Long non-coding RNA CASC19 promotes glioma progression by modulating the miR-454-3p/RAB5A axis and is associated with unfavorable MRI features

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Abstract. Glioma is one of the most common malignancies of the nervous system. Long non-coding RNAs (lncRNAs) are regulators involved in the progression of tumors. The present study aimed to determine the role of lncRNA cancer susceptibility 19 (CASC19) in glioma and its underlying molecular mechanism. Reverse transcription-quantitative PCR was performed to detect CASC19 and microRNA (miR)-454-3p expression in glioma and normal brain tissues. Ras-related protein in brain 5A (RAB5A) expression in glioma cells was also analyzed via western blotting. The relationship between CASC19 expression, clinicopathological parameters and MRI characteristics in patients with glioma was analyzed. Cell Counting Kit-8, BrdU, wound healing and Transwell assays were adopted to detect glioma cell proliferation, migration and invasion, respectively. The dual-luciferase reporter gene and RNA immunoprecipitation experiments were conducted to verify the targeting relationship between CASC19 and miR-454-3p, and between miR-454-3p and RAB5A. The results revealed that CASC19 expression was significantly upregulated in glioma tissues and cell lines. CASC19 expression was also positively associated with tumor diameter and pathological grade. Additionally, its high expression was closely associated with tumor MRI signal heterogeneity and peritumoral edema. CASC19 upregulation promoted glioma cell proliferation and metastasis, while CASC19-knockdown demonstrated the opposite effect. CASC19 sponged miR-454-3p, which indirectly increased RAB5A expression. The results demonstrated that the CASC19/miR-454-3p/RAB5A axis is involved in the promotion of glioma progression.

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Introduction

Glioma is one of the most common and invasive primary malignancies of the central nervous system that originates from glial or precursor cells, accounting for ~26.5% of all brain tumors (1,2). Despite encouraging progress in the diagnosis and treatment of this disease, the prognosis of patients remains poor, with a median overall survival of <15 months (3). Therefore, an in-depth study of the molecular mechanisms underlying glioma tumorigenesis and development may elucidate novel treatment options.

Long-chain non-coding RNAs (lncRNAs) are RNA molecules that are >200 nucleotides in length and do not encode protein (4). lncRNAs regulate gene expression at the transcriptional, post-transcriptional and epigenetic levels, thereby affecting cell differentiation, metabolism, proliferation and apoptosis (5). For instance, abhydrolase domain-containing protein 11 antisense RNA 1 (ABHD11-AS1) was found to promote ovarian cancer development by activating the endothelial growth factor receptor (EGFR) pathway and inhibiting the expression of tissue inhibitor of metalloproteinases 2 (TIMP2) (6). Nuclear receptor subfamily 2 group F member 2 antisense RNA 1 (NR2F2-AS1) was found to facilitate the proliferation of prostate cancer cells by positively regulating cyclin-dependent kinase 4 (CDK4) (7). LINC01133 was found to inhibit SRY-box transcription factor 4 (SOX4) expression by recruiting enhancer of zeste homolog 2 (EZH2) to the SOX4 promoter, inhibiting breast cancer cell metastasis. The decreased expression of LINC01133 is also associated with poor patient prognoses (8). Additionally, lncRNA cancer susceptibility 19 (CASC19) has been reported to serve an oncogenic role in gastric cancer progression (9). However, the expression of CASC19 in glioma and its underlying mechanism remains largely undefined.

It is well known that microRNAs (miRNAs or miRs) suppress the translation of specific genes through base pairing with specific target sites between the 3'-untranslated region (3'-UTR) of certain genes under physiological or pathological conditions (10,11). It has been demonstrated that the abnormal expression of miRNAs is significantly associated with the tumorigenesis and development of tumors, and serves a role

in tumor promotion or inhibition (12). For example, miR-17 functions as an oncogene to promote liver cancer progression by downregulating Smad3 (13). miR-139-5p inhibits cervical cancer cell proliferation and metastasis by negatively regulating transcription factor 4 expression and inhibiting the Wnt/β-catenin signaling pathway (14). It has been indicated that the expression of miR-454-3p is decreased in glioma, suggesting that miR-454-3p exerts a suppressive effect on glioma (15). However, the upstream mechanism that causes miR-454-3p dysregulation requires further study.

