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Original article

LASP1 promotes glioma cell proliferation and migration and is negatively regulated by miR-377-3p

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ABSTRACT

Glioma is one of the most aggressive and lethal human cancers with a low cure rate. LASP1 plays an oncogenic role in multiple human cancers; however, its role in glioma remains largely unknown. Here, we found that LASP1 was highly expressed in glioma tissue samples. Functionally, knockdown of LASP1 significantly suppressed glioma cell proliferation and migration *in vitro* and tumorigenicity *in vivo*. These effects were found to be mechanistically associated with suppression of AKT activity. Furthermore, we identified LASP1 as a direct target of miR-377-3p. Overexpression of miR-377-3p reduced the expression of LASP1 and suppressed the proliferation and migration of glioma cells. Restoration of LASP1 expression in miR-377-3p–overexpressing cells attenuated the inhibition of glioma cell malignancy and reversed the dephosphorylation of AKT. Taken together, our results suggest that LASP1 activates the PI3K/AKT signaling pathway and is downregulated by miR-377-3p during glioma progression. These data provide a new possible therapeutic target in glioma.

1. Introduction

Glioma is an aggressive and prevalent inherent tumor of the central nervous system [1]. The development of glioma is a complicated process, which involves many regulatory factors. At present, the survival of glioma patients after maximal safe resection is limited to 12–18 months [2]. One of the biggest obstacles for treatment of glioma is its high invasiveness and resistance to conventional therapies [3]. Therefore, expounding the exact molecular mechanisms of the development and progression of glioma may provide new and effective strategies to improve the treatment of this cancer.

LASP1 (LIM and SH3 protein 1) is a specific structural scaffold protein and has been primarily identified in a cDNA library from breast cancer metastases [4,5]. The *LASP1* gene is located in human chromosomal region 17q21 and encodes 261 amino acid residues that contain an N-terminal LIM domain followed by two actin-binding sites and a C-terminal SRC homology SH3 domain localized at focal contacts [6]. LASP1 functions as a signaling molecule, transducing information from the cytoplasm into the nucleus and regulating protein phosphorylation levels [7]. Increased expression of LASP1 has been found in many human malignant tumors, such as breast cancer [8], bladder cancer [9], renal cell cancer [10], liver cancer [11], colorectal carcinoma [12], and gastric cancer [13], and plays a carcinogenic role in these cancers. Nevertheless, the expression profile of LASP1 in glioma and its biological roles have not been elucidated.

MicroRNA (miRNA) is a class of conserved endogenous noncoding small RNAs 18–23 nucleotides long that negatively regulate gene expression at the post-transcriptional level. Mature miRNAs exert their function by binding to the 3′-untranslated region (3′UTR) of a target mRNA. Emerging evidence shows that miRNA dysfunction is involved in the progression of various human cancers, and miRNAs may serve as promising therapeutic targets. Recently, downregulation of miR-377-3p was frequently observed in several types of cancer, such as lung cancer [14], lymphoid cancers [15], colorectal cancer [16], and glioma [17]. Nonetheless, the latent molecular mechanisms through which miR-377-3p participates in the development and progression of glioma remain to be fully elucidated.

In the present study, we showed that LASP1 is highly expressed in glioma tissues, and knockdown of LASP1 significantly inhibits glioma cell proliferation and migration *in vitro* and attenuates tumorigenicity *in vivo*. Mechanistic experiments revealed that LASP1 knockdown suppresses the proliferation and migration of glioma cells through inhibition of the PI3K/AKT pathway. In addition, miR-377-3p was found to be a direct suppressor of *LASP1* expression and to inhibit glioma cell

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proliferation and migration.

2. Materials and methods

2.1. Tumor tissues and cell culture

Paired glioma tissue samples and adjacent normal brain tissues were obtained from the Fudan University Shanghai Cancer Center Tissue Bank (Shanghai, China). These tissue samples were snap-frozen in liquid nitrogen after surgical resection and stored at −80 °C. Informed consent was obtained from each patient, and the use of human tissues was approved by the Clinical Research Ethics Committee of Fudan University Shanghai Cancer Center. Human U87 and LN229 glioma cell lines were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China) and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). These cell lines were maintained in a medium containing 1% of a penicillin/streptomycin solution at 37 °C in a humidified atmosphere containing 5% of CO_2.

2.2. Western blotting

Proteins from tissue lysate or cells were separated by SDS-PAGE and electrophoretically transferred to a PVDF membrane. Then, the membranes were incubated with antibodies against LASP1, phospho-AKT (p-AKT), AKT, and β-actin (Proteintech, Wuhan, China) overnight at 4 °C followed by incubation with a horseradish peroxidase (HRP)-conjugated secondary antibody. Signals were detected using enhanced chemiluminescence reagents (Pierce, Rockford, IL, USA). β-actin served as an endogenous reference.

