RETRACTED ARTICLE: Silencing hsa_circ_PVT1 (circPVT1) suppresses the growth and metastasis of glioblastoma multiforme cells by up-regulation of miR-199a-5p

Guonan Chi, Fuwei Yang, Donghui Xu and Weiming Liu

Department of Neurosurgery, China-Japan Union Hospital of Jilin University, Changchun, China

ABSTRACT

Background: Glioblastoma multiforme (GBM) is one of the most prevailing primary brain tumours among adults and most aggressive cancers. Despite multiple developments in medical and surgical treatments, GBM is still a deadly disease with a high mortality rate. Here, this study was performed to investigate the function of circPVT1 on GBM.

Methods: CCK-8 and flow cytometry were utilised to estimate viability and apoptosis in both cells. qRT-PCR was performed to determine circPVT1 and miR-199a-5p expression. Western blot was conducted to determine apoptosis, migration and EMT-related proteins levels when silencing circPVT1. Subsequently, these parameters were re-tested after up-regulating miR-199a-5p.

Results: CircPVT1 was highly expressed in GBM tissues. Silencing circPVT1 raised two cells apoptosis and reduced viability and migration capacity. Moreover, EGF-induced EMT was repressed by silencing circPVT1. In addition, miR-199a-5p expression was elevated when silencing circPVT1. And silencing circPVT1 exerted above changes via up-regulating miR-199a-5p. Finally, silencing circPVT1 repressed YAP1 and PI3K/AKT pathways via up-regulating miR-199a-5p.

Conclusion: Our data suggested that silencing circPVT1 inhibited viability, migration, EGF-induced EMT and promoted apoptosis as well as repressed YAP1 and PI3K/AKT pathways by up-regulating miR-199a-5p.

HIGHLIGHTS

1. CircPVT1 expression is highly expressed in GBM tissues;
2. Si-circPVT1 represses migration and promoted apoptosis in U539 and U251 cells;
3. Si-circPVT1 represses migration and promoted apoptosis when elevating miR-199a-5p;
4. Si-circPVT1 represses EGF-induced EMT when increasing miR-199a-5p;
5. Si-circPVT1 suppresses YAP1 and PI3K/AKT pathways by up-regulating miR-199a-5p.

Introduction

Glioblastoma multiforme (GBM), also known as glioblastoma, one of the most prevailing primary brain tumours in adults and it is one of the most deadly and aggressive cancers [1]. Multiple chromosomal and genetic abnormalities are involved with GBM, leading to rapid infiltration, invasion and destruction of adjacent tissues, especially in spinal cord [2]. Low-grade GBM is generally related with neurological dysfunction [3]. Because of the increasing rate of recrudescence, infiltrative rate and limited treatment means, the prognosis of high-grade GBM is still poor [4]. Despite multiple developments in medical and surgical treatments, GBM is still a deadly disease with a high mortality rate [5]. In addition, radiotherapy for spinal GBM is often accompanied by worse outcomes [6]. Therefore, finding novel molecules participated in tumour progression is vital to improve the survival rate of GBM.

Circular RNA (circRNA) is an innovative race of RNA and is widely discovered in eukaryotes [7]. Accumulating evidence suggested that various circRNAs had cell-type specific expressions and were involved in a good deal of cancers [8]. CircRNAs could act as a sponge of diverse microRNAs (miRNAs) and play an essential part in the pathological development of GBM [9]. For instance, up-regulating circTTBK-2 in GMB was proved to be the miR-217 sponge and improved proliferation, migration and invasion [10]. Another study indicated that circ_0046701 remarkably overexpressed in GMB and exerted as a sponge of miR-142-3p to develop tumorogenesis [9]. For instance, up-regulating circTTBK-2 in GMB was proved to be the miR-217 sponge and improved proliferation, migration and invasion [10]. Another study indicated that circ_0046701 remarkably overexpressed in GMB and exerted as a sponge of miR-142-3p to develop tumorogenesis [9]. Furthermore, circRNA could also be targeted by miRNA, and miR-7 sponge CDR1-AS can be deteriorated by miR-671-5p in GMB [12]. Interestingly, several studies illustrated the function of circPVT1 in many cancers. For instance, circPVT1 facilitated growth and migration via sponging miR-125b and mobilising E2F2 pathway in non-small cell lung...
cancer (NSCLC) [13]. However, the function of circPVT1 on GBM remains unclear.

