LncRNA SNHG11 Promotes Temozolomide Resistance in Glioblastoma via Promoting MGMT Expression

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Research

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Abstract

Background

Acquired TMZ resistance is considered as the main reason for the poor prognosis of glioblastoma (GBM) patients. However, underlying mechanism remains unknown. Long noncoding RNAs (lncRNAs) have emerged as important regulators in multiple biological processes.

Methods

SNHG11 expression in cells and GBM tissues was measured using qRT-PCR. In vitro studies, including CCK-8, colony formation assay, flow cytometry and western blot, were employed to measure the role of SNHG11. Interaction between miR-7-5p, SNHG11, and IRS2 was examined by dual luciferase reporter assay, as well as RNA binding protein immunoprecipitation (RIP) assay. CHIP assays were used to measure the role of SNHG11/miR-7-5p/IRS2 axis on modulating the H3K9 acetylation of MGMT.

Results

SNHG11 overexpression in GBM tissues contributes to TMZ resistance. *In vitro* and *in vivo* studies confirmed that SNHG11 promoted TMZ resistance in GBM cells. In addition, SNHG11 conferred TMZ resistance through increasing MGMT expression. Furthermore, SNHG11 could function as ceRNA by sponging miR-7-5p, which led to increased IRS2 expression. SNHG11/miR-7-5p/IRS2 axis increased MGMT expression by promoting the acetylation of H3K9 in MGMT promoter regions.

Conclusion

Taken together, our results revealed that targeting SNHG11 is a potential therapy to overcome TMZ resistance. And SNHG11 in GBM tissues is a potential biomarker for predicting response to TMZ.

Background

Glioma is the most common tumor in the central nervous system of adults, and glioblastoma (GBM) comprises more than half of glioma^{1–3}. Despite of the aggressive therapy including maximal resection followed by radiotherapy and chemotherapy, the prognosis of GBM patients is still poor⁴. Temozolomide (TMZ) is the first-line drug for chemotherapy^{3, 5}. However, due to the extensive invasion, enforced DNA damage repair ability and other aggressive phenotype, drug resistance is very common^{6–8}. And the mechanisms that contribute to TMZ resistance remain to be explored.

Long non-coding RNAs (lncRNA) are a class of RNAs that transcript more than 200 nucleotides with no or low ability of coding protein⁹. Recent studies have revealed the multiple biological function of lncRNAs in

GBM cells, like apoptosis¹⁰, proliferation¹¹, invasion¹², immune response¹³. LncRNAs have also been proved to regulating TMZ resistance¹⁴. However, the application of lncRNAs-based therapeutic intervention on GBM recurrence needs more research results.

Here, we investigated the contribution of lncRNA SNHG11 on regulating TMZ resistance in GBM cells, and discussed the implication of SNHG11 on predicting response of GBM patients to TMZ. Our results showed that SNHG11 functions as a ceRNA by sponging miR-7-5p, resulting to increased IRS2 expression. In addition, SNHG11 overexpression leads to STAT3 activation through IRS2, and activated STAT3 promotes the remodeling of histone H3 acetylation in MGMT promoter region. Together, SNHG11/miR-7-5p/IRS2/MGMT axis enhances the development of acquired TMZ resistance in GBM cells.

Methods

Clinical samples

The clinical GBM samples and normal brain tumor samples were obtained from the Department of Neurosurgery, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China. Written informed consents were obtained from all patients. All samples were collected during surgery and were frozen in liquid nitrogen for RNA or protein extraction.

Cell Culture

Human GBM cells U87 and T98G were purchased from ATCC. Primary human GBM cells pGBM1 and pGBM2 were established from two GBM samples as previously described. All cells were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C with 5% CO₂.

Western blot assay

Protein was collected from cells using RIPA lysis buffer as previously described. The information of antibodies used in this research are listed in Supplementary Materials.

RNA extraction and quantitative real-time PCR assays

Total RNA from GBM tissues and cells was extracted using TRIzol® Reagent (Invitrogen, CA, USA) as previously described. 0.5% NP-40 (Solarbio, Nanjing, China) was used to separate the nuclear and cytoplasmic fractions with an RNAase inhibitor. cDNA was obtained using PrimeScript RT Master Mix according to the manuscript. Real-time quantitative PCR was performed using the SYBR Green Premix Ex Taq (Takara, Nanjing, China). Relative RNA levels were determined by normalizing to GAPDH. The miRNA levels were analyzed using the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) and normalized with U6 snRNA. The primers sequences were provided in Supplementary Materials.

