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MicroRNA-29b promotes cell sensitivity to Temozolomide by targeting STAT3 in glioma

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Abstract. – OBJECTIVE: This study aimed to explore the effects of microRNA-29b (miR-29b) on chemoresistance of glioma and to examine the underlying mechanisms.

MATERIALS AND METHODS: MiR-29b expression in glioma tissues and cell lines was analyzed by quantitative real time-polymerase chain reaction (qRT-PCR). The cell viability was determined by Cell Counting Kit-8 (CCK-8) assay. Cell apoptosis was analyzed by Annexin V-Fluorescein isothiocyanate (FITC) assay. The relationship between miR-29b and signal transducer and activator of transcription 3 (STAT3) was examined by the Dual-Luciferase reporter gene assay. The levels of cleaved caspase-3, Bax, Bcl-2, and STAT3 were detected by Western blotting assay.

RESULTS: The expression of miR-29b was downregulated in glioma tissues compared to normal brain tissue. In addition, the expression level of miR-29b was lower in glioma tissues from patients at late stages (III and IV) compared with early stages (I and II). Besides, miR-29b expression was significantly lower in LN229, U87MG, and U251 cells compared to normal human astrocytes (NHA) cells. Moreover, our results showed that miR-29b expression in Temozolomide (TMZ)-resistance cell lines U251/TMZ and U87MG/TMZ was markedly lower than that of TMZ-sensitivity cell lines U251 and U87MG. The protein levels of STAT3 and the phosphorylation of STAT3 were increased in U251/TMZ and U87MG/TMZ compared to U251 and U87MG. When the expression of miR-29b was repressed, cell viability was increased. Meanwhile, cell apoptosis was reduced, the protein levels of cleaved caspase-3 and (Bcl-2 Associated X Protein) Bax were decreased, whereas the protein level of B-cell lymphoma 2 (Bcl-2) was increased. Moreover, the effects of miR-29b knockdown on the cell growth and apoptosis in U251 and U87MG cells were markedly attenuated by knockdown of STAT3. In TMZ-resistant U251/TMZ and U87MG/TMZ cells, transfection with miR-29b decreased cell growth, promoted apoptotic cell death, elevated the protein levels of cleaved caspase-3, and Bax protein, while downregulated Bcl-2 protein. As expected, the effect of miR-29b upregulation on cell growth and apoptosis of TMZ-resistant glioma cells was reversed by STAT3 overexpression. The results from the Luciferase assay demonstrated miR-29b modulated STAT3 expression by directly bound with 3'-Untranslated Region (3'-UTR).

CONCLUSIONS: MiR-29b enhances the cell sensitivity to TMZ by inhibiting STAT3 in glioma. Our study might provide a novel target for treating TMZ-resistant glioma.

Key Words:
MiR-29b, Temozolomide, STAT3, Glioma.

Introduction

Due to the great ability to cross the blood-brain barrier, the methylating agent Temozolomide (TMZ) is frequently employed to treats multiple brain tumors[1]. Glioma is the most common type of malignancy of the central nervous system[2,3]. Although treatment regimens continue to improve and develop in recent years, chemoresistance often occurs in patients with glioma, leading to a high recurrence rate and poor prognosis[4,5]. Therefore, a greater understanding of the mechanisms involved in TMZ-chemoresistance is essential for improving its therapeutic efficacy in glioma.

Temozolomide is now employed as the first-line therapy for patients with glioma[6,7]. Though majority of patients show great response to TMZ at the early stage of treatment, patients often develop resistance to TMZ later, which largely compromises the therapeutic benefit of TMZ treatment. A variety factors and mechanisms have been proposed to be involved in TMZ-resistance in glioma. Mismatch repair deficiency can lead

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to TMZ-resistance in human glioblastoma cell line U251[8]. Dihydrofolate reductase (DHFR) and thymidylate synthetase (TYMS) have also been reported to function as oncogene and promote glioma cell resistance to TMZ[9]. Moreover, inhibition of miR-26a and transcription factor AP-2α (TFAP2A) increased the glioma cell stemness and TMZ resistance by accelerating Nanog/SRY-like HMG box 2 (SOX2)/Prominin-like protein 1 (CD133) and Interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) pathway[10]. Long noncoding RNAs (lncRNAs) zinc finger NFX1-type containing 1 antisense RNA 1 (ZFAS1) promotes glioma cell resistance to TMZ by regulating miR-150-5p/proteolipid protein 2 (PLP2) axis[11]. Despite numerous studies, the mechanism of TMZ-resistance in glioma is not fully understood.

