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Long non-coding RNA LINC00346 regulates proliferation and apoptosis by targeting miR-128-3p/SZRD1 axis in glioma

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Abstract. – OBJECTIVE: Long non-coding RNAs (lncRNAs) participate in multiple processes of malignant tumors, including glioma. In this study, we aimed to explore the effect of LINC00346 on glioma and its underlying mechanism.

MATERIALS AND METHODS: The Cancer Genome Atlas (TCGA) and the Chinese Glioma Genome Atlas (CGGA) databases were used to analyze the expression patterns and survival risk of LINC00346, miR-128-3p and SUZ RNA binding domain containing 1 (SZRD1) in glioma tissues. The binding sites were predicted by bioinformatic databases, and then, validated by Dual-Luciferase assay and RNA immunoprecipitation (RIP). qRT-PCR and Western blot were performed to evaluate the gene expression levels. CellTiter-Glo® and colony formation assays were used to detect the proliferation of glioma cells. Flow cytometric analysis was used to evaluate the apoptosis of glioma cells. The xenograft models were established to investigate the impact of LINC00346 on tumor growth *in vivo*.

RESULTS: We found that both LINC00346 and SZRD1 expression were negatively related to the poor overall survival rate in glioma patients. However, miR-128-3p showed the opposite effect of survival outcomes. LINC00346 knockdown remarkably restrained cell proliferation both *in vitro* and *in vivo*, as well as inducing apoptosis by acting as a molecular sponge of miR-128-3p. Moreover, miR-128-3p bound to SZRD1 3'-UTR in a sequence-specific manner. In addition, LINC00346 knockdown significantly inhibited the expression of SZRD1 and the inhibition could be reversed by miR-128-3p mimics. Furthermore, cell proliferation and apoptosis affected by LINC00346 were partially rescued by modulating miR-128-3p or SZRD1 expression.

CONCLUSIONS: LINC00346/miR-128-3p/SZRD1 axis played a crucial role in modulating the malignant progression of glioma, which may serve as a prognostic indicator and a probable therapeutic target for glioma.

Key Words:
Long non-coding RNA, LINC00346, MiR-128-3p, SZRD1, Glioma, Apoptosis.

Introduction

Glioma accounts for ~70% of malignant primary intracranial tumors in adults[1]. Although the development of three main therapeutic methods, including maximal safe resection, chemotherapy and radiotherapy have remarkably improved, the long-term survival outcomes of glioma patients remain unsatisfactory[2]. Thus, a deep understanding of the molecular mechanisms involved in tumorigenesis and progression is crucial for the treatment of glioma.

Long non-coding RNAs (lncRNAs), a group of transcripts longer than 200 nucleotides, were involved in various pathophysiological processes, such as proliferation, invasion and apoptosis[3]. MicroRNAs are transcripts of approximately 22 nucleotides, which suppress target genes expression by binding the 3' untranslated region (3'-UTR) of the target genes[4]. Both lncRNAs and microRNAs were reported to participate in the glioma progression[5,6].

LINC00346 was firstly identified as a prognosis biomarker of hepatocellular carcinoma in 2015[7]. Since then, it was reported as a tumor suppressor in the other seven different kinds of tumors[8-14]. MiR-128-3p was first discovered as an oncogenic microRNA in leukemia[15]. Qu et al[16] revealed that miR-128-3p overexpression inhibited cell proliferation and apoptosis in glioma. In addition, SZRD1, also known as C1orf144, was found as a highly conserved protein, as well as a tumor suppressor in cervical cancer according

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to the unique biological function investigation. However, the role of LINC00346 and SZRD1 in glioma and their regulatory network remained unclear.

In our study, the expression of LINC00346, miR-128-3p and SZRD1 were analyzed in glioma tissues with public databases. Besides, the effects on regulating cell proliferation and apoptosis of glioma and the regulatory mechanisms among LINC00346, miR-128-3p and SZRD1 were clarified in detail. Our findings explored a novel regulatory network of LIN00346/miR-128-3p / SZRD1 axis, which may provide a therapeutic strategy for glioma.

Materials and Methods

Bioinformatic Analysis

Differential expression and survival risk of LINC00346, miR-128-3p and SZRD1 in glioma patients were assessed by analyzing the Gene Expression Profiling Interactive Analysis (GEPIA) or CGGA databases[17,18]. The Encyclopedia of RNA Interactomes (ENCORI) was applied to analyze the correlation expression between LINC00346, miR-128-3p and SZRD1 in glioma tissues[19]. Both ENCORI and TargetScan were used to predict the potential binding sites between LINC00346 and miR-128-3p, as well as miR-128-3p and SZRD1.

Cell Culture

The human normal astrocyte cell line HEB was purchased from Beijing winter song Boye Biotechnology (Beijing, China) and the human glioma cells (U87, U251, and U343) were achieved from Dr. Xin Chen. These cells were cultured in a humidified incubator at 37°C. TT150630 and TT150714 were patient-derived diffuse intrinsic pontine glioma cell lines and cultured as previously described[20]. All of these cell lines were confirmed to be negative for mycoplasma.

