

Downregulation of lncRNA-MALAT1 Affects Proliferation and the Expression of Stemness Markers in Glioma Stem Cell Line SHG139S

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Abstract Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is among the most abundant and highly conserved lncRNAs, which has been detected in a wide variety of human tumors, including gastric cancer, gallbladder cancer, and so on. Previous research has showed that MALAT1 can activate LTBP3 gene in mesenchymal stem cells. However, the specific roles of MALAT1 in glioma stem cells (GSCs) remain unclear. In this study, we aimed to identify the effects of MALAT1 on proliferation and the expression of stemness markers on glioma stem cell line SHG139S. Our results showed that downregulation of MALAT1 suppressed the expression of Sox2 and Nestin which are related to stemness, while downregulation of MALAT1 promoted the proliferation in SHG139S. Further research on the underlying mechanism showed that the effects of MALAT1 downregulation on SHG139S were through regulating ERK/MAPK signaling activity. And we also found that downregulation of MALAT1 could activate ERK/MAPK signaling and promoted proliferation in SHG139 cells. These findings show that MALAT1 plays an important role in regulating the expression of stemness markers and proliferation of SHG139S, and provide a new research direction to target the progression of GSCs.

Keywords Glioma · lncRNA · MALAT1 · ERK/MAPK

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Introduction

Malignant glioma remains one of the most deadly cancers (Wen and Kesari 2008). With a combination of surgery, chemotherapy, and radiotherapy, the median survival duration of patients with glioblastoma multiforme, the most aggressive type of malignant glioma, was significantly improved; however, only 2- and 5-year survival outcomes were observed in a large randomized trial (Stupp et al. 2010). It underscores the urgency and importance to identify approaches that are not derivatives of current treatment modalities.

In recent years, many studies have demonstrated the existence of a stem-cell derived origin of gliomas (Vescovi et al. 2006). Glioma stem cells (GSCs) can be isolated both from human brain tumors and several glioma cell lines (Braine and Herpin 2004). Rigorous functional studies have provided evidences that GSCs promoted therapeutic resistance and relapse of glioma (Beier et al. 2007; Rosen and Jordan 2009). In this study, SHG139S glioma stem cell spheres were acquired under NSCM from SHG139 glioma cells which was gained successfully in serum-containing RPMI 1640 (Li et al. 2015; Chen et al. 2015).

In addition to glioma, cancer stem cells (CSCs) have been shown in several other types of solid tumors, such as head and neck squamous cell carcinoma (Shrivastava et al. 2015). Although there are still unanswered issues and questions on the etiology of CSCs, the importance to eliminate CSCs for achieving better antitumor efficacy is evident.

Long non-coding RNAs (lncRNAs) which are non-coding transcripts that are more than 200 nucleotides in length, have recently emerged as important molecules in several cellular processes (Chen and Carmichael 2010; Gutschner and Diederichs 2012). They were initially

thought to be spurious transcriptional noise but are emerging as new regulators in the cancer paradigm (Wilusz et al. 2009; Nagano and Fraser 2011). Emerging evidences have indicated that lncRNAs may have complex and extensive functions in the development and progression of cancer (Matouk et al. 2007; 15Huarte et al. 2010; Li and Chen 2013; Zhou et al. 2012; Pasmant et al. 2011; Du et al. 2012; Ling et al. 2013). Early in 2003, Ji et al. (2003) firstly identified long non-coding RNA -MALAT1. MALAT1 expression can serve as a potential marker of survival in stage I non-small cell lung cancer (NSCLC) patients. Furthermore, other researches have showed that MALAT1 over-expresses in liver, cervical, colon, and gallbladder cancer suggesting that MALAT1 misregulation may play a role in the development of numerous cancers (Hutchinson et al. 2007; Lin et al. 2007; Hu et al. 2015; Sun et al. 2009; Xu et al. 2011; Gupta et al. 2010; Wu et al. 2014). In our previous study, we found MALAT1 was lowly expressed in glioma tissues. In addition, MALAT1 have been reported to play a role in stem cells (Li et al. 2014). However, to date, no data are available on the detailed roles of lncRNA-MALAT1 in the proliferation and maintenance of stemness in CSCs, including GSCs.

Thus, to explore the possible function and underlying molecular mechanisms of MALAT1 in the proliferation and stemness properties of GSCs, we investigate the biological functions of MALAT1 in regulating the proliferation and maintenance of stemness of GSCs by downregulating the expression of MALAT1 in SHG139S.

Materials and Methods

Cell Culture

The SHG139 cell and glioma stem cell line SHG139S were developed and provided by the neurosurgery & brain and Nerve Research Laboratory, The First Affiliated Hospital of Soochow University, PR China. The SHG139S cell line was maintained in Dulbecco's modified Eagle's medium (DMEM)-F12 containing 20 ng/mL of epidermal growth factor, basic fibroblast growth factor (bFGF; both from R&D Systems, Minneapolis, MN, USA), nitrogen gas (1:50 dilution), and B27 (1:50 dilution; Life Technologies, Carlsbad, CA, USA). SHG139 cells were grown in DMEM (Hyclone, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10 % fetal bovine serum (FBS)(GIBCO, Invitrogen Inc., Carlsbad, CA, USA). The SHG139 cell line was maintained in 1640 (Hyclone; Thermo Fisher Scientific, Waltham, MA) supplemented with 10 % fetal bovine serum (FBS) (GIBCO, Invitrogen Inc., Carlsbad, CA).

