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Downregulation of hsa_circ_0000936 sensitizes resistant glioma cells to temozolomide by sponging miR-1294

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Glioma is one of the most aggressive forms of brain tumor and is hallmarked by high rate of mortality, metastasis and drug resistance. Herein, we explore the role of circular RNA (circRNA) hsa_circ_0000936 in the resistance of glioma cells to temozolomide (TMZ). In this study, Relative changes in gene expression levels were compared using qRT-PCR. The role of hsa_circ_0000936 was characterized by cell count kit -8 assay and flow cytometry. Luciferase reporter assay was carried out for target validation.We found that hsa_circ_0000936 was upregulated in glioma tissues as compared to their adjacent normal tissues. Increased expression of hsa_circ_0000936 was found in the glioma tissues of patients showing resistance to TMZ compared with that of patients showing sensitivity to TMZ. The upregulation of hsa_circ_0000936 was also confirmed in TMZ-resistant glioma cells. miR-1294 was downregulated in TMZ-resistant glioma cells and identified as a direct target of hsa_circ_0000936. Downregulation of hsa_circ_0000936 increased the sensitivity of TMZ-resistant glioma cells towards TMZ. Moreover, restoration of miR-1294 could abrogate the promoting effect of hsa_circ_0000936 on TMZ resistance in TMZ-resistant glioma cells. In conclusion, downregulation of hsa_circ_0000936 sensitizes TMZ-resistant glioma cells to TMZ by sponging miR-1294, suggesting that hsa_circ_0000936 may be a potential target for overcoming the resistance of glioma cells to TMZ.

Keywords. Chemoresistance; circular RNA; glioma; hsa_circ_0000936; miR-1294; temozolomide

1. Introduction

Glioma is the most common malignant primary central nervous system tumor hallmarked by strong invasiveness and extensive infiltration. Since its diagnosis is incurable, the prognosis of glioma remains poor, with a 5-year survival rate of only 5.5% (Ostrom *et al.* 2016). Chemotherapy is the most common and effective treatment for glioma at present. Temozolomide (TMZ) is an alkylating agent that can cross the blood-brain barrier to reach the lesions and is recognized as a first-line treatment for glioma (Brada *et al.* 2003). Through pH-dependent hydrolysis, TMZ is converted to 3-methyl-(triazen-1-yl) imidazole-4-carboximide responsible for alkylation/methylation of guanine residues in deoxyribonucleic acid (DNA) (van Thuijl *et al.* 2015). Attempted repair of O6-methyl-guanine can cause DNA strand breakage and thereby result in cell death (Margison *et al.* 2002). However, the emergence of chemoresistance imposes severe limitations to the antitumor efficacy of TMZ in glioma (Hombach-Klonisch *et al.* 2018). Improving our understanding of the molecular mechanism of TMZ resistance of glioma is therefore of utmost importance to overcome the barrier of TMZ resistance.

Circular RNAs (circRNAs) are novel discovered noncoding RNAs in which the 3′ tail has been back-spliced to the 5′ tail, resulting in the formation of a continuous RNA loop (Li *et al.* 2018). Their unique structure gives them the ability to become resistant to

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exonuclease-mediated degradation, providing increased stability compared with their linear counterparts (Memczak *et al.* 2013). Emerging evidence suggests that circRNA serves as a crucial regulator in cancer progression and chemoresistance (Lei *et al.* 2019). Multiple circRNAs are reportedly regarded as potential therapeutic targets for tumors (Hao *et al.* 2019). Among many circRNAs, hsa_circ_0000936 (also known as circ-SHKBP1) is an abundant circRNA in endothelial cells. Downregulation of hsa_circ_0000936 dramatically inhibited the expression of angiogenic factor with G patch and FHA domains 1 and thereby repressed the viability, migration, and tube formation of U87 glioma-exposed endothelial cells through miR-544a/forkhead box P1 and miR-379/forkhead box P2 pathways (He *et al.* 2018). However, the contribution of hsa_circ_0000936 to the resistance of glioma cells to TMZ is still uncharacterized.

The biological function of circRNA has been widely studied in the last few years, especially in cancer progression (Arnaiz *et al.* 2018). circRNAs are able to bind with RNA-binding proteins involved in various biological processes (Conn *et al.* 2015). In addition, since the circRNA sequence harbors multiple miRNA binding sites, circRNA is able to compete with messenger RNA (mRNA) for miRNA response elements (Barrett and Salzman 2016). This makes circRNAs possible to function as competitive endogenous RNA, regulators of various cellular processes (Salmena *et al.* 2011). So far, miRNAs have been extensively discussed in various cell types, including cancer cells, and some miRNAs have been established as tumor suppressors or oncogenes in cancer biology (Sun *et al.* 2010). Significantly, multiple miRNAs have been found to play a crucial role in chemoresistance (Mercatelli *et al.* 2017, Rupaimoole *et al.* 2016). Hence, studying the downstream miRNA of hsa_circ_0000936 is beneficial for acknowledging the action mechanism of hsa_circ_0000936 in TMZ resistance in glioma.

In this study, we focused on the role of hsa_circ_0000936 in the resistance of glioma cells to TMZ *in vitro*. hsa_circ_0000936 was highly expressed in glioma tissues and in glioma tissues of patients showing resistance to TMZ. Mechanistic investigations suggested that downregulation of hsa_circ_0000936 sensitizes TMZ-resistant glioma cells to TMZ by sponging miR-1294, indicating that hsa_circ_0000936 represents a potential new therapeutic target for overcoming TMZ resistance in glioma.

2. Materials and methods

2.1 *Tissues specimen*

Glioma tissues and adjacent normal tissues were collected from 30 cases of patients undergoing surgery at the First Clinical Medical College, Zhejiang Chinese Medical University. According to their clinical responses to TMZ-based chemotherapy, glioma patients were classified into TMZ-sensitive cases (n = 18) and TMZ-resistant cases (n = 12). TMZ-sensitive case is distinguished when the primary tumor was markedly reduced, otherwise, it is defined as TMZ-resistant case. Informed consent was got from each participant before study initiation and Ethical Approval was obtained from the Institutional Review Board of the First Clinical Medical College, Zhejiang Chinese Medical University.

2.2 *Cell culture and transfection*

Human glioma cell lines (U251 and U87; American Type Culture Collection, ATCC, Rockville, MD, USA) were grown in Dulbecco's Modified Eagle's medium (DMEM; Solarbio, Beijing, China), supplemented with 10% fetal bovine serum (FBS; Solarbio) and 1% streptomycin/penicillin (Solarbio) at 37°C and 5% (v/v) CO_2. Glioma cell lines (U251 and U87) were incubated in DMEM medium with increasing concentration of TMZ, and experimentally induced into TMZ-resistant glioma cell lines (U251/TMZ-R and U87/TMZ-R). Initially, U251 and U87 cells were cultured in DMEM medium containing 5 μM of TMZ for 2 weeks. Thereafter, the dose of TMZ was gradually increased, and the last dose was 400 μM. The cells were cultured for 2 weeks at each dose of TMZ, and the medium was replaced every 2 days.