Ras-related protein in brain 5A (RAB5A), belongs to the Rab GTPase family and is fundamental for the regulation of vesicle trafficking and membrane-cytoskeletal interactions (16). Recent studies have reported that RAB5A is upregulated in diverse human malignancies, including cervical cancer, pancreatic cancer and glioma (17-20), suggesting that it exerts a cancer-promoting effect. However, the mechanism that causes the upregulation of RAB5A in glioma has not yet been fully elucidated.

Bioinformatics analysis suggested that RAB5A contained potential binding sites for miR-454-3p (http://www.targetscan.org/vert_72/). Further analysis also revealed potential binding sites between CASC19 and miR-454-3p (http://starbase.sysu.edu.cn/index.php). The current study confirmed that CASC19 is significantly upregulated in glioma and its high expression is associated with unfavorable clinicopathological parameters and adverse characteristics in MRI images. Functional experiments suggested that CASC19 promotes glioma progression by sponging miR-454-3p and upregulating RAB5A expression. The present study revealed that the CASC19/miR-454-3p/RAB5A axis participates in the promotion of glioma progression, which may elucidate novel markers/agents for the diagnosis and treatment of patients with glioma.

Materials and methods

Tissue collection and clinical information. All patients enrolled in the current study provided written informed consent. The mean age ± standard deviation was 60.33±16.38 years (range, 29-86 years). Among the patients, 18 were males and 17 were females. The collection of tumor and paracancerous brain tissues of patients with glioma was approved by the Ethics Review Board of Xiangyang Central Hospital Affiliated to Hubei University of Arts and Science. Surgically removed tissue samples were immediately frozen and stored in liquid nitrogen at -196˚C for further study and analysis. Primary glioma was confirmed in all patients following clinical examination, imaging and pathological analysis (Fig. S1). Corresponding clinical information and MRI images were obtained from the Department of Neurosurgery and the Department of Radiology (Xiangyang Central Hospital), respectively.

Cell culture. Human glioma cell lines [U251, LN229, U87MG (glioblastoma of unknown origin) and A172] and normal human astrocytes (NHAs) were procured from the American Type Culture Collection. All cells were cultured in DMEM/F12 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (HyClone; Cytiva) and 100 U/ml penicillin (Sigma-Aldrich; Merck KGaA). Cells were cultured at 37˚C with 5% CO_2 and a saturated humidity.

Cell transfection. CASC19 small interfering (si)RNA (si-CASC19), negative control siRNA (si-NC), miR-454-3p mimics (miR-454-3p), miR-454-3p inhibitors (anti-miR-454-3p), miRNA negative controls, overexpressing CASC19 plasmids and empty vector plasmids were designed and constructed by Guangzhou RiboBio Co., Ltd.. According to the manufacturers' protocol, glioma cells were transfected using Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.). After 24 h, transfection efficiency was examined via reverse transcription-quantitative PCR (RT-qPCR) prior to functional experiments.

RT-qPCR. In accordance with the manufacturer's instructions, RNA was extracted using the TRIzol reagent (Thermo Fisher Scientific, Inc.). Subsequently, RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent kit (Takara Biotechnology Co., Ltd.). SYBR® Premix Ex Taq™ II (Takara Biotechnology Co., Ltd.) was used to perform RT-qPCR. The reaction conditions were as follows: Pre-denaturation at 90˚C for 10 min, followed by 40 cycles at 85˚C for 15 sec and 55˚C for 15 sec. The relative expression levels of CASC19, miR-454-3p and RAB5A were evaluated using the $2^{-\Delta\Delta Cq}$ method (21), with GAPDH and U6 as internal controls. The primer sequences were as follows: CASC19 forward, 5'-CTCAGCATTTGCCATACT ACAT-3' and reverse, 5'-TTCTAACCCAGGCACTCCAA-3'; miR-454-3p forward, 5'-GCGCGTAGTGCAATATTGCTTA-3' and reverse, 5'-AGTGCAGGGTCCGAGGTATT-3'; RAB5A forward, 5'-GCATGGGTCCCTCTCACTAA-3' and reverse, 5'-CAGTGTGGAGAAATGGGCTG-3'; U6 forward, 5'-CGC TTCGGCAGCACATATAC-3' and reverse, 5'-TTCACGAAT TTGCGTGTCAT-3'; GAPDH forward, 5'-AGTGCAGGGTCC GAGGTATT-3' and reverse, 5'-AGGCTGTTGTCATACTTC-3'.

