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# Circular RNA CircMTO1 Inhibits Proliferation of Glioblastoma Cells via miR-92/WWOX Signaling Pathway

Authors' Contribution:

Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

BCDEF 1 **Xuewen Zhang\***

BCDEF 2 **Bo Zhong\***

CF 3 **Wenjie Zhang**

AG 1 **Jie Wu**

AE 1 **Yu Wang**

1 Department of Neurosurgery, Suzhou Science and Technology Town Hospital, Suzhou, Jiangsu, P.R. China

2 Department of Neurosurgery, Yidu Central Hospital of Weifang, Weifang, Shandong, P.R. China

3 Department of Neurosurgery, Yantai Affiliated Hospital of Binzhou Medical University, Yantai, Shandong, P.R. China

\* Xuewen Zhang and Bo Zhong contributed equally to this work

**Corresponding Authors:**

Jie Wu, e-mail: xiaomingdingnt@126.com, Yu Wang, e-mail: 18762087217@139.com

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## Background:

Circular RNA circMTO1 has been reported to inhibit the progression of many types of cancers. However, the role of circMTO1 in the progression of glioblastoma remains unclear. The purpose of our study was to explore the potential involvement of circMTO1 in glioblastoma.

## Material/Methods:

The expression of circMTO1 in human glioblastoma tissues was determined via quantitative real-time polymerase chain reaction (qRT-PCR). The effect of circMTO1 on proliferation of human glioblastoma cell line U251 was assessed through the Cell Counting Kit-8 (CCK-8) and colony formation assay. The regulatory interaction between circMTO1 and miR-92 was explored by bioinformatics prediction and luciferase reporter assay.

## Results:

We showed that circMTO1 was markedly downregulated in glioblastoma tissues compared with adjacent normal tissues. Lower circMTO1 level was significantly associated with shorter overall survival among patients with glioblastoma. In addition, circMTO1 inhibited proliferation of cell U251 cells. Mechanistically, circMTO1 upregulates the expression of WWOX in U251 cells, and WWOX mediates circMTO1-induced inhibition of proliferation of U251 cells. In addition, miR-92 downregulates the expression of WWOX by the targeting its mRNA 3' UTR. More importantly, circMTO1 directly interact with miR-92, and subsequently serves as a miRNA sponge to upregulate WWOX expression.

## Conclusions:

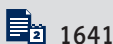
Our results demonstrate that circMTO1 inhibits the proliferation of glioblastoma cells via the miR-92/WWOX signaling pathway.

**MeSH Keywords:**

**Cell Proliferation • Glioblastoma • MicroRNAs**

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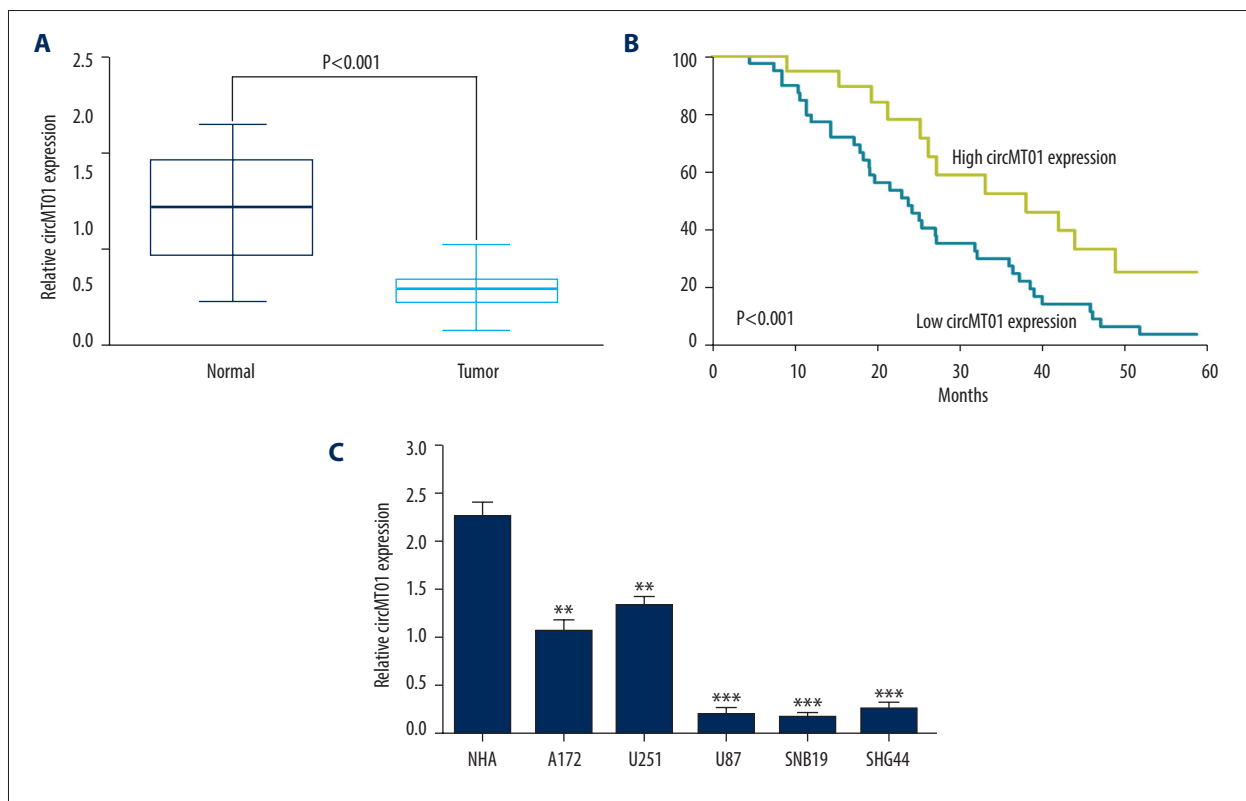
## Background