Short hairpin RNA (sh) targeting sh-hsa_circ_0000936 (sh-circ), sh-control (sh-ctrl), miR-ctrl, miR-1294, inhibitor-negative control (inhibitor-NC), miR-1294 inhibitor, hsa_circ_0000936 overexpression vector (over-circ) and empty vector (vector) were purchased from GenePharma (Shanghai, China). U251, U251/TMZ-R, U87 and U87/TMZ-R cells were transfected with corresponding plasmids using the Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) per manufacturer's instructions.

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

Total RNA was isolated from tissues and cells using TRIzol (ThermoFisher Scientific, Waltham, MA) in the light of the manufacturer's recommendations. TaqMan® MicroRNA Assay (Applied Biosystems, Foster City, CA, USA) was conducted to measure miR-583-3p, miR-1294, miR-635 and miR-665 from the cDNA, synthesized by using TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems). Quantification of hsa_circ_0000936 expression was conducted using One Step TB Green™ PrimeScript™ RT-PCR Kit (Takara, Dalian, China) and analyzed using the ABI 7900 Fast Real-Time PCR system (Applied Biosystems). The primers sequences were as follows: hsa_circ_0000936 forward, 5′-AGG TCA GGC AGA GGA AGT CA-3′ and reverse, 5′-CGC GTC ATA ACT GGT GAT GG-3′; β-actin forward, 5′-AGC CAT GTA CGT AGC CAT C-3′ and reverse, 5′-TCC CTC TCA GCT GTG GTG GTG AA-3′; miR-582-3p forward, 5′-TGC CAT AGT CTC TGC TGC TCA AG-3′ and reverse, 5′-CCA ACA CCA CGA ACA CGA AGT C-3′; miR-635 forward, 5′-TAT AGC ATA TGC AGG GTG-3′ and reverse, 5′-CGC ATT CGG AGT GCG AGT T-3′; miR-665 forward, 5′-ACCAGGAGGCTGAGGCCCCTAA-3′ and reverse, 5′- GCTGTCAACGATACGCTACCTA-3′; miR-1294 forward, 5′-TAT GAT CTC ACC GAG TCC T-3′ and reverse, 5′-CAC CTT CCT AAT CCT CAG TT-3′; U6 forward, 5′-GCT TCG GCA GCA CAT ATA CTA AAA T-3′ and reverse, 5′-CGC TTC ACG AAT TTG CGT GTC AT-3′. Gene expression was normalized to an endogenous internal control (β-actin for hsa_circ_0000936 and U6 for miRNAs) by the $2^{-\Delta\Delta Ct}$ method.

2.4 *Luciferase reporter assay*

The putative miR-1294 target binding sequences and mutant sequences in hsa_circ_0000936 were amplified and subcloned into pGL-3 luciferase reporter vector to generate the WT-circ and MUT-circ constructs. U251, U251/TMZ-R, U87 and U87/TMZ-R cells were co-transfected with WT-circ or MUT-circ constructs and miR-ctrl, miR-1294, inhibitor-NC or miR-1294 inhibitor. After 24 h of transfection, U251, U251/TMZ-R, U87 and U87/TMZ-R cells were collected and lysates were assayed for the luciferase activity with the Dual-luciferase assay system (Promega, Madison, WI, USA).

2.5 *CCK-8 assay*

Cell viability was assessed using cell count kit-8 (CCK-8). In brief, the cells in exponential growth were collected, dissociated, resuspended and seeded in a 96-well plate. After 24 h of incubation, cells were transfected with corresponding plasmids and then treated with different doses (0, 100, 200, 300, 400 and 500 μM) of TMZ for 48 h. Thereafter, 10 μl of CCK-8 solution was added into each well, and the absorbance at 450 nm was detected after 2 h of incubation using a microplate reader. The half maximal inhibitory concentration (IC50) of U251, U251/TMZ-R, U87 and U87/TMZ-R cells to TMZ was determined using GraphPad Prism software (La Jolla, CA).

2.6 *Flow cytometry*

Cell apoptosis was evaluated using the Annexin V FITC and PI kit (Thermo Fisher, San Jose, CA) following the product manual. After treatment, U251, U251/TMZ-R, U87 and U87/TMZ-R cells were rinsed in cold phosphate-buffered saline, trypsinised and suspended in Annexin V binding buffer. Afterwards, cells were stained with Annexin V and PI working solution. After 15 min of staining at room temperature, cells were analyzed using flow cytometry.

2.7 *Statistical analysis*

Data are represented as the mean ± standard deviation. Comparisons between groups were performed using Student's *t* test or one-way analysis of variance by the SPSS 20.0 software (IBM, Armonk, NY, USA). A probability value of $P<0.05$ was deemed a criterion for statistical significance. All experiments were repeated three times.

3. Results

3.1 *Ectopic expression of hsa_circ_0000936 in glioma tissues*

In this work, we focused on the role of hsa_circ_0000936 in glioma. hsa_circ_0000936 was firstly characterized by qRT-PCR. We found that the expression of hsa_circ_0000936 was much higher in glioma

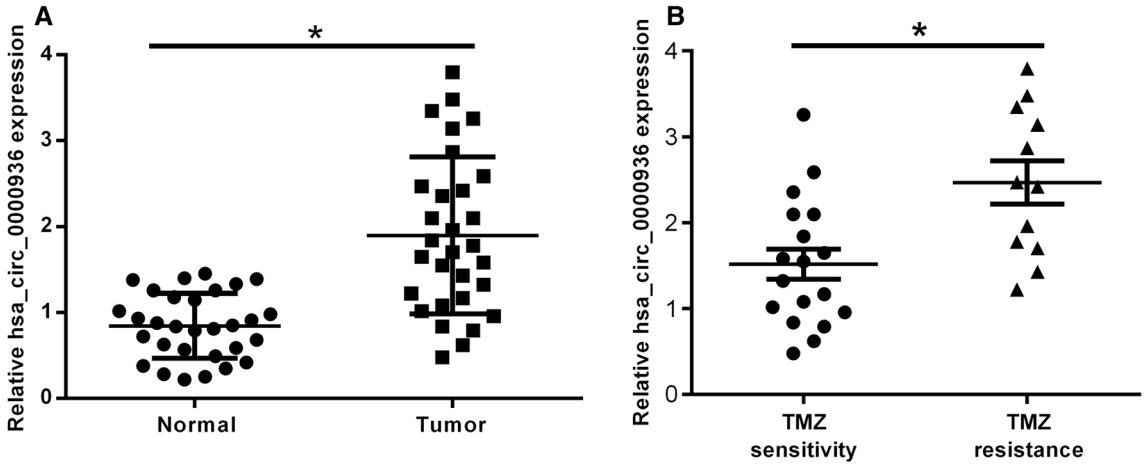

Figure 1. Ectopic expression of hsa_circ_0000936 in glioma tissues. (**A**) Comparison of hsa_circ_0000936 in glioma tissues versus their adjacent normal tissues by qRT-PCR. (**B**) Comparison of hsa_circ_0000936 in the glioma tissues of patients showing sensitivity to TMZ and in the glioma tissues of patients showing resistance to TMZ by qRT-PCR. *$P < 0.05$.