Cell transfection

Lipofectamine 2000 (Invitrogen, USA) were used for siRNAs, miRNA mimics or anti-miRNA transfection into GBM cells. For SNHG11 overexpression, full-length complementary cDNAs of SNHG11 was cloned into pcDNA 3.1 (Invitrogen). For SNHG11 down-regulation, we designed and synthesized sh-control or SNHG11 shRNAs from Genechem (Shanghai, China).

CCK-8 assay

CCK-8 assay was performed using the Cell Counting Kit 8 (Dojindo, Japan) to evaluate the sensitivity of GBM cells to TMZ. Absorbance was measured at OD 450nm.

Colony formation assay

GBM cells were seeded in six-well plates and maintained for about two weeks. Then, cells were washed twice using PBS, and fixed with 4% formaldehyde for 15 min. 0.1% crystal violet was used to stain cell colonies.

Luciferase reporter assay

To identify the connection between SNHG11 and miR-7-5p, GBM cells were co-transfected with wild-type or mutant SNHG11 reporter plasmid together with miR-7-5p. To confirm that miR-7-5p targets IRS2, we co-transfected wild-type or mutant IRS2 reporter plasmid with miR-7-5p into GBM cells. The luciferase activities were measured using the Dual Luciferase Reporter Assay System (Promega) and normalized to Renilla luciferase activity.

Apoptosis analysis

The apoptosis analysis were performed using the annexin V-fluorescein isothiocyanate apoptosis detection kit I (BD Bioscience, USA). Flow cytometry analysis was used to analysis the apoptotic rate.

RNA immunoprecipitation (RIP)

The RIP assays were performed to conform the connection between SNHG11 and miR-7-5p or IRS2 through Ago2. Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA, USA) was used according to the protocol. The RNA fraction was analyzed by qPCR.

Chromatin immunoprecipitation (ChIP) assay

CHIP assays were employed using EZ-ChIP (Millipore) according to protocol. PCR assays were performed to analyze the pull-down products. Sequences used for ChIP-PCR are provided in Supplementary Materials.

Fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) analysis

To identify the distribution of SNHG11 in GBM cells, FISH analysis was performed as previously described. The expression of cleaved caspase-3, IRS2 and MGMT in tumors were measured using IHC as previously described.

Animal study

The animal study in this study was approved by the Nanjing Medical University Animal Experimental Ethics Committee.

Female nude mice (four-week-old) were purchased from Charles River (Beijing, China). The establishment of intracranial tumor was performed as previously described. Briefly, 1×10^5 GBM cells were implanted stereo-tactically into the brain of mice. One week later, mice were treated with TMZ (60mg/kg per day for 5 days). Bioluminescence imaging system was used to monitor the growth of brain tumor. The mouse brain tumor was fixed in 4% paraformaldehyde and sectioned for further IHC analysis.

Statistical analysis

All experiments in this study were performed at least three times. Student's *t-test*, one-way ANOVA or chi-square test were used to estimate significant difference between different groups. All statistical analysis were performed using GraphPad software version 8.0 (GraphPad Software, CA, USA). All results are shown as mean $\pm$ standard error of the mean (SEM). $p < 0.05$ was considered as statistic significance.

Results

SNHG11 is highly expressed in GBM tissues and confers poor prognosis

We firstly analyzed SNHG11 expression levels in glioma by TCGA and CGGA, and the results showed that the levels of SNHG11 were significantly increased with the increase of tumor grade (Figure 1A and B). Meanwhile, we analyzed SNHG11 levels in glioma samples and got the similar results compared with analysis results of public database (Figure 1D). In addition, higher expression of SNHG11 indicated worse overall survival (OS) than low levels of SNHG11 (Figure 1C). In addition, SNHG11 expression levels were significantly higher in recurrent GBM samples than primary GBM samples (Figure 1E). Survival analysis results suggested that high SNHG11 levels indicated poor response to TMZ (Figure 1F).

