

Knockdown of long noncoding RNA AB073614 inhibits glioma cell proliferation and migration via affecting epithelial-mesenchymal transition

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Abstract. – OBJECTIVE: This study was aimed to explore the role of long noncoding RNA (lncRNA) AB073614 and its potential mechanisms in the glioma development.

MATERIALS AND METHODS: Expression levels of lncRNA AB073614 in glioma tissues and glioma cell lines were evaluated by quantitative real-time PCR (qRT-PCR). MTT assays were conducted to explore the impact of AB073614 down-regulation on the proliferation of human glioma cells. The effects of AB073614 knockdown on cell proliferation and migration were evaluated by *in vitro* assays. EMT-related molecular markers expression level was detected by qRT-PCR assay and Western blot analysis.

RESULTS: We confirmed that AB073614 was significantly upregulated in glioma tissues and cell lines ($p < 0.01$). *In vitro* studies demonstrated that knockdown of AB073614 inhibits U251 cell proliferation and migration. Moreover, knockdown of AB073614 could inhibit epithelial-mesenchymal transition (EMT) phenotype in glioma cells.

CONCLUSIONS: AB073614 functions as a tumor promoter in glioma. This finding may provide a therapeutic approach for future treatment of glioma.

Key Words:

Long noncoding RNA, AB073614, Glioma, Proliferation, Epithelial-mesenchymal transition.

the critical carcinogenic biomarkers and new and effective therapeutic strategies for glioma.

Long noncoding RNAs (lncRNAs) are a subset of noncoding RNAs >200 nucleotides that do not encode proteins and reside in the nucleus or cytoplasm⁴. They widely participate in the regulation of gene expression⁵. Accumulation data suggests that lncRNAs may play important roles in cellular development, differentiation, and many other biological processes^{6,7}. Moreover, emerging studies have revealed that some differentially expressed lncRNAs are important regulatory molecules in tumorigenesis and development^{8,9}. However, we are only beginning to understand the nature and extent of the involvement of lncRNAs on tumorigenesis.

lncRNA AB073614 was a newly found lncRNA. Hu et al¹⁰ found that lncRNA AB073614 expression was significantly up-regulated in glioma tissues compared with normal brain tissues, suggesting that AB073614 may function as a tumor promoter. In the present study, we focused on AB073614. To investigate the function of AB073614 in glioma, we explored the AB073614 expression level in glioma tissues and cells via quantitative RT-PCR. Moreover, the role of AB073614 in cellular proliferation and migration in glioma progression was also investigated using *in vitro* assays.

Introduction

Gliomas are the most common malignant primary brain tumors and are characterized by increased proliferation, robust angiogenesis, and invasion into the surrounding normal brain tissue^{1,2}. Even if the surgery combined with radiation and chemotherapy, the average survival period can be only 14 months³. Therefore, it is important to find

Patients and Methods

Patients and Tissue Samples

This study was approved by the Research Ethics Committee of Linyi People's Hospital. Written informed consent was obtained from all of the patients before his/her surgery. All specimens were

handled and made anonymous according to the ethical and legal standards. 85 glioma tissue samples for qRT-PCR were obtained from patients who underwent surgery between February 2010 and December 2013 in Department of Neurosurgery, Linyi People's Hospital. None of the patients received radiotherapy or chemotherapy before the surgery. Normal brain tissue (NBT) samples were taken from 10 patients who underwent decompressive craniectomy for severe traumatic brain injury. All samples were immediately frozen in liquid nitrogen and stored at -80°C until use.

Cell Culture

Glioma cell lines U251 were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS). Cells were maintained in a humidified atmosphere at 37°C with 5% CO_2 .

RNA Extraction and qRT-PCR Analyses

Total RNA from tissues and cells was extracted using Trizol reagent (Invitrogen Inc., Waltham, MA, USA). RNA was reverse transcribed using SuperScript First Strand cDNA System (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed using the SYBR[®] Green real-time PCR kit (Toyobo, Co., Ltd., Osaka, Japan) in a 20- μl reaction volume. The primer sequences were as follows. The primer sequences were as follows. The primers for AB073614 were 5'-TCTGCTCCTGGGTCTTACAC-3' and 5'-TGCAACCACATGTAACCACA-3'; the primers for GAPDH were 5'-CCCATCACCATCTTCCAGGAG-3' and 5'-GTTGTCATGGATGACCTTGGC-3'. Real-time PCR was performed in triplicate, and the relative expression of AB073614 was calculated using the comparative cycle threshold (CT) ($2^{-\Delta\Delta\text{CT}}$) method with GAPDH as the endogenous control to normalize the data.