Microarray Analysis

Briefly, three SHG139 cell samples were regarded as the control group and three SHG139S cell samples were regarded as the experiment group. These three-paired samples were used to synthesize double-stranded complementary DNA (cDNA) and the cDNA was labeled and hybridized to Agilent Human lncRNA4*180 K microarray (Biotechnology Corp, Shanghai, China) according to the manufacturer's instructions. The data from lncRNA microarray were used to analyze data summarization, normalization, and quality control using the GeneSpring software V11.0 (Agilent, technologies, Santa Clara, CA, US). The differentially expressed genes were selected if the change of threshold values were >2.0 or <-2.0 folds and if Benjamini-Hochberg Corrected P values were <0.05 . The data was normalized and hierarchically clustered with CLUSTER 3.0 software. The data were performed tree visualization with Java Treeview Software (Stanford University School of Medicine, Stanford,CA, USA).

Lentivirus-Mediated RNA Interference

The following short hairpin RNA (shRNA) was used to target human MALAT1: sense:5'-TGCTGTGACTATCC CATCACTGAAGGTTTTGGCCACTGACTGACCTTCATGGGGATAGTACA-3'; antisense:5'-CCTGTGACTATCCCCACTGAAGGTCAGTCAGTGGCCAAAACCTTCAGTGATGGGATAGTACAC -3'. The sequence of the negative control shRNA was Negative-F: tgctgAAATGTA CTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAGTACATTT; Negative-R: cctgAAATGTA CTGCGTGGAGACGTCAGTCAGTGGCCAAAACGCTCCACGCAGTACATTTTC. These shRNAs were synthesized and inserted into the pFHIUGW lentivirus core vector containing a cytomegalovirus (CMV)-driven enhanced green fluorescent protein (EGFP) reporter gene; expression of the shRNA was driven by the H1 promoter. Recombinant lentivirus expressing MALAT1-siRNA or control siRNA (si-MALAT1 or si-Control) was produced by Invitrogen (Carlsbad, CA, USA).

Quantitative Real-Time PCR (qRT-PCR)

Total RNA from cells was isolated using TRIzol reagent (Invitrogen Inc., Carlsbad, CA, USA). Relative levels of MALAT1 lncRNA were examined using Sybr green real-time quantitative reverse transcription-PCR(qRT-PCR) (Applied LightCycler480) and were normalized to levels of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA. The following primers were used: lncRNA MALAT1 Forward primer5'-CTAAGGTCAAGAGAAGTGTCAG-3'; Reverse primer5'-

AAGACCTCGACACCA TCGTT AC-3'/GAPDH Forward primer^{5'}- AACGGA TTTGGTCGT A TTG -3'; Reverse primer^{5'}- GGAAGA TGGTGA TGGGA TT -3'. Relative expression was calculated using the 2- $\Delta\Delta$ CT method. All qRT-PCRs analyses were performed in triplicate, and the data are presented as means \pm standard errors of the means (SEM).

Inhibition of P-ERK1/2 Expression by U0126 in Cells

SHG139S were seeded in a six-well plate and incubated at 37 °C overnight. The cells were then treated with 10 mM U0126 (Beyotime, Shanghai, China), a MAPK/ERK kinase inhibitor which inhibits MEK1/2 for downregulation of P-ERK expression for 72 h. Then, the cells were infected with lentivirus.

Western Blot Analysis

The primary antibodies used were anti-SOX2 (Abcam, Tokyo, Japan), anti-OCT-4 (Abcam, Tokyo, Japan), anti-CD133 (Boster Bioengineering Co., Wuhan, China), anti-Nestin (Boster Bioengineering Co., Wuhan, China), anti-Nanog (Abcam, Tokyo, Japan), and anti-A2B5(R&D, NASDAQ,USA). Protein samples were separated with 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Membranes were incubated with primary antibodies overnight at 4 °C. Membranes were washed and incubated for 2 h with horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibodies (Prosci Inc., Poway, CA, USA), followed by detection and visualization using Electrochemiluminescence Western blotting detection reagents (Pierce antibodies, Thermo Fisher Scientific, Waltham, MA, USA).

Cell Cycle Analysis and Cell Proliferation Assay

Cells were collected in an exponential growth phase and then fixed with ethanol. Then, RNase A treatment and propidium iodide staining were carried out. Cells were detected under flow cytometry using FACSCalibur (Becton–Dickinson, Franklin Lakes, NJ, USA). Cell populations at the G0/G1, S, and G2/M phases were quantified using Modfit software (Becton–Dickinson, Franklin Lakes, NJ, USA), excluding a calculation of cell debris and fixation artifacts. Cell proliferation was quantified using the Cell Counting Kit-8 (CCK-8; Beyotime, Shanghai, China). Briefly, 100 μ L of cells from the three groups were seeded onto a 96-well plate at a concentration of 2000 cells per well and were incubated at 37 °C. At daily intervals (days 1, 2, 3), the optical density was measured at 450 nm using

a microtiter plate reader, and the cell survival rate was expressed as the absorbance. The results represent the average of six replicates under the same conditions.

Cell Count

Seeded cells (1×10^5 SHG139S) were trypsinized and counted in the hemocytometer at different time point. Mean cell number was obtained by counting 4 replicates. Dead cells were excluded by trypan blue exclusion test.