MiRNAs as a category of small noncoding RNA acts a critical factor of gene expression by promoting mRNA degradation or preventing the translation of target mRNA [14,15]. MiRNA has been investigated in a variety of human diseases, and plays a vital part in cell proliferation, metastasis and EMT process [15]. In spite of a large amount of researches were reported on the aspects of miRNAs, little is known about the complex and various biological effects of miRNA. Numerous literatures demonstrated that miRNAs participated in the biological progresses of GBM. For instance, miR-124 prevented growth and potentiated chemosensitivity via targeting AURKA in GBM [16]. Another study indicated that up-regulating miR-338-5p inhibited proliferation, migration, invasion and improved apoptosis in GBM cells [17]. Furthermore, miR-199a-5p was investigated to play a variety of roles in cancers. Liu et al elucidated miR-199a-5p and let-7c suppressed hepatocellular carcinoma cells metastasis capacity in [18]. In the current research, our purpose was to explore circPVT1 and miR-199a-5p function and mechanism in U591 and U251 cells.

Materials and methods

Clinical specimens

Clinical human GBM tissues and normal adjacent tissues (n=25) were attained from China-Japan Union Hospital of Jilin University (Changchun, China). The whole patients received no therapy before receiving surgery. The agreement of every patient was gotten. Morethan, this work gained the support of the Medical Ethics Committee of China-Japan Union Hospital of Jilin University.

Cell culture

U539 and U251 cells were obtained from Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Cells were hatched in RPMI-1640 bought from Gibco (Grand Island, NY, USA) containing 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin (Solarbio, Beijing, China) and 100 μg/mL streptomycin (Wuhan Fortuna Chemical Co., Ltd, Wuhan, China), 50 ng/mL epidermal growth factor (EGF) in a chamber filled with 95% air as well as 5% CO₂ at 37 °C.

Transfection

CircPVT1 siRNA (si-circPVT1), si-negative control (si-NC), miR-199a-5p inhibitor and NC inhibitor were synthesised and purchased by Shanghai GenePharma Co., Ltd (Shanghai, China). All transfections were performed with the 25 nM oligonucleotides by Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) in accordance with producer’s manual. The final concentration of si-circPVT1, si-NC, miR-199a-5p inhibitor and NC inhibitor was 10 nM, 100 nM, 200 nM, 2 μg/mL respectively. After U539 and U251 cells were transfected for 48 h, all cells were harvested for follow-up investigations.

Cell counting kit-8 (CCK-8) assay

U539 and U251 cells were hatched in a 96-well plate at the concentration of 5 × 10³ cells/well. After culturing, the complete culture medium was removed and added with media including 10 μL CCK-8 (Dojindo Laboratories, Tokyo, Japan), and then the cells were contained for 1 h. The absorbance was measured at 450 nm by applying a Microplate Reader (Thermo Fisher Scientific, Waltham, MA, USA).

Apoptosis assay

After transfection, the rate of U539 and U251 cells apoptosis was assessed applying flow cytometry (Beckman Coulter, Atlanta, GA, USA) in line with the direction of Annexin V-FITC/PI apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China).

Migration assay

Migration capacity was evaluated utilising transwell chambers (Millipore, Billerica, MA, USA). The 200 μL cell serum-free suspension was placed into the upper layer of chamber. 600 μL complete growth media was added to the lower layer of chamber. After cultivating for 48 h, the migrated cells were fixed with 4% paraformaldehyde (Beyotime) as well as stained with crystal violet reagent for 15 min. A microscope was conducted to assess the number of migrated cells.