Cell Transfection

Short-hairpin RNAs (shRNAs) against LINC00346 (shLINC-1: GCTCACTACAACCTCCACTTT; shLINC-2: CCTTACGAGGCATGTTTCCTT; shLINC-3: CAAGCGGAAGGAAGTGTGTTT) and SZRD1 (shSZRD1-1: CCCATTGTGATTCAGGACGAT; shSZRD1-2: GTGATTCAGGACGATAGCCTT; shSZRD1-3: CCAGGCTTACTACTTCTAGAA) were constructed in FUGW-H1 (H1) lentiviral plasmid as previously described[21] and H1 was performed as control. Full-length LINC00346 and SZRD1 were constructed in pLVX-IRES-Zs Green1 and the vector was performed as a control. To package lentivirus, the lentiviral constructs were co-transfected using Neofect (Neofect Biotech, Beijing, China) with plasmids of psPAX2 and pMD2.G into 293FT cells and the following steps were mentioned before[22]. MiR-128-3p mimics and inhibitor, as well as their corresponding negative controls, were designed and synthesized by GenePharma Co., Ltd (Shanghai, China). The special oligonucleotides were transfected into related cell lines using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Extraction of Total RNA and qRT-PCR Analysis

Total RNA was isolated from cultured cells using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. qRT-PCR was carried out in a Bio-Rad CFX384 system (Bio-Rad, Hercules, CA, USA) and SYBR Green (CWBIO, Beijing, China) was utilized to examine the LINC00346, SZRD1 and GAPDH expression levels. TaqMan MicroRNA Reverse Transcription kit and TaqMan Universal Master Mix II (Applied Biosystems, Foster City, CA, USA) were used to detect the expression levels of miR-128-3p and U6. Relative gene expression was figured out by $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH or U6. The specific primer sequences information is listed below: LINC00346 forward: 5'-CACCATGTTGGCCAGGCTGGT-3'; LINC00346 reverse: 5'-GGCCAAAGAGTGACCATCATC-3'; SZRD1 forward: 5'-GGAAATAGACAGACGGTTGG-3'; SZRD1 reverse: 5'-CGGCAGTAGGAATACAGAAGC-3'; miR-128-3p forward: 5'- CTGGTAGGTCACAGTGAACCG-3'; miR-128-3p reverse: 5'- TCAACTGGTGTCGTGGAGTC-3'; GAPDH forward: 5'-GATCATCAGCAATGCCTCCT-3'; GAPDH reverse: 5'-TGAGTCCTTCCACGATACCA-3'; U6 forward: 5'-CTCGCTTCGGCAGCACATA-3'; U6 reverse: 5'-CGCTTCACGAATTTGCGTG-3'.

Cell Proliferation and Colony Formation Assay

U87 and U251 cells were seeded in 96-well plate (2,000 cells per well) and cultivated at 37°C. At indicated time, the CellTiter-Glo® assay (Promega, Madison, WI, USA) was used to detect

the cell viability and the luminescence signal was measured by a TECAN Infinite 2000 plate reader (TECAN, Maennedorf, Zürich, Switzerland). To perform colony formation assay, 2,000 cells/well were cultured in triplicates in 6-well plates. Seven days later, methanol was used to fix the cells and 0.5% crystal violet (Sangon Biotech, Shanghai, China) was used to stained. These plates were scanned by an electronic scanner (Fuji Xerox, Tokyo, Japan).

Flow Cytometry

U87 and U251 cells transfected with indicated plasmids were collected gently for apoptosis analysis. PE-conjugated Annexin V and 7-AAD (Beyotime Biotechnology, Shanghai, China) were added and the samples were incubated at room temperature for 20 min. Finally, CytoFLEX (Beckman Coulter, Inc, Brea, CA, USA) was used to analyze the stained cells according to the manufacturers' instructions.

Western Blot Analysis

The cells were lysed in RIPA buffer on a 4°C-shaker overnight. Following quantification using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), equal amounts of protein were separated by SDS-PAGE electrophoresis and transferred to PVDF membranes (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the membranes were probed at 4°C overnight with primary antibodies (1:1000) as below: Bax, Bcl-2, Cleaved Caspase-3 (Cell Signaling Technology, Danvers, MA, USA), SZRD1 (Novus Biologicals, Centennial, CO, USA) and GAPDH (abmgoodchina Inc, Zhenjiang, Jiangsu, China). The membrane was washed twice with TBST and incubated with HRP-labeled secondary antibodies (CWBIO). Signals were then detected using enhanced chemiluminescence reagents (ECL; Thermo Fisher Scientific, Waltham, MA, USA). GAPDH was used as a loading control.

Dual-Luciferase Reporter Assay

The predicted miR-128-3p binding sequence in LINC00346 and SZRD1 3'-UTR and their corresponding mutant sequence were cloned into pmirGLO Vector to construct Luciferase reporter vector (LINC00346-WT or -MU and SZRD1-WT or -MU). Then, U251 and U87 cells were co-transfected with miR-128-3p mimics or miR-NC by using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Luciferase activity was evaluated using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) at 48 hours post-transfection.