Confocal Microscopy Measurement

Antibodies against Sox2 (Abcam, Tokyo, Japan), Oct-4 (Abcam, Tokyo, Japan), CD133 (Boster Bioengineering Co., Wuhan, China), Nestin (Boster Bioengineering Co., Wuhan, China), Nanog (Abcam, Tokyo, Japan), and horseradish peroxidase-conjugated secondary antibody (Abcam, USA) were purchased. Cells were fixed with 4 % paraformaldehyde and were incubated with antibody according to the manufactory instruction. Cells were examined under confocal microscopy. Collection of emission was 633 nm.

Immunohistochemistry

Slides were incubated with primary antibodies against Ki-67 (Boster Bioengineering Co., Wuhan, China). Sections were subsequently incubated with the Cell & Tissue Staining Kit HRP-DAB system (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. Immunostaining was performed with known positive and negative controls and were blindly evaluated by a pathologist.

Statistical Analysis

Statistical analyses were performed using the SPSS software, Version 13.0 (SPSS, Chicago, IL, USA). Statistical significance was determined using two-tailed Student's *t* test. A *p* value of less than 0.05 was considered statistically significant.

Results

High Expression of MALAT1 in SHG139S and the Effects of MALAT1 shRNA

We carried out lncRNA array to compare the expression profiles of SHG139S and SHG139. The results of

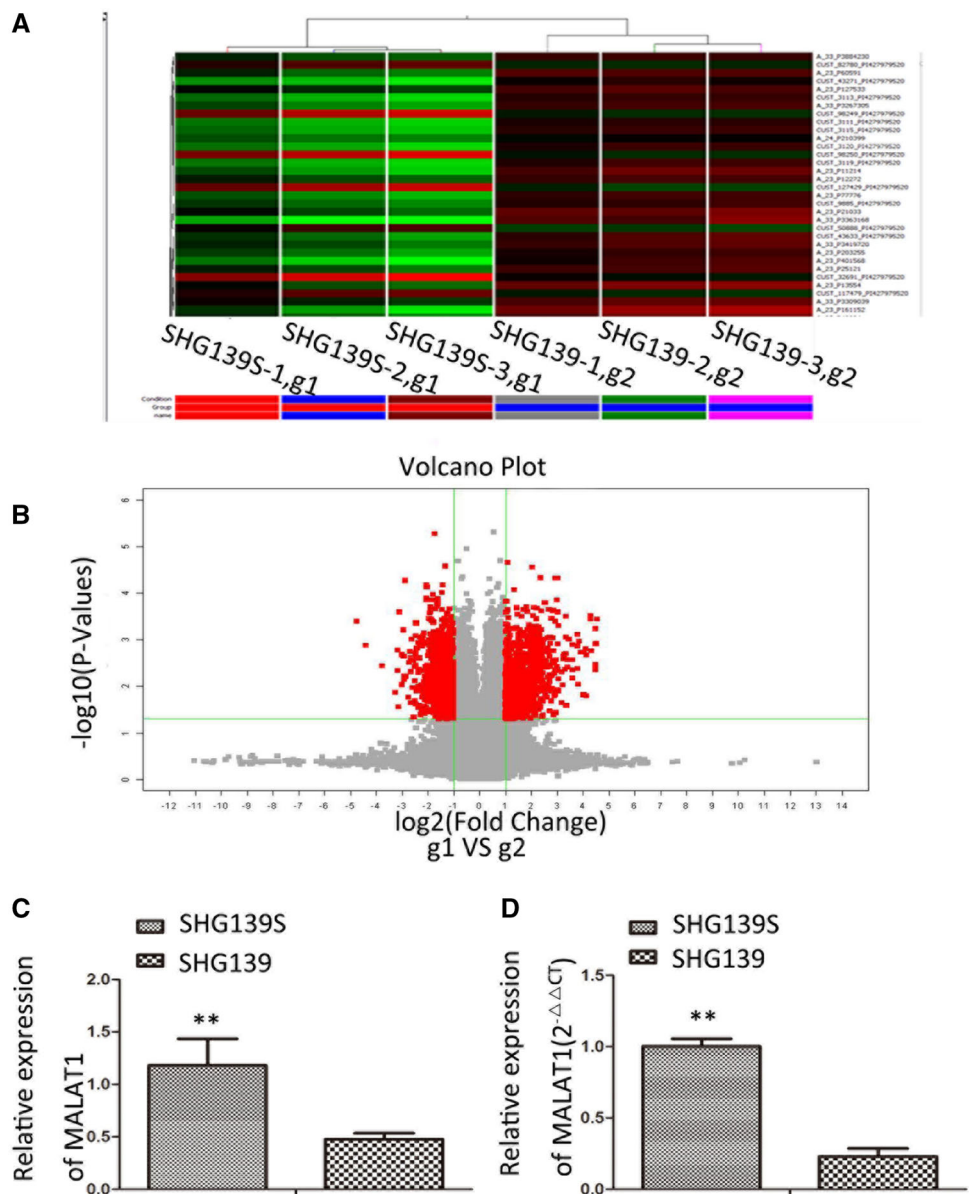
clustering analysis of the lncRNA array revealed the different expression file between the two kinds of cells (Fig. 1a, b). Among the whole lncRNA profile, lncRNA-MALAT1 is one of the most highly expressed lncRNAs in SHG139S ($P < 0.01$; Fig. 1c). Then we performed qRT-PCR to verify the high expression of MALAT1 in SHG139S compared to SHG139 ($P < 0.01$; Fig. 1d). To measure the effects of MALAT1 expression on SHG139S proliferation and the expression of stemness markers, we used chemical synthesis of specific siRNA of MALAT1 (si-MALAT1) to knockdown the expression of MALAT1 and verify its specificity by qRT-PCR. As shown in Fig. 2a, the efficiency of lentiviral transfection was more

than 85 %, and knockdown of MALAT1 in SHG139S for 48 h resulted in a significant reduction in lncRNA-MALAT1, which showed the specificity of si-MALAT1 on MALAT1 ($P < 0.01$; Fig. 2b).