Western Blot

Cells were lysed using mammalian protein extraction reagent RIPA (Beyotime, Pudong, Shanghai, China) supplemented with protease inhibitors cocktail (Pierce, Rockford, IL, USA) and PMSF (Pierce, Rockford, IL, USA). Equal amounts (30–50 μg) of the protein were electrophoresed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to NC membranes and incubated with the following primary antibodies: anti- β -actin antibody, anti-E-cadherin, anti-N-cadherin, anti-Vimentin. The lysates were then collected and subjected to ultrasonication and centrifugation at 12000 rpm for 10

min. The supernatants were collected, and protein content was determined by Bradford assay.

siRNA to Knockdown AB073614 in Glioma Cells and Transfection

siRNA targeting human AB073614 and one negative control siRNA were designed and synthesized by Genepharma (Genepharma, Pudong, Shanghai, China). The siRNAs (si-AB073614 and si-NC) were respectively transfected into U251, according to the manufacturer's instructions.

Cell Proliferation Assay

Cell proliferation was assayed using MTT (Beyotime Biotechnology, Pudong, Shanghai, China, China) according to the manufacturer's instruction. The infected cells were seeded at a density of 1×10^4 cells/well in a 96-well flat-bottom cell culture dish. OD value of cells was detected at 0, 24, 48, 72, and 96 h.

Cell Migration Assay

Migration of cells was performed using QCM[™] 24-Well Colorimetric Migration Assay Kit (Millipore, Bedford, MA, USA), according to the manufacturer's instructions. The transfected cells were seeded into the upper chambers (1×10^5 cell/well) that were incubated by Matrigel in 200 μl of serum free DMEM medium, while the bottom of the chamber was incubated with 500 μl of medium containing 10% fetal bovine serum. After 48 h incubation at 37°C , the cells migrating to the lower surface of the insert were fixed and stained with 0.1% crystal violet and counted under a microscope (200 $\times$, Olympus, Tokyo, Japan). Each experiment was repeated independently three times.

Statistical Analysis

All data were repeated at least three times and indicated that the difference was statistically significant with the t-test, $p < 0.05$ as significant difference. All the statistical analyses were performed using SPSS13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

AB073614 is Upregulated in Glioma Tissues and cell lines

Based on the RT-qPCR, we found that the expression levels of AB073614 were significantly upregulated in glioma tissues compared with adjacent non-cancerous tissues ($p < 0.01$, Figure 1A). Moreover, AB073614 was also remarkably

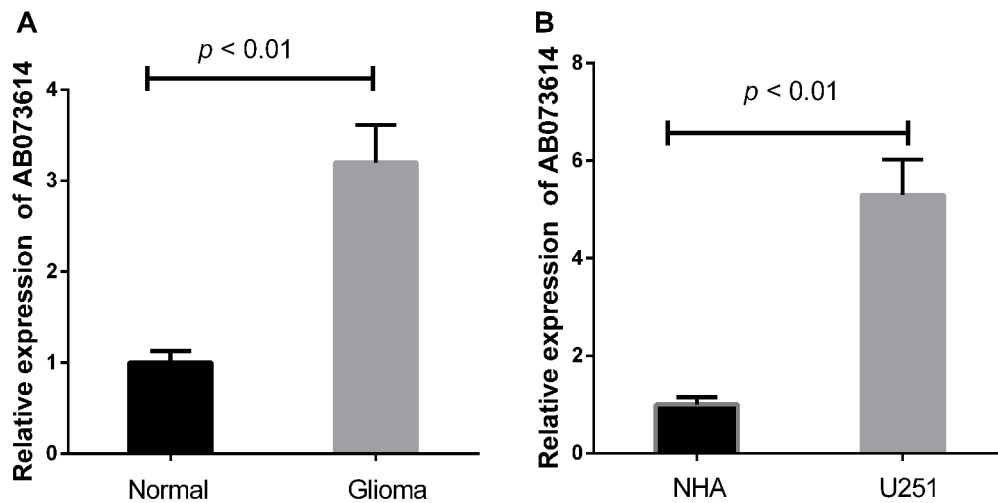


Figure 1. Expression of AB073614 in glioma tissues and cell lines. **A**, AB073614 levels were upregulated in glioma tissues compared with non-cancerous tissues, $p < 0.01$. **B**, Expression of AB073614 was found to be upregulated in U251, compared to NHA, $p < 0.01$.

increased in glioma cell lines, U251, compared with that of the normal human astrocytes (NHA) ($p < 0.01$, Figure 1B).