RNA Immunoprecipitation (RIP)

Based on the manufacturer's protocol, the EZ-Magna RIP kit (Millipore, Billerica, MA, USA) was applied to perform RIP assay. Briefly, the cells were scraped off from the plates and dissolved in RIP lysis buffer. The cell lysate was kept with RIP magnetic beads conjugated with anti-Ago2 antibody (Millipore, Billerica, MA, USA). Meanwhile, beads with anti-human immunoglobulin G (IgG) antibody (Millipore, Billerica, MA, USA) was used as control. Finally, the existence of the binding targets was verified by qRT-PCR.

Animal Experiment

All research methods were conducted strictly following the protocol of Care and Use of Laboratory Animals. Moreover, approval from the Committee on the Ethics of Animal Experiments of Beijing Tiantan Hospital was also achieved. U87 cells at the logarithmic growth phase were collected and subcutaneous injected into the four-week-old BALB/C nude mice (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China, 10^6 cells/mouse). At the end of the 4^{th} week, the tumors were completely dissected and weighted.

Statistical Analysis

All assays were conducted for at least three times. All data were expressed as the mean ± standard deviation (SD), and GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. Image Lab 4.0 (Bio-Rad, Hercules, CA; USA) was applied for the quantification of immunoblot bands. The Student *t*-test or one-way analysis of variance (ANOVA) was used to analyze the results. $p < 0.05$ was considered as statistical difference.

Results

Upregulation of LINC00346 Was Associated with Poor Prognosis of Glioma

Firstly, to estimate the clinical significance of LINC00346 in glioma, both TCGA and CGGA databases were used to determine the expression level and overall survival time. We found that

upregulation of LINC00346 predicted a higher grade of glioma (Figure 1A). Moreover, the overall survival analysis based on TCGA and CGGA indicated that patients with a higher level of LINC00346 showed a poorer outcome than patients with lower levels (Figure 1B, C). Next, qRT-PCR detected the expression of LINC00346 in five glioma cell lines U87, U251, U343, TT150630, TT150714 and a normal astrocyte cell line (Figure 1D). The five glioma cell lines displayed a higher expression level of LINC00346 compared with astrocyte HEB. Two of the most significant upregulation cell lines, U87 and U251, were selected for further investigation. Together, these results demonstrated that aberrant expression of LINC00346 was evident in glioma tissues and cells, and also remarkably associated with clinical outcome of glioma patients.

LINC00346 Regulated Cell Proliferation and Apoptosis In Vitro

To explore the role of LINC00346 in glioma cells, loss-of-function experiments were conducted in U87 and U251 cell lines by infection with lentivirus specifically targeting LINC00346. The efficiencies of silencing in these cells were evaluated with qRT-PCR (Figure 2A). Both shLINC-1 and shLINC-2 were able to decrease LINC00346 expression efficiently. Next, the CellTiter-Glo® assay suggested that suppression of LINC00346 resulted in a significant decrease of cell viability in glioma cells (Figure 2B). In line with this result, clonogenic capacity was inhibited in the LINC00346 knockdown glioma cells (Figure 2C). Moreover, LINC00346 knockdown distinctly induced cell apoptosis measured by flow cytometry (Figure 2D) and Western blot (Figure 2E).

Silencing of LINC00346 Inhibited Glioma Proliferation In Vivo

To further validate that LINC00346 could affect cell growth, the glioma xenograft mouse models were established using immunodeficient mice. Tumor weight in the LINC00346 knockdown group was remarkably decreased compared with the control (Figure 2F, G). Collectively, the knockdown of LINC00346 suppressed glioma growth both *in vitro* and *in vivo*.

LINC00346 Functions As A Competing Endogenous RNA (ceRNA) of MiR-128-3p in Glioma

Since numerous lncRNAs functioned as ceRNAs in various cancers[23], we supposed that