MicroRNAs (miRNAs) is a group of non-coding single-stranded linear small RNA with length of 18-22 nucleotides. MiRNAs regulate transcription, post transcription, and translation levels by binding to the 3'-Untranslated Region (3'-UTR) of the target gene mRNA sequences. In the context of human malignancies, miRNAs can function as an oncogene[12] and tumor suppressor gene[13]. As regards to miR-29b, it has been proposed that miR-29b can play a role as tumor suppressor in a variety of human cancers. MiR-29b represses myeloma by inhibiting pro-inflammatory activity and repressing the dendritic cells[14]. MiR-29b can also reverse bortezomib resistance of multiple myeloma by negatively regulating H19 and myeloid cell leukemia-1(Mcl-1)[15]. In gastric cancer, miR-29b suppresses cell migration and tumor growth by inhibiting matrix metalloprotein-2(MMP-2)[16]. Of note, miR-29b also functions as critical tumor suppressor in glioma by regulating multiple genes and pathways[17-21]. However, the role of miR-29b in TMZ-resistance of glioma has not been reported.

In this study, we observed the downregulation of miR-29b in glioma tissues and cell lines, and further investigated the effects and mechanism of miR-29b on TMZ-resistance in glioma.

Materials and Methods

Patients Specimens

A total of 56 glioma patients who have been diagnosed and classified according to WHO criteria were enrolled in this study. Consent forms were signed by all patients. This investigation was approved by the Institutional Ethics Committee of the Second Affiliated Hospital of Xinjiang Medical University (Urumqi, Xinjiang, China). The glioma tissues and matched non-cancerous tissues were collected from glioma patients undergoing surgery in the Second Affiliated Hospital of Xinjiang Medical University (Urumqi, Xinjiang, China) between 2014 and 2018.

Cell Lines and Cell Culture

Human glioma cell lines LN229, U87MG, and U251 were purchased from Shanghai Institute of Biochemistry and Cell Biology (CAS, Shanghai, China). The normal human astrocytes (NHA) were obtained from Shanghai Institute of Biochemistry and Cell Biology (CAS, Shanghai, China). LN229, U87MG, U251, and NHA cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplied with 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA) and 1% penicillin and streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA) in a humid incubator (Thermo Fisher Scientific, Waltham, MA, USA) with 5% CO_2 at 37°C.

Temozolomide (TMZ, purity: 99.96%, MedChem Express, Monmouth Junction, NJ, USA) resistant cell lines were established in our lab. First, U251 and U87MG cells were exposed to increasing concentration of TMZ (0-100 μM) for 3 days. Then, the floated cells were removed, and the survival cells were cultured in the complete medium containing 50 μM TMZ. Following 6-8 generation, TMZ-resistant cells (U251/TMZ and U87MG/TMZ) were established.

Cell Transfection

The miR-29b mimics and anti-miR-29b were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The signal transducer and activator of transcription 3 (STAT3) siRNA and overexpressing plasmids were designed and synthesized by GenePharma (Shanghai, China). All transfection was carried out using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol.

Cell Viability Assay

The ability of cell proliferation was analyzed by Cell Counting Kit-8 (CCK-8) assay (Beyotime,

Shanghai, China) according to the manufacturer's protocol. Following cell transfection, cells were harvested and plated in 24-well plates for 24 h at 37°C. Then, cells were treated with increasing concentration of TMZ (0, 50, and 100 μM) for 48 h. After 48 h, 10 μL CCK-8 solution was added into each well and incubated for 15 min. Then, the absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Apoptosis Assay

Cell apoptosis was analyzed by Annexin V-Fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (Beyotime, Shanghai, China) according to the manufacturer's protocol. In brief, after cell transfection, cells were harvested and rinsed with phosphate-buffered saline (PBS). Then, Annexin V-FITC and propidium iodide (PI) solution were added into cells and incubated for 15 min at room temperature in the dark. The cell apoptotic rate was measured by flow cytometry (Thermo Fisher Scientific, Waltham, MA, USA).

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA from cells and tissues was extracted using TRIzol reagent (TaKaRa, Dalian, Liaoning, China) and the RNA concentration was detected by Nanodrop 2000 (Tiangen, Beijing, China). Then, the reverse transcription was carried out using PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) abided by the manufacturer's protocol. TB Green® Fast qPCR Mix (TaKaRa, Dalian, China) was used to perform qRT-PCR in an ABI 7300 Real Time-PCR system (Applied Biosystems, Foster City, CA, USA). The primers in this study were listed as the following: miR-29b, 5'-TCAGGAAGCTGGTTTCATATGGT-3' and 5'-CCCCCAAGAACACTGATTTCAA-3'; STAT3, 5'-GTGTGACACCGTAAGTGGCT-3' and 5'-GACATCGGCAGGTCAATGGT-3'; U6, 5'-CTTGCTTCGGCAGAACATATAC-3' and 5'-AACGCTTCACGATTTTGCGT-3'; GAPDH, 5'-CCATGGGGAAGGTGAAGGTC-3' and 5'-AGTGATGGCATGGACTGTGG-3'. The expression of miR-29b was normalized by U6 and the expression of STAT3 was normalized by GAPDH. The $2^{\Delta\Delta Ct}$ method was used to quantify the relative expression in this study.