Together, these analysis results suggest that SNHG11 may be a key regulator of glioma progression, including TMZ resistance.

SNHG11 confers resistance to TMZ

As MGMT is the key element mediating TMZ resistance, the role of SNHG11 in different GBM cells with different MGMT status (Supplementary Figure 1A) was analyzed. Firstly, U87, T98G, pGBM1 and pGBM2 cells were transfected with sh-ctrl or sh-SNHG11 (Supplementary Figure 1B). As shown in Figure 2A and B, SNHG11 down-regulation resulted in a significant decreased IC50 and cell growth of T98G and pGBM1

upon TMZ treatment. However, the IC50 and cell growth of U87 and pGBM2 were not affected by SNHG11 knockdown (Supplementary Figure 1C and D). Moreover, SNHG11 knockdown contributed increased apoptosis of T98G and pGBM1, while the apoptosis of U87 and pGBM2 was not affected (Figure 2C and D, Supplementary Figure 1E and F). These results strongly suggested that SNHG11 plays an important role on mediating TMZ resistance in GBM cells.

SNHG11 confers TMZ resistance through elevating MGMT

According to the above results, we hypothesized that SNHG11 could modulate TMZ resistance in GBM cells through MGMT. To confirm the hypothesis, qPCR and WB were performed and the results showed that overexpression of SNHG11 in T98G and pGBM1 cells significantly increased the mRNA and protein levels of MGMT (Figure 3A and B). The mRNA and protein levels of MGMT decreased after down-regulation of SNHG11 (Figure 3A and B). These findings suggested that SNHG11 was responsible for MGMT expression. Further in vitro experiments indicated that MGMT blocking using O6-benzylguanine (O6-BG) restrained the increased chemoresistance caused by SNHG11 overexpression (Figure 3C-E). si-MGMT transfection conferred similar effect as O6-BG (Figure 3C-E). Together, these results implied that SNHG11 promotes TMZ resistance through MGMT.

SNHG11 serves as a sponge for miR-7-5p

One of the most important role of lncRNAs on regulating target genes is functioning as ceRNAs (competing endogenous RNAs) in the cytoplasm. To investigate the functional mechanism of SNHG11, FISH and qRT-PCR were performed to identify the distribution of SNHG11 in cells. As shown in Figure 4 A and B, most of SNHG11 was enriched in cytoplasm, which gives it the ability to function as a ceRNA. Through the analysis using starbase, miRcode and DIANA databases, we found a candidate miRNA (miR-7-5p) that has the potential to bind with SNHG11 (Figure 4C). To confirm the predicative result, dual luciferase reporter assays were performed and the results showed that miR-7-5p transfection significantly suppressed the luciferase activity driven by SNHG11. Furthermore, the mutation of SNHG11 resorted the suppressive effect caused by miR-7-5p overexpression (Figure 4D and E). In addition, we employed Ago2-based RIP assays to determine the specific interaction between SNHG11 and miR-7-5p. As shown in Figure 4F, higher enrichment of SNHG11 and miR-7-5p were detected in the IP production of AGO2 group than IgG group.

Next, we performed in vitro experiments to confirm the role of SNHG11/miR-7-5p axis on regulating TMZ resistance in GBM cells. The results of in vitro assays showed that SNHG11 depletion led to increased sensitivity, and miR-7-5p co-transfection could further increase the sensitivity, while anti-miR-7-5p co-transfection partially reversed the effect induced by SNHG11 depletion (Supplementary Figure 2A-D). Taken together, these results indicated that SNHG11 promotes TMZ resistance through sponging miR-7-5p.