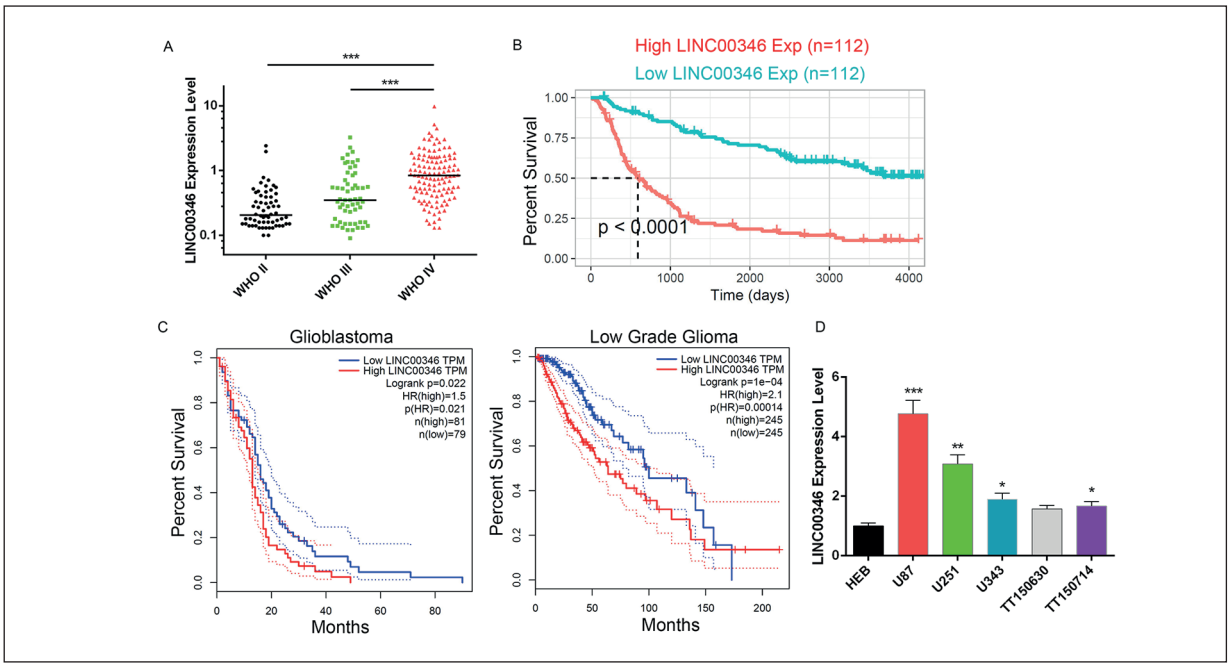

Figure 1. The clinical significance of LINC00346 in glioma. **A,** LINC00346 expression in glioma samples classified by tumor grade. **B,** Overall survival in glioma patients (n = 224) with low (n=112) or high (n=112) LINC00346 expression. Data are obtained by analyzing the CGGA database. **C,** Kaplan–Meier survival curves of patients with high and low LINC00346 expression in glioblastoma (left, n=160) or low-grade glioma (right, n=490). Data are obtained by analyzing the TCGA database through GEPIA. **D,** LINC00346 expression in human normal astrocyte and five glioma cell lines. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