tissues than that in their adjacent normal tissues (figure 1A). Moreover, hsa_circ_0000936 expression was higher in the glioma tissues of patients showing resistance to TMZ than that in the glioma tissues of patients showing sensitivity to TMZ (figure 1B).

3.2 *Establishment of TMZ-resistant glioma cell lines*

We established TMZ-resistant glioma cells lines (U251/TMZ-R and U87/TMZ-R) by stepwise exposure to increasing doses of TMZ, and the resistance of U251/TMZ-R and U87/TMZ-R was validated by CCK-8 assay. U87, U251, U251/TMZ-R and U87/TMZ-R cells were treated with increasing concentrations (0, 100, 200, 300, 400 and 500 μM) of TMZ, and assayed for cell survival. The dose-effect curve revealed that the cell survival rate of U251/TMZ-R and U87/TMZ-R cells appeared to be much higher than that of U251 and U87 cells following TMZ treatment. Stimulatenously, the IC50 of TMZ-resistant cells was markedly higher than that of parental cells, suggesting that U251/TMZ-R and U87/TMZ-R cells show much resistant to TMZ (figure 2A and B). Moreover, hsa_circ_0000936 expression was strikingly increased in U251/TMZ-R and U87/TMZ-R cells compared with that in U251 and U87 cells (figure 2C). On the contrary, miR-1294 was downregulated in U251/TMZ-R and U87/TMZ-R cells compared with that in U251 and U87 cells (figure 2D).

3.3 *hsa_circ_0000936 acts as a molecular sponge to modulate miR-1294*

According to the prediction, we found that hsa_circ_0000936 sequence contains multiple binding sites for miRNAs, including miR-582-3p, miR-1294, miR-635 and miR-665. To determine the downstream miRNA of hsa_circ_0000936, we transfected U251, U251/TMZ-R, U87 and U87/TMZ-R cells with sh-ctrl or sh-circ and analyzed the expression of miR-582-3p, miR-1294, miR-635 and miR-665 using qRT-PCR. The results showed that the expression levels of miR-582-3p and miR-1294, but not miR-635 and miR-665 were markedly increased upon sh-circ transfection. Notably, the top upregulated miRNA was found to be miR-1294 (figure 3A and B). We transfected U251, U251/TMZ-R, U87 and U87/TMZ-R cells with miR-ctrl, miR-1294, inhibitor-NC or miR-1294-inhibitor, and the transfection efficiency was determined by qRT-PCR. The expression of miR-1294 was obviously increased upon miR-1294 transfection, while decreased upon miR-1294-inhibitor transfection (figure 3C and D). As shown in figure 3E, hsa_circ_0000936 sequence has a binding site for miR-1294. Thereafter, we validated the direct binding via luciferase reporter assay. When we co-transfected cells with WT-circ and miR-1294, a marked decrease in luciferase activity of WT-circ reporter was discovered. Conversely, Co-transfection of U251, U251/TMZ-R, U87 and U87/TMZ-R cells with WT-circ and miR-1294 inhibitor caused a

