MicroRNA-433-3p enhances chemosensitivity of glioma to cisplatin by downregulating NR5A2

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Abstract
Objective: We attempted to investigate influence of microRNA-433-3p on malignant progression of glioma and identify its molecular mechanism, thus laying groundwork for glioma management.

Methods: Expression data along with clinical data of glioma were accessed from the TCGA database for differential and survival analyses to look for the target differentially expressed genes. Quantitative reverse transcriptase PCR (qRT-PCR) and western blot were utilized to assess NR5A2 mRNA and protein expression in different glioma cell lines, respectively. MTT, Transwell assay, and flow cytometry were carried out to assay the impact of NR5A2 on behaviors of glioma cells in vitro. Bioinformatics analysis was used to identify the upstream microRNA of NR5A2 in glioma, while dual-luciferase and western blot assays were used to detect binding of microRNA and NR5A2. Chemosensitivity of glioma cells was evaluated by cisplatin cytotoxicity test.

Results: NR5A2 was upregulated in both glioma tissues and cell lines. Dual-luciferase assay result showed binding site of microRNA-433-3p on NR5A2 mRNA 3′UTR, and microRNA-433-3p reduced NR5A2 expression. Cell assays revealed that silencing NR5A2 could hamper proliferation, invasion, and migration and enhance chemosensitivity to cisplatin while promoting glioma cell apoptosis and blocking glioma cells in G0/G1 phase. Rescue experiments also indicated that microRNA-433-3p suppressed glioma malignant progression via inhibiting NR5A2.

Conclusion: MicroRNA-433-3p which is significantly poorly expressed in glioma targets NR5A2 to suppress glioma malignant progression and enhance chemosensitivity to cisplatin.

KEYWORDS
glioma, malignant progression, microRNA-433-3p, NR5A2

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1 | INTRODUCTION

Glioma is a prevalent malignant tumor (Zhang et al., 2019), mainly including two subtypes: diffuse and nondiffuse glioma (Khani et al., 2019). It grows rapidly, leading to low survival rate and high mortality, and it is a serious threat to human life (Chen et al., 2019; Van Meir et al., 2010). Glioma always occurs in the brain. Glioma accounts for 30% of brain and central nervous system tumors and 80% of all brain malignancies (de Robles et al., 2015; Ostrom et al., 2014). Despite great efforts in combination therapy of glioma over the past decades, the prognosis is still unfavorable (Fukushima et al., 2013). In the treatment of malignant glioma, cisplatin has been utilized as an adjuvant therapy of carmustine and a neoadjuvant therapy of temozolomide (Y. Wang et al., 2017). But in clinical application, gliomas are less sensitive to cisplatin, and high doses of cisplatin lead to adverse reactions, which is a shortcoming of platinum-based drugs in the management of gliomas (L. Yang et al., 2018). Hence, understanding molecular markers related to chemoresistance, so as to enhance the anticancer efficacy of cisplatin-based treatments, is key to clinical cancer treatment. This study aimed to explore the pathogenesis and drug resistance mechanism of glioma and lay groundwork for clinical diagnosis and management of glioma in the future.

NR5A2 is a structurally alive orphan nuclear receptor (Stergiopoulos & Politis, 2016) that plays an important role in early development via modulating the key pluripotent factor Oct4 level in embryonic stem cells (Gu et al., 2005; Heng et al., 2010). NR5A2 is also an important mediator for liver metabolic balance (Goodwin et al., 2000; Lu et al., 2000; Sirianini et al., 2002). In addition, it is also involved in glucose metabolism, acini differentiation, and other processes (Cobo et al., 2018). In the field of tumor research, it has been reported that NR5A2 as an oncogene is involved in the development of breast cancer (S. Wang et al., 2018), gastric cancer (Liu et al., 2019), prostate cancer (Xiao et al., 2018), and pancreatic cancer (Hale et al., 2014). NR5A2 is implicated in cancer cell chemosensitivity. For example, microRNA-27b-3p directly targets NR5A2 and CREB1 to enhance tamoxifen-induced breast cancer cell toxicity (Zhu et al., 2016). Dual-inhibition of NR5A2-GATA6 and BCL-XL increases sensitivity of esophageal adenocarcinoma cells to cisplatin therapy (Duggan et al., 2016). Although NR5A2 plays a pivotal role in affecting tumor cell chemosensitivity, the role of NR5A2 in glioma and its molecular mechanism are still unclear.