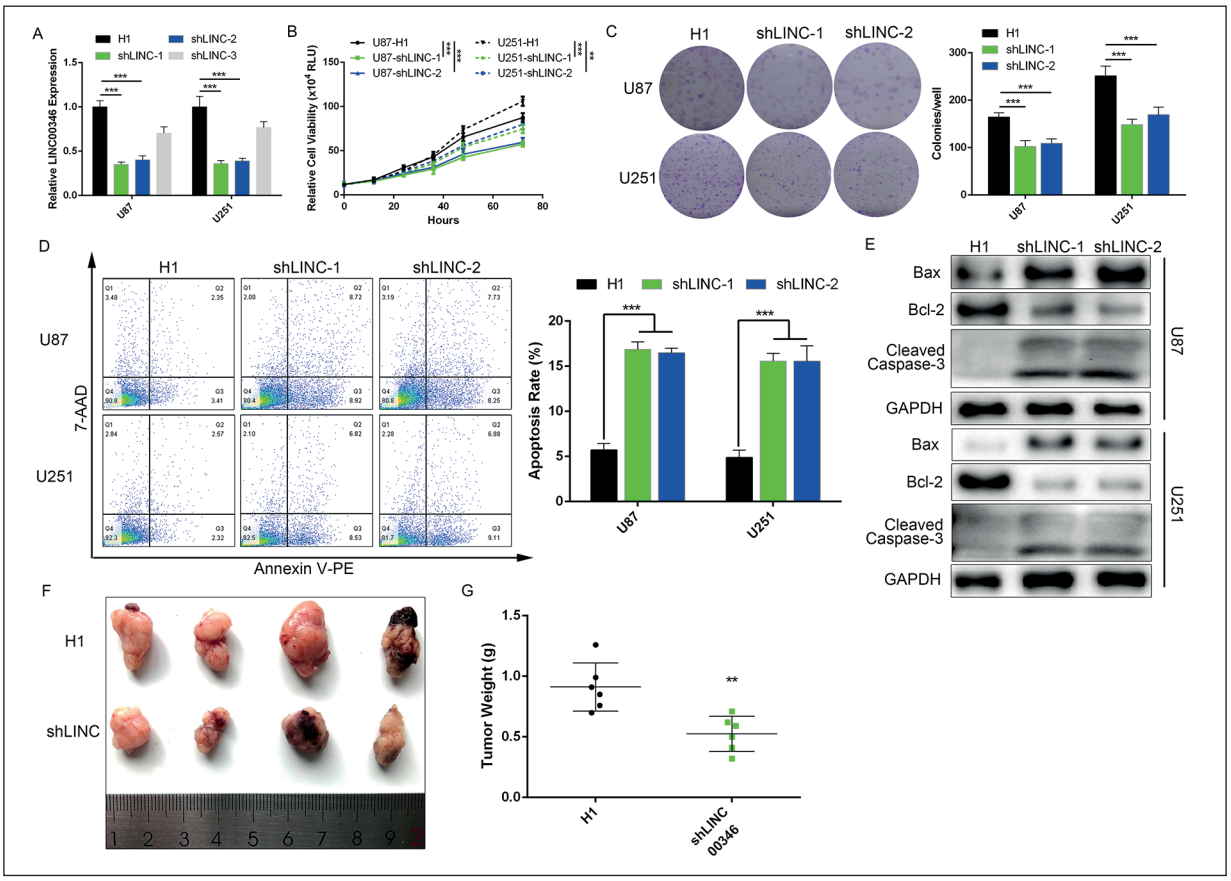

Figure 2. LINC00346 knockdown inhibited glioma cell growth both *in vitro* and *in vivo*. **A,** The expression of LINC00346 in U87 and U251 cells infected with H1 and three shLINC00346 lentiviruses. **B, C,** Celltiter-Glo **(B)** and colony formation assay **(C)** were used to determine the proliferation of U87 and U251 cells treated with LINC00346 knockdown (magnification × 10). **D,** The results of flow cytometry analysis in U87 and U251 cells treated with LINC00346 knockdown. Q2 + Q3 were considered as apoptotic cells. **E,** Western blot was applied to detect the levels of proteins related to apoptosis (Bcl-2, Bax, Cleaved Caspase-3). **F,** The representative images of xenograft tumors. **G,** The weight quantitation of xenograft tumors infected with H1 or shLINC-1 in U87 glioma cells. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

LINC00346 might also act as a ceRNA in glioma. Firstly, using online tools ENCORI and TargetScan, miR-128-3p was identified as a potential target of LINC00346 and the binding site showed in Figure 3A. Next, we analyzed the public databases to assess the clinical significance of miR-128-3p. As a result, the upregulation of miR-128-3p was positively correlated with the overall survival of glioma patients (Figure 3B). Moreover, a negative correlation between the expression of LINC00346 and miR-128-3p was identified in glioma tissues by Pearson's correlation analysis (Figure 3C). Then, the Dual-Luciferase reporter assay was performed to verify the putative binding site between LINC00346 and miR-128-3p. The result demonstrated that miR-128-3p mimic significantly decreased the Luciferase activity of LINC00346-WT rather than the LINC00346-MU group (Figure 3D). Furthermore, the RIP assay indicated that the expressions of LINC00346 and miR-128-3p were both significantly elevated in the anti-Ago2 groups compared with that in anti-IgG groups (Figure 3E). In addition, the expression level of miR-128-3p increased after the suppression of LINC00346 expression in U87 and U251 cells (Figure 3F). Taken together, these data presented competent proof to demonstrated that LINC00346 directly sponged miR-128-3p in glioma cells.

LINC00346 Regulated SZRD1 by Sponging MiR-128-3p

To further explore the ceRNA hypothesis, it was necessary to find the target gene of miR-128-3p. Through online databases, SZRD1 was predicted as the downstream target mRNA of

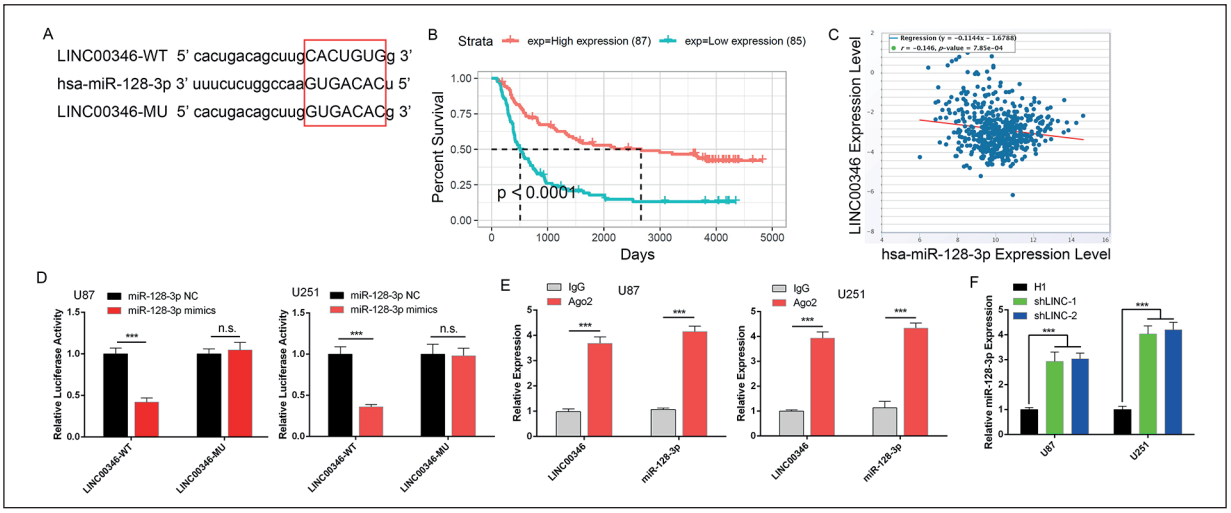

Figure 3. LINC00346 acted as a ceRNA in glioma by sponging miR-128-3p. **A,** The binding sequence between LINC00346 and miR-128-3p was predicted by ENCORI and Targetscan. **B,** The expression of miR-128-3p was positively correlated with overall survival of glioma patients. Data are obtained by analyzing the CGGA database. **C,** Pearson's correlation analysis between LINC00346 and miR-128-3p expression in glioma tissues. **D,** Luciferase reporter assays were conducted to validate the specific binding between LINC00346 and miR-128-3p in U87 and U251 cells. **E,** The complex containing LINC00346 and miR-128-3p in U87 and U251 cells were immunoprecipitated by anti-Ago2 using RIP assay. **F,** The expression of miR-128-3p in U87 and U251 cells infected with H1 or shLINC00346 lentiviruses. ($^{***}p < 0.001$, n.s. no significance).