Cell counting Kit-8 (CCK-8). Glioma cells were trypsinized with trypsin and prepared into single-cell suspensions. Samples were then seeded in 96-well plates at a density of 1,000 cells/well, with 3 replicate wells in each group. After cells had attached, 10 μl CCK-8 solution (Beyotime Institute of Biotechnology) was added to each well and a blank control well containing only medium and CCK-8 solution was set. Cells were subsequently incubated for 2 h at 37˚C. Absorbance values of each well were measured using a microplate reader (DG5032; Huadong) at a wavelength of 450 nm. The plate was measured at intervals of 24 h for a total of 4 days. A cell survival curve was plotted against time and absorbance.

BrdU assay. Glioma cells were incubated with 10 μl BrdU solution at 37˚C for 4 h. The medium and BrdU solution were subsequently discarded and cells were washed with PBS in triplicate. Cells were then incubated with fixing/denaturing solution for 30 min at room temperature, after which the solution was discarded. Cells were incubated with Alexa Fluor® 647-conjugated anti-BrdU monoclonal antibodies (Abcam; cat. no. ab220075; dilution 1:300) for 10 h at 4˚C for 2 h at room temperature. Nuclei were subsequently stained with DAPI and cells were observed and photographed under a fluorescence microscope (Olympus Corporation; magnification, x20).

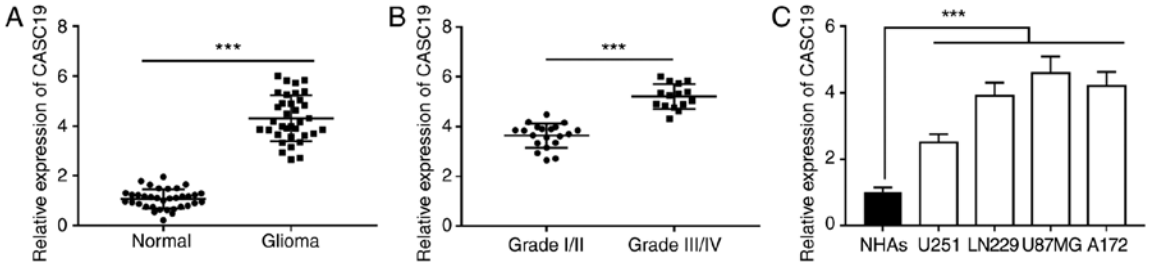

Figure 1. CASC19 expression is significantly upregulated in glioma tissues and cell lines. (A) RT-qPCR was performed to detect the expression of CASC19 in glioma and healthy brain tissues. (B) CASC19 levels in patient tissues was compared between grade I/II and III/IV gliomas. (C) CASC19 levels in glioma cell lines [U251, LN229, U87MG (glioblastoma of unknown origin) and A172] and NHAs were compared. ***P<0.001. CASC19, cancer susceptibility 19; NHAs, normal human astrocytes; RT-qPCR, reverse transcription-quantitative PCR.

Wound healing assay. Cells ($2x10^6$ cells/well) were seeded in a 12-well plate. After cells were covered the culture plate, a scratch was formed on the cell monolayer using a 10-μl pipette. Samples were then washed with PBS and the scratches were photographed. Cells were further cultured for 24 h with serum-free medium in a 37˚C and 5% CO_2 incubator. The scratches were observed and photographed a second time and the degree of wound healing was evaluated a by light microscope (CKX41; Olympus, magnification, x100).