Evaluation of MALAT1 shiRNA on the Proliferation of SHG139S In Vitro

To determine the effects of MALAT1 shiRNA on SHG139S proliferation, the flow cytometry was performed. The results indicated that downregulation of MALAT1 decreased the percentage of S cells, while there was no obvious difference in the percentage of G0/G1 cells

Fig. 1 lncRNA expression signature in SHG139S and SHG139. **a** Clustering analysis of SHG139S and SHG139. **b** Volcano map, Red areas represent differentially expressed genes. **c** Quantification of MALAT1 expression level in SHG139S and SHG139. Results are the average (\pm SD) of a single experiment run in triplicate. $**P < 0.01$, unpaired t-test, compared to SHG139. **d** Total RNA was extracted 4 days after infection, and the relative MALAT1 expression was determined using quantitative real-time PCR. GAPDH was used as an internal control. The data represent the mean \pm SD of three independent experiments ($**P < 0.01$)



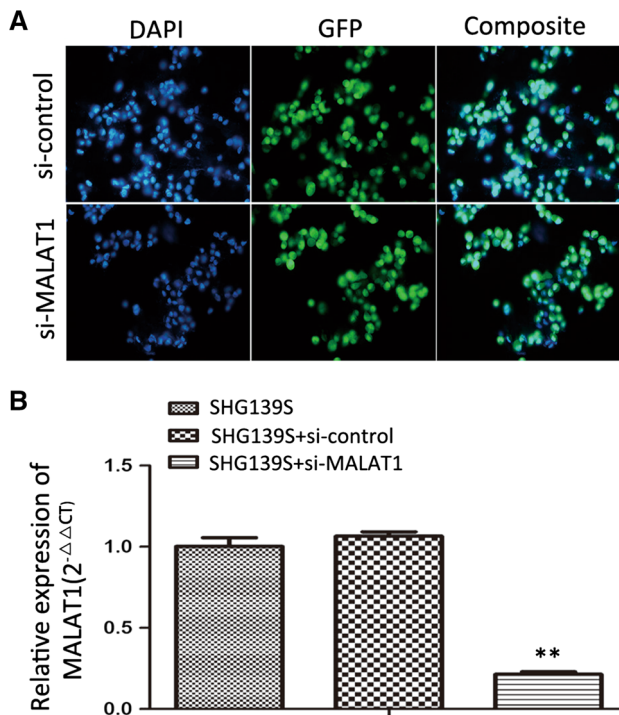


Fig. 2 Expression of MALAT1 in cells and effects of si-MALAT1 vectors on the expression of lncRNA-MALAT1. **a** The transfection efficiency was determined 3 days after incubation with lentivirus at an MOI of 20. The transfected cells labeled with GFP were observed under a fluorescence microscope (200 \times). **b** Total RNA was extracted from SHG139S and SHG139, and the relative MALAT1 expression was determined by using quantitative real-time PCR. GAPDH was used as an internal control. The data represent the mean \pm SD of three independent experiments (** $P < 0.01$)

($P < 0.01$, respectively, Fig. 3a, b). And the result of CCK-8 assay also showed that downregulation of MALAT1 promoted proliferation in SHG139S ($P < 0.01$; Fig. 3c). Then the effect of MALAT1 shRNA on SHG139S proliferation was confirmed and more evident as assessed by direct cell count ($P < 0.01$; Fig. 3d).

Downregulation of MALAT1 Decreased the Expression of Stemness Markers

To determine whether downregulation of MALAT1 in SHG139S affects the stemness markers, the protein expressions of A2B5, CD133, Nestin, SOX2, Oct-4, and Nanog which are associated with stemness were tested by confocal microscopy. Results showed that downregulation of MALAT1 decreased the protein expressions of Nestin and SOX2. (Figure 4a) To further conform this result, Western blotting was performed. And the result of Western blotting was consistent with confocal microscopy. ($P < 0.01$; Fig. 4b–d) Then the mRNA level of SOX2 and Nestin were tested by qRT-PCR ($P < 0.01$; Fig. 4e, f).

Activation of ERK/MAPK Kinase Pathways by MALAT1 Downregulation

To determine the possible mechanism by which MALAT1 regulated proliferation and expression of stemness markers in SHG139S, we performed Western blot analysis to investigate the effects of MALAT1 knockdown on the key molecular factors of cancer related pathways such as NF κ B, mTOR, Akt, and others (data not shown). The ERK/MAPK pathway is one of the most important signal transduction pathways, and downregulation of MALAT1 promoted the expression of phosphorylated ERK1/2. However, no detectable changes in total ERK1/2 protein expression were observed ($P < 0.01$; Fig. 5a, c). In order to further verify the role of signaling activation in MALAT1 aberrant expressed glioma cells, the addition of U0126 (a specific inhibitor of MEK/ERK) was used to pretreat cells. Results showed that inhibition of ERK1/2 signaling suppressed MALAT1 low expression-induced levels of phosphorylated ERK1/2, Nestin, and Sox2. ($P < 0.01$; Fig. 5b, d, e) These results indicate that MALAT1 regulates ERK/MAPK signaling activity, which regulates the expression of Nestin, Sox2, and proliferation in SHG139S.