miR-128-3p in glioma (Figure 4A). Interestingly, previous studies[24,25] have investigated that SZRD1 functions as a tumor suppressor in cervical cancer[24] and involves oligodendroglioma progression by in-depth computational analysis[25]. These studies provided us the confidence to elucidate the function of the LINC00346/miR-128-3p/SZRD1 axis. Then, the negative correlation between

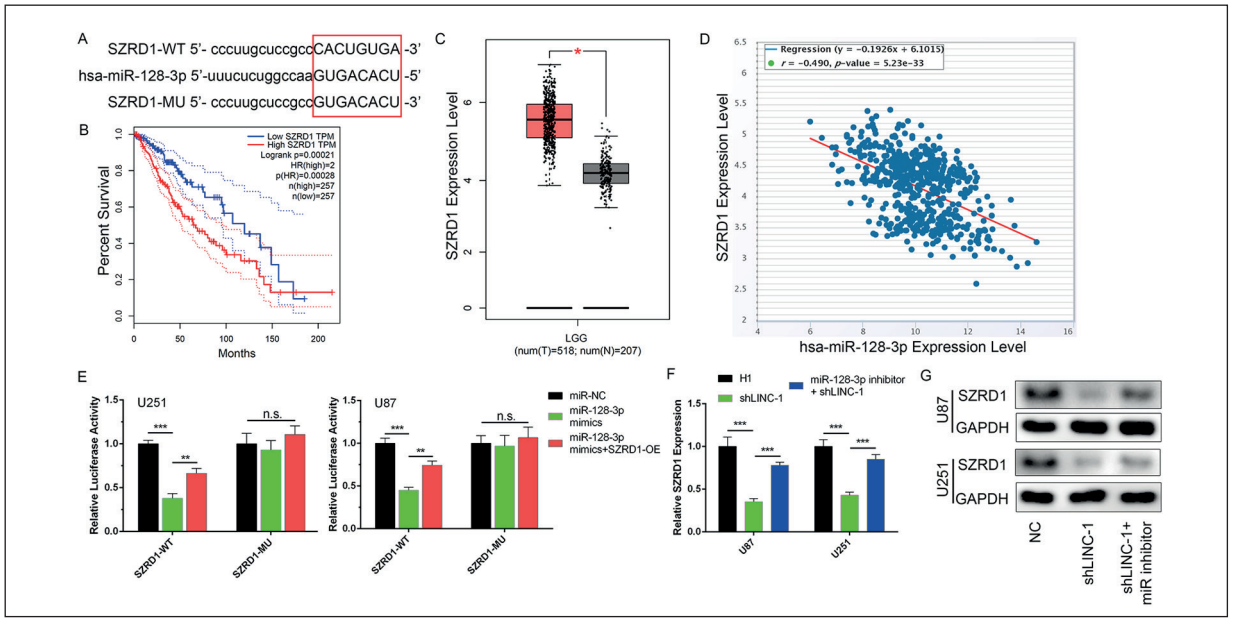

Figure 4. LINC00346 regulated SZRD1 by sponging miR-128-3p. **A,** The binding sites between miR-128-3p and SZRD1 were acquired by bioinformatics analysis. **B,** Kaplan-Meier curve of the correlation between SZRD1 expression and overall survival time in glioma patients. **C,** The expression pattern of SZRD1 in the TCGA glioma samples. **D,** Pearson's correlation analysis between miR-128-3p and SZRD1 in glioma tissues. **E,** Dual-Luciferase reporter assay exhibited the interactions among miR-128-3p and SZRD1. **F, G,** The expression of SZRD1 was inhibited by LINC00346 knockdown and reversed by co-transfection with miR-128-3p inhibitor, which was acquired by qRT-PCR **(F)** and Western blot **(G)**. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s. no significance).

the expression of miR-128-3p and SZRD1 was identified in glioma tissues from public databases (Figure 4B). Besides, the expression of SZRD1 was upregulated in glioma tissue (Figure 4C), as well as negatively correlated with the overall survival of glioma patients (Figure 4D). Subsequently, Dual-Luciferase reporter plasmids were constructed which contained the WT and MU 3'-UTR of SZRD1, based on the putative miR-128-3p binding site (Figure 4A). The results showed that the Luciferase activity of SZRD1-WT was decreased by miR-128-3p mimics and reversed under LINC00346 overexpression (Figure 4E).