Quantitative reverse transcription PCR (qRT-PCR)

The RNA of U539 and U251 cells was separated according to producer’s protocols of Trizol purchased from Invitrogen (San Diego, CA, USA). The cDNA of RNA was synthesised through applying the MultiscribeRTkit (Biosystems, Barcelona, Spain). CircPVT1 and miR-199a-5p expression was detected utilising QuantiNova SYBR Green PCR Kit (Qiagen, Hilden, Germany). The relative expression was estimated applying the 2−ΔΔCt method.

Western blot assay

Proteins of U539 and U251 cells were extracted by using RIPA lysis buffer (Beyotime, Shanghai, China) containing protease-inhibitor (Beyotime). The protein concentration was evaluated by BCA™ Protein Assay Kit (Pierce, Rockford, IL, USA). The 10% SDS-PAGE was collected to isolate every protein and a class of appropriate membrane (Millipore) was chose to transfer proteins from gel. Sequentially, the membranes carrying with proteins were hatched with primary antibodies. The time and temperature was overnight and 4 °C. Primary antibodies were as following: cleaved-Caspase-3 (ab2302, Abcam, Cambridge, MA, USA), cleaved-Caspase-9 (ab2324, Abcam), MIMP-9 (ab73734, Abcam), E-cadherin (ab15148, Abcam), N-cadherin (ab18203, Abcam), Vimentin.
CircPVT1 was highly expressed in GBM tissues
To explore the abnormal expression of circPVT1 in GBM tissues, qRT-PCR was adapted to quantify circPVT1. As the results displayed in Figure 1, circPVT1 levels were dramatically raised in GBM tissues (p < .001).

CircPVT1 expression was silenced by vector transfection in U539 and U251 cells
The qRT-PCR was performed to examine if it is successful for si-circPVT1 transfection. The results suggested that circPVT1 expression was remarkably reduced in U539 and U251 cells through transferring si-circPVT1 (p < .001, Figure 2).

Silencing circPVT1 suppressed viability, migration, and improved apoptosis in U539 and U251 cells
For identifying the function circPVT1, the proliferation and migration of U539 and U251 cells transferred by si-circPVT1 were detected. As the evidence showed in Figure 3(A), viability was notably reduced in U539 and U251 cells when circPVT1 was silenced in U539 and U251 cells (p < .01). Oppositely, the number of apoptotic cells was prominently enhanced when silencing circPVT1 (p < .001, Figure 3(B)). In addition, apoptosis-associated proteins were quantified in both experimental cells. The cleaved-Caspase-3 and cleaved-Caspase-9 expression was significantly raised in U539 cells (p < .001, Figure 3(C–D)). Consistently, the above two proteins were profoundly augmented in U251 cells when silencing circPVT1 (p < .001, Figure 3(E–F)). Furthermore, migration capacity was theatrically flattened via silencing circPVT1 expression (p < .01, Figure 3(G)). Sequentially, migration-associated proteins levels were also researched utilising western blot. MMP-9 expression was strongly declined in both U539 and U251 cells when silencing circPVT1 (p < .01, Figure 3(H–I)).

Silencing circPVT1 repressed EGF-induced EMT in U539 and U251 cells
In next study, the impact of circPVT1 on was explored in GBM. Firstly, the expression of N-cadherin, Vimentin and Zeb1 was observably escalated in U539 cells by EGF, and E-cadherin expression was remarkably decreased by EGF (p < .001, Figure 4(A–B)). Subsequently, silencing circPVT1 declined N-cadherin, Vimentin and Zeb1 expression as well as elevated E-cadherin expression in U539 cells (p < .05 or p < .01, Figure 4(A–B)). Consistently, the expression of above-mentioned proteins showed the same trend in U251 cells as in U539 cells (p < .05, p < .01 or p < .001, Figure 4(C–D)).