We investigated the mechanism of NR5A2 underlying malignant progression of glioma through assays and further studied the sensitivity of glioma to cisplatin, laying groundwork for future clinical diagnosis and management.

2 | MATERIALS AND METHODS

2.1 | Bioinformatics methods

Data of mature microRNAs (normal: 5; tumor: 530) and mRNAs (normal:5; tumor:698) at expression level were downloaded from the TCGA database. Expression differences in normal and glioma tissues were assessed by t-test, with benign tissue as control. R package “survival” was utilized to conduct survival analysis on NR5A2. R package “edgeR” (|logFC| > 2, padj < 0.01) was conducted to screen differentially expressed microRNAs (DEmicroRNAs). R package “survival” was utilized to conduct survival analysis on DEmicroRNAs. To select the microRNA of interest that had significant correlation with NR5A2, mirDB (http://mirdb.org/), starBase (http://starbase.sysu.edu.cn/), and mirDIP (http://ophid.utoronto.ca/mirDIP/index.jsp) databases were used to predict microRNAs regulated by NR5A2 in upstream, which were overlapped with downregulated DEmicroRNAs.

2.2 | Cell culture and transfection

Human normal glial cell line HEB (BNCC338123) and human glioma cell lines T98G (BNCC338721) were accessed from BeNa Culture Collection. HS683, U87, and glioma resistant cell lines HS683/DDP and U87/DDP were purchased from KeygeneBio (Nanjing, China). Cell lines were grown in DMEM with 10% fetal bovine serum (FBS) at 37°C and 5% CO2.

MicroRNA-433-3p mimic (100 nmol/L), microRNA-433-3p inhibitor (100 nmol/L), vectors with overexpressed NR5A2 (oe-NR5A2) (100 nmol/L), small interfering RNAs (siRNAs) targeting NR5A2 (si-NR5A2) (100 nmol/L), and negative control (NC mimic, NC inhibitor, oe-NC, si-NC) were synthesized by Gima (Shanghai, China). Cells were transfected with the Lipofectamine 2000 transfection reagent (Invitrogen, USA).

2.3 | qRT-PCR

Total RNA from glioma cells was separated by Trizol (Invitrogen). Complementary DNA was reversely transcribed with the PrimeScript RT kit, and qRT-PCR was done with the SYBR Premix Ex TaqTM II kit on the Roche LC480 real-time quantitative PCR system (Roche). The conditions for qRT-PCR were as follows: 95°C for 10 s, 60°C for 20 s, 72°C for 30 s, 45 cycles, and qRT-PCR was conducted using a Roche Lightcycler 480 real-time PCR machine. U6 and GAPDH were assessed by 2-ΔΔCt method. Primer sequences shown in Table 1 were synthesized by RiboBio company (Guangzhou, China). The assay was repeated three times.

2.4 | MTT assay

Cell viability was examined by MTT assay. Glioma cells were plated in 96-well plates with 5 × 103 cells/well 12 h before transfection. MTT assay was introduced after transfection for 24, 48, and 72 h. Specifically, 10 μl MTT (5 mg/ml) was added to each well at the above time points for cell incubation in a 37°C incubator for 4 h. After supernatant was discarded, 200 μl dimethyl sulfoxide was added. The optical
density (OD) values at 490 nm were measured using a microplate reader (ThermoFisher Scientific, USA) to examine cell viability. The assay was repeated three times. Cell growth curves were drawn with time as the horizontal axis and the ratio of absorbance values of cells in each group at each time point as the vertical axis.

2.5 Flow cytometry assay

Glioma cells were plated into 6-well plates, and the Annexin V-FITC apoptosis assay kit (BD Biosciences, USA) was implemented to evaluate apoptosis of cells in different treatment groups. At room temperature, cells were digested by trypsin and washed. Afterward, the FACSCanto II flow cytometry instrument (BD Biosciences) was applied for cell apoptosis analysis, with the FITC-labeled Annexin V as the fluorescence probe. The assay was repeated three times.