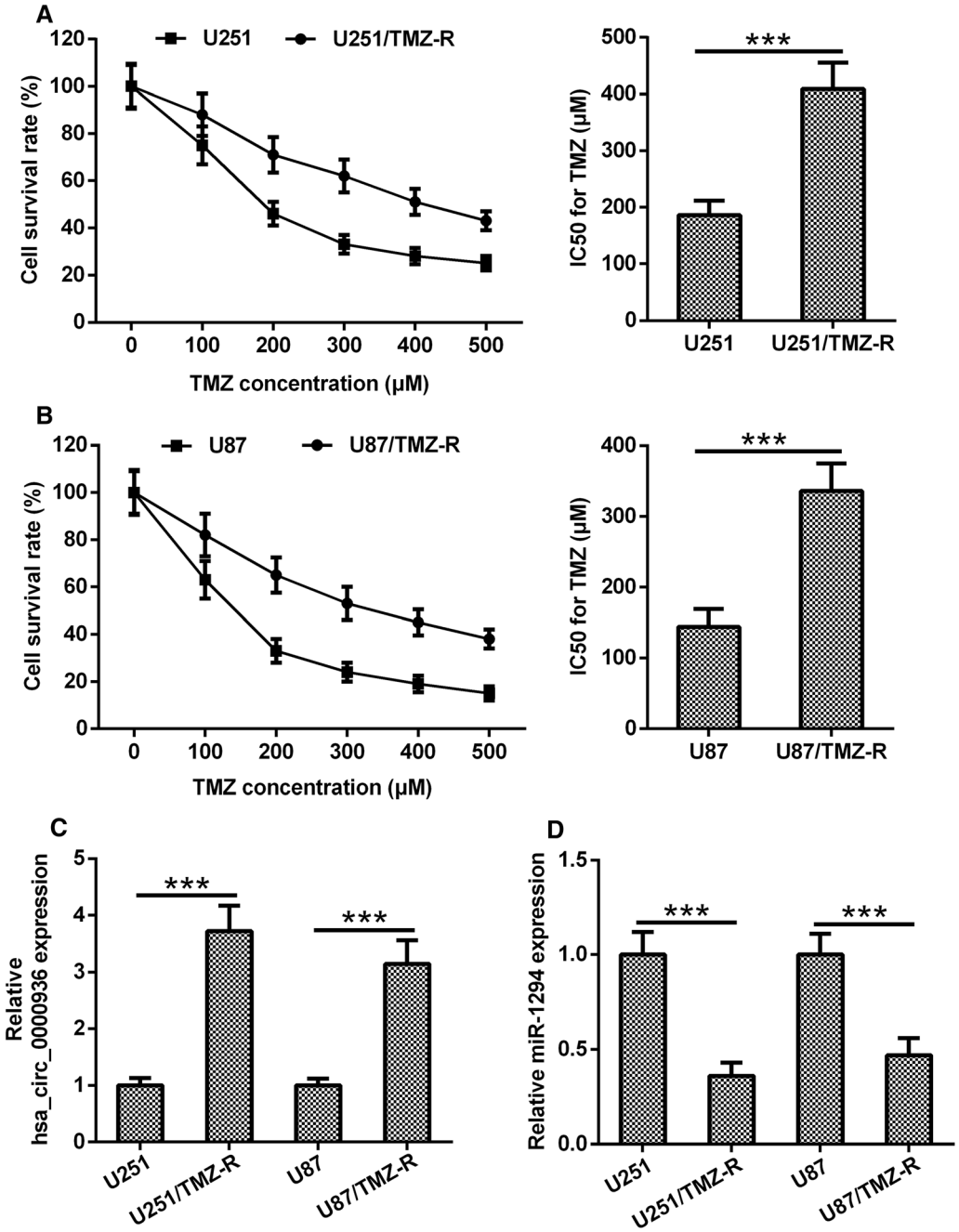

Figure 2. The establishment of TMZ-resistant glioma cell lines. (**A**) U251 and U251/TMZ-R cells were treated with increasing concentrations (0, 100, 200, 300, 400 and 500 μM) of TMZ. CCK-8 assay was used to detected cell viability and calculate the IC50 for TMZ. (**B**) U87 and U87/TMZ-R cells were treated with increasing concentrations (0, 100, 200, 300, 400 and 500 μM) of TMZ, and the cell viability and IC50 for TMZ were analyzed by CCK-8 assay. (**C**) Comparison of hsa_circ_0000936 expression in U251 and U251/TMZ-R cells. (**D**) Comparison of hsa_circ_0000936 expression in U87 and U87/TMZ-R cells. ***$P < 0.001$.

substantial increase in luciferase activity of WT-circ reporter. Besides, we found that the luciferase activity of MUT-circ, which includes seven mutations in the miR-1294 binding site of hsa_circ_0000936, was unaffected by miR-1294 or miR-1294-inhibitor transfection (figure 3F and G).

3.4 *Downregulation of hsa_circ_0000936 increases TMZ sensitivity in TMZ-resistant glioma cell lines*

To further characterize the role of hsa_circ_0000936 in TMZ resistance mechanisms, we transfected U251,

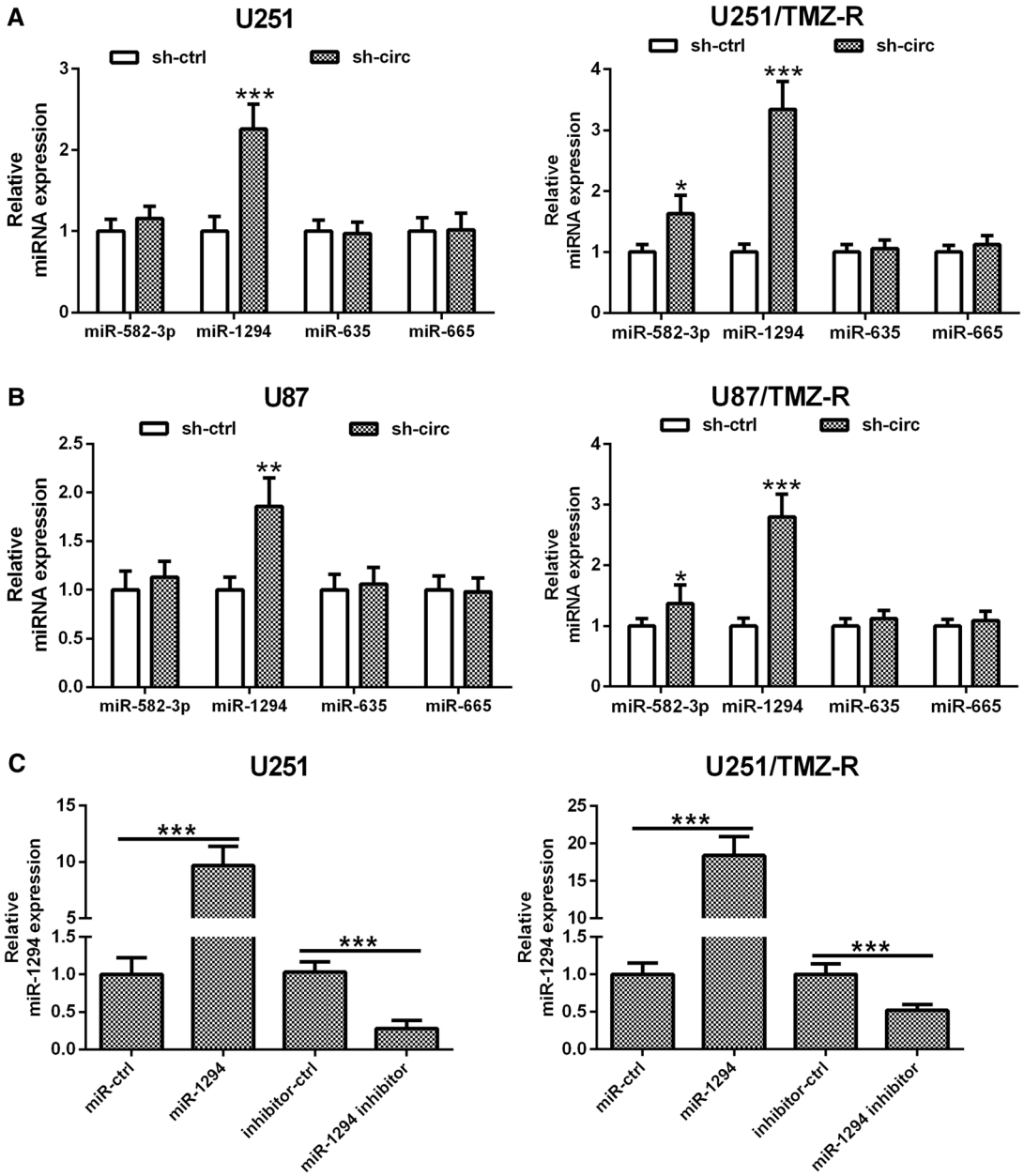

Figure 3. hsa_circ_0000936 acts as a molecular sponge to modulate miR-1294. (**A** and B) Evaluation of miR-1294 expression after transfection of U251, U251/TMZ-R, U87 and U87/TMZ-R cells with sh-circ or sh-ctrl. (**C** and **D**) Evaluation of miR-1294 expression after transfection of U251, U251/TMZ-R, U87 and U87/TMZ-R cells with miR-ctrl, miR-1294, inhibitor-NC or miR-1294-inhibitor. (**E**) The sequences for hsa_circ_0000936 and miR-1294. (**F** and **G**) Luciferase reporter assay was conducted to show the direct interaction between miR-1294 and hsa_circ_0000936. $^{**}P < 0.01$ and $^{***}P < 0.001$.