IRS2 is a direct target of miR-7-5p

Next, we used 5 prediction tools (microT, miRanda, PITA, RNA22 and PicTar) to identify the target genes of miR-7-5p. As shown in Figure 5A, seven common genes were identified (IRS2, MBD2, ASXL1, AMBRA1, PATL1, RPS6KB1 and NFAT5). Thus, we selected these seven genes as potential downstream targets of miR-7-5p. RT-PCR results showed that upregulation of miR-7-5p greatly suppressed IRS2 expression in GBM cells, while down-regulation of miR-7-5p greatly promoted IRS2 expression (Figure 5B). In addition, we found that miR-7-5p overexpression decreased IRS2 protein levels, while decreased miR-7-5p induced increased IRS2 protein levels (Figure 5C). The expression of other six proteins were not affected by dysregulation on miR-7-5p. To further confirm the direct target of miR-7-5p to IRS2, we constructed wild-type and mutant IRS2 3'UTR luciferase reporter plasmids (Figure 5D). Then, dual luciferase reporter assays were performed, and the results showed that miR-7-5p overexpression significantly inhibited luciferase activity of GBM cells transfected with wild-type luciferase reporter plasmids; however, mutation in the miR-7-binding seed region of the IRS2 3'UTR abolished the repressive effect of miR-7-5p (Figure 5D). In addition, RNA-ChIP assays were employed, and the results suggested that miR-7-5p overexpression led to increased IRS2 mRNA abundance in the RISC complex (Figure 5E-G). These data suggested that IRS2 is a direct target of miR-7-5p.

IRS2 is regulated by SNHG11

Next, we co-transfected SNHG11 and miR-7-5p in GBM cells, and found that miR-7-5p could restore the increased IRS2 levels induced by SNHG11 overexpression (Figure 6A). Meanwhile, we co-transfected SNHG11 shRNA and anti-miR-7-5p in GBM cells, and found that down-regulation of miR-7-5p could rescue the decreased IRS2 levels induced by down-regulation of SNHG11 (Figure 6A). We then studied whether SNHG11 regulated IRS2 through miR-7-5p. Wild-type and mutant IRS2 luciferase reporter plasmids were constructed. As shown in Figure 6B, the luciferase activity of wild-type IRS2 reporter was increased with SNHG11 overexpression, while the activity of mutant reporter was not affected. On the contrary, the luciferase activity of wild-type IRS2 reporter was decreased with SNHG11 down-regulation. As expected, mutant reporter was not affected by SNHG11 knockdown (Figure 6C). Moreover, Ago2-based RIP assays were performed. The RIP results showed that IRS2 could bind to Ago2, and qRT-PCR results suggested that overexpression of SNHG11 led to decreased IRS2 enrichment in IP production of Ago2, while down-regulation of SNHG11 contributed to increased IRS2 enrichment in IP production of Ago2 (Figure 6D and E).

Next, we tend to figure out the SNHG11/IRS2 axis on regulating TMZ resistance on GBM cells. Colony formation assays, FACS and western blots were performed, and the results suggested that IRS2 overexpression could significantly reverse the increased sensitivity of GBM cells to TMZ induced by SNHG11 knockdown (Supplementary Figure 3A-C). These results demonstrated that SNHG11 could positively regulate IRS2 through sponging miR-7, which promotes TMZ resistance in GMB cells.

SNHG11 increases MGMT by activating STAT3

MGMT levels were proved to be regulated by histone modifications. Chuanlu Jiang et.al pointed out that Lnc-TALC increases MGMT expression by mediating the H3K9/27/36 acetylation in MGMT promoter

region. Therefore, we tend to investigate whether SNHG11/IRS2/STAT3 axis could increase MGMT through modifying histone acetylation. We totally examined H3K4, H3K9 and H4K27 and H3K36, which were reported to be the regulating sites, acetylation levels in MGMT promoter region. The results showed that SNHG11 overexpression significantly increased H3K9Ac level instead of the other H3 sites (Figure 7A and Supplementary Figure 4A-C). While down-regulation of SNHG11 led to a loss of H3K9Ac in the MGMT promoter region (Figure 7A). And, no significant changes were observed of H3K4Ac, H3K27Ac and H3K36Ac in GBM cells transfected with sh-SNHG11 (Supplementary Figure 4A-C). These results suggested that SNHG11 promotes MGMT expression through modulating the acetylation of H3K9Ac.