Western Blotting Assay

The proteins were isolated from cells using Radio-Immunoprecipitation Assay (RIPA) Lysis Buffer (Beyotime, Shanghai, China) and the concentration of the protein was measured by Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China) according to the manufacturer's protocol. Then, proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then, transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were then blocked by non-fat milk for 2 h at room temperature. Following blockade, the membrane was incubated with primary antibodies overnight at 4°C. After a rinsing process, the membranes were then incubated with anti-rabbit IgG horseradish peroxidase (HRP)-linked secondary antibody (Beyotime, Shanghai, China) for 1 h at room temperature. The bands were visualized using BeyoECL Moon (Beyotime, Shanghai, China) according to the manufacturer's protocol in autoradiographs (Bio-Rad, Hercules, CA, USA). Specific primary antibodies in this study are: anti-cleaved caspase-3 antibody (1:500, ab32042), anti-Bax antibody (1:2000, ab182733), anti-Bcl-2 antibody (1:1000, ab32124), stat3 (1:1000, ab119352), anti-stat3 (phosphor-S727) antibody (1:1000, ab32143), GAPDH (1:10000, ab181602). All primary antibodies were purchased from Abcam (Cambridge, MA, USA).

Luciferase Activity Assay

The relationship between miR-29b and stat3 was performed using Dual-Luciferase Reporter Gene Assay Kit (Beyotime, Shanghai, China) abided the manufacturer's protocol. The *Renilla* Luciferase activity of each well was normalized by firefly Luciferase activity.

Statistical Analysis

SPSS 22.0 (IBM, Armonk, NY, USA) and GraphPad Prism Software (Version 4.0, San Diego, CA, USA) were used to analyze data in this study. Data are showed as means ± standard deviation (SD) from three independent experiments. Student's *t*-test and one way-analysis of variance (ANOVA) were used to analyze the difference between two groups and among multiple groups. The correlation between miR-29b and STAT3 was analyzed by Pearson correlation methods. $p<0.05$ considered a significant difference in this study.

Results

MiR-29b Is Downregulated in Glioma

The level of miR-29b in glioma tissue was first measured and we found that miR-29b was significantly down-regulated in glioma tissues (Figure 1A). Besides, miR-29b expression was markedly lower in glioma tissues from patients at advanced stages (III and IV) compared to early stages (I and II; Figure 1B). Meanwhile, our results showed that miR-29b was remarkably down-regulated in glioma cell lines (LN229, U87MG, and U251) compared to normal human astrocytes (NHA) (Figure 1C). Furthermore, miR-29b expression was strongly down-regulated in TMZ-resistant glioma cell lines U251/TMZ and U87MG/TMZ compared to their parental cells U251 and U87MG (Figure 1D). Our findings suggested that miR-29b functioned as tumor suppressor in glioma, and downregulation of miR-29b correlated with the resistance of glioma cells to TMZ.

Knockdown of MiR-29b Renders the Glioma Cells Resistant to TMZ

To determine the effects of miR-29b in the resistance of glioma cells to TMZ, the expression of miR-29b was repressed by transfection with anti-miR-29b. Results from qRT-PCR showed that miR-29b expression was markedly repressed (Figure 2A). We found that miR-29b knockdown remarkably reduced the sensitivity of glioma cells to TMZ (Figure 2B). In addition, the pro-apoptotic activities of TMZ in both U251 and U87MG cells were significantly compromised by miR-29b knockdown (Figure 2C). As expected, the levels of pro-apoptotic protein cleaved caspase-3 and Bax were increased by TMZ whereas the effects of TMZ were reversed by knockdown of miR-29b (Figure 2D). On the contrary, the levels of anti-apoptotic protein Bcl-2 were reduced by TMZ whereas the inhibitory effects of TMZ were reversed by knockdown of miR-29b (Figure 2D). We indicated that knockdown of miR-29b renders the glioma cells resistant to TMZ.