Transwell assays. Cell migration and invasion assays were performed using Transwell chambers (Corning Inc.; 8 μM pore diameter), coated with Matrigel (invasion assay) or without Matrigel (migration assay). A total of $2x10^5$ cells suspended in serum-free medium were added into the upper chamber and 800 μl medium containing 10% FBS was added into the lower chamber. After 48 h of culture, cells remaining on the surface of the upper membrane were removed and cells passing through pores were fixed with 4% paraformaldehyde for 5 min at room temperature and stained with 0.1% crystal violet for 15 min at room temperature. The membranes were cut and observed under an Olympus microscope (magnification, x100). In each group, migrated or invaded cells were counted in five randomly selected fields.

Western blotting. After cells were lysed with RIPA lysis buffer containing phenylmethylsulfonyl fluoride (Sigma-Aldrich; Merck KGaA), the resulting supernatant was collected via high-speed centrifugation (12,000 x g for 10 min at 4˚C). The supernatant was subsequently denatured by heating in a water bath at 100˚C for 15 min. SDS-PAGE was performed after proteins were quantified using the bicinchoninic acid method (Beyotime Institute of Biotechnology). Samples were then transferred onto PVDF membranes. Primary antibodies (anti-RAB5A; Abcam; product code ab18211; dilution 1:1,000) were added and the membranes were incubated at 4˚C overnight. After washing samples with Tris-buffered saline with Tween-20 (TBST), goat anti-rabbit IgG secondary antibody (Abcam; product code ab205718; dilution 1:2,000) was added and incubated at room temperature for 1 h. Following additional rinsing with TBST, hypersensitive ECL (Hubei Biossci Biotechnology Co., Ltd.) was added to the membranes and enhanced chemiluminescence (Biozym, Inc.) was detected. The western blotting results were quantified by ImageJ software (version 1.52p; National Institutes of Health).

Luciferase reporter assay. The luciferase reporter gene assay was conducted using the dual-luciferase reporter assay system (Promega Corp.). The target fragments of wild-type and mutant CASC19 were constructed and integrated into pGL3 vectors (Promega Corp.) to construct pGL3-CASC19 wild-type (CASC19-WT) and pGL3-CASC19-mutant (CASC19-MUT) reporter vectors. Cells were co-transfected with CASC19-WT or CASC19-MUT and miR-454-5p mimics or negative controls. After 48 h of transfection, luciferase activity was determined in accordance with the manufacturer's protocol.

RNA immunoprecipitation (RIP) assay. The RNA immunoprecipitation assay was performed using the EZ-Magna RIP kit (EMD Millipore) according to the manufacturer's protocol. Human anti-Ago2 antibodies were obtained from EMD Millipore and IgG was used as the control. RNA concentration was measured using a NanoDrop spectrophotometer and purified RNA was measured via RT-qPCR.

Statistical analysis. All statistical analyzes were conducted using SPSS 22.0 (IBM Corp.) and GraphPad Prism 5.0 software (GraphPad Software, Inc.), and all data are expressed as the means ± standard deviation. Whether or not the data were normally distributed the data were examined using the Kolmogorov-Smirnov test. For normally distributed data, Student's t-tests was used to analyze differences between two groups. A one-way analysis of variance followed by Tukey's post hoc test was used to analyze differences among multiple groups. For data that were not normally distributed, comparisons between 2 groups were performed by a paired sample Wilcoxon signed-rank test. Spearman's correlation among CASC19, miR-454-3p and RAB5A expression levels in glioma tissues was calculated. A Chi-square test was used to analyze the relationship between CASC19, clinicopathological parameters and features of MRI images. P<0.05 was considered to indicate a statistically significant difference.

Results

CASC19 expression and significance in glioma. To investigate the role of CASC19 in glioma, CASC19 expression in tumor and paracancerous tissue of 35 patients with glioma were examined via RT-qPCR. The results demonstrated that CASC19 expression was significantly upregulated in glioma tissue (Fig. 1A). Additionally, CASC19 expression was

significantly higher in grade III/IV gliomas compared with grade I/II gliomas (Fig. 1B). A high expression of CASC19 was also associated with increased tumor volume, heterogeneity of MRI signals and severe peritumoral edema in MRI images (Tables I and II). Furthermore, CASC19 expression in human glioma cell lines (U251, LN229, U87MG and A172 cells) was also significantly upregulated compared with the NHA cells (Fig. 1C). These data suggest that CASC19 expression is abnormally and highly expressed in glioma, possibly contributing to glioma progression.