To further investigate the regulatory relationship between miR-128-3p and SZRD1, qRT-PCR and Western blot were utilized to measure the expression of SZRD1 in U87 and U251 cells transfected with shLINC-1 or miR-128-3p inhibitor together with shLINC-1. The results indicated that SZRD1 expression was inhibited by shLINC-1, and the inhibitory effect was partially rescued by suppression of miR-128-3p (Figure 4F, G). The above findings revealed that LINC00346 could act as a ceRNA in regulating SZRD1 by competitively binding to miR-128-3p in glioma.

SZRD1 and MiR-128-3p Participated in LINC00346-Mediated Cell Proliferation and Apoptosis

In order to explore the exact impact of LINC00346/miR-128-3p/SZRD1 axis on glioma cell activities, SZRD1 was silenced by three specific shRNAs (shSZRD1-1, shSZRD1-2, shSZRD1-3). The shSZRD1-1 showed the best knockdown efficiency and was selected for further experiments (Figure 5A). Next, colony formation assay indicated that the suppression of cell growth caused by LINC00346 knockdown was partially reversed by miR-128-3p or SZRD1 expression (Figure 5B). Meanwhile, cell growth inhibited by miR-128-3p mimics or SZRD1 knockdown alone

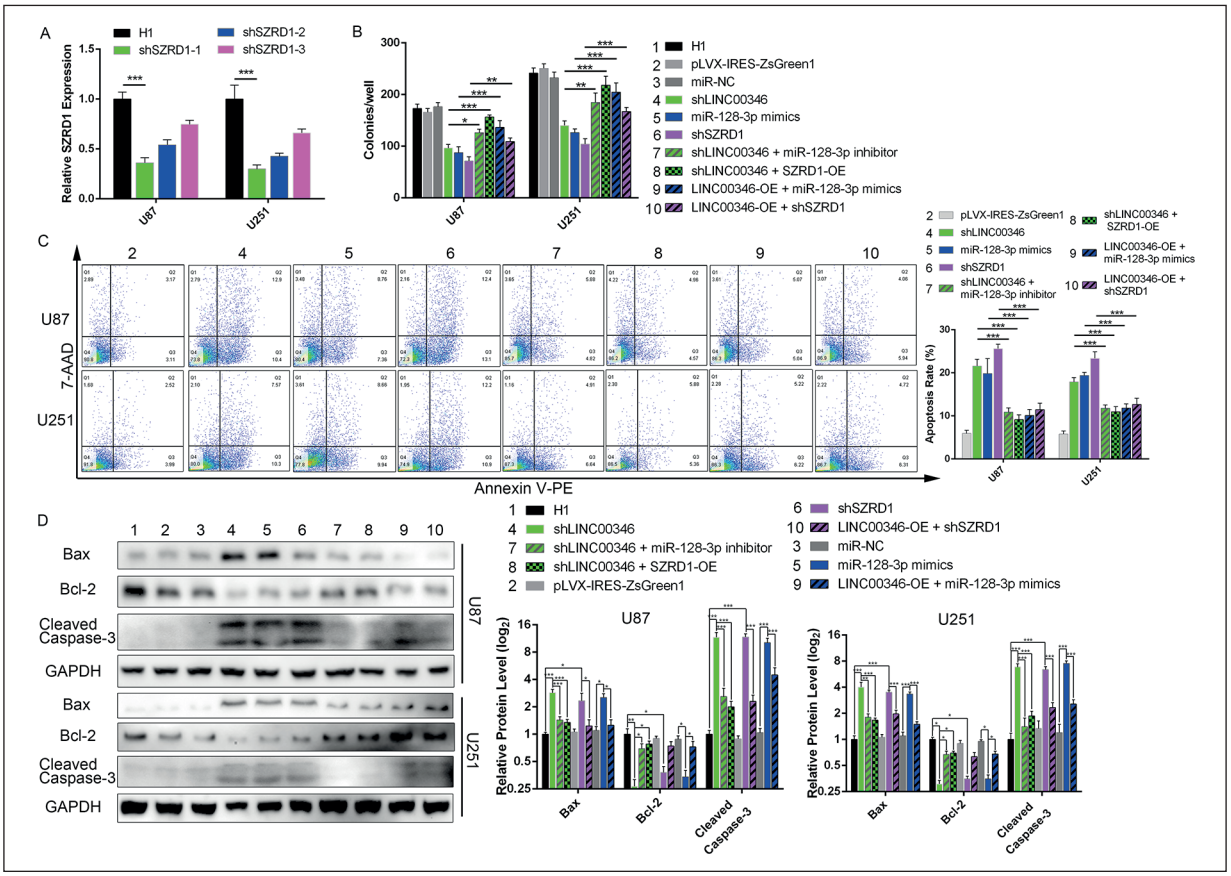

Figure 5. miR-128-3p and SZRD1 involved in LINC00346-mediated cell proliferation and apoptosis. **A,** Knockdown efficiency of SZRD1 in U87 and U251 cells measured by qRT-PCR. **B,** The effect of miR-128-3p mimics, inhibitor or shSZRD1, SZRD1-OE on LINC00346-mediated cell proliferation was detected in U87 and U251 cells. **C, D,** LINC00346-induced apoptosis was partially rescued by miR-128-3p or SZRD1 in glioma, which was detected by flow cytometry **(C)** and Western blot **(D)**. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