Next, we tend to figure out how SNHG11 modulate the acetylation of H3K9Ac. Previous studies indicated that the interaction between IRS2 and JAK2 protein could further activate JAK/STAT3 signaling pathway. Our results also proved that SNHG11 knockdown significantly inhibited STAT3 activation, while SNHG11 overexpression promoted STAT3 activation and SH-4-54 (STAT3 inhibitor) abolished this effect (Figure 7B and Supplementary Figure 4D). These results suggested that SNHG11 could positively modulate STAT3 phosphorylation levels. Meanwhile, as shown in Figure 7B and Supplementary Figure 4D, we found that SH-4-54 treatment significantly abolished the increased level of MGMT caused by SNHG11 overexpression, indicating SNHG11 regulating MGMT through activating STAT3. Previous results indicated that STAT3 could post-transcriptionally regulate MGMT¹⁵. And STAT3 could interact with p300, which is a histone acetyltransferase, to modulating the histone acetylation of MGMT¹⁶. We found that SNHG11 overexpression significantly increased the interaction between pSTAT3 and p300 in GBM cells, and SNHG11 knockdown decreased the binding of p300 to pSTAT3 (Figure 7C and Supplementary Figure 4E). Moreover, SNHG11 overexpression elevated the enrichment of p300 in the MGMT promoter, and SNHG11 knockdown decreased the enrichment of p300 in the MGMT promoter (Figure 7D and Supplementary Figure 4F). Collectively, these results suggested that SNHG11 increases MGMT expression by modulating STAT3/p300 complex-mediated H3K9 acetylation.

SNHG11 knockdown increases TMZ sensitivity *in vivo*

To measure the effect of SNHG11 on modulating TMZ resistance in GBM, we established control or SNHG11 depleted pGBM1 cells transfected with luciferase plasmid. Then, pGBM1 cells were orthotopically injected into nude mice. Two weeks after injection, tumor-bearing mice were treated with TMZ (60mg/kg/day per mouse) or placebo. In vivo bioluminescence imaging system was used to trace the tumor progression. As shown in Figure 8A and B, knockdown of SNHG11 significantly increased the sensitivity of GBM to TMZ. And mice with SNHG11 depleted tumor possessed longer survival (Figure 9C). Meanwhile, IHC results showed that tumors derived from SNHG11 depleted cells expressed decreased pSTAT3 and MGMT levels, and cleaved caspase-3 levels were increased (Figure 9D). These results confirmed the effect of SNHG11 on promoting TMZ resistance in GBM cells.

Discussion

TMZ resistance is considered as the main reason to GBM recurrence¹⁷. Therefore, figuring out the molecular mechanisms of TMZ resistance could markedly improve the prognosis of GBM patients. TMZ exerts its function through methylating DNA adenine and guanine residues, which inducing DNA damage and cell death^{18, 19}. MGMT is the DNA repair enzyme, which could remove the methylation of the O6 position of guanine to itself, and repair the DNA damage²⁰. Previous studies have demonstrated that MGMT expression is higher in TMZ resistance GBM cells²¹. However, the mechanisms of MGMT regulation in GBM cells has not been fully clarified. Aberrant expression of lncRNAs, like H19 and HOXD-AS2²², have been reported to be involved in TMZ resistance through MGMT. However, more lncRNAs are needed to identify the ncRNAs network that regulating MGMT. In this research, we found that knockdown of SNHG11 significantly decreased MGMT expression, while overexpression of SNHG11 improved MGMT expression. These results indicated the potential regulation of SNHG11 on MGMT.

lncRNAs have emerged as biological regulators for different kinds of function of GBM cells. One of the most popular mechanism of lncRNAs is that acting as ceRNAs, competing for miRNAs binding. For example, lncRNA SNHG12 confers TMZ resistance through binding with miR-129-5p¹⁴; Silencing lncRNA FOXD-AS1 inhibits GBM progression via sponging miR-98-5p²³. Using several predictive tools, we found that miR-7-5p is a candidate miRNA binding with SNHG11. Luciferase reporter assays and RIP assays confirmed the direct binding relation between SNHG11 and miR-7-5p. In addition, using several predictive tools, we identified 7 potential targets of miR-7-5p. Further results indicated that IRS2 is the direct target of miR-7-5p, and SNHG11 could positively regulate IRS2 through sponging miR-7-5p. Rescue experiments showed that IRS2 could significantly impair the increased sensitivity caused by SNHG11 knockdown. Together, these results demonstrated that SNHG11 could function as a ceRNA by sponging miR-7-5p to regulate IRS2 expression. And SNHG11/miR-7-5p/IRS2 axis plays an important role in promoting TMZ resistance.