In the central nervous system, glioblastoma is the most common tumor with highly malignancy [1]. Despite significant improvements in standard treatment for glioblastoma, the overall survival of patients with glioblastoma is only 15–18 months [2,3]. Thus, it is imperative to explore the genetic regulatory networks associated with the progression and development of glioblastoma.

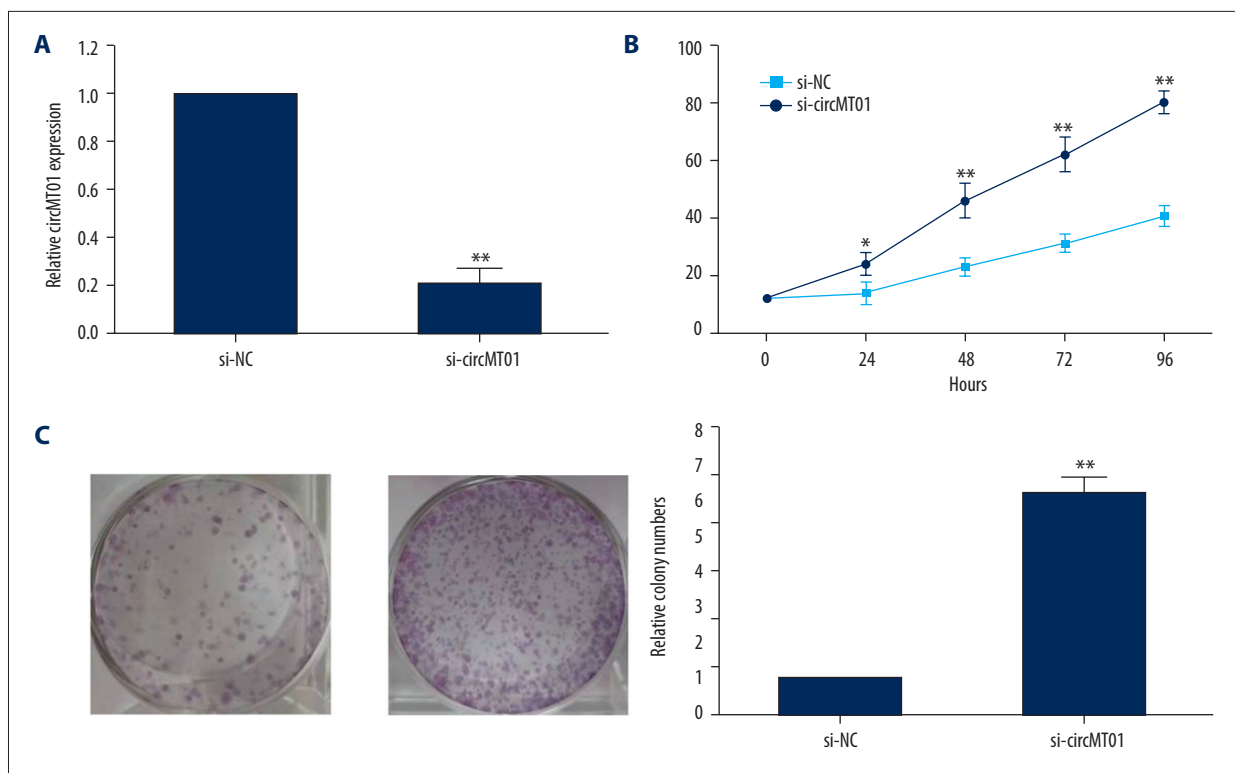
Circular RNAs (circRNAs), a new member of the endogenous non-coding RNAs family, have been reported to play important roles in the occurrence and progression of many types of cancers [4–7], such as breast cancer [8], colorectal cancer [9], ovarian cancer [10], gastric cancer [11], hepatocellular carcinoma [12], and glioblastoma [13]. Accumulating evidence indicates that circRNAs serve as a tumor suppressor or oncogenic gene and are involved in regulating cancer cell differentiation, proliferation, metastases, and chemosensitivity [14,15]. CircMTO1 (mitochondrial translation optimization 1 homologue) has been demonstrated to act as a tumor suppressor

in many human cancers [16–19]. Recently, it has been reported that circMTO1 is significantly downregulated in human hepatocellular carcinoma tissues, and inhibits the progression of hepatocellular carcinoma by acting as the sponge of oncogenic miR-9 to promote p21 expression [16]. In addition, circMTO1 suppresses the proliferation and invasion of colorectal cancer cells through regulating the Wnt/ $\beta$ -catenin signaling pathway [19]. However, the functional roles of circMTO1 in the pathogenesis of glioblastoma and the molecular mechanisms underlying these roles remain unclear.

