Neuroscience Letters 590 (2015) 40–46

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

# miR-29s inhibit the malignant behavior of U87MG glioblastoma cell line by targeting DNMT3A and 3B

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## HIGHLIGHTS

- DNMT3A and 3B are targets of miR-29s in U87MG glioblastoma cell line.
- miR-29s suppress DNMT3A/3B expression primarily by degrading their mRNAs.
- miR-29s block proliferation, migration/invasion but induce apoptosis in U87MG.
- DNMT3A/3B siRNA mimics miR-29s' effects while exogenous DNMT3A/3B reverse them.
- miR-29s exert the above anti-glioblastoma effects by targeting DNMT3A/3B.

## ARTICLE INFO

*Article history:*
Received 4 November 2014
Received in revised form 6 January 2015
Accepted 23 January 2015
Available online 24 January 2015

*Keywords:*
Glioblastoma
miR-29 family
DNA methyltransferase
Proliferation
Invasion
Apoptosis

## ABSTRACT

miR-29s (including miR-29a–c) have been confirmed to be effective tumor suppressors for a variety of malignant tumors including glioblastoma. Promoter hypermethylation resulting from DNMT3A and 3B overexpression is an important epigenetic mechanism for tumor suppressive gene silencing. Bioinformatics predicts both DNMT3A and 3B are targets of miR-29s, but the anti-glioblastoma effects of miR-29s induced DNMT3A/3B downregulation deserve further investigation. We herein demonstrated that miR-29s effectively blocked DNMT3A and 3B expression by degrading their mRNAs in U87MG glioblastoma cell line. Exogenous miR-29s substantially inhibited the proliferation, migration and invasion of U87MG cells, and promoted their apoptosis. These effects could be perfectly mimicked by a small interfering RNA against DNMT3A and 3B, and partially compromised by DNMT3A/3B expression plasmids co-transfection, suggesting that miR-29s exerted the above tumor suppressive effects at least partly by silencing DNMT3A/3B. These findings provide a rationale for miR-29s based therapeutic strategies against glioblastoma.



## 1. Introduction

Glioblastoma multiforme (GBM) is the most frequent primary malignant brain tumor with a median survival time of only 15 months. Despite the great advances in neurosurgery and radiochemotherapy, the prognosis of GBM patients remains poor, mostly due to the vigorous proliferation, high aggressiveness and apoptotic resistance of the tumor cells [1].

The mature members of human miR-29 family, including miR-29a–c, are expressed at aberrantly low levels in a variety of tumors including GBM [2–8]. Increasing evidence associates low miR-29s expression with tumorigenesis and progression, indicating the tumor suppressive nature of these miRNAs.

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http://dx.doi.org/10.1016/j.neulet.2015.01.060
0304-3940/