IRS2 belongs to the insulin receptor substrate (IRS) family, which is composed of six members²⁴. IRS2 was firstly identified in IRS1-knockout mice and was considered as a phosphoprotein which reacts to insulin²⁵. IRS2 protein participates in multiple cellular process via transducing extracellular signals to intracellular environment, like PI3K/AKT signaling pathway²⁶. IRS2 could also be regulated by ncRNAs, like miRNAs. For example, IRS2 is a direct target of let-7 miRNA, and let-7/IRS2 is essential for the telencephalic neuroepithelium²⁷. Here, we identified that IRS2 is also a direct target of miR-7-5p. Further investigation results suggested that SNHG11 significantly increased IRS2 level through sponging miR-7-5p. Further, we found that SNHG11/miR-7-5p/IRS2 axis could activate STAT3 signaling in GBM cells.

Consistent activation of STAT3 is considered as the key regulator in tumor progression. And, STAT3 inhibition has been implicated as an attractive focus for GBM intervention²⁸. STAT3 activation is also recognized to be associated with TMZ resistance. This contribution of STAT3 activation to TMZ resistance was found to be essential for MGMT elevation in GBM cells¹⁵. Although STAT3 is a well-known transcriptional factor, the increased MGMT expression in TMZ resistant GBM cells did not rely on transcriptional activity of STAT3. Chuanlu Jiang et. al found that STAT3 activation led to elevated

acetylation of histone H3 within the MGMT promoter regions¹⁶. In this study, we also found that STAT3 activation induced by SNHG11 overexpression could significantly raise the H3K9 acetylation and increased recruitment of p300 to MGMT promoter regions.

Conclusion

In conclusion, we identified the new function of SNHG11 in GBM cells, promoting TMZ resistance. In addition, SNHG11 mediated TMZ resistance relies on MGMT expression. Bio-informational analysis together with followed experiments indicated that SNHG11 could function as a ceRNA to positively regulate IRS2 by sponging miR-7-5p. Furthermore, STAT3 activation induced by SNHG11 overexpression significantly increased H3K9 acetylation level in the MGMT promoter regions. At last, we found that SNHG11 could serve as a biomarker for response to TMZ treatment.

Abbreviations

GBM

glioblastoma; TMZ:temozolomide; SNHG11:small nucleolar RNA host gene 11; miRNA:microRNA; lncRNA:long non-coding RNA; TCGA:the Cancer Genome Atlas; RIP:RNA binding protein immunoprecipitation; MGMT:O⁶-methylguanine-DNA-methyltransferase.

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by the Ethics Board of The First Affiliated Hospital of Nanjing Medical University and carried out in accordance with institutional guidelines. Informed consent was obtained from the patients undergoing surgery. The animal study was approved by Nanjing Medical University

Consent for publication

Not applicable

Availability of data and material

The datasets supporting the conclusions of this article are available from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TJ and YY designed the study. TJ and XL participated in the manuscript preparation and carried out the experiments in vitro and in vivo. All authors read and approved the final manuscript.

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Figures

Figure 1

SNHG11 is highly expressed in GBM tissues and confers poor prognosis A. Relative expression of SNHG11 in GBM and normal brain tissues were analyzed using TCGA dataset. B. Relative expression of SNHG11 in different grades of glioma were analyzed using CGGA dataset. C. Kaplan Meier survival analysis of OS in GBM patients were analyzed using TCGA dataset. D. Relative expression of SNHG11 in GBM and normal brain tissues were analyzed using clinical samples. E. Relative expression of SNHG11 in

recurrent and primary GBM tissues were analyzed by qRT-PCR. F. Kaplan Meier survival analysis of OS in GBM patients with TMZ chemotherapy. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (* $P < .05$, ** $P < .01$.)

Figure 2

SNHG11 confers resistance to TMZ A. Cell growth of transfected GBM cells was evaluated using CCK-8 assay. B. The proliferation of transfected GBM cells was evaluated using colony formation assay. C. The apoptosis of transfected GBM cells was evaluated using flow cytometric analysis. D. The expression of apoptosis related proteins in transfected GBM cells was evaluated using western blot. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (* $P < .05$, ** $P < .01$.)

