MicroRNA-450a/PPM1L Axis Contributes to The Malignant Phenotype of Glioma

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Abstract

MicroRNAs play an important role in glioma, and the function of miR-450a in glioma is unknown. We aimed to clarify the role and underlying mechanism of miR-450a in glioma. We analyzed the miR-450a expression and prognosis data of glioma patients from TCGA and CGGA databases. CCK8, colony formation, nude tumor formation, transwell and wound healing assay investigated glioma cells transfected with miR-450a inhibitor growth, invasion and migration, respectively. Bioinformatics and luciferase reporter assay were applied to predict and verify the direct target gene of miR-450a. In this study, we found that miR-450a expression was significantly higher in glioma tissues and cells than the normal tissues and cells, and there is a positive correlation between miR-450a expression and histopathological grade of gliomas. In addition, the glioma patients with high miR-450a expression exhibited poorer prognosis. Downregulation of miR-450a remarkably suppressed glioma cells growth in vitro and vivo, and inhibited glioma cells invasion and migration. Luciferase reporter assay verified that PPM1L is a direct target of miR-450a. PPM1L silencing partially rescued miR-450a knockdown-induced suppressive effects on glioma cells. Therefore, our data demonstrated that miR-450a/PPM1L axis could mediate the malignant phenotype of glioma, which provided a feasible target for glioma therapy.

Introduction

Glioma is the most common tumor of the central nervous system, of which glioblastoma (GBM, WHO grade IV) is the most aggressive malignant brain tumor with a poor survival rate and is highly aggressive and invasive, exhibiting rapid cell growth [Louis et al. 2016]. Currently, the most effective treatment strategy for high grade glioma is surgical resection, followed by radiotherapy and chemotherapy [Stupp et al. 2005]. However, the prognosis of patients with high grade glioma remains poor, with a median survival of about one and half years due to therapeutic resistance and tumor recurrence [Chen et al. 2012]. Thus, it is necessary to obtain a better understanding of the molecular mechanisms and to investigate new therapeutic strategies for glioma is needed.

MicroRNAs (miRNAs, approximately 19–25 nucleotide in length) are a class of non-coding RNA, and regulate gene expression at the post-transcriptional level by complementary binding to the 3′ UTR region of the target gene [Wang et al. 2018]. Sequencing of the human genome revealed that only 2% of the entire genome can encode proteins in the human genome and the remaining 98% of DNA is only transcribed into RNA, without continuing to translate into proteins [Salta and Strooper 2017]. Numerous of studies confirmed that miRNAs play an important role in cancers [Anastasiadou et al. 2018], nerve injury disease [Bhalala et al. 2013], developmental [Pitkänen et al. 2016], cardiovascular diseases [Barringhaus and Zamore 2009] and so on. Importantly, miRNAs have been identified to be a novel tumor biomarker and promising therapeutic targets, like miR-10b in breast cancer [Gee et al. 2008], miR-375 in prostate cancer and miR-205 in non-small-cell lung cancer [Lebanony et al. 2009].

Recently, some RNA-sequence found differential expression of miR-450a in tumors, including endometrial carcinosarcoma, rectal cancer, lymphoblastic leukemia and adrenocortical tumor [Castilla et al. 2011;
Svoboda et al. 2012; Almeida et al. 2019; Koperski et al. 2017]. Chen et al. reported that miR-450a inhibits autophagy and enhances radiosensitivity by targeting DUSP10 in esophageal cancer [Chen et al. 2020]. Weng et al. reported that overexpression miR-450a inhibits the progression of hepatocellular carcinoma by targeting Dnmt3a [Weng et al. 2011]. Bruna et al. reported that miR-450a acts as a tumor suppressor in ovarian cancer by regulating energy metabolism [Muys et al. 2019]. However, the role of miR-450a in glioma remains unknown.

