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Long noncoding RNA LINC00460 targets miR-539/MMP-9 to promote meningioma progression and metastasis

Hongshun Xing[a,b], Shilei Wang[c], Quancai Li[b], Yongqian Ma[b], Peng Sun[d,*]

[a] Qingdao University, Qingdao, Shandong, 266003, China
[b] Department of Neurosurgery, Weifang People's Hospital, Weifang, Shandong, 261041, China
[c] Department of Neurosurgery, Liaocheng People's Hospital, Liaocheng, Shandong, 252000, China
[d] Department of Neurosurgery, Affiliated Hospital of Qingdao University, Qingdao, Shandong, 266003, China

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ABSTRACT

Emerging evidence reveal that long noncoding RNAs (lncRNAs) participates in the epigenetic regulation of pathophysiological process. However, the deepgoing role of lncRNAs on meningioma is still unclear. In present study, we investigate the roles of lncRNA LINC00460 in meningeoma tissue and uncover its molecular mechanism. Results revealed that LINC00460 expression level was significantly up-regulated in meningeoma tissues and malignant meningeoma cell lines (IOMM-Lee, CH157-MN). Mechanically, loss-of-function assays showed that LINC00460 knockdown significantly suppressed the proliferation ability, increased the apoptosis and decreased the proteins (MMP-2, MMP-9, ZEB1) expression. Bioinformatics tools predicted that miR-539 both targeted with the 3'-UTR of LINC00460 and MMP-9 mRNA, which was confirmed by luciferase reporter assay and western blot analysis. In summary, our study reveals that LINC00460 promotes MMP-9 expression through targeting miR-539, acting as an oncogenic RNA in the meningeoma malignancy and accelerating the proliferation and metastasis of meningeoma.

1. Introduction

Meningeoma is one of the most frequent primary tumors occurs in central nervous system with 30–40% morbidity[1,2]. Fortunately, the vast majority of meningeoma is benign tumour. According to the classification of World Health Organization (WHO), meningeoma is classified into WHO grade I, grade II, grade III [3]. Meningioma is a type of common neoplasms originated from the meningeal coverings of the brain or spinal cord. Although large portion of meningioma is classified as benign tumors in primary qualitative classification, clinical research found that malignant meningiomas always occur accompanied by rapid growth, invasive and metastasis [4]. In recent years, although significant progress have achieved on diagnosis and treatment for meningeoma, the recurrence rate is still pessimistic due to the ambiguous pathogenesis [5].

Among the transcriptome, the vast portion of products is the non-coding RNAs (ncRNAs), besides, these ncRNAs are divided into short non-coding RNAs and long non-coding RNAs (lncRNAs) according to their length [6,7]. Increasing evidences have manifested the extensive biological role of lncRNAs on multiple tumor pathological process, for example proliferation, invasion, metastasis and recrudesce [8–10]. For the tumorigenesis of meningeoma, more and more lncRNAs have been identified to modulate the pathological process.

LINC00460 has been reported to act as oncogenic RNA in several human cancers, e.g. lung cancer, nasopharyngeal carcinoma nasopharyngeal carcinoma [11,12]. However, the role of lncRNA LINC00460 in meningioma is still unclear. In consideration of the vital role of ncRNAs in the meningeoma tumorigenesis, we choose LINC00460 as the target and explore its role in meningioma cell phenotype. We performed series of validation experiments to verify the underlying role of LINC00460 in meningeoma tumorigenesis. We found the aberrant high-expression of LINC00460 in meningeoma tissue samples and cells, moreover, it targets miR-539/MMP-9 to accelerate the meningeoma.

2. Materials and methods

2.1. Clinical samples

A total of 33 cases of human meningeoma tissues specimens and 10 cases of normal meninges tissues specimens were collected at Affiliated Hospital of Qingdao University between 2015 and 2017. The tumor

* Corresponding author at: Department of Neurosurgery, Affiliated Hospital of Qingdao University, Jiangsu Road, No. 16, Qingdao, Shandong, 266003, China.
E-mail address: sunpeng_neurosu@yeah.net (P. Sun).

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specimens were immediately frozen stored as soon as excision at liquid nitrogen. This study was approved by the Ethics Committees of Affiliated Hospital of Qingdao University. All written informed consents had been collected from every patient before surgery.

2.2. Cell culture and transfection

The human benign meningeoma cell line (Ben-Men-1) and malignant meningeoma cell lines (IOMM-Lee, CH157-MN) were obtained from the Chinese Academy of Medical Sciences (Beijing, China). Cells were cultured in Dulbecco's Modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) at 37 °C in a humidified incubator containing 5% CO_2. For LINC00460 silencing, miR-539 silencing or up-regulation, miR-539 mimics, miR-539 inhibitor and siRNAs targeting LINC00460 were provided by GenePharma (Shanghai, China). Transfection was performed using Lipofectamine2000 (Invitrogen, USA) following the instructions of manufacturer. The sequences were presented as following: si-LINC00460-1, 5′-GUGUCAACAACCUGUUUAAUU-3′, si-LINC00460-2: 5′-UUAAGUUCAGAAUUGGCACUU-3′, si-LINC00460-3, 5′-GUAACAACUUCUAGAGCUUUU-3′. miR-539 mimics, forward, 5′-UGGCAGUGUCUUAGCUGGUUG-3′; reverse, 5′-ACCAGCUAAGACA CUGCCAUU-3′. miR-539 inhibitor, 5′-ACAUGGUUAGAUCAAGCA CAA-3′.