Upregulation of MiR-29b Enhances the Sensitivity of TMZ-Resistant Glioma Cells to TMZ

To further analyze the effects of miR-29b in TMZ-resistance of glioma cells, TMZ-resistant glioma cells were transfected with miR-29b. Our results showed that miR-29b was markedly up-regulated in U251/TMZ and U87MG/TMZ cells (Figure 3A). We found that miR-29b upregulation significantly enhanced the sensitivity of

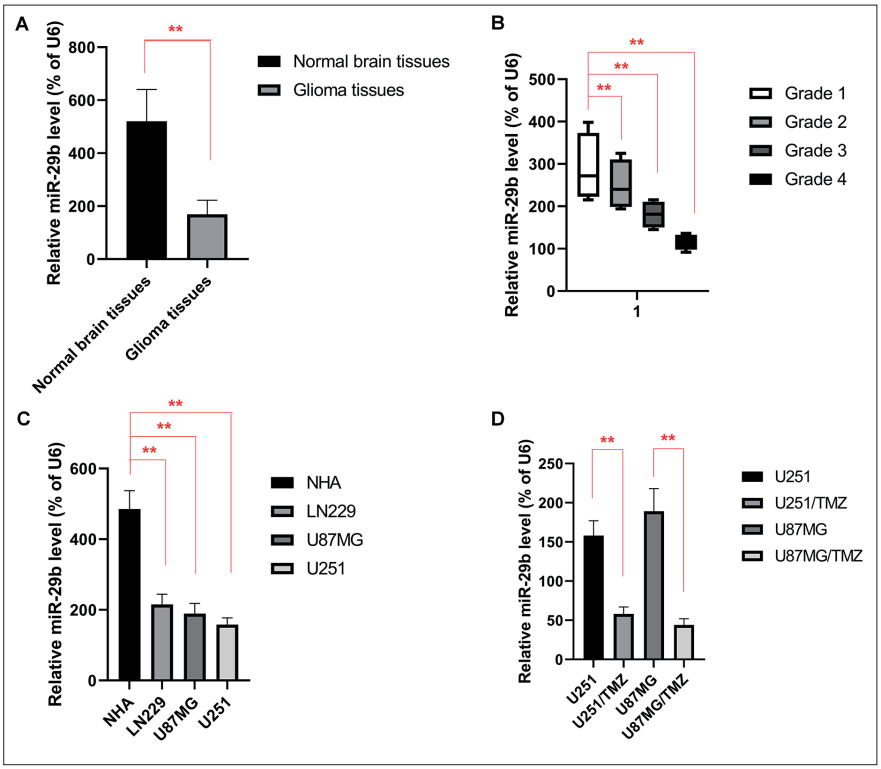

Figure 1. MiR-29b is down-regulated in glioma. **A,** MiR-29b expression in glioma tissues and paired normal brain tissues was measured by qRT-PCR (n=56). **B,** MiR-29b expression in different glioma stages was analyzed by qRT-PCR (stage I, n=8; stage II, n=11; stage III, n=17; stage IV, n=20). **C,** MiR-29b expression in human glioma cell lines LN229, U87MG, and U251, and the normal human astrocytes (NHA) were measured by qRT-PCR. **D,** MiR-29b expression in U251/TMZ and U87MG/TMZ cells was measured by qRT-PCR. ** $p<0.01$.