CASC19 enhances glioma cell proliferation, migration and invasion. The aforementioned results revealed that among the five glioma cells assessed in the current study, CASC19 expression was the lowest in U251 cells and highest in U87MG cells. Therefore, the overexpression CASC19 plasmid and the empty plasmid were transfected into U251 cells, respectively. Additionally, CASC19 siRNA and control siRNA were transfected into U87MG cells. The results of RT-qPCR confirmed the successful construction of CASC19 overexpression and knockdown models (Fig. 2A). The results of CCK-8 and BrdU experiments revealed that CASC19 overexpression promoted the proliferation of U251 cells compared with the control group (Fig. 2B-D). Wound-healing and Transwell assays were also performed. The results demonstrated that CASC19 upregulation markedly enhanced the movement, migration and invasion of U251 cells compared with the control group (Fig. 2E-H). Conversely, CASC19 knockdown in U87MG cells demonstrated the opposite effect (Fig. 2B-H). The results indicated that CASC19 enhances glioma cell proliferation, migration and invasion.

CASC19 directly targets miR-454-3p. In recent years, non-coding RNAs (ncRNAs), including lncRNAs, have become the focus of cancer research. As an miRNA sponge, lncRNA is involved in regulating the biological behavior of cells. StarBase V3.0 (http://starbase.sysu.edu.cn/index.php) was used to predict the miRNAs that interact with CASC19. The results elucidated a potential binding site between CASC19 and miR-454-3p (Fig. 3A). To validate this prediction, a dual luciferase assay was performed. It was revealed that miR-454-3p mimics significantly inhibited CASC19-WT luciferase activity, but did not significantly affect CASC19-MUT (Fig. 3B). RIP experiments confirmed that CASC19 and miR-454-3p were enriched in Ago2-containing microribonucleoproteins compared with control IgG-treated cells (Fig. 3C). Furthermore, CASC19 overexpression in U251 cells significantly inhibited miR-454-3p expression, whereas CASC19 knockdown in U87MG cells resulted in increased miR-454-3p expression (Fig. 3D). Taken together, the results indicated that CASC19 adsorbed and suppressed miR-454-3p in glioma cells.

miR-454-3p suppresses glioma cell proliferation and metastasis. To investigate the role of miR-454-3p in glioma, RT-qPCR was performed to detect miR-454-3p expression in glioma tissues and cell lines. The results indicated that miR-454-3p expression was downregulated in glioma tissues and cell lines when compared with normal tissues and NHAs, repectively (Fig. 4A). To explore the biological role of miR-454-3p in glioma, miR-454-3p mimics and their controls were transfected into U87MG cells, respectively. Additionally, anti-miR-454-3p and its controls were transfected into U251 cells. Transfection was successfully verified via RT-qPCR (Fig. 4B). Glioma cell proliferation and metastasis was assessed by CCK-8, BrdU, wound healing

Table I. Association between CASC19 expression and clinical characteristics in glioma patients.

Variables	No. of cases	CASC19 expression		χ^2	P-value
		High	Low		
All cases	35	19	16		
Age, years					
<60	14	6	8	1.228	0.268
≥60	21	13	8		
Sex					
Male	18	12	6	2.289	0.130
Female	17	7	10		
Mean tumor diameter					
<3 cm	13	4	9	4.609	0.031
≥3 cm	22	15	7		
Pathological type					
Astrocytoma	11	4	7	2.076	0.354
Oligodendroglioma	16	10	6		
Others	8	5	3		
Tumor location					
Frontal lobe	6	4	2	1.908	0.591
Temporal lobe	11	7	4		
Parietal lobe	12	6	6		
Others	6	2	4		

CASC19, lncRNA cancer susceptibility 19.

Table II. Association between CASC19 expression and MRI features in glioma patients.