FACSCanto II flow cytometry instrument (BD Biosciences, USA) was introduced for cell cycle assay at 48 h after mimic transfection. Note that \(1 \times 10^6\) cells were collected, followed by washing with cold PBS, and fixing with 70% cold ethanol at 4°C for 48 h. Next, after rinsing cells with cold PBS again, propidium iodide (PI) was used for staining nucleus followed by measuring DNA amount using flow cytometry. At least \(1 \times 10^4\) cells were counted, and the cell cycle distribution was analyzed using ModFit software.

2.6 Transwell migration and invasion assays

In cell migration assay, \(5 \times 10^5\) glioma cells were added into the top insert without coating Matrigel. In the cell invasion assay, \(1 \times 10^5\) glioma cells were added into the top chamber coated with Matrigel matrix. Cells were added into the top insert with serum-free medium, and medium with 20% serum (Gibco, USA) was added into the bottom chamber as a chemical inducer in both assays. The cells were maintained under routine conditions. After 16 h, the cells on the surface of the membrane were removed with a cotton swab, the migrated/invading cells under the membrane were stained with crystal violet, and five areas were randomly selected for photographing and cell counting.

2.7 Western blot assay

After transfection for 48 h, cells were lysed in radioimmunoprecipitation buffer (Solaibo, China) to extract total proteins. BCA protein assay kit was utilized to quantify the proteins. The proteins were dispersed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transferring the proteins to a polyvinylidene fluoride (PVDF) membrane. The membrane was sealed with 5% skim milk at room temperature for 1 h and maintained with relevant antibodies overnight. After incubation with secondary antibodies (horseradish peroxidase-conjugated IgG), protein bands were developed by ECL. Finally, Image J software was used to quantify the protein bands. The assay was repeated three times. \(\beta\)-Actin was taken as an internal control. The relative level of proteins was calculated by the ratio of the gray value of the target band to the internal reference band. Detailed information on relevant antibodies is presented in Table 2.

2.8 Dual-luciferase assay

To identify the targeting relationship between microRNA-433-3p and NR5A2, the 3′UTR of NR5A2 was cloned into a pcMV6 vector containing luciferase gene. The luciferase vector fused with wild-type or mutant NR5A2 3′UTR was transfected into glioma cells together with NC mimic or microRNA-433-3p mimic with Lipofectamine 2000 (Invitrogen). In the end, luciferase activity was assayed with luciferase assay reagent (Promega, USA). Firefly luciferase was normalized to Renilla luciferase.

2.9 Cisplatin cytotoxicity test

Glioma cells were planted to 96-well plate (\(1 \times 10^4\) cells/well), and at 48 h after transfection, cisplatin with a final concentration of 0.5, 1.0, 2.0, 4.0, or 8.0 \(\mu\)g/ml was added to three replicate wells for 24 h. Cell viability was determined by MTT method. Next, 20 \(\mu\)l 5 mg/ml MTT was supplemented for 4 h of incubation at 37°C. A microplate reader was utilized to read the absorbance at 490 nm. All experiments were conducted at least three times.
NR5A2 is increased in glioma cells and tissue. (a) Box plot of NR5A2 differential expression in TCGA; (b) the effect of NR5A2 on patient’s survival detected by survival analysis; (c) NR5A2 mRNA level in HEB, HS683, T98G, and U87, cells and drug-resistant glioma cell lines HS683/DDP and U87/DDP assayed through qRT-PCR; (d) NR5A2 protein level in HEB, HS683, T98G, and U87 cells, and drug-resistant glioma cell lines HS683/DDP and U87/DDP detected by western blot; * compared with HEB group, #compared with corresponding parent cells, p < 0.05.

2.10 Statistical analysis

All the data were processed by SPSS 22.0 statistical software. The analyzed results were presented as mean ± standard deviation, and the comparison of two groups was performed by t-test, p < 0.05.

3 RESULTS

3.1 NR5A2 is increased in glioma cells and tissue

The glioma data downloaded from the TCGA database indicated that NR5A2 was significantly increased in glioma tissue (Figure 1a), and survival analysis result showed that patients with high NR5A2 expression in glioma suffered poor prognosis (Figure 1b). Several studies have revealed that NR5A2 plays a crucial role in cancer progression (Liu et al., 2019; Z. Luo et al., 2017). In this study, NR5A2 was taken as the object of interest, and its biological role in progression of glioma was clarified. qRT-PCR and western blot were introduced to evaluate NR5A2 level in HEB, T98G, HS683, and U87 cell lines. NR5A2 was increased in glioma cells (p < 0.05, Figure 1c,d). In addition, we found that NR5A2 level in glioma resistant cell lines HS683/DDP and U87/DDP was conspicuously higher than that in its parental cells (p < 0.05, Figure 1c,d). Thus, NR5A2 was increased in glioma cells and tissue.