Effects of MALAT1 on SHG139 Cells

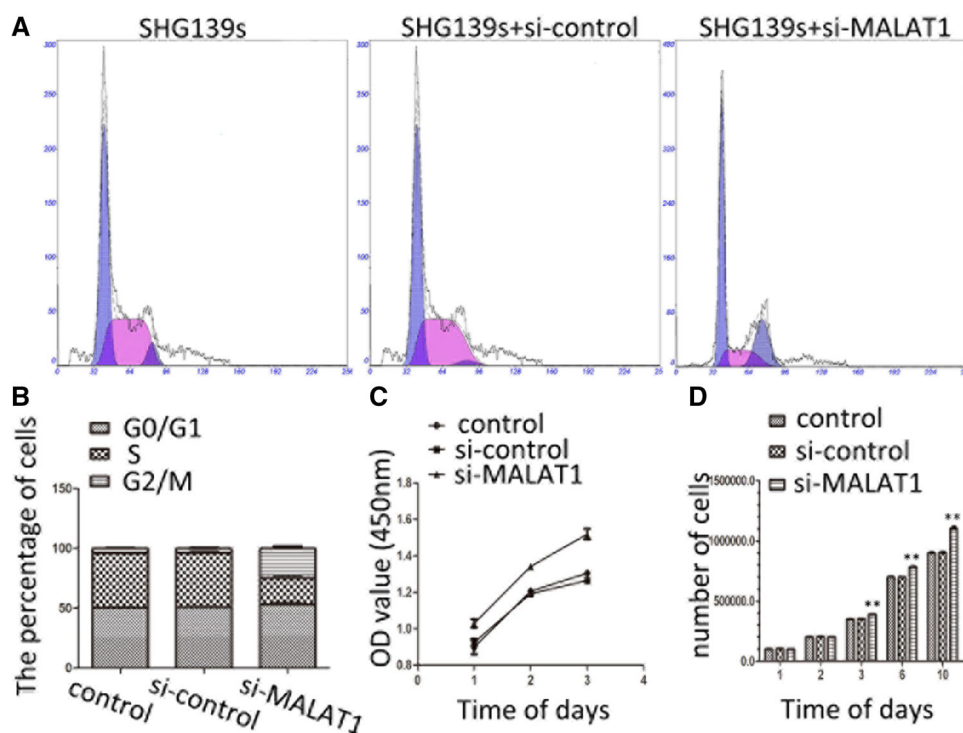
In order to explore the effects of MALAT1 on SHG139 cells, Western blotting was performed, and results showed that downregulation of MALAT1 could also promoted the expression of phosphorylated ERK1/2 ($P < 0.01$; Fig. 6a, b). Then addition of U0126 was used to pretreat SHG139 cells. Results showed that inhibition of ERK1/2 signaling suppressed MALAT1 low expression-induced levels of phosphorylated ERK1/2 ($P < 0.01$; Fig. 6c, d). And immunohistochemistry (IHC) testing the expression of ki-67 and CCK-8 assay were performed to explore the effects of MALAT1 downregulation on SHG139 cells which showed that downregulation of MALAT1 promoted proliferation ($P < 0.01$; Fig. 6e–g).

Discussion

Cell culture is one of the most powerful tools in cancer research, with 60 years of history so far (Gangoso et al. 2014). SHG-44 is the first glioma cell line generated in China in our laboratory, and more recently, we have successfully used NSCM to culture the glioma stem cell line SHG-139S. In this study, SHG-139S was used to study the effect of lncRNA-MALAT1 in GSCs.

Glioma is a highly invasive and rapidly proliferative cancer with a poor prognosis (Yang et al. 2014). Therefore, the identification of novel methods that can effectively

Fig. 3 Effects of si-MALAT1 on cell proliferation in vitro. **a** Untransfected or transfected SHG139S were stained by propidium iodide and analyzed by flow cytometry. **b** The percentage of cells in the G0/G1, S, and G2/M phases of the cell cycle was calculated. Results are expressed as mean \pm SD from three independent experiments ($P < 0.01$). **c** Cellular proliferation of untransfected or transfected SHG139S were measured using a CCK-8 assay daily for 3 days. **d** Histograms represent mean \pm SE cell counting at each day



inhibit glioma cell growth and metastasis is needed. Cancer stem cells have become a popular field in cancer research in recent years.