PPM1L (Protein Phosphatase Mg2+/Mn2+-dependent 1L), which is a member of the serine/threonine phosphatase family, can help regulate ceramide transport from the endoplasmic reticulum to the Golgi apparatus, as well as can prevent excessive inflammatory responses and cardiac dysfunction after myocardial infarction [Wang et al. 2019; Lu et al. 2013; Shinoda et al. 2012]. In addition, PPM1L relates to protein serine/threonine phosphatase activity and acts as a suppressor of the SAPK signaling pathways by associating with and dephosphorylating MAP3K7/TAK1 and MAP3K5 [Wang et al. 2019]. Recently, a study reported that PPM1L exhibited copy number variation and exerted tumor suppressive effect on colorectal cancer [Thean et al. 2010]. However, the relation of PPM1L and glioma is not clarified in previously study. Hence, we investigated that the role of miR-450a-PPM1L axis on glioma.

In this study, we found that miR-450a expression shows different in gliomas, which miR-450a expression in high-grade glioma tissues is significantly higher than that in low-grade gliomas tissues from TCGA and CGGA databases. Moreover, there is correlation between miR-450a expression and prognosis in glioma patients. Then, we performed a series of experiments and assays in vitro and in vivo, and found that miR-450a knockdown can inhibit the glioma cells proliferation, invasion and migration. Importantly, we found PPM1L is a direct target of miR-450a. Interfering with the expression of PPM1L effectively reversed the tumor-promoting effect of miR-450a. Further, we found miR-450a and PPM1L are inversely correlated in clinical samples. Hence, we confirmed the role of miR-450a-PPM1L axis on glioma and may provide potential therapeutic targets for improving poor prognosis of glioma patients.

**Materials And Methods**

**Ethics statement**

All animal experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Southwest Medical University.

**Analyses of transcriptome data from TCGA and CGGA databases**

miRNA expression profiles, mRNA expression profiles and corresponding clinical information (WHO grades and survival data) of glioma patients were obtained from TCGA and CGGA database (https://portal.gdc.cancer.gov and http://cgga.org.cn). The expression data of miR-450a were analyzed by the R package edgeR.
Cell culture and transfection

Human glioma cell lines U87, U251, U118, T98, SNB19, LN229 and control cell NHA were originated from American Type Culture Collection (Manassas, VA), and they were cultivated in DMEM medium supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 0.1 mg/mL streptomycin. All the cells were incubated in a humidified atmosphere of 5% CO2 at 37 °C. miR-450a mimics, miR-450a inhibitors and negative control (NC) were synthesized by GenePharma (Suzhou, China), lentivirus-based shPPM1L and luciferase PPM1L plasmids were obtained from GeneChem (Shanghai, China). The 20 nmol/L miRNA products and luciferase plasmids transfection were performed with lipofectamine 3000 reagent according to the manufacturer's instructions.

Cell proliferation assay

Cell proliferation assay was performed by CCK8 kit. Transfected cells (4 × 10^3 cells/well) were seeded in 96-well plates and incubated at 37 °C overnight. Then, CCK-8 solution (10 µL/well) was added to each well. After incubation for 1 h at 37 °C, the absorbance at 450 nm was measured in a microplate reader.

Cell colony assay

The transfected cells (1 × 10^3 cells/well) were planted in 6-well plates and cultured for 10–12 days. The cells were fixed with methanol and stained with 1% crystal violet. Colonies of more than 50 cells were counted. Photos were obtained using a digital camera.

Cell cycle analysis

Cell cycle distribution was measured using a cell cycle kit. The transfected cells (5 × 10^5 cells/well) were seeded in 6-well plates and cultured overnight. Then, the cells were serum starved with serum-free media for 12 h. After treatment, the cells were fixed in ice-cold 70% ethanol and stored at 4 °C overnight. The fixed cells were washed three times with pre-cold PBS, and stained with a solution consisting of staining buffer with 100 mg/mL RNase A and 50 mg/mL propidium iodide (PI) in the dark at 37 °C for 30 min. Cell cycle was analyzed with a flow cytometric.

Transwell assay

The transwell chamber for cell invasion assay was purchased from Corning (Corning, USA). Cells were allowed to invade the matrigel-coated filters toward the lower chamber. The transfected cells (1.0 × 10^5 cells per well) were seeded with 200 µL FBS-free medium in the upper chamber and 600 µL of DMEM with 10% FBS. After 24 h, the cells in the lower chamber were fixed by methanol, stained with 0.1% crystal violet in methanol. Invasive cells were counted and photographed using a microscope.

