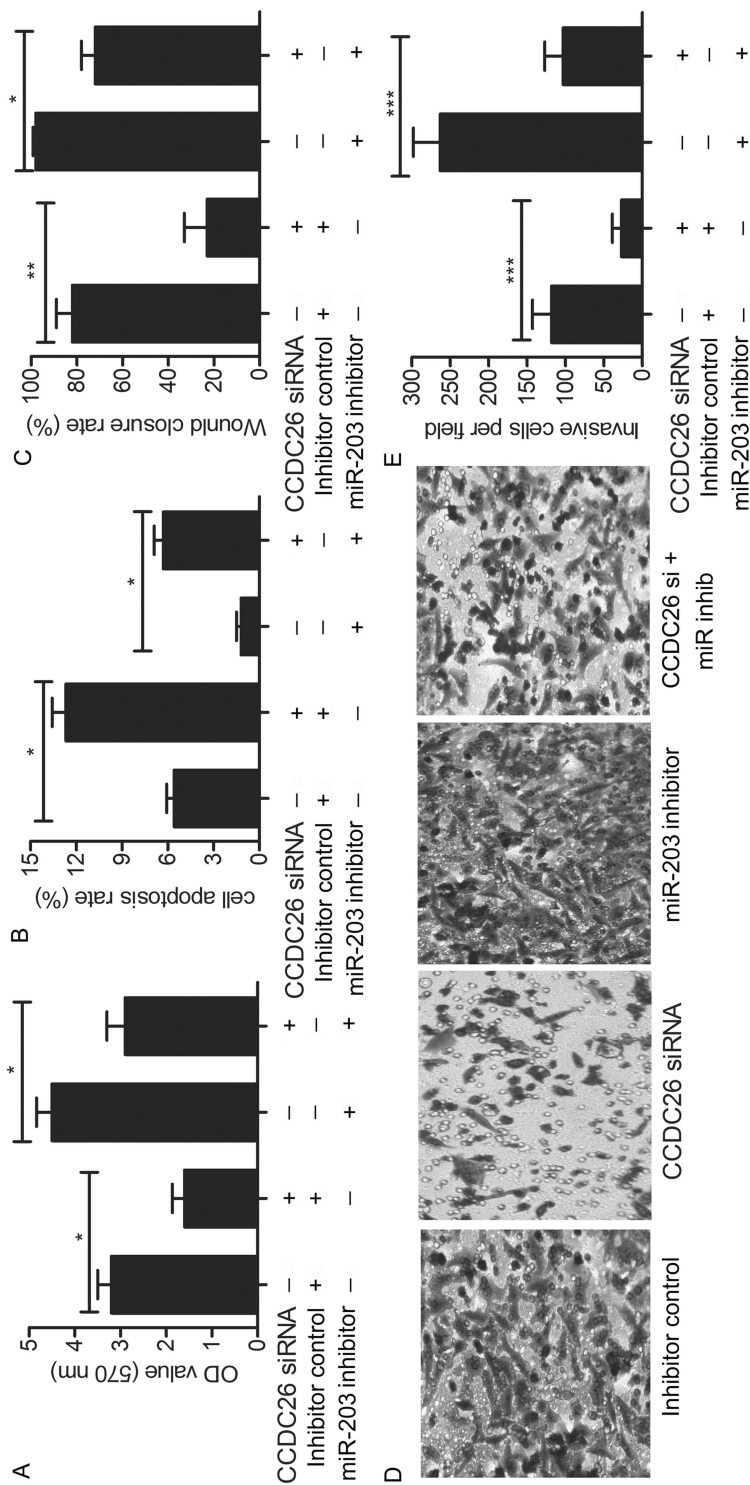
Figure 5. CCDC26-siRNA neutralizes the role of miR-203 on cell viability and motility in gliomas. U-251 cells were transfected with CCDC26-siRNA and/or siRNA inhibitor or inhibitor control. (A) Cell proliferation in U-251 cells was detected through the CCK-8 assay, and the OD value was measured at 570 nm. The statistical results are shown in the form of a histogram (*$p<0.05$). (B) Cell apoptosis in U-251 cells was detected through flow cytometry. Histogram represents the statistical analysis of cell apoptosis rate (*$p<0.05$, **$p<0.01$). (C) The migration rate of U-251 cells was examined through wound healing assays. The histogram represents the statistical analysis of wound healing assays (*$p<0.05$, **$p<0.01$). (D) Invasive cells in U-251 cells were observed through Transwell invasion assay. (E) Histogram represents the statistical analysis of Transwell invasion assay (***$p<0.001$). The bars show means±SD of three independent experiments.