3.2 NR5A2 promotes the malignant progression of glioma

si-NR5A2 was transfected into glioma cells HS683 and U87, respectively. qRT-PCR assay result disclosed that NR5A2 level was noticeably decreased after cells transfected si-NR5A2 (p < 0.05, Figure 2a). MTT assay was used to assess the impact of NR5A2 on proliferative ability of glioma cells. The result showed that interference with NR5A2 expression prominently repressed HS683 and U87 cell proliferation after transfection for 48 and 72 h (p < 0.05, Figure 2b). The effect of NR5A2 on cell apoptosis was investigated by flow cytometry assay, and the apoptosis rate was detected by flow cytometry at 48 h after transfection. Results showed that interference with NR5A2 significantly promoted apoptosis (p < 0.05, Figure 2c). Flow cytometry assay was also utilized to assess cell cycle at 48 h after transfection, and the result indicated that silencing NR5A2 induced HS683 and U87 cell arrest at G0/G1 phase, indicating that NR5A2 may accelerate cell cycle progress and promote cell proliferation (p < 0.05, Figure 2d). Hence, NR5A2 promoted growth of glioma cells. In addition, effects of
NR5A2 promotes the malignant progression of brain glioma. (a) Changes in NR5A2 expression in group si-NC, si-NR5A2 in HS683 and U87 cells, respectively, assayed through qRT-PCR; (b) changes in the proliferative ability of glioma cells in different groups assessed by MTT assay; (c and d) changes in the apoptosis and cell cycle of glioma cells in various groups assessed by flow cytometry assay; (e) changes in the invasive and migratory abilities of glioma cells in various groups assessed by Transwell assay; (f) response curve of HS683/DDP and U87/DDP cells to cisplatin dose assessed by MTT; *compared with si-NC group, \( p < 0.05 \).

3.3 MicroRNA-433-3p targets and restrains the expression of NR5A2

Bioinformatics method was used to find the microRNA with binding sites on NR5A2 mRNA. The result of the differential analysis performed by using R package “edgeR” showed that a total of 78 differential microRNAs were obtained (43 upregulated and 35 downregulated) (Figure 3a). Upstream microRNA prediction for NR5A2 was performed using bioinformatics databases, and an intersection of the predicted upstream microRNAs with the 35 differentially down-regulated microRNAs was taken to get two microRNAs with targeted binding sites to NR5A2 (microRNA-433-3p, microRNA-381-3p) (Figure 3b). Pearson correlation analysis revealed that NR5A2 and microRNA-433-3p had the highest Pearson correlation coefficient (Figure 3c), suggesting that microRNA-433-3p might be able to target NR5A2 to regulate the malignant progression of glioma. Meanwhile, Kaplan–Meier survival analysis showed that patients with high expression of microRNA-433-3p shared a better prognostic status (Figure 3d).

Western blot and qRT-PCR results suggested that forced microRNA-433-3p level hindered NR5A2 level, while interference with microRNA-433-3p promoted NR5A2 expression, with statistical significance (\( p < 0.05 \), Figure 3e). Luciferase reporter vectors containing NR5A2 3′UTR fragments were transfected into HS683 cells with microRNA-433-3p mimic or NC mimic. Different 3′UTR fragments were used to validate their putative functional sites, including wild-type and mutant 3′UTR (Figure 3f). Dual-luciferase assay result indicated that forced microRNA-433-3p level hindered luciferase activity of the cells with wild-type NR5A2 3′UTR but had no impact on that of cells with mutant NR5A2 3′UTR, with statistical significance (\( p < 0.05 \), Figure 3g). Hence, microRNA-433-3p directly targeted NR5A2 3′UTR to reduce NR5A2 expression.