U251/TMZ-R, U87 and U87/TMZ-R cells with sh-ctrl or sh-circ, followed by treatment with 300 μM of TMZ. The results of CCK-8 assay showed that knockdown of hsa_circ_0000936 is associated with TMZ resistance, causing a substantial decrease in the IC50 for TMZ in U251 and U251/TMZ-R cells. While, U87 and U87/TMZ-R cells expressing sh-circ yielded similar results (figure 4A and B). Simultaneously, flow cytometry analysis revealed that inhibition of hsa_circ_0000936 led to a marked increase in the apoptotic rate of U251, U251/TMZ-R, U87 and U87/TMZ-R (figure 4C and D).

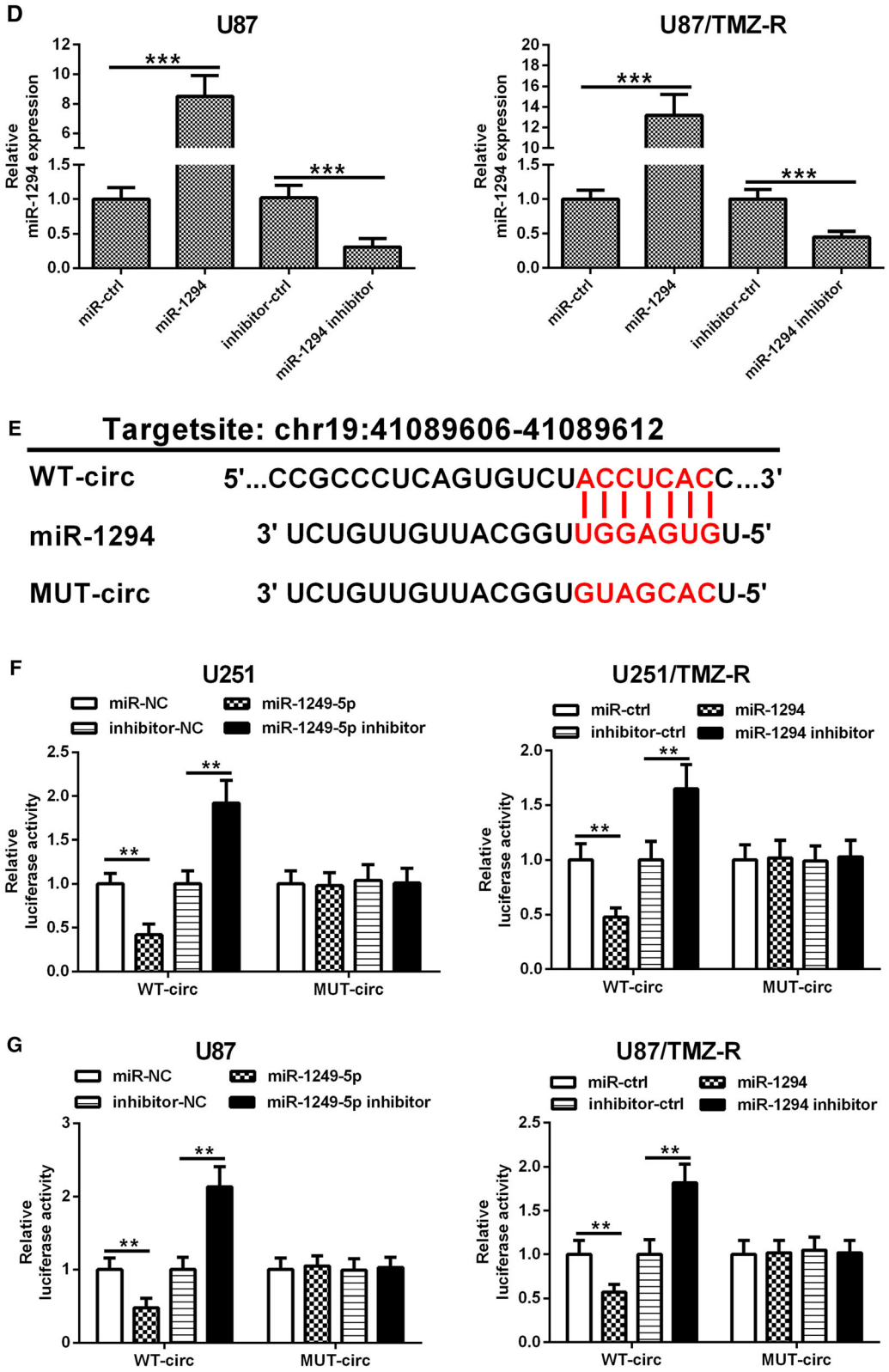

Figure 3. continued

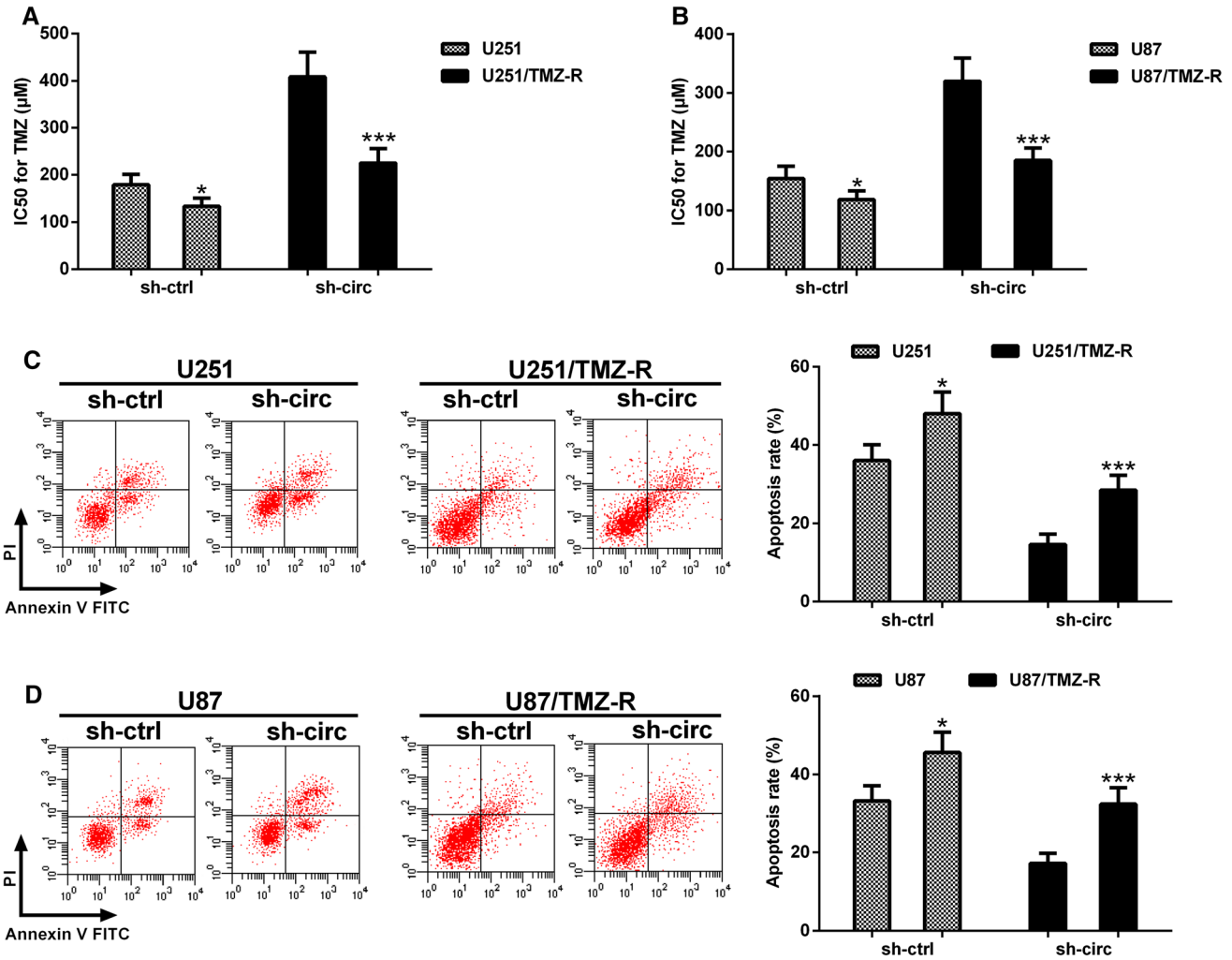

Figure 4. Downregulation of hsa_circ_0000936 increases TMZ sensitivity in TMZ-resistant glioma cell lines. CCK-8 assay was performed to evaluate the impact of hsa_circ_0000936 knockdown in U251 and U251/TMZ-R (**A**), U87 and U87/TMZ-R (**B**) cells exposed to 300 μM of TMZ. Flow cytometry was exploited to assess the effect of hsa_circ_0000936 knockdown in U251 and U251/TMZ-R (**C**), U87 and U87/TMZ-R (**D**) cells exposed to 300 μM of TMZ. $*P < 0.05$ and $***P < 0.001$.

3.5 *Restoration of miR-1294 abrogates the promotion effect of hsa_circ_0000936 on TMZ resistance in TMZ-resistant glioma cell lines*