Wound healing assay

The transfected cells (5.0 × 10^5 cells/well) were seeded in 6-well plates and grown to 90% confluence. Five neat and straight scratches were drawn using a 200 µL sterile plastic pipette tip in each well. Then, the cells were washed twice with PBS to remove debris, and cultivated in serum-free DMEM. Five fields of
each wound were monitored at 0 and 24 h to evaluate the migration of cells. The distance between the two edges was calculated with Image J software.

**Quantitative real-time PCR**

TRIzol was used to extract the cell total RNA, and reverse transcription was performed in accordance with the instructions of the PrimeScript™ RT Reagent Kit (Takara). Real-time PCR was performed in a 20-ul reaction volume using Fast SYBR® Green Master Mix (Takara). U6 and ACTIN were used as internal references, and the data were analyzed using the \(2^{-\Delta\Delta Ct}\) method. The sequences of the primer for gene were listed in Table 1.

| Gene      | Sequence (5'-3')                                                                 |
|-----------|---------------------------------------------------------------------------------|
| miR-450a-1-3p | F GCCGAGTATGTACGTTTTAC  
|           | R CAGTGCCTGCTGAGGACTGAGT  
|           | RT GTCGTATCCAGTGAGTGGAGACCTGGCAATTGCCACTGGATACGACTAACCCTT |
| NC        | F UUCUCCGAACGUGUCAGUTT  
|           | R ACGUGACACGUCGGAGAATT  
|           | RT GTCGTATCCAGTGAGTGGAGACCTGGCAATTGCCACTGGATACGACTAACCCTT |
| U6        | F CTCGCTTCCGCAGACACA  
|           | R AACGCTTCACGAATTTCGCT  
|           | RT GTCGTATCCAGTGAGTGGAGACCTGGCAATTGCCACTGGATACGACTAACCCTT |
| PPM1L     | F ACCCGTCCATCTTCGGGT  
|           | R GCCTCTGGAGGTCAGGAGATTTT  
| ACTIN     | F TGCCATCCACGAAACTACC  
|           | R GTGTTGGCCTACAGGCTT |

**Western blotting**

RIPA lysis buffer was used to extract the cell total protein. The concentration of protein was measured with a BCA kit. Equal amounts of protein (30 µg/lane) were loaded on SDS–PAGE gel and transferred on to PVDF membranes. The blots were probed with primary antibodies-PPM1L and ACTIN at 1:1000 dilution followed by incubation with species specific horseradish peroxidase conjugated secondary antibodies (1:10000). The bands of the target protein were obtained using Super Signal ECL, and the intensity of the target bands was quantified by Image J Software.
Dual reporter luciferase assay

Cells were seeded in 6-well plates and cultured overnight. Then, cells were co-transfected with the wild type or mutated PPM1L-reporter luciferase vector plasmid and miR-450a mimics or NC. After 48 h, luciferase assays were performed by a dual luciferase reporter kit, and absorbance was measured using a GloMax microplate reader.

Xenograft mouse model

Female BALB/c nude mice (6–8 weeks) were obtained from HFK Bioscience Company (Beijing, China) and were housed in a pathogen-free animal facility with access to food and water ad libitum. U87 cells (1 × 10^6) with LV-miR-450a inhibitor/NC suspended in 100 μl PBS were injected subcutaneously into the flanks of the mice. The tumor size was measured in two orthogonal directions using calipers, and the tumor volume (mm^3) was calculated using the equation: 1/2 × length × width^2 [Wang et al. 2018]. When the tumors grew for one week, tumor sizes and body weights were measured once every four days. The blank control mice were not transplanted tumor cells.

Software and statistics

Data are expressed as the mean ± SD. SPSS and GraphPad prism software were used for data analysis and statistical analysis. One-way ANOVA, Student’s t-test and Pearson correlation followed by Dunnett’s multiple comparisons were used to evaluate statistical significance. Log-rank test was used to the survival analysis, and survival curve was drew by GraphPad prism software. R and Perl program were used to extract and process the transcriptome raw data and clinical information. TBtools software was used for the drawing of a Vann diagram. P-values < 0.05 were considered significant.