2.3. RNA isolation, reverse transcription, and quantitative PCR

Total-RNA samples were extracted using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Briefly, 1 μg of total RNA was reverse-transcribed with the Script cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) to produce cDNA. Real-time PCR was performed on an ABI 7500 H T system. PCR reactions were carried out in a final volume of 10 μl of a mixture consisting of the FastStart DNA Master SYBR Green I Kit (Roche Diagnostics). Fold changes were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.4. Lentivirus transduction, plasmid construction, and transfection

The short hairpin RNA (shRNA)-mediated knockdown of LASP1 was performed by means of shRNA-carrying lentiviral particles. Lentivirus with shLASP1 and a corresponding control were purchased from Hanbio (Shanghai, China). Cells were infected with recombinant lentivirus plus 5 mg/ml Polybrene (Sigma, St Louis, Missouri, USA). The full-length *LASP1* cDNA was obtained from Hanbio and cloned into the pcDNA3.1 vector. The miR-377-3p mimics, and a nonspecific miRNA negative control (miR-control) were purchased from RiboBio (Guangzhou, China). Target cells were transfected with a plasmid or oligonucleotide using Lipofectamine 2000 (Invitrogen). Cells were collected 48 h after transfection.

2.5. Immunohistochemical (IHC) assay

This assay was performed as described previously [8]. LASP1 and Ki67 expression in tissues was evaluated according to the staining procedure with antibodies against LASP1 and Ki67 (Proteintech).

2.6. Cell proliferation analysis

Cell proliferation assays were performed using the Cell Counting Kit 8 (DOJINDO, Japan). Cells were seeded in 96-well plates at 2×10^3 per well. Cell proliferation was assessed at 24, 48, and 72 h. To this end, 10 μl of CCK-8 was added into each well and incubated for 3 h. Absorbance was measured at 490 nm on a microplate reader.

2.7. Colony-formation assay

Cells were seeded at a density of 300 cells per well in a six-well culture plate. After 12 days, the cells were fixed with 4% paraformaldehyde for 10 min and stained with 0.05% crystal violet for 15 min. The colonies with a diameter greater than 1 mm were counted in each well.

2.8. In vitro cell migration assay

This assay was carried out in 24-well Transwell chambers with 8.0 μm membrane filter inserts (Corning, Cambridge, MA, USA). Cells resuspended in 100 μl of a serum-free medium were seeded in the upper chamber, and 600 μl of the medium containing 10% of FBS was added into the lower chamber. After 48 h of incubation, migrating cells on the lower membrane surface were fixed with 4% paraformaldehyde for 10 min, and then stained with a 0.4% crystal violet solution. Five randomly selected visual fields were imaged in each well by microscopy.

2.9. A subcutaneous xenograft model

A total of 2×10^6 U87 cells infected with a lentivirus expressing either shLASP1 or negative control were injected subcutaneously into the flank of BALB/c nude mice (4–6 weeks old, female, n = 5 per group). Tumor growth was monitored every 5 days for 25 days. The tumor volume was calculated using the following formula: V (mm^3) = $width^2 \times length \times 0.5$. After euthanasia, the tumors were excised, and the wet weight of each tumor was determined. IHC staining for Ki67 and LASP1 was also performed. Animal studies were approved by the Animal Care and Use Committee of Fudan University.

2.10. Dual-luciferase assay

A 200 bp fragment of LASP1 3′UTR, which contains two putative binding sites for miR-377-3p, was amplified by PCR and cloned downstream of the *Renilla* luciferase gene in the psiCHECK-2 vector (Promega, Madison, WI, USA). This vector was named wild-type (WT) 3′UTR. The mutant 3′UTR of *LASP1* had mutated sequences in the complementary site for the seed region of miR-377-3p. All the constructs were verified by direct sequencing. HEK293 cells were co-transfected with an appropriate reporter vector and miR-377-3p mimics or miR-control mimics by means of Lipofectamine 2000. The cells were harvested 48 h after transfection, and luciferase activity was detected via a dual-luciferase assay system (Promega). Each experiment was conducted in triplicate.

2.11. Statistical analysis

SPSS 12.0 and Graph Pad Prism 5.0 software were used for statistical analysis. Data are presented as mean ± SD of at least three independent experiments. Student's *t* test or one-way analysis of variance was carried out for statistical analysis when appropriate. Data with $P < 0.05$ were considered statistically significant.

3. Results

3.1. LASP1 is overexpressed in glioma tissue samples

To explore the expression and significance of LASP1 in glioma carcinogenesis, we measured LASP1 expression in eight glioma tissue samples and samples of their adjacent normal tissue by western blotting and qRT-PCR. As shown in Fig. 1A, seven of eight glioma tissue samples