3.4 MicroRNA-433-3p represses malignant progression of glioma through binding NR5A2

To investigate the effect of microRNA-433-3p targeting NR5A2 on glioma cells, NR5A2 overexpression vectors were constructed, and the
NR5A2 belongs to the superfamily of nuclear receptors (Z. Luo et al., 2017) and is crucial in many biological processes. It has been found that NR5A2 can accelerate the development of intestinal tumors through regulating cell cycle and inflammation (Petruzzelli et al., 2016) and promote the invasive ability of breast cancer cells via changing actin cytoskeleton or reducing the transcription of cyclin-dependent kinase inhibitors independent of ERα and p53 state (Bianco et al., 2015; Chand et al., 2010). In addition, NR5A2 is a direct target for microRNA-376c to inhibit cell proliferation and invasion via Wnt signaling pathway in nonsmall cell lung cancer (Jiang et al., 2016). Liang et al. (2015) illustrated that microRNA-381 can promote progression of colon cells by upregulating NR5A2. Our study showed that NR5A2 was increased in glioma cells. Moreover, silencing NR5A2 hindered cell cycle progression, proliferation, invasion, and migration of glioma cells and induced cell apoptosis. In addition, NR5A2 can modulate chemosensitivity of cancer cells to cisplatin. For example, forced expression of NR5A2 weakens the cytotoxicity of cisplatin in breast cancer cells and xenograft models (S. Wang et al., 2018). We manifested that NR5A2 was activated in drug-resistant glioma cell line while silencing NR5A2 notably inhibiting cell resistance to cisplatin. As such Q. Yang, Deng, et al. (2020) manifested that aberrant level of NR5A2 results in unfavorable clinical prognosis and overall survival of patients, and upregulation of NR5A2 enhances cell resistance to temozolomide glioma. Collectively, NR5A2 could facilitate glioma cell malignant progression and enhance cell resistance to cisplatin.

Many microRNAs have been proved to have positive or negative effects on brain glioma (Geng et al., 2019). For example, microRNA-499a-5p fosters development of glioma via regulating the expression of KPNA2 (Z. Yang, Li, et al., 2020). MicroRNA-30a inhibits self-renewal vectors (or empty vectors) were cotransfected with microRNA-433-3p mimic (or NC mimic) into HS683 cells. Western blot and qRT-PCR assays confirmed that forced NR5A2 level rescued the repressive impact of microRNA-433-3p on NR5A2 (p < 0.05, Figure 4a). MTT assay displayed that forced microRNA-433-3p level conspicuously repressed increased cell proliferation induced by NR5A2 (p < 0.05, Figure 4b). Flow cytometry result exhibited that overexpression of microRNA-433-3p notably reverses inhibition of NR5A2 on cell apoptosis (p < 0.05, Figure 4c), increases tumor cells in G0/G1 phase, delays cell cycle progression, and inhibits cell proliferation (p < 0.05, Figure 4d). Transwell assay result denoted that overexpression of microRNA-433-3p noticeably hampered cell invasion and migration induced by NR5A2, and to some extent rescued promoting impact of NR5A2 on the cell invasion and migration of glioma (p < 0.05, Figure 4e). Cisplatin chemosensitivity assay showed that forced expression of microRNA-433-3p in HS683/DDP cell line markedly promoted sensitivity of glioma cells to cisplatin, while NR5A2 could reverse the promotion of microRNA-433-3p on glioma cell sensitivity to cisplatin (Figure 4f). The above experimental results indicated that microRNA-433-3p repressed NR5A2 level and thus inhibited malignant progression of glioma.

4 DISCUSSION

NR5A2 could facilitate glioma cell malignant progression and enhance cell resistance to cisplatin.