Results

miR-450a expression is upregulated in glioma and correlated with poor prognosis

The expression of miR-450a in glioma is still unknown. To determine the expression of miR-450a in glioma, we analyzed the miR-450a expression profile in 198 glioma samples of different grades from CGGA database and 507 normal brain tissues and different grades glioma specimens from TCGA database. Compared the normal brain tissues, we found that miR-450a had a much higher expression level in glioma tissues. In addition, miR-450a expression was highest in glioblastoma (GBM) samples (IV grade glioma) (Fig. 1a, b). Then, we explored the relationship between the expression of miR-450a and the prognosis of glioma patients. As shown in Fig. 1c, d, the patients with higher miR-450a expression had poorer survival. RT-PCR detected the miR-450a expression in GBM cell lines, we found that miR-450a was expressed higher level in GBM cell lines compared with normal human astrocytes (NHA) (Fig. 1e). These results indicated that miR-450a is also expressed higher levels in glioma cell lines, in line with the observations in glioma tissues. As such, we chose U87 and U251 cells for subsequent research. We
transfected with miR-450a inhibitor to downregulated the miR-450a level, the cell growth status as shown in Fig. 1f, and RT-PCR results confirmed that miR-450a expression had been inhibited (Fig. 1g).

**miR-450a promotes glioma cell growth in vitro and in vivo**

To investigate the role of miR-450a in glioma cell growth in vitro, we preformed the CCK8 assay and colony formation assay. Glioma cells knockdown miR-450a exhibited a decreased growth compared with the NC group (Fig. 2a). Consistently, colony formation assay showed that cell colony counts significantly decreased in miR-450a knockdown treatment cells compared with the NC group (Fig. 2b).

To further explore the effect of miR-450a on the glioma growth in vivo, LV-miR-450a inhibitor and NC-infected U87 cells were injected subcutaneously into the flanks of nude mice, respectively. The tumors volume in the miR-450a knockdown group were markedly reduced compared with the NC group, but no significant changes in body weights during 31 days (Fig. 2c-e). These results indicated that miR-450a knockdown significantly inhibits glioma cells growth in vitro and in vivo.

**miR-450a knockdown inhibits cell cycle, invasion and migration in glioma cell**

We previously confirmed that miR-450a knockdown can inhibit the proliferation of glioma cells, and cell proliferation is associated with cell division and growth. Thus, we analyzed the cell cycle distribution of glioma cells treated with miR-450 inhibitor and NC. The results showed that miR-450a downregulation significantly affected the cell cycle of glioma cells causing arrest at the G0/G1 phase compared with the control group (Fig. 3a, b).

To further examine whether tumor metastasis is related to miR-450a, we performed cell transwell assay and cell wound healing assay. Glioma cells transfected with miR-450a inhibitor presented remarkably reduced invasive capacity and wound healing capacity compared with the NC group (Fig. 3c-f). These results suggested that miR-450a downregulation can inhibit glioma progression.

**miR-450a directly targets PPM1L**

To understand the mechanism of miR-450a in glioma, we applied the bioinformatic algorithms and mRNA profiling of glioma patients to identify potential target genes of miR-450a. We analyzed four miRNA target gene prediction databases (miRDB, miRTarBase, TargetScan and miRWalk), and we got 18 candidate genes in the overlapping regions of Venn diagram (Fig. 4a). Then, we analyzed the expression correlation between miR-450a and the candidate target genes by the Spearman test, and we found that PPM1L has the strongest negative correlation with miR-450a (Fig. 4b). Additionally, there was a binding site to miR-450a in the 3'UTR region of PPM1L by TargetScan database screening (Fig. 4c). To verify whether miR-450a directly targeted PPM1L mRNA, we performed the luciferase reporter assay after co-transfected glioma cells with wild type or mutate PPM1L-luciferase plasmid and miR-450a or NC. The results demonstrated that miR-450a inhibited the luciferase activity of the wild type 3'UTR instead of the mutate 3'UTR (Fig. 4d), and indicated that miR-450a directly targeted PPM1L. Furthermore, we detected
PPM1L expression of glioma cells transfected with miR-450a inhibitor by RT-PCR and western blot. The results showed that miR-450a knockdown glioma cells exhibited a higher protein expression of PPM1L than the control cells (Fig. 4f). However, the mRNA levels of PPM1L in miR-450a knockdown glioma cells had no different compared with the control cells (Fig. 4e). This result is consistent with the functional characteristics of miRNA, which the mechanism of miRNA action is always at the posttranscriptional level by inhibiting protein translation instead of degrading mRNA.