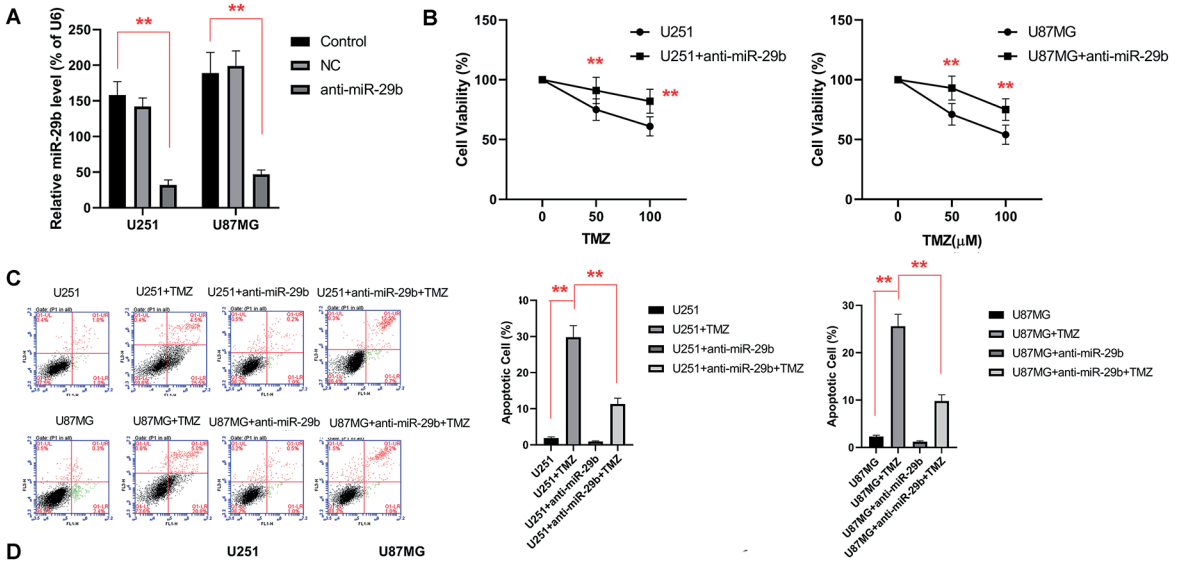

Figure 2. Knockdown of miR-29b enhances the glioma cells resistance to TMZ. Knockdown of miR-29b in U251 and U87MG cells. **A**, MiR-29b expression in U251 and U87MG cells was detected by qRT-PCR. **B**, Proliferation of cells was determined by CCK-8 assay. **C**, Cell apoptosis was detected by Annexin-V FITC/PI assay. **D,** Protein levels of cleaved caspase-3, Bcl-2, and Bax were measured by Western blotting. ** $p<0.01$.

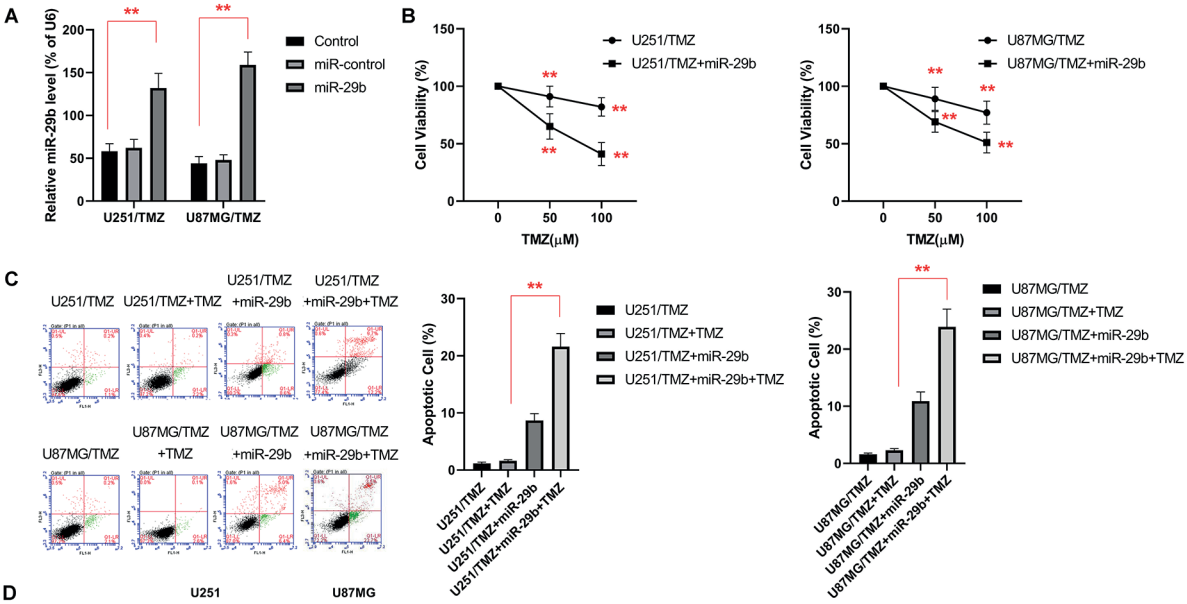

Figure 3. Upregulation of miR-29b enhances the TMZ-resistant glioma cells sensitivity to TMZ. Upregulation of miR-29b in U251/TMZ and U87MG/TMZ cells. **A**, MiR-29b expression in U251/TMZ and U87MG/TMZ cells were determined by qRT-PCR. **B,** Proliferation of cells was analyzed by the CCK-8 assay. **C,** Cell apoptosis was measured by Annexin-V FITC/PI assay. **D,** Protein levels of cleaved caspase-3, Bcl-2, and Bax were detected by Western blotting. ** $p<0.01$.

U251/TMZ and U87MG/TMZ cells to TMZ (Figure 3B). Moreover, the pro-apoptotic activities of TMZ in both U251/TMZ and U87MG/TMZ cells were increased by overexpression of miR-29b (Figure 3C). Furthermore, the levels of pro-apoptotic protein cleaved caspase-3 and Bax were increased by TMZ and the effects of TMZ were enhanced by upregulation of miR-29b (Figure 3D). Meanwhile, the levels of anti-apoptotic protein Bcl-2 were reduced by TMZ and the inhibitory effects of TMZ were enhanced by upregulation of miR-29b (Figure 3D). These results indicated that upregulation of miR-29b enhances the sensitivity of TMZ-resistant glioma cells to TMZ.