In the present study, we first investigated the expression of circMTO1 in human glioblastoma tissues, and the effects of circMTO1 on proliferation of human glioblastoma cells were examined. Furthermore, we also explored the molecular mechanisms underlying these effects.



**Figure 1.** The expression of circMTO1 is decreased and is associated with poor prognosis of glioblastoma patients. **(A)** The expression of circMTO1 in 59 paired glioblastoma tissues and corresponding adjacent normal tissues was assessed via qRT-PCR.  $P < 0.001$ . **(B)** The relationship between circMTO1 level and glioblastoma patients' overall survival was determined by Kaplan-Meier survival analysis.  $P < 0.001$ . **(C)** The expression of circMTO1 in human glioblastoma cancer cell lines (A172, U251, U87, SNB19, and SHG44) and normal human astrocytes (NHA) cells was measured through qRT-PCR. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  vs. NHA group.



**Figure 2.** CircMTO1 inhibits glioblastoma cells proliferation. (A–C) U251 cells were transfected with si-circMTO1 or si-NC. The expression of circMTO1 was explored by qRT-PCR. CCK-8 assay and colony formation assay were performed to assess U251 cells proliferation. \*  $P < 0.05$ , \*\*  $P < 0.01$ .

## Material and Methods

### Clinical samples

Our study was approved by the Research Ethic Committee of Yidu Central Hospital of Weifang (2015-50). Glioblastoma tissues and corresponding normal tissues derived from 59 patients with glioblastoma were collected from the Department of Neurosurgery, Yidu Central Hospital of Weifang, between January 2015 and January 2018. Written informed consent was obtained from all patients.

### Cell culture

Normal human astrocytes (NHA) cells and human glioblastoma cancer cell lines (A172, U251, U87, SNB19, and SHG44) were purchased from ATCC (American Type Culture Collection). These cell lines were cultured in DMEM (Dulbecco's modified Eagle's medium) (HyClone, USA) at 37°C with 5% CO<sub>2</sub>. This medium was supplemented with 10% FBS (fetal bovine serum) (Gibco, USA).

### Quantitative real-time PCR

The total RNA was extracted by using TRIzol purchased from Invitrogen (Thermo Fisher Scientific, Inc.). According to the

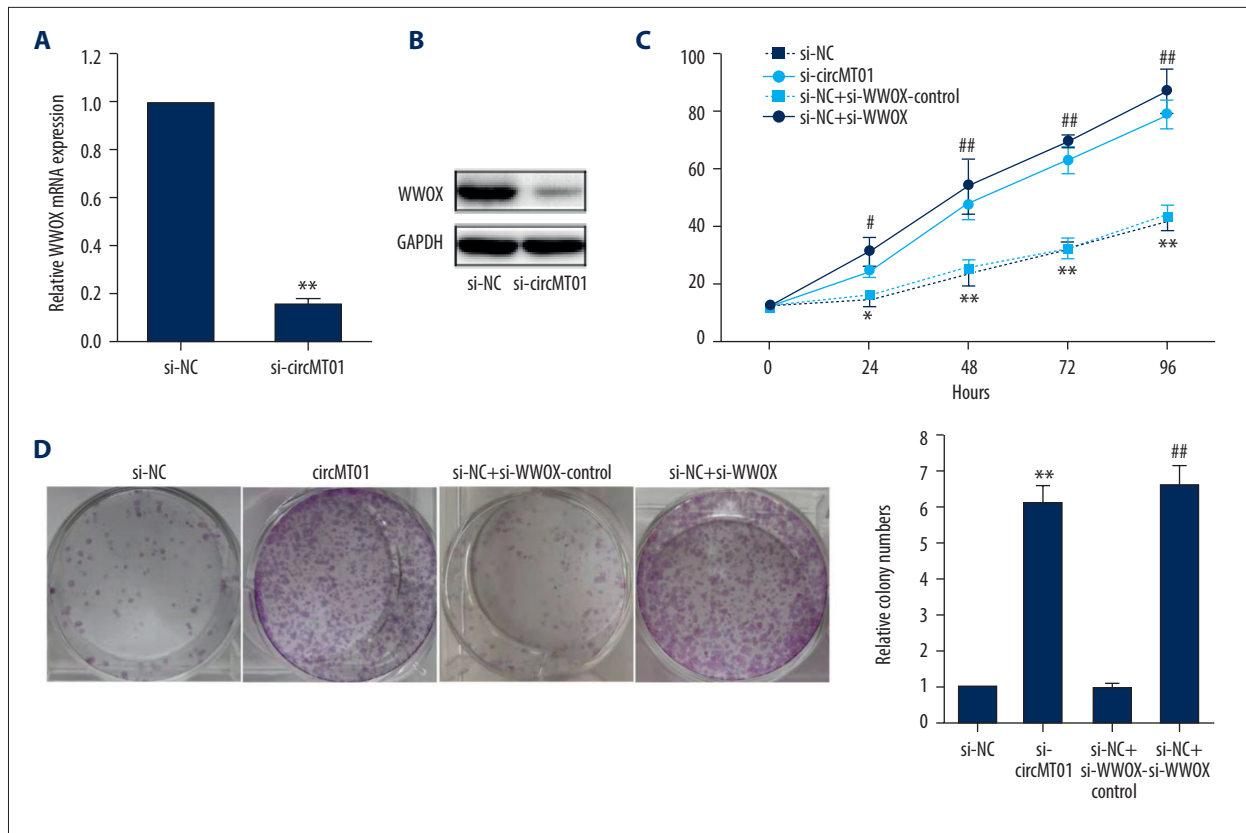
manufacturer's instructions, the extraction of RNA was performed. Primers for circMTO1, miR-92, and WWOX were obtained from Invitrogen Bioengineering Corporation (Shanghai, China). qRT-PCR was carried out as previously described [20]. The relative mRNA expression levels of genes were calculated via using the 2<sup>-ΔΔCt</sup> method.