Figure 6. CCDC26 depresses tumor growth and metastasis in vivo. U-251 cells were transfected with CCDC26-siRNA or siRNA scramble. A glioma xenograft mouse model was created by subcutaneous injection of U-251 cells to SPF nude mice. (A) Representative images of glioma xenograft tissues in each group ($n=5$). (B) The diameter of glioma xenograft tissues in each group was measured every 6 days from tumor formation to 30 days. Tumor growth curve is shown as means±SD. (C) Relative expression of CCDC26 in glioma xenograft tissues from each group was detected through qRT-PCR. (D) Relative expression of miR-203 in glioma xenograft tissues from each group was detected through qRT-PCR. (E) Expression of CCDC26 and miR-203 in glioma xenograft tissues from each group was detected through Northern blotting. GAPDH was used as an endogenous reference. (F) Expression of migration marker protein vascular endothelial cell growth factor (VEGF) in formalin-fixed, paraffin-embedded glioma tumors from each group was detected through IHC analysis. $*p<0.05$, $**p<0.01$, $***p<0.001$ versus the scramble group. The bars show means±SD of three independent experiments.

Respective tumors in each group were removed and photographed ($n = 5$) (Fig. 6A). Average tumor volume was obviously smaller in the CCDC26-siRNA group compared with the siRNA scramble group ($p < 0.05$, $p < 0.01$) (Fig. 6B). In addition, relative expression of CCDC26 was suppressed, and relative expression of miR-203 was elevated in the CCDC26-siRNA group tumor tissues compared with the scramble tumor tissues ($p < 0.001$) (Fig. 6C–E). Moreover, the expression level of migration marker proteins VEGF was also significantly decreased in the CCDC26-siRNA group compared with the scramble group through IHC analysis (Fig. 6F). On the basis of the results, we deduced that CCDC26-siRNA depresses tumor growth and metastasis in vivo.

DISCUSSION

Gliomas, as the most common primary malignant tumor, result in high morbidity due to unlimited growth and strong invasive ability. Despite efforts being made to improve therapeutic strategies, the prognosis of gliomas is still poor. In this study, lncRNA CCDC26 was found upregulated in gliomas and regulated the progression of gliomas by targeting miR-203, providing new sights and targets for glioma treatment. In previous reports, a number of lncRNAs acted as predictive biomarkers in gliomas or regulated the pathogenesis of gliomas. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)[23], highly upregulated in liver cancer (HULC)[24], and urothelial carcinoembryonic antigen 1 (UCA1)[25] are all lncRNAs associated with the malignant status and poor prognosis in gliomas. lncRNA taurine-upregulated gene 1 (TUG1)[26], MALAT1[27], and colorectal neoplasia differentially expressed (CRNDE)[28] acted as cancer promotors or suppressors by regulating glioma cell growth and invasion. The function of the lncRNA-CCDC26 used in this study has not been widely studied until now. The expression of CCDC26 was found upregulated in pancreatic cancer (PC), and the knockdown of CCDC26 suppressed proliferation and induced apoptosis in PC cells[9]. Simultaneously, others reported that the CCDC26 gene was amplified in acute myeloid leukemia (AML) cells, and upregulated CCDC26 was involved in the growth of AML cells[29]. However, to date whether and how CCDC26 is related to the progression of gliomas have not been clearly elucidated. In this study, we investigated the regulating role of CCDC26 in gliomas for the first time. In support of a previous concept, the expression of CCDC26 was found upregulated in glioma tissues and cell lines (U-251 and M059J) compared with normal tissues and human astrocyte cells. The aberrant expression of CCDC26 indicated a certain relationship between CCDC26 and gliomas.