To further delineate the role of miR-1294 in hsa_circ_0000936-mediated resistance to TMZ, restoration experiment was performed in U251, U251/TMZ-R, U87 and U87/TMZ-R cells. U251, U251/TMZ-R, U87 and U87/TMZ-R cells were transfected with over-circ alone or with miR-1294, followed by treatment with 300 μM of TMZ. The IC50 for TMZ was obviously increased after transfection of U251 and U251/TMZ-R cells with over-circ, however, this increase was blocked by miR-1294 transfection (figure 5A). Similar results were also observed in U87 and U87/TMZ-R cells (figure 5B). Simultaneously, overexpression of hsa_circ_0000936 reduced the apoptotic rate of U251, U251/TMZ-R, U87 and U87/TMZ-R cells, whereas upregulation of miR-1294 abolished this action (figure 5C and D).

4. Discussion

TMZ resistance has become the great challenge in glioma treatment, yet its molecular mechanism is still dimness. Recently, aberrant expression of circRNAs has been found to be associated with the development of chemoresistance (Xu *et al.* 2018). Therefore, investigation of the function of circRNAs in therapeutic drug resistance is of great importance, and it may provide new insights for overcoming the resistance of glioma cells to TMZ.

Discoveries of circRNAs have provided a new vision for understanding the underlying mechanism of