FIGURE 3 MicroRNA-433-3p targets NR5A2 to decrease NR5A2 level. (a) Volcano map of differential microRNAs in the glioma dataset of the TCGA database; (b) Venn diagram of the putative upstream microRNAs and the differential microRNAs; (c) Pearson correlation analysis of NR5A2 and its predicted upstream microRNAs; (d) survival curves show the effect of the expression of microRNA-433-3p on the prognosis of patients; (e) changes of NR5A2 protein and mRNA levels upon forced and silenced expression of microRNA-433-3p, respectively; (f) the binding sites of microRNA-433-3p on NR5A2 3′UTR were predicted; (g) dual-luciferase activity was used to detect whether there is an interaction between microRNA-433-3p and NR5A2; * compared with NC mimic group, # compared with NC inhibitor group, p < 0.05.
FIGURE 4  MicroRNA-433-3p hampers malignant progression of glioma via binding to NR5A2. (a) Changes of NR5A2 protein and mRNA levels in transfection groups detected by western blot and qRT-PCR; (b) the impact of simultaneous overexpression of microRNA-433-3p and NR5A2 on proliferation of glioma cells detected by MTT assay; (c and d) the effect of simultaneous forced microRNA-433-3p and NR5A2 on apoptosis level and cell cycle of glioma cells assayed by flow cytometry; (e) the impact of simultaneous overexpression of microRNA-433-3p and NR5A2 on invasion and migration of glioma cells assayed by Transwell assay; (f) response curve of U87/DDP cells to cisplatin dose assessed by MTT; *microRNA-433-3p+oe-NC group and NC-mimic+oe-NR5A2 group compared with NC-mimic+oe-NC group, respectively, #NC-mimic+oe-NR5A2 group and microRNA-433-3p+ oe-NR5A2 group compared with microRNA-433-3p+ oe-NC group, p < 0.05
of glioma stem cells through blocking NTSE-dependent Akt signaling (Peng et al., 2020). MicroRNA-433 plays different roles in the progression of human tumors. MicroRNA-433 is decreased in gastric cancer (H. Luo et al., 2009), lung cancer (Li et al., 2019), visceral adipose tissue of patients with nonalcoholic fatty liver disease (Estep et al., 2010), and hepatocellular carcinoma with hepatitis B virus infection (W. Wang et al., 2012). In addition, microRNA-433 negatively modulates 5-fluorouracil-sensitive thymidylate synthetase level in HeLa cells (Gotanda et al., 2013). Moreover, microRNA-433-3p is implicated with cancer cell drug resistance. For example, binding of NORAD and miRNA-433-3p induces gastric cancer cell resistance to oxaliplatin (J. Wang et al., 2021). Nonetheless, the exact role of microRNA-433 in glioma is less studied. Herein, we proved that the expression of NR5A2 was negatively correlated with that of microRNA-433-3p in glioma and confirmed that NR5A2 was the direct target of microRNA-433-3p by luciferase reporter assay. We further investigated that microRNA-433 was decreased in glioma cells. After upregulating microRNA-433-3p level in glioma cell line U87, microRNA-433-3p decreased NR5A2 level. MicroRNA-433-3p plays a tumor repressor role in glioma by targeting NR5A2, thus regulating cell behaviors, and enhancing chemosensitivity to cisplatin. As such, a study described that microRNA-433-3p is underexpressed in glioma tissue and cells and activates apoptotic-signaling pathway to enhance glioma chemosensitivity to temozolomide by targeting CREB (Sun et al., 2017). Thus, microRNA-433-3p may enhance chemosensitivity of glioma to cisplatin via modulating cell cycle distribution, activating cell apoptosis, and downregulating NR5A2 level.

Overall, our study unveiled that microRNA-433-3p may be a possible repressive gene in glioma. MicroRNA-433-3p hindered malignant progression of glioma via binding NR5A2 and enhanced glioma chemosensitivity to cisplatin. This discovery may lay groundwork for management of brain glioma. Despite the finding that microRNA-433-3p could restrain glioma progression via targeting NR5A2, other targets of microRNA-433-3p may also affect the carcinogenesis of gliomas. The molecular mechanism and signaling pathway of microRNA-433-3p in glioma, therefore, need to be further studied.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Jun Li contributed to the study design. Jingshun Gu and Juntong Wang conducted the literature search. Aiwu You and Yuyan Zhang acquired the data. Guoming Rao and Shuang Li wrote the article. Xuehua Ge and Kun Zhang performed data analysis. Jun Li revised the article. Dongchun Wang gave the final approval of the version to be submitted.

DATA AVAILABILITY STATEMENT
The data and materials in the current study are available from the corresponding author upon reasonable request.