could be rescued by LINC00346 overexpression. Moreover, cell apoptosis affected by LINC00346 expression showed a similar trend as cell growth (Figure 5C). Meanwhile, several apoptosis-related proteins were detected by Western blot. As a result, apoptosis induced by shLINC could be partially reversed by co-transfection with miR-128-3p inhibitor or SZRD1-OE. Similarly, apoptosis promoted by miR-128-3p overexpression was partially rescued by co-transfection with LINC00346-OE. In addition, shSZRD1-induced apoptosis was partially rescued by LINC00346 overexpression. Conversely, the expression level of Bax showed the opposite trend (Figure 5D). Taken together, LINC00346 regulated glioma proliferation apoptosis through modulating miR-128-3p/SZRD1 axis.

Discussion

Multiple lncRNAs serve as crucial modulators in the progression of various tumors including glioma[26-29]. NEAT1 promotes glioma proliferation and migration[30], while Gas5 acts as a tumor suppressor in glioma cells[31]. It has been reported[12,32-34] that LINC00346 is upregulated in bladder cancer, non-small cell lung cancer, colorectal cancer and hepatocellular carcinoma. Peng et al[9] reported that LINC00346 acts as a prognostic biomarker and promotes pancreatic cancer progression. All these studies have demonstrated that LINC00346 functions as an oncogene in different tumors.

In this study, we focused on the biological function and underlying mechanism of LINC00346 in glioma progression. Upregulation of LINC00346 was observed in high-grade glioma tissues and glioma cell lines. Importantly, the negative correlation between LINC00346 expression and the overall survival time was observed in glioma patients. Functionally, LINC00346 knockdown significantly suppressed cell proliferation and induced cell apoptosis *in vitro*. Besides, we used several classical apoptotic biomarkers, Bax, Bcl-2 and Cleaved Caspase-3, as well as Annexin V to detect cell apoptosis. Bcl-2 could prevent apoptosis *via* distinct pathways[35]. Whereas Bax induces the loss in membrane potential and the release of cytochrome c, which results in apoptosis[36]. Cleaved caspase-3, the marker of caspase-3 activation, exerts function *via* either dependent or independent on mitochondrial cytochrome c release[37]. Moreover, the suppression of LINC00346 inhibited glioma growth *in vivo*. Thus, these results suggested that LINC00346 could act as a prognostic biomarker, as well as an oncogene in glioma.

Some lncRNAs could exert biological roles as ceRNAs. These lncRNAs sequester miRNAs and regulate miRNAs expression in various tumors[38]. To ascertain the carcinogenic mechanism of LINC00346 in glioma, we conducted bioinformatic analysis and predicted that miR-128-3p was a potential therapeutic target of LINC00346. MiR-128-3p exhibits a low expression level and suppresses tumor progression by regulating distinct signal pathways in several tumors, including glioma[39-41]. Our further investigation revealed that miR-128-3p expression was downregulated in glioma tissues and was negatively related to LINC00346 expression. In contrast to LINC00346, the downregulation of miR-128-3p was correlated to the poor overall survival of glioma patients. MiR-128-3p expression was increased after LINC00346 knockdown in glioma cells. In addition, Dual-Luciferase reporter assay and RIP assay verified that miR-128-3p could directly bound to LINC00346. These results indicated that miR-128-3p was a direct target of LINC00346.

MiRNAs could regulate the expression of target genes through binding the 3'-UTR. MiR-128-3p directly binds to Kruppel-like factor 4, pyruvate dehydrogenase kinase 1, sirtuin 1 and LIM domain kinase 1 in tumor progression or tissue recovery[16,42-44]. In our study, bioinformatic analysis and Dual-Luciferase assay determined that SZRD1 was a direct target of miR-128-3p. Gladitz et al[25] reported that SZRD1 induced glioma development using network-based analysis. We found that overexpression of SZRD1 in glioma tissues could predict the poor dismal prognosis of glioma patients. Furthermore, a significant negative correlation was observed between SZRD1 and miR-128-3p expression. The expression of SZRD1 was repressed by LINC00346 knockdown and could be reversed by co-transfection with miR-128-3p inhibitor in glioma cells. Cell proliferation and apoptosis affected by LINC00346 were partially rescued by the intervention of miR-128-3p or SZRD1. Taken together, our results demonstrated that SZRD1 and miR-128-3p involved LINC00346-mediated cell growth and apoptosis.

Conclusions

Our study demonstrated that LINC00346 upregulation could predict the poor overall survival

outcomes of glioma patients. To our best knowledge, this was the first study that demonstrated the ceRNA function of LINC00346 on glioma proliferation and apoptosis via regulating the miR-128-3p/SZRD1 axis. Our study verified LINC00346/miR-128-3p/SZRD1 as a new regulatory network in glioma progression. The axis might contribute to precise therapy for glioma.

Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper..

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