Figure 3

SNHG11 confers TMZ resistance through elevating MGMT A. Relative expression of MGMT expression in transfected GBM cells were analyzed using RT-PCR. B. The expression of MGMT in transfected GBM cells was evaluated using western blot. C. The proliferation of transfected GBM cells was evaluated using colony formation assay. D. The apoptosis of transfected GBM cells was evaluated using flow cytometric analysis. E. The expression of apoptosis related proteins in transfected GBM cells was evaluated using

western blot. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (* $P < .05$, ** $P < .01$.)

Figure 4

SNHG11 serves as a sponge for miR-7-5p A. The distribution of SNHG11 (green) in GBM cells was analyzed using FISH analysis. Nuclei were stained by DAPI (blue). B. Relative expression of SNHG11 in nuclear and cytosolic fractions of GBM cells was analyzed using qRT-PCR. C. Schematic drawing of the screening procedure of candidate miRNAs. D. Schematic representation of the miR-7-5p binding sites in

SNHG11 and the site mutagenesis. E. Relative luciferase activity of GBM cells co-transfected with luciferase reporter plasmid carrying wild type (WT) or mutant (Mut) SNHG11 and miR-NC or miR-7-5p. F. Relative enrichment of SNHG11 and miR-7-5p in IP product of Ago2 or IgG was analyzed using qRT-PCR. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (* $P < .05$, ** $P < .01$.)

Figure 5

IRS2 is a direct target of miR-7-5p A. Schematic drawing of the screening procedure of candidate targets. B. Relative expression of potential targets of miR-7-5p in transfected GBM cells was analyzed using qRT-PCR. C. Relative expression of IRS2 in transfected GBM cells was analyzed using western blot. D. Up: Schematic representation of the IRS2 binding sites in miR-7-5p and the site mutagenesis. Down: Relative luciferase activity of GBM cells co-transfected with luciferase reporter plasmid carrying wild type (WT) or mutant (Mut) IRS2 and miR-NC or miR-7-5p. E. Immunoprecipitation of the Ago2/RISC using the Pan-Ago2 antibody in GBM cells transfected with miR-NC or miR-7-5p. F. Relative miR-7-5p enrichment in the IP product of GBM cells was analyzed using qRT-PCR. G. Relative IRS2 enrichment in the IP product of GBM cells was analyzed using qRT-PCR. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (*P< .05, **P< .01.)

Figure 6

IRS2 is regulated by SNHG11. A. Relative expression of IRS2 in transfected GBM cells was evaluated using western blot. B. Relative luciferase activity of reporters containing wild-type or mutant IRS2 3'UTR in GBM cells co-transfected with control or SNHG11. C. Relative luciferase activity of reporters containing wild-type or mutant IRS2 3'UTR in GBM cells co-transfected with sh-control or sh-SNHG11. D. Relative enrichment of SNHG11 and IRS2 in IP product of Ago2 from GBM cells transfected with control or

SNHG11 was analyzed using qRT-PCR. E. Relative enrichment of SNHG11 and IRS2 in IP product of Ago2 from GBM cells transfected with sh-control or sh-SNHG11 was analyzed using qRT-PCR. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (*P< .05, **P< .01.)

Figure 7

SNHG11 increases MGMT by activating STAT3 A. The enrichment of H3K9ac in the MGMT promoter region in transfected GBM cells was analyzed using CHIP assays. B. STAT3 signaling activation in transfected GBM cells was evaluated using western blot. C. Direct binding of p300 to pSTAT3 in transfected GBM cells was evaluated using IP. D. The enrichment of p300 in the MGMT promoter region in transfected pGBM1 cells was analyzed using CHIP assays. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (*P< .05, **P< .01.)

Figure 8

SNHG11 knockdown increases TMZ sensitivity in vivo A. Bioluminescent images of nude mice. B. Quantification of bioluminescent imaging signal intensities in nude mice. C. Kaplan Meier survival curve of nude mice is shown. D. The expression of Cleaved caspase-3, IRS2 and MGMT in tumors was evaluated using IHC staining. In all experiments, bars represent mean $\pm$ SD from three independent experiments. (* $P < .05$, ** $P < .01$.)

Supplementary Files

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