### Western blot analysis

The protein expression of WWOX was measured via Western blot analysis, performed as previously described [21]. Antibodies against WWOX (ab189410) and GAPDH (ab181602) were obtained from Abcam (Cambridge, UK). Electrochemiluminescence material was obtained from Millipore.

### Cell proliferation assay

The effect of circMTO1, miR-92, and WWOX on glioblastoma cell proliferation were assessed via CCK-8 assay and colony formation assay. Cell Counting Kit-8 was obtained from Dojindo. CCK-8 and colony formation assays were carried out as previously described [22].



**Figure 3.** WWOX is involved in glioblastoma cells proliferation inhibited by circMTO1. (**A, B**) The protein and mRNA expression of WWOX among U251 cells transfected with si-circMTO1 or si-NC were examined via Western blot and qRT-PCR. \*\*  $P < 0.01$ . (**C, D**) U251 cells were transfected with si-circMTO1 or si-NC and si-WWOX or si-WWOX-control. CCK-8 assay and colony formation assay were performed. \*  $P < 0.05$ , \*\*  $P < 0.01$  vs. si-NC group. #  $P < 0.05$ , ##  $P < 0.01$  vs. si-NC + si-WWOX-control group.

### Dual-luciferase reporter assay

The regulatory interaction between circMTO1 and miR-92 was investigated via dual-luciferase reporter assay. The vectors of circMTO1-WT (wild-type), circMTO1-MT (mutant-type), WWOX-WT, and WWOX-MT were constructed. Then, these vectors were transfected into U251 cells with miR-92-mimics or NC-mimics. Luciferase reporter assays were performed as previously described [23].

### Statistical analysis

All data are presented as mean  $\pm$  standard deviation (SD). The overall survival of patients with glioblastoma was assessed with Kaplan-Meier method. The difference between the 2 groups was determined via the *t* test.  $P < 0.05$  was considered statistically significant.