Due to their stemness properties, these cells are thought to be one of the most important methods for the treatment of glioma patients (Herrmann et al. 2014). Since glioma stem cells were firstly isolated, researchers have focused on their stemness properties and on the molecular pathways. However, so far, there are no unified, generally accepted therapies that can target glioma stem cells. Lathia et al. (2010) reported that integrin alpha 6 could regulate glioblastoma stem cells and it may be a promising anti-glioblastoma therapy (Lathia et al. 2010). Guryanova et al. (2011) reported that BMX could maintain the self-renewal and tumorigenic potential of glioma stem cells by activating STAT3 and develop an effective therapy against GSCs to improve the treatment of glioma (Guryanova et al. 2011). In recent years, an increasing number of studies have focused on the roles of lncRNAs in human tumors. LncRNAs such as PRNCR1 (prostate cancer non-coding RNA 1), HOTAIR (HOX antisense intergenic RNA), PRINS (psoriasis-associated RNA induced by stress), have been shown to be involved in the biological hallmarks of cancer, including promoting proliferative signaling, evading growth suppressors, enabling replicative immortality, activating invasion and metastasis, inducing angiogenesis, resisting cell death, and so on (Prensner et al. 2014; Zhang et al. 2015; Szegedi et al. 2010). MALAT1, which is also known as HCN, NEAT2, PRO2853, and NCRNA00047, is

located at chromosome 11q13.1 and encodes a polyadenylated non-coding RNA (ncRNA) of ~ 8 kb (Ji et al. 2003; Hutchinson et al. 2007). This lncRNA is highly expressed in many human cell types and is highly conserved across several species, which implies its functional importance (Bernard et al. 2010). Ji's work has demonstrated that MALAT1 could influence metastasis and patient survival in non-small cell lung cancer (NSCLC) (Ji et al. 2003). In addition, Wu et al. (2014) found that MALAT1 was upregulated in gallbladder cancer, and promoted the proliferation and metastasis of gallbladder cancer cells. MALAT1 could also control the activation of LTBP3 gene in Mesenchymal stem cells (Li et al. 2014). However, MALAT1 has not so far been linked to GSCs. In this study, we found that MALAT1 was highly expressed in SHG139S compared with SHG139. To understand its biological role in SHG139S, we used lentivirus-mediated siRNA to knock down MALAT1. We observed that in SHG139S, MALAT1 downregulation significantly promoted cell proliferation in vitro according to the CCK-8 assay, cell count, and flow cytometry. Additionally, the protein expressions of Nestin and Sox2 which are associated with stemness were inhibited in vitro by MALAT1 knockdown.

However, the exact mechanisms of MALAT1 functions are not clear so far. It has been proved that MALAT1 specifically localizes to nuclear speckles and regulates the alternative splicing of pre-mRNAs by modulating the levels of active serine/arginine splicing factors (Tripathi