Variables	No. of cases	CASC19 expression		χ^2	P-value
		High	Low		
All cases	35	19	16		
Tumor signal					
Uniform	13	4	9	4.609	0.031
Non-uniform	22	15	7		
Peritumoral edema					
Mild	17	6	11	4.804	0.028
Moderate to severe	18	13	5		

CASC19, lncRNA cancer susceptibility 19; MRI, magnetic resonance imaging.

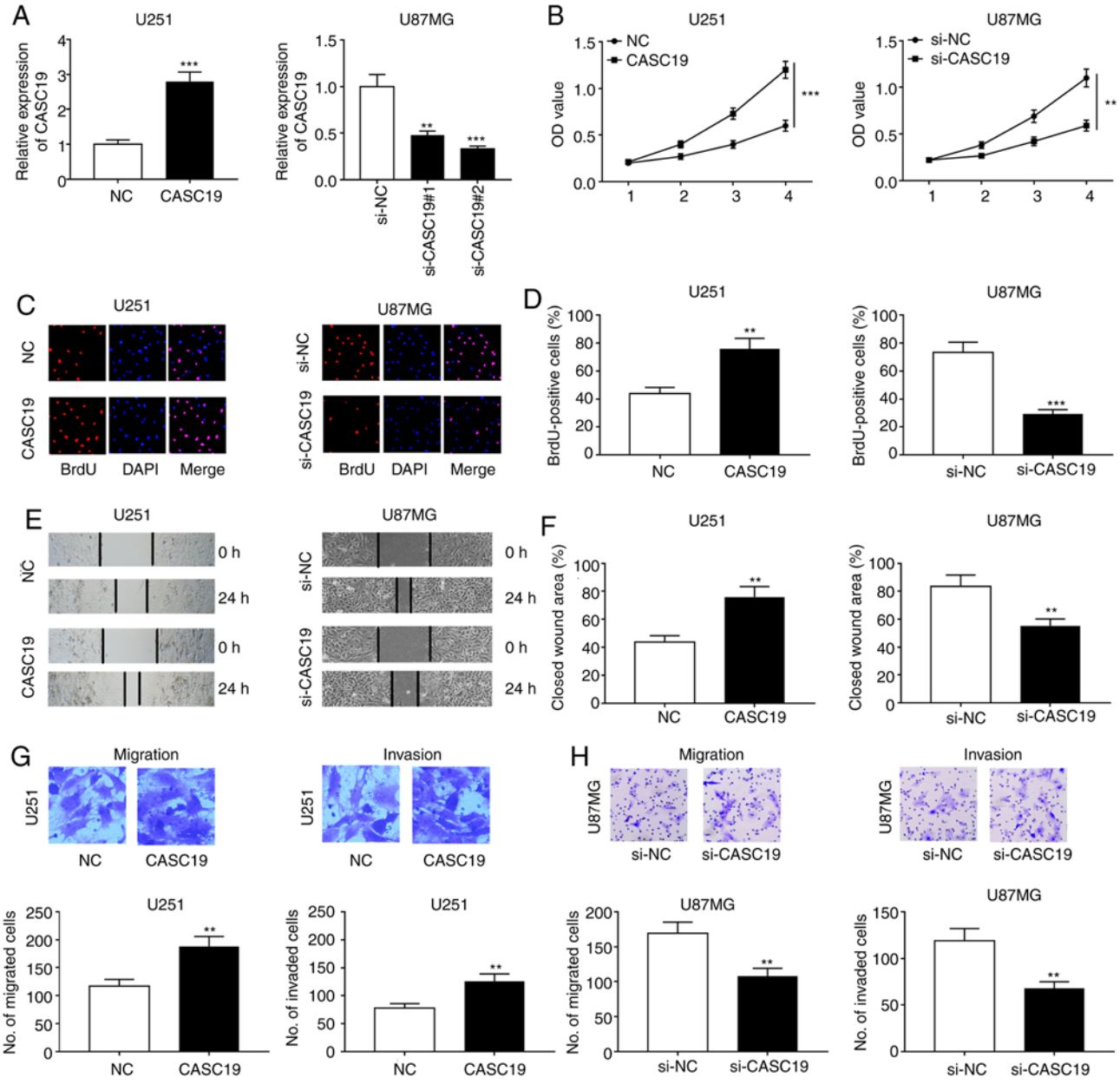

Figure 2. CASC19 promotes glioma cell proliferation, migration and invasion. (A) Transfection efficacy of U251 cells transfected with CASC19 overexpression plasmid and U87MG cell transfected with si-CASC19#1 and #2 was confirmed via RT-qPCR. **P<0.01 and ***P<0.001 vs. the NC or si-NC group. (B) Glioma cell proliferation was detected via Cell Counting Kit-8 in both transfected cell lines. **P<0.01 vs. the NC or si-NC group. (C and D) Glioma cell proliferation was detected by BrdU assays. **P<0.01 and ***P<0.001 vs. the NC or si-NC group. (E and F) Wound healing experiments were performed to detect glioma cell movement. **P<0.01 vs. the NC or si-NC group. (G and H) Transwell assays was performed to detect glioma cell migration and invasion. **P<0.01 vs. the NC or si-NC group. CASC19, cancer susceptibility 19; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR; siRNA or si, small interfering RNA.

