MicroRNA-448 suppresses the proliferation, migration, and invasion of glioma cell line U251 by targeting B-cell lymphoma-2

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To the Editor: Glioma is one of the most common malignant tumors in the central nervous system, which is highly aggressive and has a median survival of less than 2 years after diagnosis.[1] As an important regulator in apoptosis signaling, B-cell lymphoma-2 (BCL-2) is an adverse prognostic factor of glioma.[2] Overexpression of BCL-2 blocks cell apoptosis, which has been identified in various types of cancer.[3] Researchers have uncovered a complex web that regulates apoptosis. Inhibitors of BCL-2 are showing great promise in clinical trials. In addition, identification of the upstream signaling mechanisms that control the expression and function of BCL-2 also provides further drug targets.[4] MicroRNA (miRNA) is a kind of non-coding RNA consisting of 18 to 25 nucleotides, which can regulate gene expression through binding to the 3′-untranslated region (UTR) of the target genes. The miRNA/mRNA base-pairing leads to the translational repression and/or direct cleavage of the target mRNA.[5] miRNAs are excellent biomarker candidates and potential therapeutic tools.[6]

In this study, glioma tissues and peritumoral tissues were collected in the Third Affiliated Hospital of Soochow University from 2010 to 2015. Peritumoral tissues were obtained from patients who received glioma resection. The present study was approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University. Informed consent was obtained from all the patients.

We used miRDB, TargetScan, miRNA, and Draw Venn Diagram to predict the miRNAs that target BCL-2. Only miR-448 and miR-204-5p were predicted to target BCL-2 in the three databases. It has been reported that miR-204-5p inhibits the progression of glioma by targeting BCL-2.[7] Recently, Hu *et al*[8] have found that miR-448 down-regulates the cancer gene *CTTN* to inhibit cell proliferation and promote apoptosis in glioma cell lines. However, the expression of miR-448 in glioma tissue and whether it can target BCL-2 in glioma remains to be elucidated.

We measured the expression of BCL-2 and miR-448 in 20 glioma tissues and 20 peritumoral tissues, as well as U251 and U373 MG Uppsala glioma cell lines and normal human astrocyte cell line (HEB) by the quantitative real-time polymerase chain reaction. Besides, BCL-2 expression was upregulated [Figure 1A, and 1B], whereas miR-448 was down-regulated in both the glioma tissues and the cell lines in comparison with normal tissues [Figure 1C]. Furthermore, there existed a negative correlation between miR-448 and BCL-2 expression in the glioma tissues [Figure 1D].

We propose that the down-regulation of miR-488 contributes to the overexpression of BCL-2. To verify our hypothesis, 3′-UTR of BCL-2 containing two putative miR-448 binding sites and mutant sites were cloned into the psi-check2 vector [Figure 1E]. BCL-2 coding sequences without the 3′-UTR were cloned into the pcDNA 3.1 vector. Then, psi-check2-BCL2 and mutant vector and miR-448 mimic or negative control were transfected into Hela cells. Using a dual-luciferase reporter analysis system, luciferase activities were analyzed. The relative luciferase activity was normalized to renilla luciferase activity. The results confirm that miR-448 can directly target BCL-2 in glioma cells [Figure 1F]. Furthermore, we transfected glioma cell line U251 with miR-448 mimic, or together with pcDNA3.1-BCL-2, cell proliferation, colony formation, and invasion ability were evaluated.

Our results show that miR-448 was down-regulated in glioma tissues and glioma cell lines. Moreover, the expression level of miR-448 was negatively associated with the expression of BCL-2. The BCL-2 expression was down-regulated in U251 cells after transfection with miR-448 mimic. The proliferation, colony formation, and