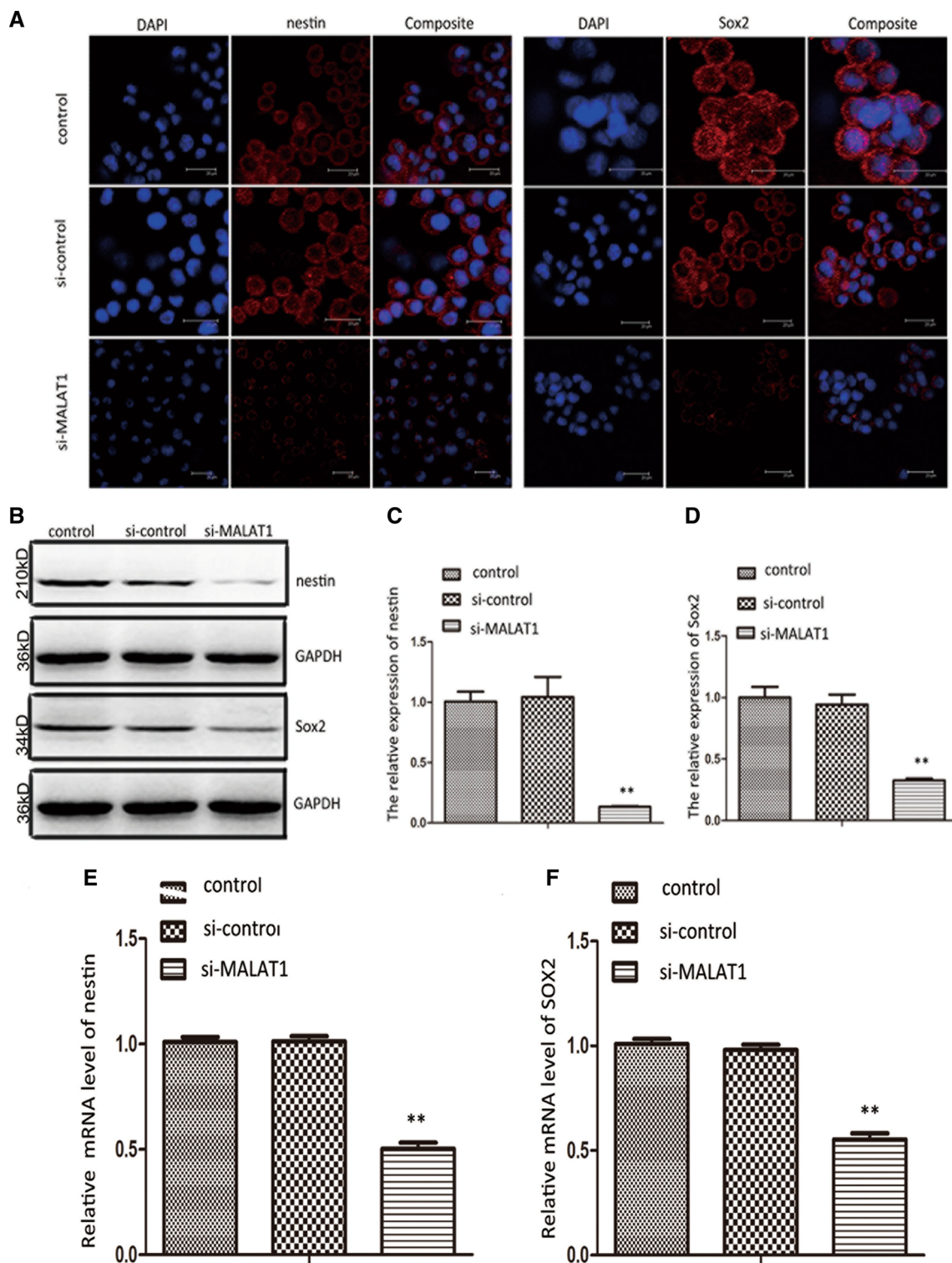


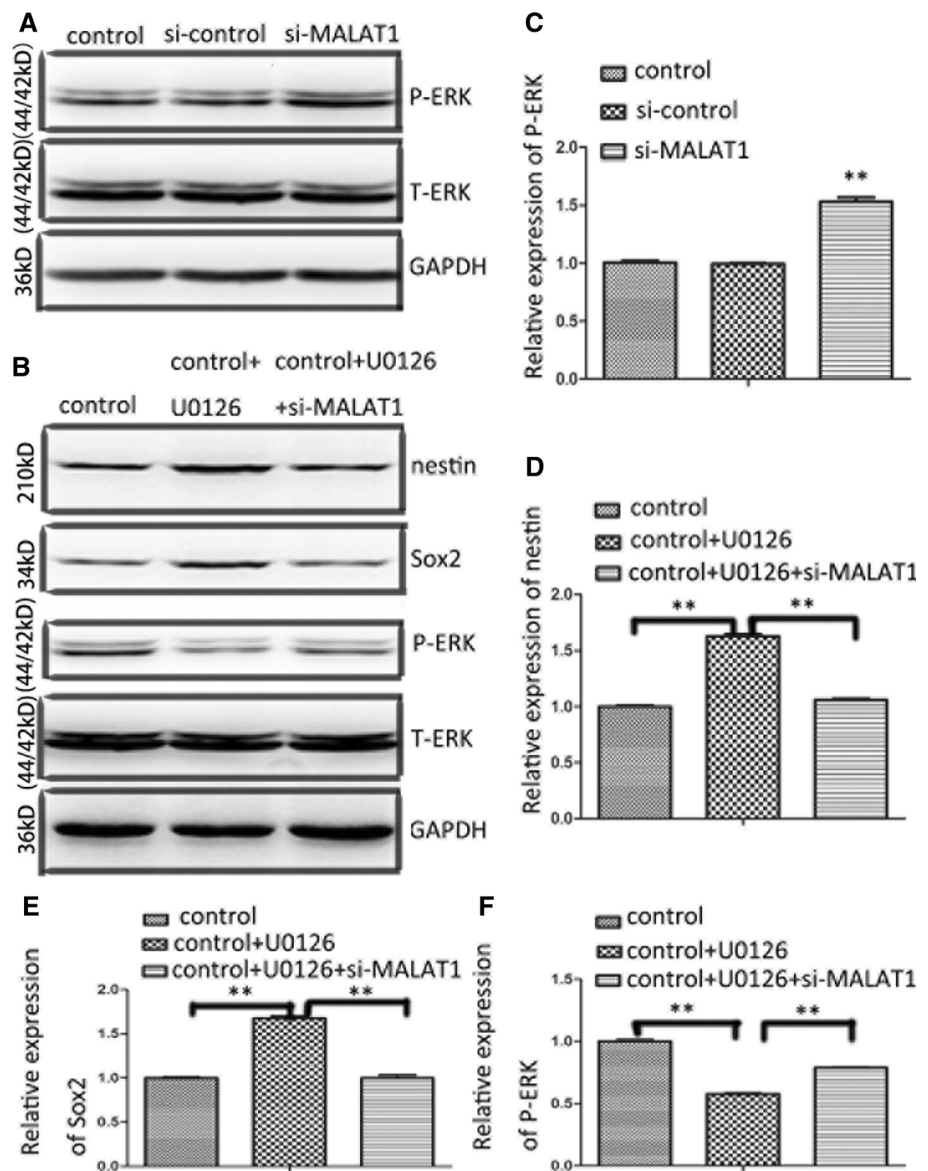
Fig. 4 Downregulation of MALAT1 reduced the expression of proteins associated with stemness. **a** Confocal microscopy measurements showed that si-MALAT1 reduced the expression of Nestin and Sox2. **b, c** The expression of Nestin and Sox2 were obviously

inhibited by downregulation of MALAT1. Each *bar* represents mean values \pm SD from six independent experiments. **d, e** The relative mRNA level of SOX2 and Nestin were determined using quantitative real-time PCR (** $P < 0.01$)

et al. 2010). Depletion of MALAT1 changes the processing of many pre-mRNAs that have important roles in cancer biology (Birney et al. 2007). This evidence means that

MALAT1 could be a regulator of posttranscriptional RNA processing or modification. Moreover, MALAT1 was found to promote cancer cell proliferation and metastasis