To further analyzed the expression of PPM1L in glioma tissues and its relationship with glioma patient prognosis from TCGA and CGGA databases. The results revealed that the expression of PPM1L was significantly lower high-grade glioma (grade III and IV) compared with NBT and grade II glioma from TCGA database (Fig. 4g). Consistently, similar results are also shown in the CGGA database (Fig. 4h). In addition, the glioma patients expressing lower level of PPM1L were associated with poorer survival relative to those with higher PPM1L expression in both TCGA and CGGA database (Fig. 4i, j). These data suggested that PPM1L is a direct target of miR-450a, and low expression of PPM1L correlates with a poorer survival outcome in glioma patients.

**miR-450a promotes malignant phenotype of glioma by targeting PPM1L**

To further investigate whether miR-450a promotes proliferation, invasion and migration of glioma cells through PPM1L. First, we cotransfected with miR-450a inhibitor and shPPM1L in glioma cells, and detected the transfection efficiency by western blot. The results revealed that shPPM1L can partially rescue the knockdown effect of miR-450 inhibitor (Fig. 5a). Then, co-transfected cells were examined for glioma cells proliferation, invasion, and migration ability by CCK8 assay, transwell assay, and wound healing assay, respectively. Compared with the only miR-450a knockdown group, reducing PPM1L can promote the glioma cells proliferation (Fig. 5b). Moreover, transwell and wound healing assay results demonstrated that the loss of PPM1L rescued the miR-450a knockdown glioma cells invasion and migration (Fig. 5c-f). These results indicated that miR-450a promotes glioma cells proliferation, invasion and migration by targeting PPM1L.

**Discussion**

Glioma is the most common brain malignancy in adults. Although the incidence of glioma is relatively low, the degree of malignancy is high, and the degree of malignancy increases following with the grade of glioma [Alexander and Cloughesy 2017]. Unfortunately, molecular mechanisms underlying of glioma remain incompletely clarified, and effective therapeutic strategies are limited. Extensive evidence suggests that miRNAs contribute to the progression of various tumors, including lung cancer [Dimitrova et al. 2016], breast cancer [Dvinge et al. 2013], gastric cancer [Ueda et al. 2010] and glioma [Sumazin et al. 2011]. miR-450 has been reported that affects the biological behavior of HepG2 cells by targeting DNMT3a [Wang et al. 2019]. Liu et al. reported that upregulation of miR-450 inhibits the progression of lung cancer in vitro and in vivo by targeting IRF2 [Liu et al. 2016]. These results suggested that miR-450 is
a functional miRNA in tumors. However, the role and molecular mechanisms of miR-450a in glioma are less reported.

TCGA database is the largest and most comprehensive public database of tumors over the world. We analyzed miR-450a expression of approximately 500 case glioma patients and clinical information, and found that miR-450a expression was higher in glioma tissues than normal brain tissues. Additionally, miR-450a had the highest expression in GBM patients. However, only 5 case GBM patients had miRNA expression profile data in the recently updated TCGA database glioma data, and then we combined the analysis with the CGGA database. Consistently, similar results are also showed in the CGGA database, and these results indicated that miR-450a expression was strongly linked to glioma grade. Furthermore, glioma patients with higher miR-450a expression obtained poorer prognosis. Thus, we deemed that miR-450a may play an important role in glioma.