The role of lncRNAs in regulating cell viability and motility has been evidenced in many cancers. As reported, the lncRNA-CPS1 intronic transcript 1 (CPS1-IT1) suppressed cell proliferation and metastasis in human lung cancer and was used as a biomarker for early diagnosis[30]. Besides, downregulation of lncRNA-PEG10 inhibited cell proliferation and invasion and promoted cell apoptosis in esophageal cancer[31]. To further investigate the possible biological function of CCDC26 in glioma tumorigenesis, the high expression level of CCDC26 was knocked down by specific siRNA in our study. The results of the CCK-8 assay and flow cytometry indicated that CCDC26-siRNA suppressed cell viability and motility in vitro. Simultaneously, siRNA-mediated knockdown of CCDC26 resulted in diminished wound closing rate and reduced number of invasive cells in U-251 and M059J cells. The results above identify that CCDC26-siRNA inhibits cell viability and motility in glioma cells and would be a novel target for gliomas treatment.

According to literature retrieval, miR-203 is involved in a variety of cancers such as human gastric cancer[32], non-small cell lung cancer[33], breast cancer[34], and gliomas[17]. miR-203 acted as an important predictive biomarker by regulating cell proliferation, invasion, and migration. The regulating role of miRNAs depends on the combination with 3′-UTR of their targeted mRNAs to cause gene degradation or translational repression[35]. For example, miR-203 acted as the target of lncRNA UCA1 to promote hepatocellular carcinoma progression by regulating the expression of Snail2[36]. Others demonstrated that miR-203 played tumor-suppressive roles by downregulating the expression of lncRNA HULC in hepatocellular carcinoma[37]. Interestingly, the expression of miR-203 was found suppressed in glioma tissues and cell lines compared with the control in our study. Besides that, the expression of miR-203 was upregulated by CCDC26-siRNA in U-251 and M059J cells. The targeting relationship between miR-203 and CCDC26 was further supported by bioinformatics analyses and the luciferase reporter assay. The results above indicate that miR-203 is a direct target of CCDC26 in gliomas.

In previous reports, miR-203 regulated cell viability and motility in kinds of cancers. For example, miR-203 was reported to facilitate tumor growth and metastasis by targeting fibroblast growth factor 2 in breast cancer[38]. Others also demonstrated that miR-203 functioned as a tumor suppressor to control cell proliferation, migration, and invasion by targeting of fibroblast growth factor 2 (FGF2) in human renal cancer[39]. Moreover, overexpressed miR-203 inhibited cell proliferation, migration, and invasion and increased cell apoptosis in esophageal cancer cells by downregulating the expression of miR-21[40]. Similarly, in our study, cell proliferation rate was elevated, and cell apoptosis rate was suppressed in the miR-203 inhibitor group, but the cotransfection of CCDC26-siRNA weakened the facilitating effect of the miR-203 inhibitor

on cell viability. Apart from that, increased wound closing rate and number of invasive cells were also decreased by adding CCDC26-siRNA into U-251 cells transfected with the miR-203 inhibitor. The results above suggest that CCDC26-siRNA counteracts the role of the miR-203 inhibitor on cell viability and motility in gliomas.

The regulating effects of lncRNAs on tumor growth and metastasis have also been proven in different in vivo tumor models. For example, the lncRNA MIR4697 host gene promoted tumor growth and metastasis in a xenograft model of ovarian cancer via the ERK and AKT signaling pathways[41]. lncRNA SPRY4-IT1 was overexpressed in bladder cancer and regulated tumor growth and metastasis in bladder cancer[42]. Therefore, the effect of CCDC26 on glioma growth and metastasis in vivo was further explored. In our study, glioma volume and growth rate were obviously decreased by CCDC26-siRNA. Expression of miR-203 and CCDC26 was also suppressed by CCDC26-siRNA in vivo. The expression of migration marker protein VEGF was largely decreased in the CCDC26-siRNA group tissues compared with the scramble group tissues. The results demonstrate that CCDC26-siRNA suppresses the growth and metastasis of gliomas in vivo.

Taken together, our research found that the expression of lncRNA CCDC26 was upregulated in glioma tissues and cell lines. Cell viability and motility were suppressed by CCDC26-siRNA in glioma cell lines U-251and M059J. Further researches revealed that miR-203 was a direct target of CCDC26, and CCDC26-siRNA counteracted the role of the miR-203 inhibitor on cell viability and motility. Moreover, CCDC26-siRNA was identified to suppress glioma growth and metastases in vivo. Our research may provide a new perspective and new targets for glioma treatment.

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