Silencing circPVT1 promoted miR-199a-5p expression in U539 and U251 cells
As a next step, the relation of miR-199a-5p and circPVT1 was assessed. MiR-199a-5p expression was facilitated via silencing circPVT1 (p < .01, Figure 5).
Silencing circPVT1 suppressed viability, migration, and improved apoptosis via up-regulating miR-199a-5p

To analyse whether miR-199a-5p inhibitor was transfected into cells successfully, miR-199a-5p expression was determined using qRT-PCR. The level of miR-199a-5p was remarkably declined via transferring miR-199a-5p inhibitor in experimental cells (p < .01, Figure 6(A)). To discover the impacts of miR-199a-5p on si-circPVT1-induced viability and migration of U539 and U251 cells, we added miR-199a-5p inhibitor. We found that viability was raised via reducing miR-199a-5p (p < .05, Figure 6(B)). Conversely, apoptosis was attenuated by down-regulating miR-199a-5p in both U539 and U251 cells (p < .05, Figure 6(C)). Subsequently, expression of cleaved-Caspase-3 and cleaved-Caspase-9 was notably relieved in both cells (p < .05 or p < .01, Figure 6(D–F)). In addition, migration capacity was partially elevated in two cells added with miR-199a-5p inhibitor (p < .05, Figure 6(G)). Consequently, MMP-9 expression in both cells was markedly raised by down-regulating miR-199a-5p (p < .05 or p < .01, Figure 6(H–I)).

Silencing circPVT1 repressed EGF-induced EMT via up-regulating miR-199a-5p

As a next step, we further explored whether miR-199a-5p took part in circPVT1-inhibited EMT of U539 and U251 cells. As the data indicated in Figure 7(A–B), expression of N-cadherin, Vimentin and Zeb1 was higher in U539 cells by down-regulating miR-199a-5p (p < .05 or p < .01). And E-cadherin expression was alleviated by down-regulating miR-199a-5p (p < .05, Figure 7(A–B)). Similarly, changes of these proteins expression in U251 presented consistent trend as in U539 cells (p < .05 or p < .01, Figure 7(C–D)).

Silencing circPVT1 repressed VAP1 and PI3K/AKT pathways by up-regulating miR-199a-5p

To uncover the function of circPVT1 on VAP1 and PI3K/AKT pathways, the expression of VAP1, PI3K and AKT was examined. VAP1 expression was dramatically reduced when silencing circPVT1 in both U539 and U251 cells (p < .001). Then the reduction was partially reversed via down-regulating miR-199a-5p in two cells (p < .05, Figure 8(A–D)). Moreover, PI3K and AKT phosphorylation was profoundly debilitated via silencing circPVT1 (p < .001). Sequentially, the phosphorylation degrees of above two proteins were elevated via declining miR-199a-5p in two cells (p < .01, Figure 8(E–H)).

Discussion

GBM is listed as the most prevalent type of cancer in China, and its incidence is rising every year, and will remain to advance in the coming years [19]. So far, the prognosis of GBM is still very poor. Early diagnosis and prognosis of GMB require the identification of sensitive bookmakers [20]. Nevertheless, the molecular biological mechanisms of GBM
are complex and not yet been entirely disentangled, making it difficult to screen for key biomarkers. Thus, understanding the molecular mechanism of GBM is critical to improving the anti-cancer strategies of patients with GBM.