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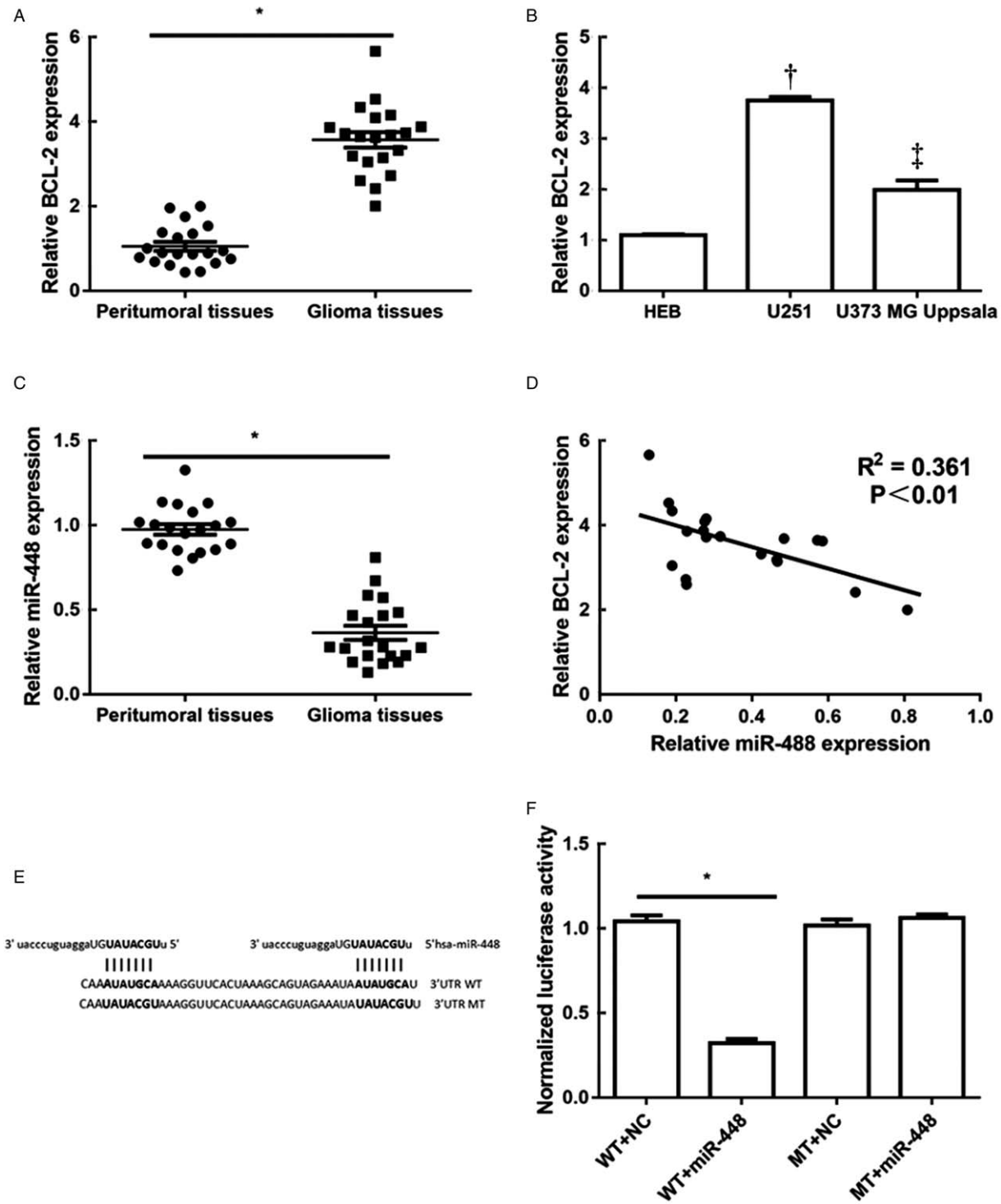

Figure 1: Quantitative real-time PCR was used to detect the expression of BCL-2 (A) and miR-448 (C) in 20 glioma tissues and 20 peritumoral brain tissues. (B) Quantitative real-time PCR was employed to detect the expression of BCL-2 in two glioma cell lines and peritumoral tissue. (D) There existed a negative relationship between the expression level of miR-448 and BCL-2 in the glioma tissues. (E) The putative binding sites between miR-448 and BCL-2 were predicted based on the TargetScan database. (F) After transfection, the relative luciferase activities of BCL-2 3′-UTR (wild type and mutant type) reporter in Hela cells were measured using the Dual-Luciferase Reporter System. $^{*}P < 0.01$. $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.05$, compared with normal human astrocyte cell line (HEB). BCL-2: B-cell lymphoma-2; MT: Mutant type; NC: Negative control; PCR: Polymerase chain reaction; WT: Wild type.

invasion were suppressed in U251 cells after miR-448 mimic treatment. Over-expressing of BCL-2 rescued miR-448-mediated suppression on U251 cell proliferation, colony formation, and invasion.

This study reported that miR-448 can directly target BCL-2. Besides, miR-448 is down-regulated in glioma tissues. miR-448 served as a tumor suppressor miRNA by inhibiting BCL-2 expression, which may be employed as

an effective biomarker and therapeutic strategy for glioma patients in the future.

Conflicts of interest

None.

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