Fig. 5 MALAT1 downregulation activated the ERK/MAPK pathway in the glioma stem cell line. **a**, **c** Downregulation of MALAT1 increased the expression of P-ERK. Each bar represents the mean values \pm SD from three independent experiments (** $P < 0.01$). **b**, **d**, **e**, **f** Western blot analysis showed that inhibition of ERK/MAPK signaling abrogated downregulation of MALAT1 induced expression of P-ERK, but increased the expression of Nestin and Sox2. Each bar represents the mean values \pm SD from three independent experiments (** $P < 0.01$)



by activating the ERK/MAPK signaling pathway (Wu et al. 2014). In addition, MALAT1 was proved to interact with the unmethylated form of CBX4, which was shown to control the relocation of growth-control genes between the polycomb bodies and interchromatin granules, sites of silent or active gene expression, respectively (Yang et al. 2011). To elucidate the possible mechanism by which MALAT1 regulates SHG139S proliferation and expression of stemness markers, Western blot analysis of the key molecular factors of cancer-related pathways, such as Akt, mTOR, NF κ B, and others (data not shown), was performed. The ERK/MAPK pathway is one of the most important signal transduction pathways, MALAT1 promotes SHG139S proliferation and inhibits the protein expression associated with stemness by activating this signaling cascade.

We observed that in SHG139S MALAT1 knockdown significantly increased the expression of phosphorylated ERK1/2, while no detectable changes in total ERK 1/2 was observed. In order to further verify the role of signaling activation in MALAT1 aberrant expressed glioma cells, the addition of U0126 (a specific inhibitor of MEK/ERK) was used to pretreat cells. Results showed that inhibition of ERK1/2 signaling suppressed MALAT1 low expression-induced levels of phosphorylated ERK1/2, Nestin, and Sox2. And in SHG139 cells, MALAT1 downregulation could also activate ERK/MAPK signaling and promote proliferation. However, the direct link between MALAT1 and the ERK/MAPK pathway remains to be further studied.

In conclusion, we found that MALAT1 was significantly upregulated in SHG139S. Knockdown of MALAT1 could

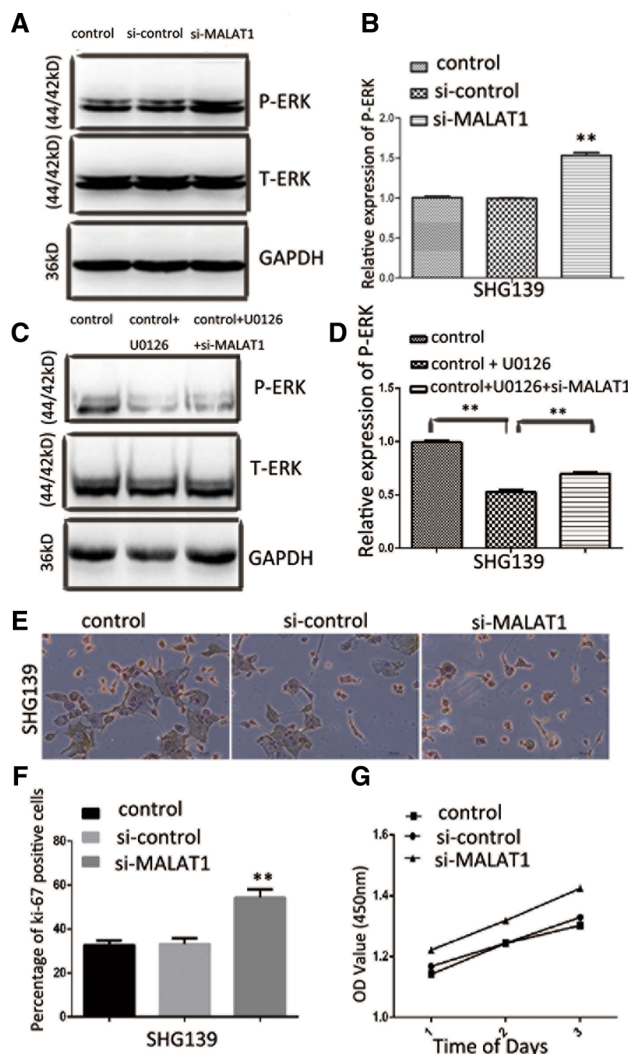


Fig. 6 Effects of MALAT1 downregulation on SHG139 cells. **a**, **b** Downregulation of MALAT1 increased the expression of P-ERK. Each bar represents the mean values \pm SD from three independent experiments (** $P < 0.01$). **c**, **d** Western blot analysis showed that inhibition of ERK/MAPK signaling abrogated downregulation of MALAT1 induced expression of P-ERK. Each bar represents the mean values \pm SD from three independent experiments (** $P < 0.01$). **e**, **f** Downregulation of MALAT1 increased the expression of ki-67 in SHG139. **g** Cellular proliferation of untransfected or transfected SHG139 were measured using a CCK-8 assay daily for 3 days

promote SHG139S and SHG139 proliferation and decrease the expression of stemness-associated proteins. Moreover, knockdown of MALAT1 led to the activation of the ERK/MAPK pathway. These results mean MALAT1 probably by inducing stem cells to adherent cells to promote cell proliferation. Cells with rapid proliferation are more sensitive to chemotherapeutic drugs. Therefore, MALAT1 might serve as a new therapeutic target for GSCs. But we only studied the role of MALAT1 in SHG139S, the role of

MALAT1 in other glioma stem cell lines remains to be studied.

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Compliance with Ethical Standards

Conflict of interest None.

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