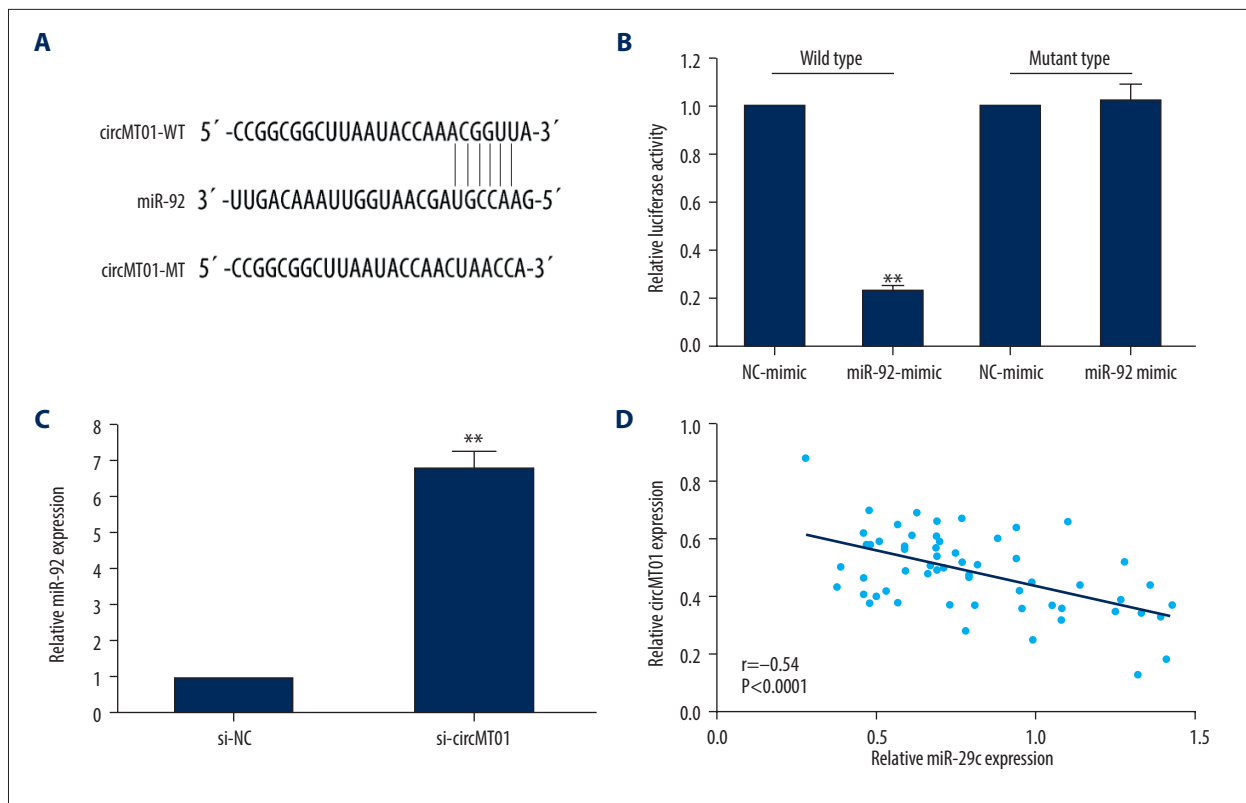
## Results

### The expression of circMTO1 is decreased and is associated with poor prognosis of glioblastoma patients

To assess the expression of circMTO1 in glioblastoma, we detected its expression in 59 paired glioblastoma tissues and corresponding adjacent normal tissues via qRT-PCR. As shown in Figure 1A, compared with normal tissues, the expression of circMTO1 was decreased in glioblastoma tissues. In addition, the overall survival of patients with lower expression of circMTO1 was decreased (Figure 1B). Meanwhile, the expression of circMTO1 was downregulated in human glioblastoma cancer cell lines (A172, U251, U87, SNB19, and SHG44) relative to normal human astrocytes (NHA) (Figure 1C). These findings indicate that the low expression of circMTO1 is associated with poor prognosis of glioblastoma patients.

### CircMTO1 inhibits glioblastoma cells proliferation

To investigate the effect of circMTO1 in glioblastoma cell proliferation, the CCK-8 assay and colony formation assay were



**Figure 4.** CircMTO1 sponges miR-92 in glioblastoma cells. (A) The potential binding site of miR-92 in circMTO1. (B) Luciferase reporter assays were carried out among U251 cells transfected with miR-92-mimic or NC-mimic. \*\*  $P < 0.01$  vs. NC-mimic group. (C) The expression of miR-92 in U251 cells transfected with si-circMTO1 or si-NC was detected by qRT-PCR. \*\*  $P < 0.01$ . (D) The relationship between circMTO1 and miR-92 in 59 glioblastoma tissues was determined by Pearson correlation analysis.  $P < 0.0001$ .

performed in U251 cells transfected with si-circMTO1 or si-NC. QRT-PCR indicated that the expression of circMTO1 in U251 cells transfected with si-circMTO1 was obviously reduced compared with the si-NC group (Figure 2A). The results of CCK-8 assay showed that silencing of circMTO1 significantly promoted U251 cell proliferation (Figure 2B), and a similar result was provided by colony formation assay (Figure 2C). These data show that circMTO1 suppresses glioblastoma cell proliferation.