REFERENCES
Bianco, S., Jangal, M., Garneau, D., & Gevry, N. (2015). LRH-1 controls proliferation in breast tumor cells by regulating CDK11A gene expression. Oncogene, 34, 4509–4518. https://doi.org/10.1038/onc.2014.382
Chand, A. L., Herridge, K. A., Thompson, E. W., & Clyne, C. D. (2010). The orphan nuclear receptor LRH-1 promotes breast cancer motility and invasion. Endocrine-Related Cancer, 17, 965–975. https://doi.org/10.1677/ERC-10-0179
Chen, X., Yang, F., Zhang, T., Wang, W., Xi, W., Li, Y., Zhang, D., Huo, Y., Zhang, J., Yang, A., & Wang, T. (2019). MR-9 promotes tumorigenesis and angiogenesis and is activated by MYC and OCT4 in human glioma. Journal of Experimental & Clinical Cancer Research, 38, 99. https://doi.org/10.1186/s13046-019-1078-2
Cobo, I., Martíneili, P., Flández, M., Bakiri, L., Zhang, M., Carrillo-de-Santa-Pau, E., Jia, J., Sánchez-Arévalo Lobo, V. J., Megías, D., Felipe, I., Del Pozo, N., Millán, I., Thommesen, L., Bruland, T., Olson, S. H., Smith, J., Schoonjans, K., Lamlet, W. R., Petersen, G. M.,... Real, F. X. (2018). Transcriptional regulation by NR5A2 links differentiation and inflammation in the pancreas. Nature, 554, 533–537. https://doi.org/10.1038/nature25751
de Robles, P., Fiest, K. M., Frolikis, A. D., Pringsheim, T., Atta, C., St Germaine-Smith, C., Day, L., Lam, D., & Jette, N. (2015). The worldwide incidence and prevalence of primary brain tumors: A systematic review and meta-analysis. Neuro-Oncology, 17, 776–783. https://doi.org/10.1093/neuonc/nou283
Duggan, S. P., Behan, F. M., Kirca, M., Zaheer, A., McGarrigle, S. A., Reynolds, J. V., Vaz, G. M., Senge, M. O., & Kelleher, D. (2016). The characterization of an intestine-like genomic signature maintained during Barrett’s-associated adenocarcinogenesis reveals an NR5A2-mediated promotion of cancer cell survival. Science Reports, 6, 32638. https://doi.org/10.1038/srep32638
Estep, M., Armistead, D., Hossain, N., Elarainy, H., Goodman, Z., Baranova, A., Chandhoke, Y., & Younossi, Z. M. (2010). Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. Alimentary Pharmacology & Therapeutics, 32, 487–497. https://doi.org/10.1111/j.1365-2036.2010.04366.x
Fukushima, S., Narita, Y., Miyakita, Y., Ohno, M., Takizawa, T., Kasugai, T., Takusagawa, Y., Mori, M., Ichimura, K., Tsuda, H., & Shibui, S. (2013). A case of more than 20 years survival with glioblastoma, and development of cavernous angioma as a delayed complication of radiotherapy. Neuropathology, 33, 576–581. https://doi.org/10.1111/neup.12022
Geng, F., Yang, W., Song, D., Hou, H., Han, B., Chen, Y., & Zhao, H. (2019). MicroRNA-26b-3p/ANTXR1 signaling modulates proliferation, migration, and apoptosis of glioma. American Journal of Translational Research, 11(12), 7568–75780.
Goodwin, B., Jones, S. A., Price, R. R., Watson, M. A., McKee, D. D., Moore, L. B., Galardi, C., Wilson, J. G., Lewis, M. C., Roth, M. E., Maloney, P. R., Willson, T. M., & Kliever, S. A. (2000). A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Molecular Cell, 6, 517–526. https://doi.org/10.1016/s1056-8223(00)00051-4
Gotanda, K., Hirota, T., Matsumoto, N., & Ieiri, I. (2013). MicroRNA-433 negatively regulates the expression of thymidylate synthase (TYMS) responsible for 5-fluourouracil sensitivity in HeLa cells. BMC Cancer, 13, 369. https://doi.org/10.1186/1471-2407-13-369
Gu, P., Goodwin, B., Chung, A. C., Xu, X., Wheeler, D. A., Price, R. R., Galardi, C., Peng, L., Latour, A. M., Koller, B. H., Gossen, J., Kliever, S. A., & Cooney, A. J. (2005). Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development. Molecular and Cellular Biology, 25, 3492–3505. https://doi.org/10.1128/MCB.25.9.3492-3505.2005