Lately, accumulating evidence indicated that circRNAs acted an essential part in the development of GBM. Additionally, abnormal regulation of circRNAs can be observed in cancer tissues, indicating that circRNAs can be a prognostic marker and possible target for GBM therapy [11,21]. Currently, researches have illustrated that circPVT1 as a newly explored circRNA is involved in lung cancer, oral squamous cell carcinoma (OSCC), and osteosarcoma [13,22,23]. For instance, two studies have reported that there is the high expression of circPVT1 in patients suffering from NSCLC and NSCLC lines [13,24]. It was confirmed that reducing circPVT1 expression could weaken cell proliferation and invasion as well as facilitate apoptosis in NSCLC cells [13,24]. The report of OSCC discovered a decision similar to NSCLC. The expression of circPVT1 in OSCC cells and specimens was elevated, and cells growth was alleviated via decreasing circPVT1 expression [23]. Our experimental results were similar to above results of NSCLC and OSCC cancers. We found the levels of circPVT1 expression in GBM tissue were outstandingly increased, as well as silencing circPVT1 decreased viability and migration capacity, while promoted apoptosis in both U539 and U251 cells. More than that, silencing circPVT1 contributed to alleviating EGF-induced EMT in both cells. In this study, the function of circPVT1 was explored in GBM for the first time.

Up to now, miRNAs have been revealed that they are involved in a large amount of biological processes, including tumour formation and inflammatory responses in many human diseases [25–27]. It is well known that miR-199a-5p has been studied to take part in the progress of cancer [28]. In glioma cells, there are the lower levels of miR-199a-5p than in normal cells, as well as glioma cells proliferation and

Figure 4. Silencing circPVT1 repressed EGF-induced EMT in U539 and U251 cells. (A–D) Expression of N-cadherin, Vimentin and Zeb1 was escalated by EGF, and the escalation was attenuated by silencing circPVT1. Conversely, E-cadherin was declined by EGF and elevated by silencing PVT1 in U539 and U251 cells. **p < .05; ***p < .01 or ****p < .001 compared to labelled group.

Figure 5. Silencing circPVT1 promoted miR-199a-5p expression in U539 and U251 cells. MiR-199a-5p was elevated by silencing circPVT1. **p < .01 compared to labelled group.
Figure 6. Silencing circPVT1 suppressed viability, migration, and improved apoptosis via up-regulating miR-199a-5p. (A) MiR-199a-5p was lessened by miR-199a-5p inhibitor. (B) Viability was relieved with adding miR-199a-5p inhibitor. (C) Apoptosis was attenuated by down-regulating miR-199a-5p. (D-F) The cleavage of Caspase-3 and Caspase-9 was alleviated when declining miR-199a-5p in U539 and U251 cells. (G) Migration ability was facilitated via reducing miR-199a-5p. (H-I) MMP-9 levels were elevated by down-regulating miR-199a-5p in U539 and U251 cells. *p < .05, **p < .01 or ***p < .001 compared to labelled group.

Figure 7. Silencing circPVT1 repressed EGF-induced EMT via up-regulating miR-199a-5p in U539 and U251 cells. (A-D) Expression of N-cadherin, Vimentin and Zeb1 was relieved by down-regulating miR-199a-7p, while the expression of E-cadherin expression was impeded by down-regulating miR-199a-5p in both U539 and U251 cells. *p < .05, **p < .01 or ***p < .001 compared to labelled group.
invasion were retarded when overexpressing miR-199a-5p [29,30]. Our results were similar to the results of previous studies, we discovered overexpressing miR-199a-5p attenuated U539 and U251 cells proliferation and migration. In addition, a number of researches have expounded that circPVT1 could effectively sponge miR-125b, miR-145 and miR-497 to promote cell growth and metastasis in colorectal cancer and NSCLC [13,24,31]. Hence, we verified the

Figure 8. Silencing circPVT1 repressed VAP1 and PI3K/AKT pathways by up-regulating miR-199a-5p. (A–D) VAP1 expression was reduced by silencing circPVT1 and attenuated with the transfection of miR-199a-5p inhibitor. (E–H) The degrees of PI3K and AKT phosphorylation were decreased via reducing circPVT1 and partially reversed when down-regulating miR-199a-5p in both cells. **p < .01 or ***p < .001 compared to labelled group.
correlation of circPVT1 and miR-199a-5p as well as the impact of circPVT1 and miR-199a-5p on human glioma cell lines. Our results demonstrated that silencing circPVT1 decreased viability and migration and promoted apoptosis by aggregansing miR-199a-5p in U539 and U251 cells. Furthermore, silencing circPVT1 restrained EGF-induced EMT via miR-199a-5p augment.