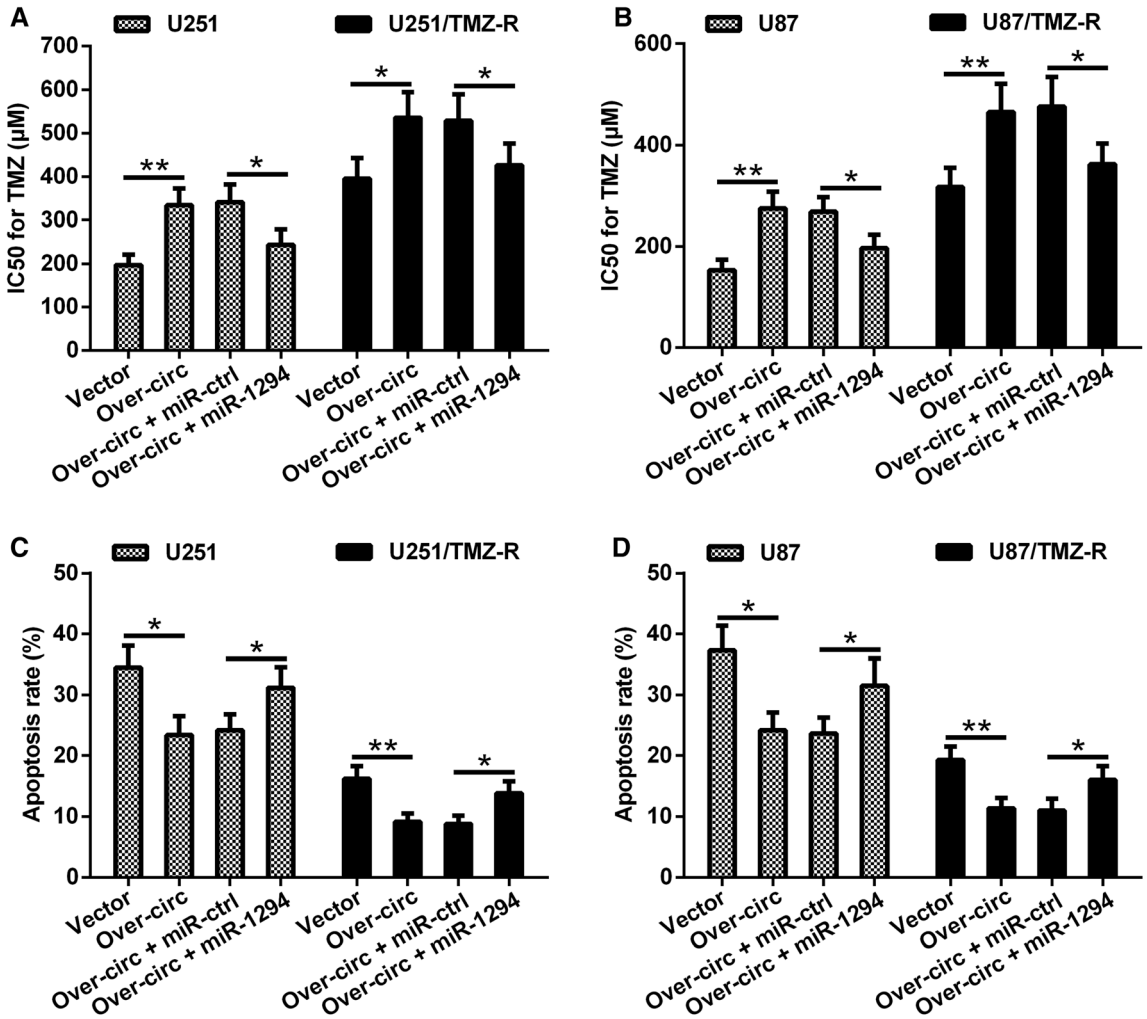

Figure 5. Restoration of miR-1294 abrogates the promotion effect of hsa_circ_0000936 on TMZ resistance in TMZ-resistant glioma cell lines. U251, U251/TMZ-R, U87 and U87/TMZ-R cells were transfected with over-circ alone or with miR-1294, followed by treatment with 300 μM of TMZ. (**A** and **B**) CCK-8 cell viability assay was performed in U251, U251/TMZ-R, U87 and U87/TMZ-R cells. (**C** and **D**) Flow cytometry analysis of U251, U251/TMZ-R, U87 and U87/TMZ-R cells. $*P < 0.05$ and $**P < 0.01$.

chemoresistance and new inspiration for overcoming chemoresistance (Geng *et al.* 2018). A substantial body of evidence has been accumulated suggesting that circRNAs serve as a crucial regulator in the development of chemoresistance (Gao *et al.* 2017). As an example, increased expression of circPVT1 has been associated with poor prognosis of osteosarcoma, implying the potential diagnostic role of circPVT1. Moreover, knockdown of circPVT1 decreased the resistance of osteosarcoma cells to doxorubicin and cisplatin by inhibiting the expression of ATP-binding cassette subfamily B member 1 (Kun-Peng *et al.* 2018). Similarly, studies in cisplatin-resistant gastric cancer cells (SGC7901CDDP) suggest that circAKT3 functions as a sponge of miR-198 to restore the expression of phosphatidylinositol 3-kinase regulatory subunit alpha and activate the phosphoinositide 3-kinase/ATK signaling, ultimately facilitating cisplatin resistance (Huang *et al.* 2019). Furthermore, circRNA-MTO1 was found to be upregulated in monastrol-resistant breast cancer cells, and overexpression of circRNA-MTO suppressed the viability of MCF-7 and MDA-MB-231 cells, and weakened the resistance of MCF-7R and MDA-MB-231R cells towards to monastrol through regulating the TNF receptor-associated factor 4/kinesin-5 axis (Liu *et al.* 2018). Nevertheless, no previous implication of hsa_circ_0000936 in responsiveness to TMZ resistance has been reported. In this study, we identified the upregulation of hsa_circ_0000936 in glioma tissues, as well as in the glioma tissues of

patients showing resistance to TMZ, suggesting the association between hsa_circ_0000936 and TMZ resistance. In addition to the findings from glioma tissues, hsa_circ_0000936 was also found to be upregulated in U251/TMZ-R and U87/TMZ-R cells compared with their parental cells. It is important to underscore that knockdown of hsa_circ_0000936 increased the sensitivity of TMZ-resistant glioma cells to TMZ, suggesting the potential therapeutic target of hsa_circ_0000936 in TMZ resistance in glioma.

Evidence has emerged indicating that circRNAs typically function as miRNA sponges to participate in the development of chemoresistance (Wang *et al.* 2018a; Wu *et al.* 2019). Hence, we determine the miRNAs downstream of hsa_circ_0000936 in glioma. According to the bioinformatics analysis, we found that hsa_circ_0000936 sequence contains multiples putative binding sites for miRNA, including miR-583-3p, miR-1294, miR-635 and miR-665. As determined by qRT-PCR, downregulation of hsa_circ_0000936 strikingly increased the expression of miR-583-3p and miR-1294, but not miR-635 and miR-665. Remarkably, the top upregulated miRNA was found to be miR-1294, which might be the direct target of hsa_circ_0000936. There is also evidence to suggest that miR-1294 is implicated in cancer progression and chemoresistance (Shi *et al.* 2018). For instance, miR-1294 was found to be downregulated in various human malignancies, including osteosarcoma, esophageal squamous cell carcinoma and oral squamous cell carcinoma. Decreased expression of miR-1294 has been proposed to be associated with the International Federation of Gynecology and Obstetrics stage, lymph node metastasis and shorter overall survival rate in patients with epithelial ovarian cancer, suggesting the prognostic indicator role of miR-1294 (Guo *et al.* 2018). Upregulation of miR-1294 inhibited the proliferation and invasion of osteosarcoma cells through targeting homeobox A9 (Zhang *et al.* 2018b). In addition, miR-1294 reportedly repressed cell proliferation, migration and invasion through inhibiting the expression of c-Myc (Liu *et al.* 2015; Wang *et al.* 2018b). Notably, the downregulation of miR-1294 was observed in cisplatin-resistant ovarian cancer tissues and cells, and overexpression of miR-1294 inhibited cell proliferation, migration and invasion, and reversed the epithelial-mesenchymal transition phenotype, as well as re-sensitized SKOV3/DDP cells towards cisplatin through targeting insulin-like growth factor 1 receptor, suggesting the involvement of miR-1294 in chemoresistance (Zhang *et al.* 2018a). In glioma, miR-1294 has been documented to downregulated in glioma tissues and cells. Gain-of-function experiments revealed that forced expression of miR-1294 in U87 and U251 cells suppressed cell proliferation, migration and invasion, and reinforced chemosensitivity to TMZ (Chen *et al.* 2018). However, whether hsa_circ_0000936 regulated the chemoresistance of glioma cell to TMZ through sponging miR-1254 is largely unknown. The interaction of hsa_circ_0000936 and miR-1294 has been confirmed by luciferase reporter assay. More importantly, restoration of miR-1294 abrogated the promotion effect of hsa_circ_0000936 on TMZ resistance in U251/TMZ-R and U87/TMZ-R cells. This suggested that downregulation of hsa_circ_0000936, sponging miR-1294, re-sensitized TMZ-resistant glioma cells to TMZ, implying a novel mechanism of chemoresistance.

In conclusion, hsa_circ_0000936 defined as a miR-1294 sponge to confer TMZ resistance in glioma. This study is the first to identify the role of hsa_circ_0000936 in chemoresistance, and provides new insights into mechanisms of chemoresistance. Our work indicates that targeting hsa_circ_0000936 may be used to overcome resistance to TMZ in glioma.