and Transwell assays. It was demonstrated that miR-454-3p upregulation inhibited glioma cell proliferation, migration and invasion (Fig. 4C-F). Conversely, miR-454-3p downregulation enhanced the aforementioned biological behaviors of glioma cells (Fig. 4C-F).

CASC19 is involved in the regulation of glioma cell proliferation and metastasis by sponging miR-454-3p. To explore the mechanism of the CASC19/miR-454-3p axis in glioma progression, miR-454-3p mimics and CASC19 overexpression plasmids were co-transfected into U87MG cells. The results of RT-qPCR indicated that combined transfection of the miR-454-3p mimics and CASC19 overexpression plasmids significantly suppressed the miR-454-3p expression in U87MG cells compared with miR-454-3p mimic-treated cells alone (Fig. 5A). CCK-8, BrdU, wound healing and Transwell assays were then performed. The results revealed that co-transfection of miR-454-3p mimics and CASC19 overexpression plasmids markedly enhanced cell proliferation, migration and invasion compared with miR-454-3p mimic transfection alone group (Fig. 5B-E). Furthermore, anti-miR-454-3p and CASC19 siRNA co-transfection significantly suppressed the aforementioned malignant biological behaviors of U251 cells compared with anti-miR-454-3p alone (Fig. 5B-D and F). The results indicated that CASC19 was involved in the regulation of glioma progression by adsorbing miR-454-3p.