YAP is a proline-rich phosphoprotein and encoded by YAP1 gene. The activation of YAP1 may be associated with stem cells, organ sized and cancer. With the increase of YAP1 expression, the invasiveness of cancer cells is enhanced. In Orr’s study, YAP1 was transfected with shRNA plasmid into U87, U373 and HSR-GBM cells, the data indicated that the knockdown of YAP1 remarkably declined GBM cells growth [32]. And the down-regulation of YAP1 may provide a new approach to treat GBM [32]. PI3K/AKT pathway is a generally uncontrolled pathway in cancer; 86% of GBM clinical specimens indicate the changes of the receptor tyrosine kinases/PI3K pathway [33,34]. In addition, the sustained activation of AKT1 endothelial cells has been shown to induce the formation of structurally abnormal blood vessels, thereby regenerating tumour vascular abnormalities [33]. PI3K/AKT pathway is a complicated central regulator of a quantity of basic cellular processes that mediates all phenotypes that conduce to human cancer progression, including GBM [35]. The AKT pathway was proved to be mobilised in EC tumours in the previous study [36]. Growth factor signalling, intracellular metabolism, and extracellular signal molecules regulates the growth and proliferation of malignant GBM via PI3K/AKT pathway [37,38]. Moreover, PI3K/AKT pathway was considered to be a key regulator of growth and proliferation in GBM cells [39]. Consistent with previous studies, our data implied that silencing circPVT1 repressed YAP1 and PI3K/AKT pathways. Additionally, these two pathways were partially activated via impeding miR-199a-5p, which displayed that circPVT1 exerted its protective effect on YAP1 and PI3K/AKT pathways by up-regulating miR-199a-5p. Although our study made major breakthroughs in the molecular mechanism of GBM and provided a good theoretical basis for the treatment of GBM, it was mainly limited to in vitro experiments and lacked clinical application.

**Conclusion**

On the whole, we first investigated the function and interrelation of circPVT1 and miR-199a-5p in GBM. The results indicated a protective effect of circPVT1 on U539 and U251 cells in vitro. Firstly, silencing circPVT1 declined viability, migration and EGF-induced EMT, as well as facilitated apoptosis in U539 and U251 cells. Subsequently, silencing circPVT1 could raise the level of miR-199a-5p in both cells, as well as silencing circPVT1 reduced cells viability, migration, EGF-induced EMT and accelerated apoptosis via promoting miR-199a-5p expression. Moreover, further researches indicated that silencing circPVT1 repressed YAP1 and PI3K/AKT pathway via up-regulating miR-199a-5p. The evidence uncovered an innovative molecular mechanism of the impacts of circPVT1 and miR-199a-5p on GBM, proving an advanced target for clinical therapy.

**Disclosure statement**

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**ORCID**

Weiming Liu https://orcid.org/0000-0001-9098-3570

**References**

[1] Yang TQ, Lu XJ, Wu TF, et al. MicroRNA-16 inhibits glioma cell growth and invasion through suppression of BCL2 and the nuclear factor-kappaB1/MMP9 signaling pathway. Cancer Sci. 2014;105(3):265–271.

[2] Kimura H, Zhang L, Zhao M, et al. Targeted therapy of spinal cord glioma with a genetically modified Salmonella typhimurium. Cell Proliferation. 2010;43(1):41–48.

[3] Liu X, Song B, Li S, et al. Identification and functional analysis of the risk microRNAs associated with cerebral low-grade glioma prognosis. Mol Med Rep. 2017;16(2):1173–1179.