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Sirianni, R., Seely, J. B., Attia, G., Stocco, D. M., Carr, B. R., Pezzi, V., & Rainey, W. E. (2002). Liver receptor homologue-1 is expressed in human steroidogenic tissues and activates transcription of genes encoding steroidogenic enzymes. *Journal of Endocrinology*, 174, R13–R17. https://doi.org/10.1677/joe.0.174r013

Stergiopoulos, A., & Politis, P. K. (2016). Nuclear receptor NRSa2 controls neural stem cell fate decisions during development. *Nature Communications*, 7, 12230. https://doi.org/10.1038/ncomms12230

Sun, S., Wang, X., Xu, X., Di, H., Du, J., Xu, B., Wang, Q., & Wang, J. (2017). NRSa2 enhances chemosensitivity of glioma by regulating CREB in human glioma. *Oncotarget*, 8(3), 5057–5068. https://doi.org/10.18632/oncotarget.13789

Van Meir, E. G., Hadjipanayis, C. G., Norden, A. D., Shu, H. K., Wen, P. Y., & Olson, J. J. (2010). Exciting new advances in neuro-oncology: The avenue to a cure for malignant glioma. *CA: A Cancer Journal for Clinicians*, 60, 166–193. https://doi.org/10.3322/caac.20069

Wang, J., Sun, Y., Zhang, X., Cai, H., Zhang, C., Qu, H., Liu, L., Zhang, M., Fu, J., Zhang, W., & Zhang, G. (2021). Oxidative stress activates NORAD expression by H3K27ac and promotes oxaliplatin resistance in gastric cancer by enhancing autophagy flux via targeting the miR-433-3p. *Cell Death & Disease*, 12(1), 90. https://doi.org/10.1038/s41419-020-03368-y

Wang, S., Zou, Z., Luo, X., Mi, Y., Chang, H., & Xing, D. (2018). LRH1 enhances cell resistance to chemotherapeutic agents by transcriptionally activating MDC1 expression and attenuating DNA damage in human breast cancer. *Oncogene*, 37, 3243–3259. https://doi.org/10.1038/s41418-018-0193-4

Wang, W., Zhao, L. J., Tan, Y. X., Ren, H., & Qi, Z. T. (2012). Identification of deregulated miRNAs and their targets in hepatitis B virus-associated hepatocellular carcinoma. *World Journal of Gastroenterology*, 18, 5442–5453. https://doi.org/10.3748/wjg.v18.i38.5442

Wang, Y., Kong, X., Guo, Y., Wang, R., & Ma, W. (2017). Continuous dose-intense temozolomide and cisplatin in recurrent glioblastoma patients. *Medicine*, 96, e6261. https://doi.org/10.1097/MD.0000000000006261

Xiao, L., Wang, Y., Xu, K., Hu, H., Xu, Z., Wu, D., Zhang, W., You, W., Ng, C. F., Yu, S., & Chan, F. L. (2018). Nuclear receptor LRH-1 functions to promote castration-resistant growth of prostate cancer via its promotion of intratumoral androgen biosynthesis. *Cancer Research*, 78, 2205–2218. https://doi.org/10.1158/0008-5472.CAN-17-2341

Yang, L., Li, N., Yan, Z., Li, C., & Zhao, Z. (2018). MiR-29a-mediated CD13 expression contributes to cisplatin resistance in CD13(+)+ glioblastoma stem cells. *Journal of Molecular Neuroscience*, 66, 369–377. https://doi.org/10.1007/s12031-018-1177-0

Yang, Q., Deng, L., Li, J., Miao, P., Liu, W., & Huang, Q. (2020). NRSa2 promotes cell growth and resistance to temozolomide through regulating notch signal pathway in glioma. *Oncotargets and Therapy*, 13, 10231–10244. https://doi.org/10.2147/OTT.S243833

Yang, Z., Li, C., Fan, X. Y., & Liu, L. J. (2020). Circular RNA circ_0079593 promotes glioma development through regulating KPN2A expression by sponging miR-499a-5p. *European Review for Medical and Pharmacological Sciences*, 24, 1288–1301. https://doi.org/10.26355/eurrev_202001_20186

Zhang, F., Mai, S. R., & Zhang, L. (2019). Circ-ZNF264 promotes the growth of glioma cells by upregulating the expression of miR-4493 target gene SOX4. *Brain and Behavior*, 9, 2007–2018. https://doi.org/10.1002/brb3.2632

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