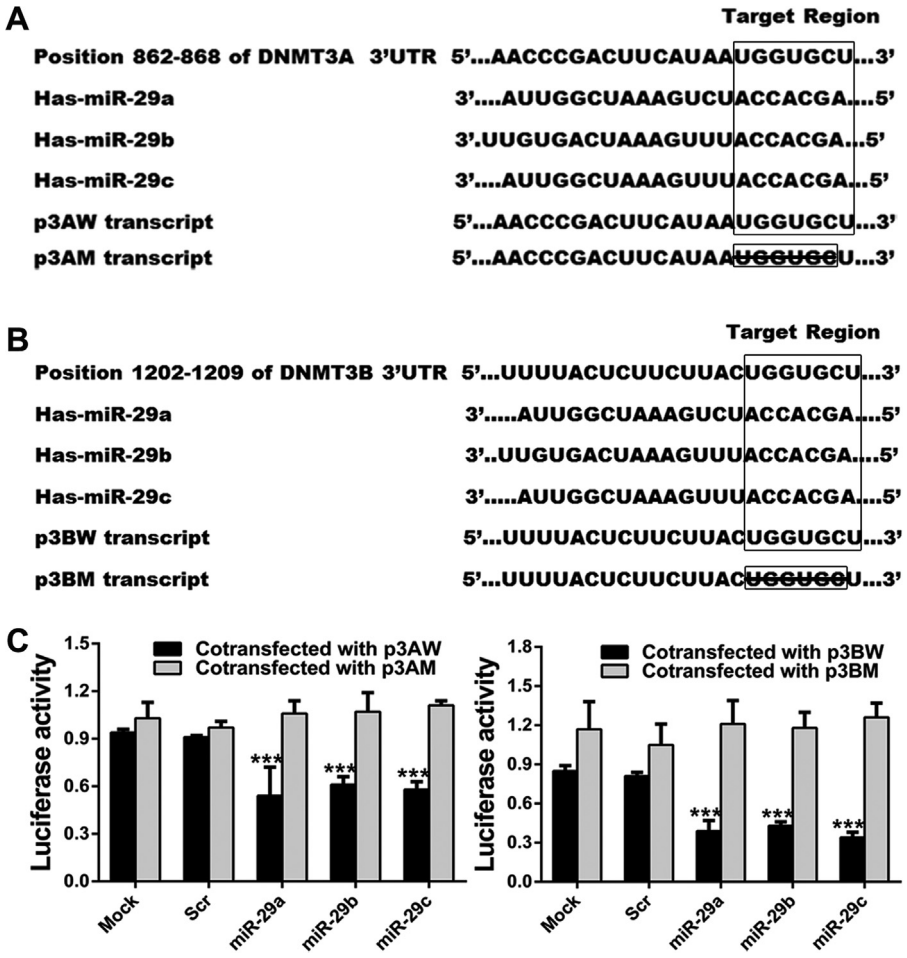


**Fig. 1.** DNMT3A and 3B are natural targets of miR-29a/b/c. (A and B) The target regions of miR-29s on the 3'UTRs of DNMT3A and DNMT3B. The target regions also exist in the recombinant luciferase mRNA transcribed from the wild-type plasmids p3AW and p3BW, but the first 6 nucleotides of these regions are deleted in the transcripts of the mutant plasmids p3AM and p3BM. (C) Dual luciferase assay results. U87MG cells were co-transfected with the recombinant luciferase plasmids indiated by the color of the column and the dsRNA oligonucleotides indicated by the labels of the horizontal axis (for the mock groups, cells were transfected with the recombinant luciferase plasmids only). The firefly luciferase activities were normalized by the renilla luciferase activities. ***: compared with the corresponding Mock groups, $P < 0.001$.

DNA methylation is an important modulating mechanism for gene expression. Aberrant DNA methylation induced by DNA methyltransferases (DNMTs) overexpression participates in oncogenesis directly. Compared with normal cells, tumor cells are characterized by global hypomethylation of genomic DNA and specific hypermethylation of tumor suppressor gene promoters [9]. DNMTs overexpression has been found in gliomas and GBM cell lines [10], and it is responsible for excessive methylation of MGMT, PTEN, RASSF1A and p16 promoters [11–13]. Since DNA methylation is reversible, DNMTs have become new targets for cancer therapy.

Recent studies suggest that DNMT3A and 3B are direct targets of miR-29s in several extracranial malignant tumors, but their actual relationship in GBM is still unknown. In the present study, we demonstrated that DNMT3A/3B were targets of miR-29s, miR-29s suppressed the proliferation, migration and invasion of U87MG cells and induce their apoptosis partly by blocking DNMT3A/3B expression.

## 2. Materials and methods

### 2.1. Oligonucleotides and plasmids

Mimics for miR-29a–c, and a scrambled control oligonucleotide (Scr) were purchased from GenePharma (Shanghai, China). A small interfering RNA complementary to the common sequence of DNMT 3A and 3B mRNAs (siDNMT3AB) was also synthesized by GenePharma. The eukaryotic expression plasmids for DNMT3A and 3B (pCDNA-DNMT3A and 3B) with the corresponding open reading frames inserted into pCDNA3.0 were purchased from GenScript (Nanjing, China).

### 2.2. Cell culture and transfection

U87MG GBM cell line purchased from the American Type Culture Collection (ATCC) was maintained in DMEM (Gibco, USA) with 10% fetal bovine serum (Gibco) at 37 °C and 5% $CO_2$. Cells ($5 \times 10^4$) were plated in 6-well plates and transfected with 100 pmol miR-29a–c mimics, siDNMT3AB and Scr respectively, using Lipofectamine 2000 (Invitrogen, USA). A parallel control (Mock) was established by treating the cells with the transfection reagent only. Cells were harvested or collected 48 h after transfection.

### 2.3. miR-29s target prediction

Candidate targets of miR-29a/b/c were predicted by miRBase (http://www.mirbase.org/) and TargetScan (http://www.Targetscan.org/).

### 2.4. Dual-luciferase assay

For wild type plasmids p3AW and p3BW, fragments of DNMT3A (nucleotide 782–948) and 3B 3'UTR (nucleotide 1122–1282)