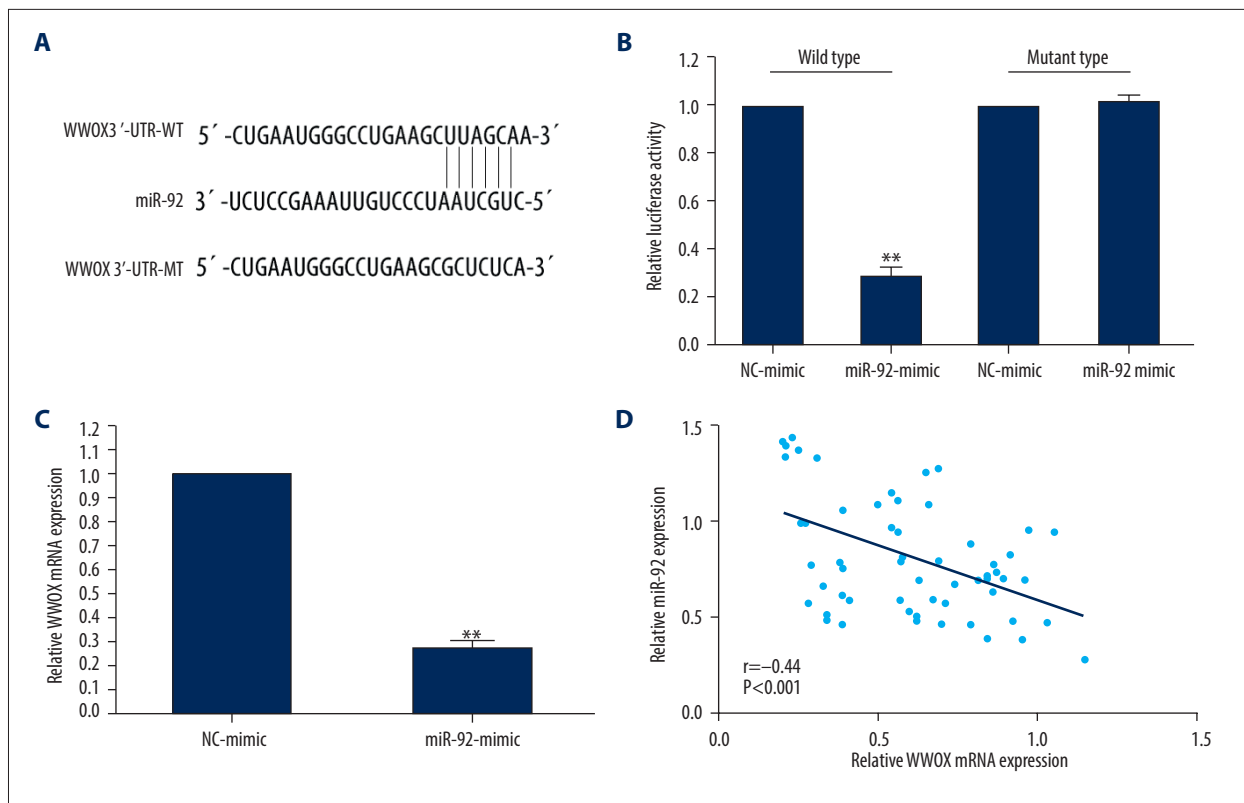
#### WWOX is involved in glioblastoma cells proliferation inhibited by circMTO1

WWOX, a tumor suppressor gene, inhibits the proliferation of various cancer cells [24–26], including glioblastoma cells [27]. To investigate the effect of circMTO1 on the expression of WWOX, its expression in U251 cells transfected with si-circMTO1 or si-NC was determined by qRT-PCR and Western blot. The results showed that silencing of circMTO1 significantly downregulated the mRNA and protein expression of WWOX (Figure 3A, 3B). To further explore the role of WWOX in glioblastoma cell proliferation inhibited by circMTO1, the CCK-8 assay and colony formation assay were performed in U251

cells transfected with si-WWOX or si-WWOX-control. We found that silenced WWOX blocked the inhibitory effect of circMTO1 on proliferation of U251 cells (Figure 3C, 3D). These observations indicate that WWOX is involved in glioblastoma cell proliferation inhibited by circMTO1.

#### CircMTO1 sponges miR-92 in glioblastoma cells

CircRNAs play important roles in the development and progression of cancer by acting as a competing endogenous RNA (ceRNA) to interact with miRNAs [28]. To further explore the molecular mechanisms of circMTO1-induced WWOX expression, the potential circRNA-miRNA interactions were predicted via StarBase prediction software. As shown in Figure 4A, miR-92 was found to have a potential binding site with circMTO1. The dual-luciferase reporter assays verified these findings (Figure 4B). Moreover, ablation of circMTO1 led to a significant increase in miR-92 expression in U251 cells (Figure 4C). Correlation analysis showed a negative relationship between circMTO1 and miR-92 in glioblastoma tissues (Figure 4D). These results suggest that circMTO1 sponges miR-92 in glioblastoma cells.



**Figure 5.** WWOX is a target of miR-92. **(A)** The predicted binding site of miR-92 in the 3'-UTR of WWOX. **(B)** Luciferase reporter assays were performed among U251 cells transfected with miR-92-mimic or NC-mimic. \*\*  $P < 0.01$  vs. NC-mimic group. **(C)** The mRNA expression of WWOX in U251 cells transfected with miR-92-mimic or NC-mimic was assessed by qRT-PCR. \*\*  $P < 0.01$ . **(D)** The relationship between WWOX and miR-92 in 59 glioblastoma tissues was measured via Pearson correlation analysis.  $P < 0.001$ .

### WWOX is a target of miR-92

To further investigate the role of miR-92 in the expression of WWOX induced by circMTO1, the potential miR-92 binding sites in the 3'-UTR of WWOX were predicted through StarBase prediction software. The results indicated that miR-92 can bind to WWOX mRNA (Figure 5A) and a binding interaction between them was shown by luciferase reporter assays (Figure 5B). Additionally, miR-92 remarkably downregulated the expression of WWOX in U251 cells (Figure 5C), which is consistent with the inverse correlation of miR-92 and WWOX in glioblastoma tissues (Figure 5D). In conclusion, these data demonstrate that WWOX is a target of miR-92 in glioblastoma cells.