[4] Wu DM, Han XR, Wen X, et al. Long Non-Coding RNA LINCO1260 inhibits the proliferation, migration and invasion of spinal cord glioma cells by targeting CARD11 via the NF-kappaB signaling pathway. Cell Physiol Biochem. 2018;48(4):1563–1578.

[5] Yu K, Fan J, Ding X, et al. Association study of a functional copy number variation in the WWOX gene with risk of gliomas among Chinese people. Int J Cancer. 2014;135(7):1687–1691.

[6] Milano MT, Johnson MD, Sul J, et al. Primary spinal cord glioma: a Surveillance, Epidemiology, and End Results database study. J Neurooncol. 2010;98(1):83–92.

[7] Yang P, Qiu Z, Jiang Y, et al. Silencing of cZNF292 circular RNA suppresses human glioma tube formation via the Wnt/beta-catenin signaling pathway. Oncotarget. 2016;7(9):63449–63455.

[8] Chen B, Huang S. Circular RNA: An emerging non-coding RNA as a regulator and biomarker in cancer. Cancer Lett. 2018;418:41–50.

[9] Panda AC, Grammatikakis I, Munk R, et al. Emerging roles and context of circular RNAs. Wiley Interdiscip Rev RNA. 2017;8(2).

[10] Zheng J, Liu X, Xue Y, et al. TTBK2 circular RNA promotes glioma malignancy by regulating miR-217/HNF1beta/Denlin-1 pathway. J Hematol Oncol. 2017;10(1):52.

[11] Li G, Yang H, Han K, et al. A novel circular RNA, hsa_circ_0046701, promotes carcinogenesis by increasing the expression of miR-142-3p target ITGB8 in glioma. Biochm Biophys Res Commn. 2018; 498(1):254–261.

[12] Barbagallo D, Condorelli A, Ragusa M, et al. Dysregulated miR-671-5p/CDR1-AS/CDR1/ VSNL1 axis is involved in glioblastoma multiforme. Oncotarget. 2016;7(4):4746–4759.

[13] Li X, Zhang Z, Jiang H, et al. Circular RNA circPVT1 promotes proliferation and invasion through sponging miR-125b and activating E2F2 signaling in non-small cell lung cancer. Cell Physiol Biochem. 2018;51(5):2324–2340.

[14] Yachi K, Tsuda M, Kohsaka S, et al. miR-23a promotes invasion of glioblastoma via HOXD10-regulated glial-mesenchymal transition. Sig Transduct Target Ther. 2018;3(1):33.

[15] Zeng W, Zhu JF, Liu JY, et al. miR-133b inhibits cell proliferation, migration and invasion of esophageal squamous cell carcinoma by targeting EGFFR. Biomed Pharmacother. 2019;111:476–484.

[16] Qiao W, Guo B, Zhou H, et al. miR-124 suppresses glioblastoma growth and potentiates chemosensitivity by inhibiting AURKA. Biochem Biophys Res Commn. 2017;486(1):43–48.
[17] Lei D, Zhang F, Yao D, et al. MiR-338-5p suppresses proliferation, migration, invasion, and promote apoptosis of glioblastoma cells by directly targeting EFEMP1. Biomed Pharmacother. 2017;89:957–965.

[18] Liu L, Lu L, Zheng A, et al. MiR-199a-5p and let-7c cooperatively inhibit migration and invasion by targeting MAP4K3 in hepatocellular carcinoma. Oncotarget. 2017;8(8):13666–13677.

[19] Hou L, Jiang J, Liu B, et al. Smoking and adult glioma: a population-based case-control study in China. Neuro Oncol. 2016;18(1):105–113.

[20] Ludwig K, Kornblum HI. Molecular markers in glioma. J Neurooncol. 2017;134(3):505–512.

[21] Li F, Ma K, Sun M, et al. Identification of the tumor-suppressive function of circular RNA ITCH in glioma cells through sponging miR-214 and promoting linear ITCH expression. Am J Transl Res. 2018;10(5):1373–1386.