MiR-29b Directly Targets STAT3

To fully appreciate the role of miR-29b in glioma, the downstream target gene of miR-29b was predicted by the TargetScan. As shown in Figure 4A, miR-29b could directly bind to the 3'-Untranslated Region (3'-UTR) of STAT3. The relationship between miR-29b and STAT3 was analyzed using the Dual-Luciferase reporter gene. MiR-29b mimics were co-transfected into U251 and U87MG cells with a Luciferase reporter gene containing STAT3 wild-type or mutant 3'-Untranslated Region (3'-UTR). The results showed that the Luciferase activities reduced in miR-29b overexpressing cells, supporting that STAT3 was a direct target of miR-29b (Figure 4B). In addition, upregulation of miR-29b remarkably reduced the STAT3 mRNA expression (Figure 4C). Moreover, the STAT3 mRNA expression was negatively associated with miR-29b mRNA expression in glioma tissues (Figure 4D). Our results suggest that STAT3 was a direct target of miR-29b and negatively regulated by miR-29b in glioma.

MiR-29b Regulates the Sensitivity of Glioma Cells to TMZ Through Targeting STAT3

Experiments were carried out to examine whether miR-29b regulates TMZ-resistance in glioma cells by negatively modulating STAT3. We found that the protein levels of STAT3 and the phosphorylation of STAT3 of U251 and U87MG cells were significantly lower compared to U251/TMZ and U87MG/TMZ cells (Figure 5A). In addition, TMZ-induced apoptosis in U251 and U87MG cell apoptosis were reduced by miR-29b knockdown while this effect was reversed by knockdown of STAT3, the U251 and U87MG cell apoptosis was significantly increased by knockdown of STAT3 (Figure 5B). The elevating effect of TMZ on the levels of pro-apoptotic protein cleaved caspase-3 and Bax were dampened by knockdown of miR-29b whereas knockdown of STAT3 reversed the effect of anti-miR-29b (Figure 5C). Meanwhile, the repressing effects of TMZ on the levels of anti-apoptotic protein Bcl-2 were attenuated by miR-29b knockdown while knockdown of STAT3 reversed the effect of anti-miR-29b (Figure 5C). In contrast, overexpression of STAT3 significantly reduced the potentiating

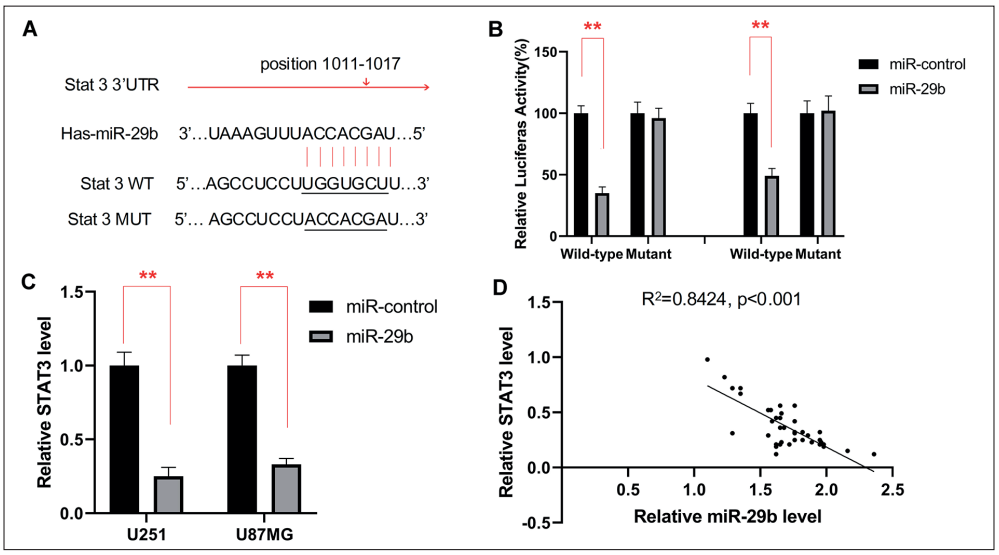

Figure 4. MiR-29b directly targets STAT3. **A,** Target gene of miR-29b was predicated by TargetScan. **B,** Luciferase activity was detected by Dual-Luciferase reporter gene assay. **C,** The mRNA expression of STAT3 was measured by qRT-PCR. **D,** Correlation between miR-29b and STAT3 in glioma tissues was determined by Pearson correlation methods. ** $p<0.01$.