To investigate the effect of miR-450a on glioma cells, we downregulated the miR-450a expression in U87 and U251 cells by transfected miR-450a inhibitor. Subsequently, we performed a series of assay, including CCK8, cell cycle assay, colony formation assay, transwell assay and wound healing assay, to explore the effect of miR-450a on the malignant phenotype of glioma cells. These results demonstrated that miR-450a promotes glioma cells proliferation, invasion and migration. Animals experiment also confirmed that miR-450a contribute to glioma growth. Furthermore, we explored the miR-450a underlying molecular mechanism to promote malignant phenotype of glioma. We predicted the target genes of miR-450a using miRDB, miRTarBase, miRWalk and TargetScan databases, and obtained 18 intersection genes. Then, we analyzed the correlation between these 18 candidate target genes and miR-450a expression in TCGA database, and found that PPM1L had the strongest correlation with miR-450a. Luciferase report assay also verified that PPM1L mRNA 3’UTR exists complementarity sequence with miR-450a. PPM1L expression and prognosis analysis in glioma samples from TCGA and CGGA databases indicated that PPM1L may act as a glioma suppress gene. Then, we co-transfected with miR-450a inhibitor and PPM1L shRNA to investigate the malignant phenotype of glioma cells again, and revealed that PPM1L downregulation effectively reversed the tumor-promoting effect of miR-450a. Therefore, our data verified that downregulation miR-450a inhibits glioma cells growth, invasion and migration by targeting PPM1L. But, Liu et al. and Wang et al. reported that miR-450 acts as a tumor suppressor in lung cancer and liver cancer [Wang et al. 2019; Liu et al. 2016]. We deem that miR-450a may play a dual regulatory role in tumors.

PPM1L is a member of the serine/threonine phosphatase family and involves in immune regulation after myocardial infarction [Wang et al. 2019]. However, rarely reported role of PPM1L in tumors. Although PPM1L expression and prognosis data from online databases indicated that PPM1L is downregulated in glioma, the functional role of PPM1L in glioma remains unclear. In the future, we will continue to explore the function of PPM1L in gliomas.

In conclusion, our data revealed miR-450a plays an essential role on promoting glioma cells proliferation, invasion and migration by directly targeting PPM1L, indicating miR-450a/PPM1L axis may as potential
molecular biomarker for glioma patients. Thus, miR-450a/PPM1L may act as a novel diagnostic or treatment target in glioma.

Declarations

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Authors’ contributions

JY and XC performed the experiments and wrote the first draft of the article. XC, JZ and LP analysed the data. YM and LL participated in the execution of the experiments. RX and LC conceptualized the project and revised the article. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Louis DN, Perry A, Reifenberger G, Deimling AV, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW (2016) The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol 131(6): 803-820

2. Stupp R, Mason WP, Ben MJ, Weller M, Fisher B, Taphoorn MJB, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10): 987-996

3. Chen J, Li YJ, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488(7412): 522-526

4. Wang J, Xu ZM, Chen XF, Li Y, Chen C, Wang CW, Zhu JW, Wang ZT, Chen WJ, Xiao ZY, Xu RX (2018) MicroRNA-182-5p attenuates cerebral ischemia-reperfusion injury by targeting Toll-like receptor 4. Biochem Biophys Res Commun 505(3): 677-684

5. Salta E, Strooper BD (2017) Noncoding RNAs in neurodegeneration. Nat Rev Neurosci 18(10): 627-640

6. Anastasiadou E, Jacob LS, Slack FJ (2018) Non-coding RNA networks in cancer. Nat Rev Cancer 18(1): 5-18

7. Bhalala OG, Srikanth M, Kessler JA (2013) The emerging roles of microRNAs in CNS injuries. Nat Rev Neurol 9(6): 328-339

8. Pitkänen A, Löscher W, Vezzani A, Becker AJ, Simonato M, Lukasiuk K, Gröhn O, Bankstahl JP, Friedman A, Aronica E, Gorter JA, Ravizza T, Sisodiya SM, Kokaia M, Beck H (2016) Advances in the development of biomarkers for epilepsy. Lancet Neurol 15(8): 843-856

9. Barringhaus KG, Zamore PD (2009) MicroRNAs: regulating a change of heart. Circulation 119(16): 2217-2224

10. Gee HE, Camps C, Buffa FM, Colella S, Sheldon H, Gleadle JM, Ragoussis J, Harris AL (2008) MicroRNA-10b and breast cancer metastasis. Nature 455(7216): E8-E9

11. Lehanony D, Benjamin H, Gilad S, Ezagouri M, Mov A, Ashkenazi K, Gefen N, Izrael  S, Rechavi G, Pass H, Nonaka D, Li J, Spector Y, Rosenfeld N, Chajut A, Cohen D, Aharonov R, Mansukhani M (2009) Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol 27(12): 2030-2037