2.3. Quantitative real-time polymerase chain reaction

Total RNA was extracted from meningeoma tissues and cell lines using TRIzol Reagent (Invitrogen, USA) accordance with the protocol of manufacturer. RNAs were reverse transcribed into cDNAs using PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). Reaction system (20 μL) was used for the analysis. Then, the quantitative real-time polymerase chain reaction (qRT-PCR) was performed using SYBR Premix Ex Taq (TaKaRa, Dalian, China). The primers used in this study were as follows: LINC00460, 5′-AATGGTGGTAGGAGGGAGGA-3′ (forward) and 5′-CAAGGGGAATGAACACGAGG-3′ (reverse); β-actin: 5′-AAGCCCACTTCTCTCCACCTAA-3′ (forward) and 5′-AATGCTATCA CCT CCCCTGTGT-3′ (reverse). β-actin acted as the endogenous controls. Each expression level was calculated with the $2^{-\triangle\triangle Ct}$ method and every data was performed in triplicate.

2.4. Cell proliferation assay

Cell proliferation ability of meningeoma cells were detected using Cell Counting Kit-8 assay (CCK-8, Dojin, Japan). For CCK-8 assay, meningeoma cells (IOMM-Lee, CH157-MN) (2×10^3 cells/well) were transfected with siRNAs and then seeded in 96-well plates. 72 h later, CCK-8 (10 μL) was added into each well and treated for 2 h at 37 °C. Finally, the absorbance was tested at 450 nm.

2.5. Flow cytometry analysis

Meningeoma cells (IOMM-Lee, CH157-MN) (2×10^3 cells/well) were digested with trypsin and washed with phosphate-buffered saline. 48 h after transfection, cells were seeded into 6-well plates and then treated with 400 μl binding buffer, 5 μl Annexin V-FITC and 5 μl propidium iodide (PI) using Annexin V-FITC Apoptosis Detection Kit (Invitrogen, Carlsbad, Calif, USA) according to manufacturer's instruction. FlowJo Version 6.1 software (TreeStar, Asland, OR, USA) was used to calculated the apoptotic rate.

2.6. Transwell invasion assay

For invasion assay, transwell assay was performed using chamber with a pore size of 8 μm (Sigma). Briefly, after transfection, meningeoma cells (IOMM-Lee, CH157-MN) were re-suspended in serum-free medium then seeded in the upper chamber. The upper chambers were pre-coated with Matrigel solution (BD, Franklin Lakes, NJ, USA) and incubated at 37 °C for 4 h. The lower chamber was filled with DMEM supplemented with 20% FBS. After 24 h of incubation, cells in the upper membrane surface were removed using a cotton swab. The filters were fixed with 95% ethanol and stained with 0.2% crystal violet solution (Sigma) and then counted.

2.7. Western blotting

Total protein was extracted from the cells using RIPA buffer. The protein lysates were centrifuged at 4 °C for 40 min and the protein concentration of the supernatant extract was determined by BCA protein assay kit (Beyotime, Shanghai, China). Protein was separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to PVDF membranes. Then, samples were incubated in tris-buffered saline containing 5% nonfat milk for 2 h at room temperature and incubated with primary antibody (anti-MMP-2, 1:1000; anti-MMP-9, 1:1000; anti-ZEB1, 1:1000; Abcam, UK, GAPDH, 1:5000, Proteintech, Rosemont, IL, USA). Then, membrane was probed with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz, Dallas, TX, USA). Immunoblots were visualized by enhanced chemiluminescence detection system (Pierce, Rockford, IL, USA) and scanned using ChemImager 5500 V2.03 software (Alpha Innotech, San Leandro, CA). Anti-GAPDH antibody was used to monitor the loading amount.

2.8. Luciferase reporter assay

The sequences of LINC00460 and MMP-9 wild type with potential miR-539 binding sites or mutant of each sites were amplified and cloned into the luciferase reporter vector pGL3 (Promega, Madison, WI, USA), named as LINC00460 wide-type plasmid (LINC00460 WT, MMP-9 WT) and mutant-type plasmid (LINC00460 Mut, MMP-9 Mut). HEK293 cells were co-transfected with the pGL3 vector with either WT or Mut, and miR-539 or control miRNA using Lipofectamine 2000. After 48 h, cells were collected and examined for β-galactosidase and luciferase activities using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

2.9. Statistical analysis

All the statistical analyses were performed using SPSS 18.0 software package and graphs were generated using GraphPad Prism 6.0 software. Differences among groups were analyzed by one-way analysis of variance (ANOVA) or Student's two-tailed *t*-test Tukey was used as post-hoc-test for ANOVA. All p values < 0.05 were regarded as statistical significance.

3. Results

3.1. LncRNA LINC00460 was over-expressed in meningeoma tissue and cells

In the previous stage of experiments, we measured the expression levels of lncRNA LINC00460 in meningeoma tissue and control meninges tissue using RT-PCR. Data showed that LINC00460 expression was significantly over-expressed in meningeoma tissues compared with normal meninge tissue (Fig. 1A). Moreover, RT-PCR revealed that lncRNA LINC00460 was also increased in malignant meningeoma cell lines (IOMM-Lee, CH157-MN) compared with benign meningeoma cell line (Ben-Men-1) (Fig. 1B). Overall, results indicated that lncRNA LINC00460 was over-expressed in meningeoma tissue and cells, indicating the potential oncogenic role of LINC00460 in malignant meningeoma.