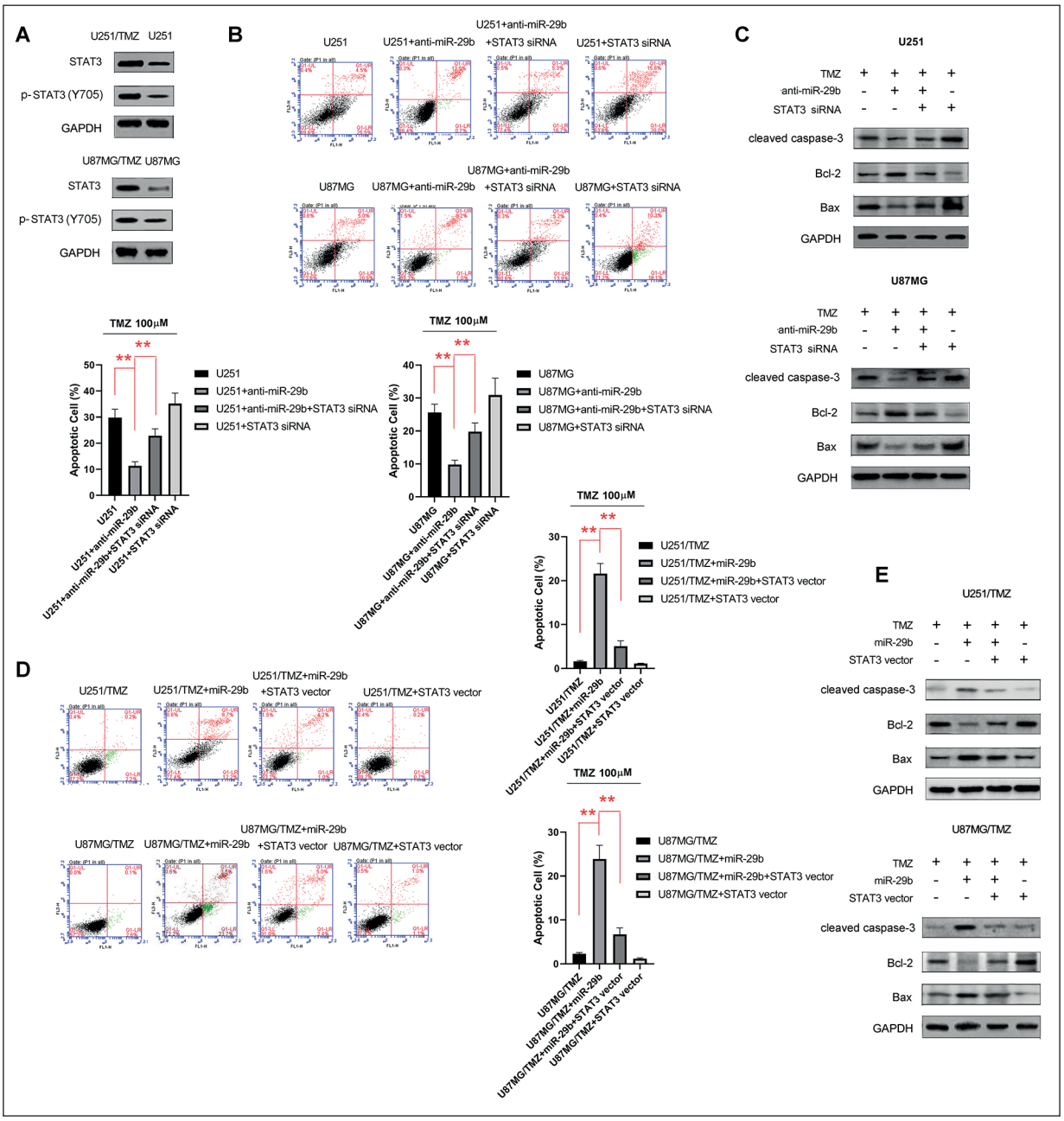

Figure 5. MiR-29b regulates TMZ-resistant glioma cells and their parental cells sensitivity to TMZ through targeting STAT3. **A,** Protein and phosphorylation levels of STAT were analyzed by Western blotting. Knockdown of miR-29b and STAT3 in U251 and U87MG cells. **B**, Cell apoptosis was determined by Annexin-V FITC/PI assay. **C,** Protein levels of cleaved caspase-3, Bcl-2, and Bax were analyzed by Western blotting. Upregulation of miR-29b and STAT3 in U251/TMZ and U87MG/TMZ cells. **D,** Cell apoptosis was measured by Annexin-V FITC/PI assay. **E,** Protein levels of cleaved caspase-3, Bcl-2, and Bax were evaluated by Western blotting. ** $p<0.01$.