### Discussion

Accumulating evidence demonstrates that circRNAs play important roles in the occurrence and progression of many types of cancers [4–7]. Glioblastoma is the most common primary cancer derived from the central nervous system [29]. However, little is known about the functional roles of circMTO1 in the pathogenesis of glioblastoma and the molecular mechanisms

underlying these roles. In this study, we found that circMTO1 was markedly downregulated in glioblastoma tissues. The lower expression of circMTO1 was significantly associated with shorter overall survival of patients with glioblastoma. Moreover, circMTO1 suppressed glioblastoma cell proliferation by regulating the expression of WWOX. More importantly, circMTO1 can directly interact with miR-92, and subsequently serve as a miRNA sponge to upregulate WWOX expression.

CircMTO1 been shown to act as tumor suppressor in many human cancers [16–19]. Recently, it has been reported that circMTO1 is significantly downregulated in human hepatocellular carcinoma tissues and inhibits the progression of hepatocellular carcinoma [16]. Additionally, circMTO1 can suppresses the proliferation and invasion of colorectal cancer cells through regulating the Wnt/ $\beta$ -catenin signaling pathway [19]. However, the functional roles of circMTO1 in the pathogenesis of glioblastoma remain unknown. Similar to previous findings, we showed that circMTO1 also served as a tumor suppressor in glioblastoma. Our results indicate that the lower expression of circMTO1 was significantly associated with shorter overall survival of patients with glioblastoma. In addition, circMTO1 suppressed glioblastoma cell proliferation. However, a recent

study confirmed that circMTO1 can also act as a cancer-promoting gene and accelerate tumorigenesis and chemoresistance of cervical cancer through regulating miR-6893 [30].

The mechanisms for the functional roles circRNAs in the occurrence and development of cancer are not completely clear. However, emerging evidence confirms that circRNAs can regulate the expression of tumor-suppressive or oncogenic genes through serving as miRNA sponges and forming the circRNA-miRNA-mRNA axis [31–34]. It has been reported that circMTO1 inhibits the growth of hepatocellular carcinoma by acting as the sponge of oncogenic miR-9 to promote p21 expression [16]. Furthermore, circMTO1 inhibits bladder cancer metastasis by suppressing epithelial-to-mesenchymal transition through sponging miR-221 [18]. WWOX, a tumor-suppressor gene, has inhibitory effects on proliferation of various cancer cells [24–26], including glioblastoma cells [27]. In the present study, we demonstrated that circMTO1 directly interacts with miR-92 and subsequently serve as a miRNA sponge to upregulate WWOX expression.