12. Castilla MÁ, Moreno-Bueno G, Romero-Pérez L, Vijver KVD, Biscuola M, López-García MÁ, Prat J, Matías-Guiu X, Cano A, Oliva E, Palacios J (2011) Micro-RNA signature of the epithelial-mesenchymal transition in endometrial carcinosarcoma. J Pathol 223(1): 72-80

13. Svoboda M, Sana J, Fabian P, Kocakova I, Gombozova J, Nekvindova J, Radova L, Vyzula R, Slaby O (2012) MicroRNA expression profile associated with response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients. Radiat Oncol 7: 195
14. Almeida RS, Silva MC, Coutinho LL, Gomes RG, Pedrosa F, Massaro JD, Donadi EA, Lucena-Silva N (2019) MicroRNA expression profiles discriminate childhood T- from B-acute lymphoblastic leukemia. Hematol Oncol 37(1): 103-112

15. Koperski Ł, Kotlarek M, Świerniak M, Kolanowska M, Kubiak A, Górnicka B, Jaźdżewski K, Wójcicka A (2017) Next-generation sequencing reveals microRNA markers of adrenocortical tumors malignancy. Oncotarget 8(30): 49191-49200

16. Chen H, Yao XJ, Di XK, Zhang YX, Zhu HC, Liu S, Chen TT, Yu DY, Sun XC (2020) MiR-450a-5p inhibits autophagy and enhances radiosensitivity by targeting dual-specificity phosphatase 10 in esophageal squamous cell carcinoma. Cancer Lett 483: 114-126

17. Weng ZH, Wang DD, Zhao WY, Song MQ, You FP, Yang L, Chen LB (2011) MicroRNA-450a targets DNA methyltransferase 3a in hepatocellular carcinoma. Exp Ther Med 2(5): 951-955

18. Muys BR, Sousa JF, Plaça JR, Araújo LF, Sarshad AA, Anastasakis DG, Wang XT, Li XL, Molfetta GA, Ramão A, Lal A, Vidal DO, Hafner M, Silva WA (2019) MiR-450a acts as a tumor suppressor in ovarian cancer by regulating metastasis. Cancer Res 79(13): 3294-3305

19. Wang B, Zhou QQ, Bi Y, Zhou WH, Zeng QY, Liu ZM, Liu XG, Zhan ZZ (2019) Phospatase PPM1L prevents excessive inflammatory responses and cardiac dysfunction after myocardial infarction by inhibiting IKKβ activation. J Immunol 203(5): 1338-1347

20. Lu G, Ota A, Ren SX, Franklin S, Rau CD, Ping PP, Lane TF, Zhou ZH, Reue K, Lusis AJ, Vondriska T, Wang YB (2013) PPM1L encodes an inositol requiring-protein 1 (IRE1) specific phosphatase that regulates the functional outcome of the ER stress response. Mol Metab 2(4): 405-416

21. Shinoda Y, Fujita K, Saito S, Matsui H, Kanto Y, Nagaura Y, Fukunaga K, Tamura S, Kobayashi T (2012) Acyl-CoA binding domain containing 3 (ACBD3) recruits the protein phosphatase PPM1L to ER-Golgi membrane contact sites. FEBS Lett 586(19): 3024-3029

22. Thean LF, Loi C, Ho KS, Koh PK, Eu KW, Cheah PY (2010) Genome-wide scan identifies a copy number variable region at 3q26 that regulates PPM1L in APC mutation-negative familial colorectal cancer patients. Genes Chromosomes Cancer 49(2): 99-106

23. Wang ZT, Liu Z, Yu GY, Nie XH, Jia WQ, Liu RE, Xu RX (2018) Paeoniflorin inhibits migration and invasion of human glioblastoma cells via suppression transforming growth factor β-induced epithelial-mesenchymal transition. Neurochem Res 43(3): 760-774

24. Alexander BM, Cloughesy TF (2017) Adult glioblastoma. J Clin Oncol 35(21): 2402-2409

25. Dimitrova N, Gocheva V, Bhutkar A, Resnick R, Jong RM, Miller KM, Bendor J, Jacks T (2016) Stromal expression of miR-143/145 promotes neoangiogenesis in lung cancer development. Cancer Discov 6(2): 188-201