Aberrant Expression of miR-325-3p Regulates Cell Proliferation in vitro

To assess the biological role of AB073614 in glioma, we investigated the effect of targeted knockdown of AB073614 on cell proliferation. As shown in Figure 2A, knockdown of AB073614 significantly decreased AB073614 mRNA expres-

sion in U251 cells, as compared with the si-NC group. Next, MTT assay was conducted to analyze the proliferation of transfected cells. data showed that knockdown of AB073614 significantly inhibited the proliferation of U251 cells 72 hours after transfection (Figure 2B).

Knockdown of AB073614 inhibits Migration of glioma cells

To investigate the function of AB073614 in the metastasis, the effects of AB073614 on the

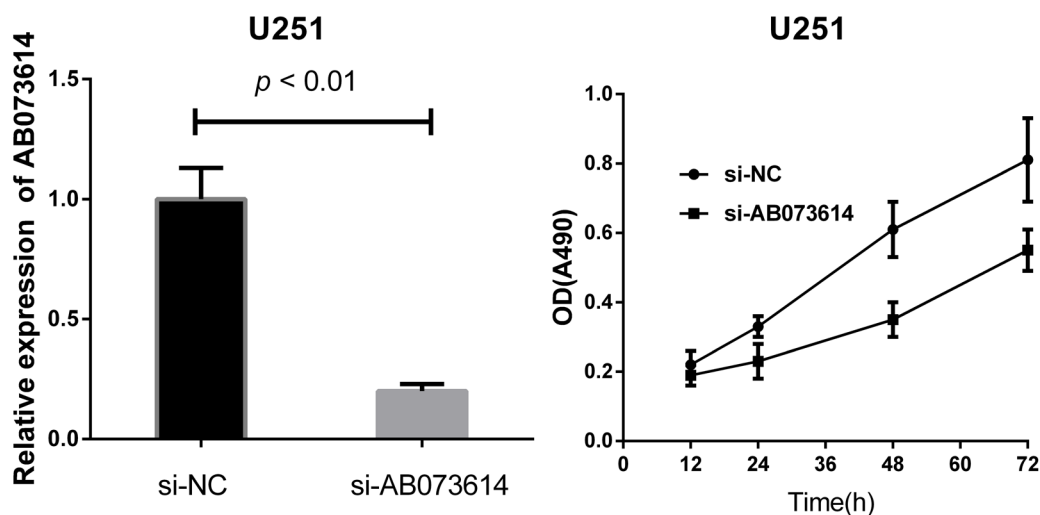


Figure 2. Effects of AB073614 expression on glioma cell proliferation. **A**, The relative levels of AB073614 normalized to GAPDH in two glioma cell lines; U251 was detected after the cells transfected with si-AB073614 or si-NC using real-time PCR. **B**, MTT assay was performed in U251. Si-AB073614-transduced U251 cells exhibited significantly lower growth rates than control cells at day 3 after plating ($p < 0.01$).

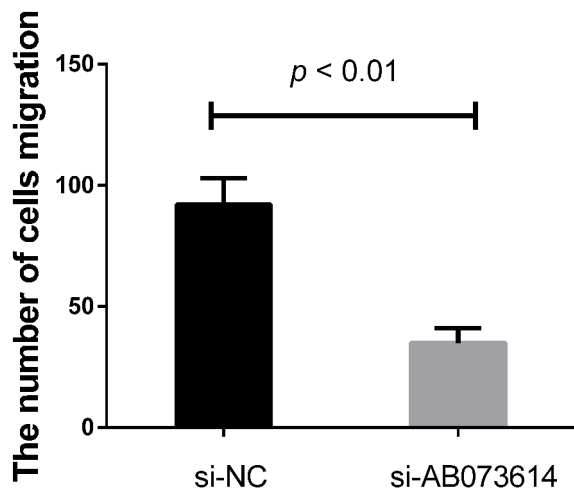


Figure 3. U251 cells transfected with si-AB073614 exhibited a lower migratory capacity in comparison with those transfected with si-NC ($p < 0.01$).

migrated ability of glioma cells were assessed by migration assays. As shown in Figure 3, silencing of AB073614 in U251 cells caused a significant reduction in cell migration.

Effects of AB073614 expression on Glioma cell EMT

To measure the effect of AB073614 on EMT of glioma cells, Western blot was performed to examine the expression of EMT-related markers in U251 cells after transfection with si-AB073614. As shown in Figure 4, downregulation of AB073614 in U251 cells remarkably increased

the expression of E-cadherin (a marker of epithelial cells) and meanwhile greatly decreased the expression of Vimentin (markers of mesenchymal cells), in comparison with control groups. These data suggested that AB073614 contributes to glioma cells metastasis may partly via affecting EMT process, and further experiments are needed to elucidate the potential mechanism.