effect of miR-29b on the sensitivity of U251/TMZ and U87MG/TMZ cells to TMZ (Figure 5D). In addition, the enhancing effect of miR-29b on TMZ-induced expression elevation of pro-apoptotic protein cleaved caspase-3 and Bax were decreased by STAT3 overexpression (Figure 5E). We also found that the enhancing effect of miR-29b on TMZ-induced downregulation of anti-apoptotic protein Bcl-2 was attenuated by STAT3 upregulation (Figure 5E). Our results indicate that miR-29b promotes cell sensitivity to TMZ by down-regulating STAT3 in glioma.

Discussion

It has been demonstrated that microRNAs exert a crucial role in tumorigenesis and cancer development by modulating cell growth, cell cycle, cell survival, apoptosis, migration, invasion, and stemness[22,23]. Moreover, miRNAs have also been reported to regulate chemoresistance in a variety of human malignancies. Upregulation of miR-101 promotes liver cancer cell sensitivity to cisplatin by inhibiting DNA-dependent protein kinase catalytic subunit (DNA-PKcs) signaling pathway[24]. Inhibition of miR-5787 reduces the sensitivity of tongue squamous cell carcinoma to cisplatin by promoting glucose metabolism reprogramming and reducing mitochondrial cytochrome c oxidase subunit 3 (MT-CO3)[25]. MiR-29b has been reported to regulate chemoresistance in multiple cancers. Inhibition of miR-29b promotes paclitaxel resistance in ovarian cancer by suppressing BAG family molecular chaperone regulator 3 (BAG3) and myeloid cell leukemia-1(Mcl-1)[26]. Di Fiore et al[27] showed that miR-29b reverses doxorubicin, cisplatin, and etoposide resistance in osteosarcoma by targeting Octamer-binding transcription factor 3/4 (Oct3/4), SRY-like HMG box 2 (SOX2) and Nanog. However, the effects of miR-29b on chemoresistance in glioma have not been explored.

In this study, our findings showed that miR-29b was remarkably down-regulated in glioma tissues and cell lines. In addition, miR-29b was down-regulated in TMZ resistant cell lines U251/TMZ and U87MG/TMZ compared to their parental U251 and U87MG cells. Knockdown of miR-29b enhanced TMZ resistance of U251 and U87MG cells while miR-29b upregulation increased TMZ sensitivity of U251/TMZ and U87MG/TMZ cells. Mechanistically, we found that miR-29b regulated chemoresistance of TMZ *via* transcription and translation and posttranslational modification of STAT3 by directly targeting STAT3 gene. We demonstrated that the downregulation of STAT3 reversed the reducing effects of knockdown of miR-29b on the sensitivity of U251 and U87MG cells to TMZ. On the other hand, overexpression of STAT3 reversed the enhancing effects of the upregulation of miR-29b on the sensitivity of U251/TMZ and U87MG/TMZ cells to TMZ.

STAT3 is one member of the family of signal transducers and activators of transcription. The SH2 and SH3 domains of STAT3 could bind to specific peptides containing phosphorylated tyrosine[28]. Following phosphorylation, the activated transcription factors enter the nucleus to promote transcription[29]. It has been reported that STAT3 is involved in progression of multiple malignant tumor by modulating a variety of biological functions of cancerous cells, such as cell proliferation, differentiation, and apoptosis[30]. In fact, serine-phosphorylated STAT3 induces tumorigenesis by repressing RNA polymerase transcriptional activity[31]. It has also been found that cisplatin inhibits tumor growth of lung cancer mouse model by suppressing Janus kinase 2 (JAK2)/STAT3 signaling pathway[32]. Moreover, STAT3-miR-92a-Dickkopf 1 (DKK1) pathway promotes tumor development in ovarian cancer[33]. The involvement of STAT3 in chemoresistance in human malignancies has also been evidenced. It has been documented that JAK2/STAT3 promotes breast cancer cell chemoresistance by inducing lipid metabolic genes carnitine palmitoyltransferase 1B (CPT1B) expression[34]. Inhibition of STAT3 by docetaxel-induced autophagy promotes chemoresistance in prostate cancer[35]. Our results are consistent with previous studies showing that STAT3 enhanced chemoresistance of TMZ in glioma.

Conclusions

In summary, miR-29b acts as a tumor suppressor to modulate TMZ-resistance of glioma by directly targeting STAT3. Our findings provide novel insights and potential therapeutic targets for the treatment of glioma, especially TMZ-resistance glioma.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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