Acknowledgements

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References

Arnaiz E, Sole C, Manterola L, Iparraguirre L, Otaegui D and Lawrie CH 2018 CircRNAs and cancer: Biomarkers and master regulators. *Semin. Cancer Biol.* **58** 90–99

Barrett SP and Salzman J 2016 Circular RNAs: analysis, expression and potential functions. *Development* **143** 1838–1847

Brada M, *et al.* 2003 Phase II study of primary temozolomide chemotherapy in patients with WHO grade II gliomas. *Ann. Oncol.* **14** 1715–1721

Chen H, Liu L, Li X, Shi Y and Liu N 2018 MicroRNA-1294 inhibits the proliferation and enhances the chemosensitivity of glioma to temozolomide via the direct targeting of TPX2. *Am. J. Cancer Res.* **8** 291–301

Conn SJ, Pillman KA, Toubia J, Conn VM, Salmanidis M, Phillips CA, Roslan S, Schreiber AW, Gregory PA and Goodall GJ 2015 The RNA binding protein quaking regulates formation of circRNAs. *Cell* **160** 1125–1134

Gao D, Zhang X, Liu B, Meng D, Fang K, Guo Z and Li L 2017 Screening circular RNA related to chemotherapeutic resistance in breast cancer. *Epigenomics* **9** 1175–1188

Geng Y, Jiang J and Wu C 2018 Function and clinical significance of circRNAs in solid tumors. *J. Hematol. Oncol.* **11** 98

Guo TY, Xu HY, Chen WJ, Wu MX and Dai X 2018 Downregulation of miR-1294 associates with prognosis and tumor progression in epithelial ovarian cancer. *Eur. Rev. Med. Pharmacol. Sci.* **22** 7646–7652

Hao Z, Hu S, Liu Z, Song W, Zhao Y and Li M 2019 Circular RNAs: Functions and prospects in glioma. *J. Mol. Neurosci.* **67** 72–81

He Q, *et al.* 2018 circ-SHKBP1 Regulates the Angiogenesis of U87 glioma-exposed endothelial cells through miR-544a/FOXP1 and miR-379/FOXP2 pathways. *Mol. Ther. Nucleic Acids* **10** 331–348

Hombach-Klonisch S, *et al.* 2018 Glioblastoma and chemoresistance to alkylating agents: Involvement of apoptosis, autophagy, and unfolded protein response. *Pharmacol. Ther.* **184** 13–41

Huang X, *et al.* 2019 Circular RNA AKT3 upregulates PIK3R1 to enhance cisplatin resistance in gastric cancer via miR-198 suppression. *Mol. Cancer.* **18** 71

Kun-Peng Z, Xiao-Long M and Chun-Lin Z 2018 Overexpressed circPVT1, a potential new circular RNA biomarker, contributes to doxorubicin and cisplatin resistance of osteosarcoma cells by regulating ABCB1. *Int. J. Biol. Sci.* **14** 321–330

Lei B, Huang Y, Zhou Z, Zhao Y, Thapa AJ, Li W, Cai W and Deng Y 2019 Circular RNA hsa_circ_0076248 promotes oncogenesis of glioma by sponging miR-181a to modulate SIRT1 expression. *J. Cell Biochem.* **120** 6698–6708

Li X, Yang L and Chen LL 2018 The biogenesis, functions, and challenges of circular RNAs. *Mol. Cell.* **71** 428–442

Liu K, Li L, Rusidanmu A, Wang Y and Lv X 2015 Down-regulation of MiR-1294 is related to dismal prognosis of patients with esophageal squamous cell carcinoma through elevating C-MYC expression. *Cell Physiol. Biochem.* **36** 100–110

Liu Y, Dong Y, Zhao L, Su L and Luo J 2018 Circular RNAMTO1 suppresses breast cancer cell viability and reverses monastrol resistance through regulating the TRAF4/Eg5 axis. *Int. J. Oncol.* **53** 1752–1762

Margison GP, Santibanez Koref MF and Povey AC 2002 Mechanisms of carcinogenicity/chemotherapy by O6-methylguanine. *Mutagenesis* **17** 483–487

Memczak S, *et al.* 2013 Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* **495** 333–338

Mercatelli N, Galardi S and Ciafre SA 2017 MicroRNAs as Multifaceted Players in Glioblastoma Multiforme. *Int. Rev. Cell Mol. Biol.* **333** 269–323

Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C and Barnholtz-Sloan JS 2016 CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009–2013. *Neuro. Oncol.* **18** v1–v75

Rupaimoole R, Calin GA, Lopez-Berestein G and Sood AK 2016 miRNA Deregulation in cancer cells and the tumor microenvironment. *Cancer Discov.* **6** 235–246

Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP 2011 A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146** 353–358

Shi YX, Ye BL, Hu BR and Ruan XJ 2018 Expression of miR-1294 is downregulated and predicts a poor prognosis in gastric cancer. *Eur. Rev. Med. Pharmacol. Sci.* **22** 5525–5530

Sun W, Julie Li YS, Huang HD, Shyy JY and Chien S 2010 microRNA: a master regulator of cellular processes for bioengineering systems. *Annu. Rev. Biomed. Eng.* **12** 1–27

van Thuijl HF, *et al.* 2015 Evolution of DNA repair defects during malignant progression of low-grade gliomas after temozolomide treatment. *Acta Neuropathol.* **129** 597–607

Wang R, Zhang S, Chen X, Li N, Li J, Jia R, Pan Y and Liang H 2018a CircNT5E acts as a sponge of miR-422a to promote glioblastoma tumorigenesis. *Cancer Res.* **78** 4812–4825

Wang Z, Yan J, Zou T and Gao H 2018b MicroRNA-1294 inhibited oral squamous cell carcinoma growth by targeting c-Myc. *Oncol. Lett.* **16** 2243–2250

Wu Y, *et al.* 2019 Circular RNA circTADA2A promotes osteosarcoma progression and metastasis by sponging miR-203a-3p and regulating CREB3 expression. *Mol. Cancer* **18** 73

Xu C, Yu Y and Ding F 2018 Microarray analysis of circular RNA expression profiles associated with gemcitabine resistance in pancreatic cancer cells. *Oncol. Rep.* **40** 395–404

Zhang Y, Huang S, Guo Y and Li L 2018a MiR-1294 confers cisplatin resistance in ovarian cancer cells by targeting IGF1R. *Biomed. Pharmacother.* **106** 1357–1363

Zhang ZF, Li GR, Cao CN, Xu Q, Wang GD and Jiang XF 2018b MicroRNA-1294 targets HOXA9 and has a tumor suppressive role in osteosarcoma. *Eur. Rev. Med. Pharmacol. Sci.* **22** 8582–8588

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