26. Dvinge H, Git A, Gräf S, Salmon-Divon M, Curtis C, Sottoriva A, Zhao YJ, Hirst M, Armisen J, Miska EA, Chin SF, Provenzano E, Turashvili G, Green A, Ellis I, Aparicio S, Caldas C (2013) The shaping and functional consequences of the microRNA landscape in breast cancer. Nature 497(7449): 378-382

27. Ueda T, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM (2010) Relation between
microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. Lancet Oncol 11(2): 136-146

28. Sumazin P, Yang XR, Chiu HS, Chung WJ, Lyer A, Llobet-Navas D, Rajbhandari P, Bansal M, Guarnieri P, Silva J, Califano A (2011) An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. Cell 147(2): 370-381

29. Wang Y, Wang L, Yu X, Duan JP (2019) Overexpression of miR-450 affects the biological behavior of HepG2 cells by targeting DNMT3a. Onco Targets Ther 12: 5069-5076

30. Liu FB, Yu XB, Huang HH, Chen X, Wang J, Zhang XM, Lin Q (2016) Upregulation of microRNA-450 inhibits the progression of lung cancer in vitro and in vivo by targeting interferon regulatory factor 2. Int J Mol Med 38(1): 283-290

Figures

Figure 1

Correlation between miR-450a expression and the clinic-pathologic features of glioma patients from TCGA and CGGA. a and b miR-450a expression is showed according to the histopathologic grade of TCGA and CGGA glioma patients. c and d Prognostic value of miR-450a in TCGA and CGGA glioma patients. e The relative expressions of miR-450a in glioma cell lines (U87, U251, U118, T98, SNB19, LN229) and normal cell line (NHA). f Representative micrograph of glioma cells transfected with miR-
miR-450a inhibitor or NC. miR-450a expression in glioma cells transfected with miR-450a inhibitor or NC. * P < 0.05, ** P < 0.01, **** P < 0.0001, ns means no significance.

**Figure 2**

Effect of miR-450a knockdown on glioma cells growth in vitro and in vivo. a Cell viability of glioma cells transfected with miR-450a inhibitor or NC. b Effect of miR-450a knockdown on glioma cells colony formation. c Representative photos of the xenografted U87 tumors. d and e Tumor volumes and mouse body weights were measured on the indicated days. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.
Figure 3

Effect of miR-450a knockdown on glioma cells cycle, invasion and migration. a and b miR-450a knockdown glioma cells cycle arrested at G0/G1 phase. c and d Invasive capacity of glioma cells transfected with miR-450a inhibitor or NC. e and f Effect of miR-450a knockdown on glioma cells migration. **** P < 0.0001.
miR-450a directly targets PPM1L in glioma cells. a Venn diagram showing four different prediction databases (miRDB, miRWalk, miRTarBase and TargetScan) jointly calculate 18 candidate target genes. b Spearman correlation test of miR-450a expression with PPM1L expression from TCGA glioma patients. c miR-450a target regions in the 3'UTR of PPM1L mRNA as predicted by the TargetScan database. d Dual-luciferase reporter assays results. e PPM1L protein expression in glioma cells transfected with miR-450a inhibitor or NC. f PPM1L mRNA level in miR-450a knockdown glioma cells. g and h PPM1L expression are showed according to the histopathologic grade of TCGA and CGGA glioma patients. i and j Prognostic value of PPM1L in TCGA and CGGA glioma patients. *** P < 0.001, **** P < 0.0001, ns means no significance.
miR-450a mediates malignant phenotype of glioma by targeting PPM1L. 

**a** PPM1L protein expression in glioma cells cotransfected with miR-450a inhibitor and shPPM1L. 

**b** Cell viability of miR-450a knockdown glioma cells after transfected with shPPM1L. 

**c and d** Transwell assay showed invasive capacity of glioma cells cotransfected with miR-450a inhibitor and shPPM1L. 

**e and f** Wound healing assay evaluated cell migration rate of miR-450a knockdown glioma cells after treated with shPPM1L. 

* P < 0.05, ** P < 0.01, miR-450a inhibitor + shPPM1L group vs inhibitor NC group, # P < 0.05, ## P < 0.01, miR-450a inhibitor + shPPM1L group vs miR-450a inhibitor group, *** P < 0.001, **** P < 0.0001.