[22] Kun-Peng Z, Xiao-Long M, Chun-Lin Z. Overexpressed circPVT1, a potential new circular RNA biomarker, contributes to doxorubicin and cisplatin resistance of osteosarcoma cells by regulating ABCB1. Int J Biol Sci. 2018;14(3):321–330.

[23] He T, Li X, Xie D, et al. Overexpressed circPVT1 in oral squamous cell carcinoma promotes proliferation by serving as a miRNA sponge. Mol Med Report. 2019;20(4):3509–3518.

[24] Qin S, Zhao Y, Lim G, et al. Circular RNA PVT1 acts as a competing endogenous RNA for miR-497 in promoting non-small cell lung cancer progression. Biomed Pharmacother. 2019;111:244–250.

[25] Jiang Y, Wang D, Ren H, et al. MiR-145-targeted HBXIP modulates human breast cancer cell proliferation. Thorac Cancer. 2019;10(1):71–77.

[26] Yin Q, Han Y, Zhu D, et al. mir-145 and miR-497 suppress TGF-beta-induced epithelial-mesenchymal transition of non-small cell lung cancer by targeting MTDH. Cancer Cell Int. 2018;18(1):105.

[27] Pan Y, Ye C, Tian Q, et al. miR-145 suppresses the proliferation, invasion and migration of NSCLC cells by regulating the BAX/BCL-2 ratio and the caspase-3 cascade. Oncol Lett. 2018;15(4):4337–4343.

[28] Huang GH, Shan H, Li D, et al. MiR-199a-5p suppresses tumorigenesis by targeting clathrin heavy chain in hepatocellular carcinoma. J Cell Biochem. 2017;135(2):98–104.

[29] Zhang C, Chen Q, Zhu JW, et al. MicroRNA-199a-5p regulates glioma progression by targeting MARCH8. Eur Rev Med Pharmacol Sci. 2019;23(17):7482–7487.

[30] Zhang H, Qin D, Jiang Z, et al. SNHG9/miR-199a-5p/Wnt2 axis regulates cell growth and aerobic glycolysis in glioblastoma. J Neurooncol. 2019;78(10):939–948.

[31] Wang Z, Su M, Xiang B, et al. Circular RNA PVT1 promotes metastasis via miR-145 sponging in CRC. Biochem Biophys Res Commun. 2019;512(4):716–722.

[32] Orr BA, Bai H, Odia Y, et al. Yes-associated protein 1 is widely expressed in human brain tumors and promotes glioblastoma growth. J Neurooncol. 2011;70(7):568–577.

[33] Karar J, Maity A. PI3K/AKT/mTOR pathway in angiogenesis. Front Mol Neurosci. 2011;4:51.

[34] Prasad G, Sottero T, Yang X, et al. Inhibition of PI3K/mTOR pathways in glioblastoma and implications for combination therapy with temozolomide. Neuro-oncology. 2011;13(4):384–392.

[35] Arcaro A, Guerreiro AS. The phosphoinositide 3-kinase pathway in human cancer: genetic alterations and therapeutic implications. Curr Genomics. 2007;8(5):271–286.

[36] Keunen O, Johansson M, Oudin A, et al. Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma. Proc Natl Acad Sci USA. 2011;108(9):3749–3754.

[37] Dimitrova V, Arcaro A. Targeting the PI3K/AKT/mTOR signaling pathway in medulloblastoma. Curr Mol Med. 2015;15(1):82–93.

[38] Li X, Wu C, Chen N, et al. PI3K/Akt/mTOR signaling pathway and targeted therapy for glioblastoma. Oncotarget. 2016;7(22):33440–33450.

[39] Huang BS, Luo QZ, Han Y, et al. MiR-223/PAX6 axis regulates glioblastoma stem cell proliferation and the chemoresistance to TMZ via regulating PI3K/Akt pathway. J Cell Biochem. 2017;118(10):3452–3461.