Discussion

Recently, many lncRNAs have been identified, and the participation of lncRNAs in a wide repertoire of biological processes has been a topic of intense contemporary research¹¹. So far, clinical significance and biological functions of lncRNAs in glioma have been identified¹². For instance, Qin et al¹³ suggested that lncRNA TSLC1-AS1, the antisense transcript of tumor suppressor gene TSLC1, serves as a tumor suppressor in the tumorigenesis of glioma through modulating the expression of TSLC1. Xiang et al¹⁴ showed that silencing of long non-coding RNA MALAT1 could promote apoptosis of glioma cells. Yue et al¹⁵ found that MDC1-AS inhibit glioma cell proliferation and cycle through up-regulation of its antisense tumor-suppressing gene MDC1. Furthermore, many lncRNAs have been identified as diagnostic and prognostic biomarkers in glioma^{16,17}. However, the specific mechanism about lncRNAs regulating the tumor cells remain largely unknown.

LncRNA AB073614 is a new lncRNA transcript which first has been considered as a Homo sapiens primary hepatoblastoma cDNA (clone:

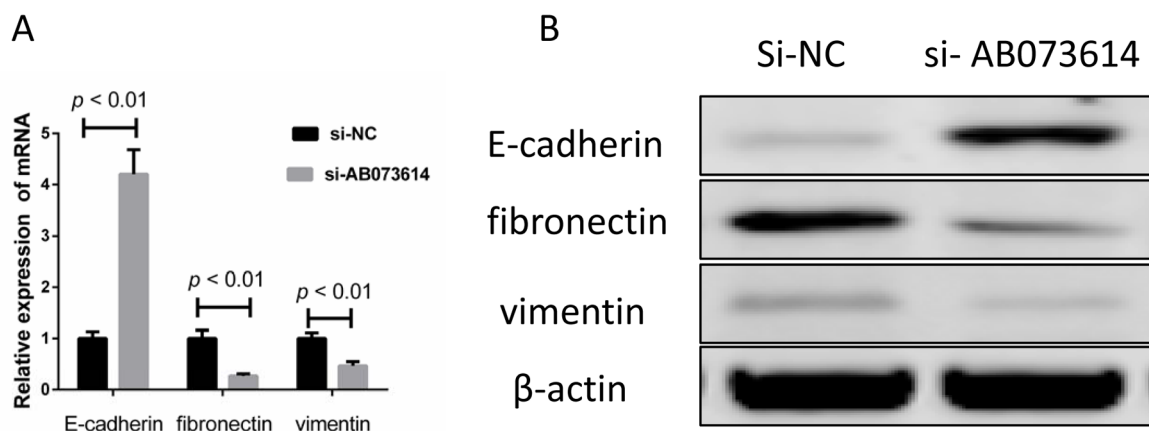


Figure 4. Effects of AB073614 expression on glioma cell EMT. A, qRT-PCR was used to analyzed the expression of EMT-related molecular markers in U251 cells. B, Western blot to detect the influence of AB073614 on the expression of EMT-related protein in U251 cells. Compared with NC group.

HMFN1050)¹⁸. Cheng et al¹⁹ firstly showed that AB073614 expression was significantly up-regulated in ovarian cancer tissues compared with normal counterparts. Furthermore, they found that knockdown of AB073614 expression significantly inhibited ovarian cancer cell proliferation and invasion. Hu et al¹⁰ reported that lncRNA AB073614 expression was significantly up-regulated in cancerous tissues compared with normal brain tissues. Moreover, their study suggested that high expression of AB073614 was involved in glioma progression and could be a novel biomarker of poor prognosis in the patient with glioma. Those results informed that AB073614 might serve as a tumor promoter in glioma. However, the mechanism of AB073614 in regulating glioma was not reported.

In the present study, we confirmed that AB073614 was significantly upregulated in glioma-tissues and cell lines. Furthermore, we found that knockdown of AB073614 resulted in an obvious reduction in cell proliferation rate and migratory capacity. EMT is a key step toward cancer metastasis, a biological process where epithelial cells lose their polarity and undergo transition into a mesenchymal phenotype²⁰. The EMT process plays a vital role in cancer invasion and metastasis²¹. Thus, we examined the expression levels of hallmarks of EMT in glioma cells with AB073614 knockdown. Our findings showed that AB073614 knockdown upregulates E-cadherin and downregulates vimentin in glioma cell lines. Taken together, these data indicated that AB073614 is a critical regulator in preventing EMT in glioma.

Conclusions

Our data not only supply novel insights regarding AB073614 function and the potential mechanisms of the glioma cell proliferation, but also suggest AB073614 may serve as a potential therapeutic target of glioma patients.

Conflict of Interests

The Authors declare that they have no conflict of interests

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