## References:

1. Van Meir EG, Hadjipanayis CG, Norden AD et al: Exciting new advances in neuro-oncology: The avenue to a cure for malignant glioma. *Cancer J Clin*, 2010; 60: 166–93
2. Stupp R, Hegi ME, Mason WP et al: Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*, 2009; 10: 459–66
3. Bai RY, Staedtke V, Riggins GJ: Molecular targeting of glioblastoma: Drug discovery and therapies. *Trends Mol Med*, 2011; 17: 301–12
4. Vo JN, Cieslik M, Zhang Y et al: The landscape of circular RNA in cancer. *Cell*, 2019; 176: 869–81.e13
5. Su M, Xiao Y, Ma J et al: Circular RNAs in cancer: Emerging functions in hallmarks, stemness, resistance and roles as potential biomarkers. *Mol Cancer*, 2019; 18: 90
6. Shang Q, Yang Z, Jia R, Ge S: The novel roles of circRNAs in human cancer. *Mol Cancer*, 2019; 18: 6
7. Han B, Chao J, Yao H: Circular RNA and its mechanisms in disease: From the bench to the clinic. *Pharmacol Ther*, 2018; 187: 31–44
8. Chen N, Zhao G, Yan X et al: A novel FLI1 exonic circular RNA promotes metastasis in breast cancer by coordinately regulating TET1 and DNMT1. *Genome Biol*, 2018; 19: 218
9. Zeng K, Chen X, Xu M et al: CircHIPK3 promotes colorectal cancer growth and metastasis by sponging miR-7. *Cell Death Dis*, 2018; 9: 417
10. Chen Q, Zhang J, He Y, Wang Y: hsa\_circ\_0061140 knockdown reverses FOXM1-mediated cell growth and metastasis in ovarian cancer through miR-370 sponge activity. *Mol Ther Nucleic Acids*, 2018; 13: 55–63
11. Liu H, Liu Y, Bian Z et al: Circular RNA YAP1 inhibits the proliferation and invasion of gastric cancer cells by regulating the miR-367-5p/p27 (Kip1) axis. *Mol Cancer*, 2018; 17: 151
12. Li Q, Pan X, Zhu D et al: Circular RNA MAT2B promotes glycolysis and malignancy of hepatocellular carcinoma through the miR-338-3p/PKM2 axis under hypoxic stress. *Hepatology*, 2019 [Epub ahead of print]
13. Zhang M, Zhao K, Xu X et al: A peptide encoded by circular form of LINC-PINT suppresses oncogenic transcriptional elongation in glioblastoma. *Nat Commun*, 2018; 9: 4475
14. Meng S, Zhou H, Feng Z et al: CircRNA: functions and properties of a novel potential biomarker for cancer. *Mol Cancer*, 2017; 16: 94
15. Cui X, Wang J, Guo Z et al: Emerging function and potential diagnostic value of circular RNAs in cancer. *Mol Cancer*, 2018; 17: 123
16. Han D, Li J, Wang H et al: Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression. *Hepatology*, 2017; 66: 1151–64
17. Rao J, Cheng X, Zhu H et al: Circular RNA-0007874 (circMTO1) reverses chemoresistance to temozolomide by acting as a sponge of microRNA-630 in glioblastoma. *Cell Biol Int*, 2018 [Epub ahead of print]
18. Li Y, Wan B, Liu L, Zhou L, Zeng Q: Circular RNA circMTO1 suppresses bladder cancer metastasis by sponging miR-221 and inhibiting epithelial-to-mesenchymal transition. *Biochem Biophys Res Commun*, 2019; 508: 991–96
19. Ge Z, Li LF, Wang CY, Wang Y, Ma WL: CircMTO1 inhibits cell proliferation and invasion by regulating Wnt/beta-catenin signaling pathway in colorectal cancer. *Eur Rev Med Pharmacol Sci*, 2018; 22: 8203–9
20. Wang R, Zhang S, Chen X et al: EIF4A3-induced circular RNA MMP9 (circMMP9) acts as a sponge of miR-124 and promotes glioblastoma multiforme cell tumorigenesis. *Mol Cancer*, 2018; 17: 166
21. Tan Y, Zhang T, Zhou L et al: MiR-34b-3p represses the multidrug-chemoresistance of bladder cancer cells by regulating the CCND2 and P2RY1 genes. *Med Sci Monit*, 2019; 25: 1323–35
22. Hou PF, Jiang T, Chen F et al: KIF4A facilitates cell proliferation via induction of p21-mediated cell cycle progression and promotes metastasis in colorectal cancer. *Cell Death Dis*, 2018; 9: 477
23. Wang Y, Du J, Niu X et al: MiR-130a-3p attenuates activation and induces apoptosis of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis by directly targeting TGFB1 and TGFB2. *Cell Death Dis*, 2017; 8: e2792
24. Abu-Remaileh M, Khalailah A, Pikarsky E, Aqeilan RI: WWOX controls hepatic HIF1alpha to suppress hepatocyte proliferation and neoplasia. *Cell Death Dis*, 2018; 9: 511
25. Gourley C, Paige AJ, Taylor KJ et al: WWOX gene expression abolishes ovarian cancer tumorigenicity *in vivo* and decreases attachment to fibronectin via integrin alpha3. *Cancer Res*, 2009; 69: 4835–42
26. Kurek KC, Del Mare S, Salah Z et al: Frequent attenuation of the WWOX tumor suppressor in osteosarcoma is associated with increased tumorigenicity and aberrant RUNX2 expression. *Cancer Res*, 2010; 70: 5577–86
27. Chiang MF, Chou PY, Wang WJ et al: Tumor suppressor WWOX and p53 alterations and drug resistance in glioblastomas. *Front Oncol*, 2013; 3: 43
28. Zhong Y, Du Y, Yang X et al: Circular RNAs function as ceRNAs to regulate and control human cancer progression. *Mol Cancer*, 2018; 17: 79
29. Lim M, Xia Y, Bettgowda C, Weller M: Current state of immunotherapy for glioblastoma. *Nat Rev Clin Oncol*, 2018; 15: 422–42

## Conclusions

We identified for the first time that circMTO1 is significantly downregulated in glioblastoma tissues and is associated with poor prognosis of patients. In addition, we also demonstrate that circMTO1 can inhibit the proliferation of glioblastoma cells by serving as a miR-92 sponge to induce WWOX expression. The present data verified that the circMTO1-miR-92-WWOX axis may be a therapeutic target for the management of glioblastoma.

## Conflict of interest

None.

30. Chen M, Ai G, Zhou J et al: circMTO1 promotes tumorigenesis and chemoresistance of cervical cancer via regulating miR-6893. *Biomed Pharmacother*, 2019; 117: 109064
31. Memczak S, Jens M, Elefsinioti A et al: Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*, 2013; 495: 333–38
32. Du WW, Yang W, Liu E et al: Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res*, 2016; 44: 2846–58
33. Hansen TB, Jensen TI, Clausen BH et al: Natural RNA circles function as efficient microRNA sponges. *Nature*, 2013; 495: 384–88
34. Bian A, Wang Y, Liu J et al: Circular RNA complement factor H (CFH) promotes glioma progression by sponging miR-149 and regulating AKT1. *Med Sci Monit*, 2018; 24: 5704–12