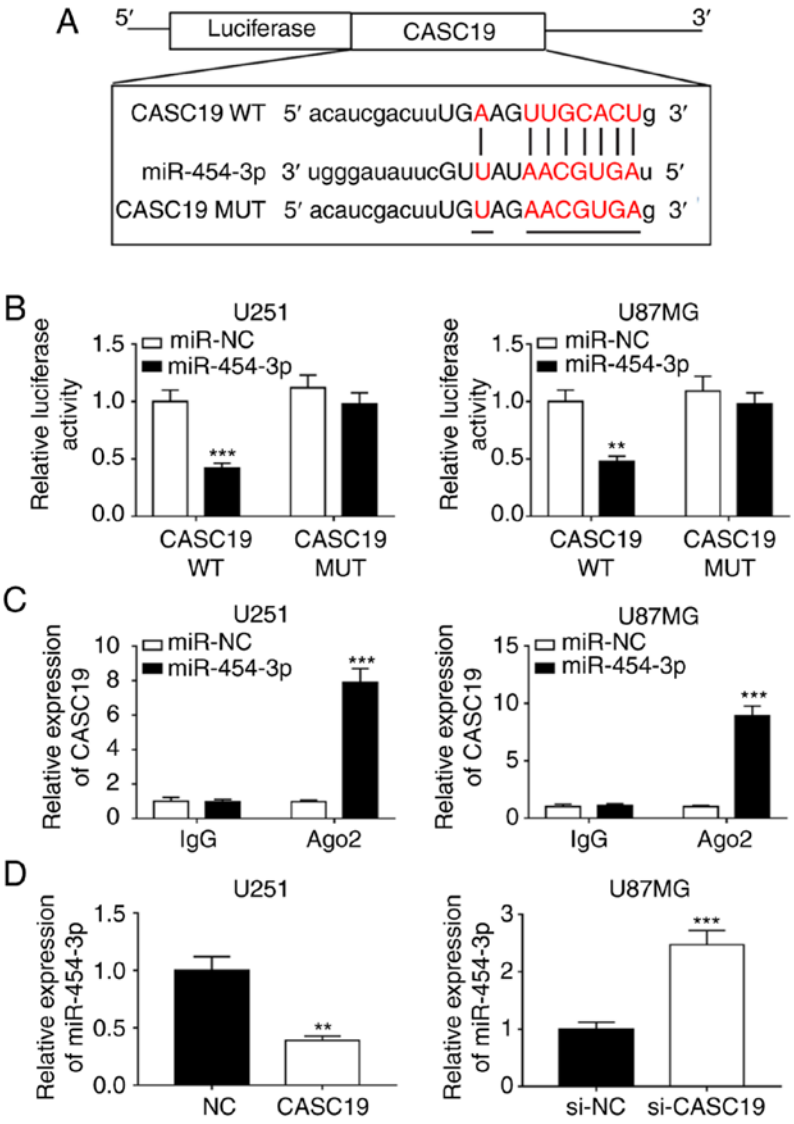

Figure 3. miR-454-3p is a target for CASC19 in gliomas. (A) StarBase 3.0 (http://starbase.sysu.edu.cn/index.php) was used to predict the potential binding site between CASC19 and miR-454-3p. (B) Dual luciferase reporter assay results demonstrated that miR-454-3p negatively regulated the luciferase activity of wild-type (WT) CASC19, but had no significant effect on mutant (MUT) CASC19. **P<0.01 vs. the miR-NC group. (C) An RIP assay was performed to validate the direct interaction between CASC19 and miR-454-3p. ***P<0.001 vs. the miR-NC group. (D) Expression of miR-454-3p was detected via reverse RT-qPCR after CASC19 upregulation or downregulation in glioma cells. **P<0.01 and ***P<0.001 vs. the NC or si-NC group. miR, microRNA; CASC19, cancer susceptibility 19; RIP, RNA immunoprecipitation; RT-qPCR, reverse transcription-quantitative PCR; siRNA or si, small interfering RNA.

miR-454-3p targets RAB5A in glioma. To screen the downstream targets of miR-454-3p, TargetScanHuman (http://www.targetscan.org/vert_72/) and miRDB (http://mirdb.org/miRDB/) databases were searched. The results revealed that RAB5A was a candidate target of miR-454-3p (Fig. 6A). To determine whether miR-454-3p interacts with the 3'-UTR of RAB5A, a luciferase reporter assay was performed. The results demonstrated that miR-454-3p markedly decreased the luciferase activity of wild-type RAB5A, but did not significantly affect the luciferase activity of mutant RAB5A (Fig. 6B). Additionally, RT-qPCR and western blotting revealed that miR-454-3p overexpression significantly inhibited RAB5A mRNA and protein expressions, while knockdown of miR-454-3p significantly increased RAB5A expression in glioma cells (Fig. 6C and D). Additionally, CASC19 overexpression induced RAB5A upregulation in U251 cells, while CASC19 knockdown significantly inhibited RAB5A expression in U87MG cells (Fig. 6E and F). Compared with CASC19 overexpression, RAB5A levels were significantly decreased after CASC19 plasmid and miR-454-3p mimic co-transfection (Fig. S2). The correlations among CASC19, miR-454-3p and RAB5A in glioma tissues was subsequently assessed in glioma samples. The results indicated that there was a negative association between CASC19 and miR-454-3p, which further validated the regulatory relationship between them. It was further demonstrated that the expression of miR-454-3p was negatively associated with RAB5A, while CASC19 was positively associated with RAB5A (Fig. S3). These data indicated that RAB5A was a target gene of miR-454-3p and that it was positively and negatively regulated by CASC19 and miR-454-3p, respectively.

Discussion

Glioma is the most common malignancy of the brain and is characterized by rapid proliferation, invasive growth, intracranial

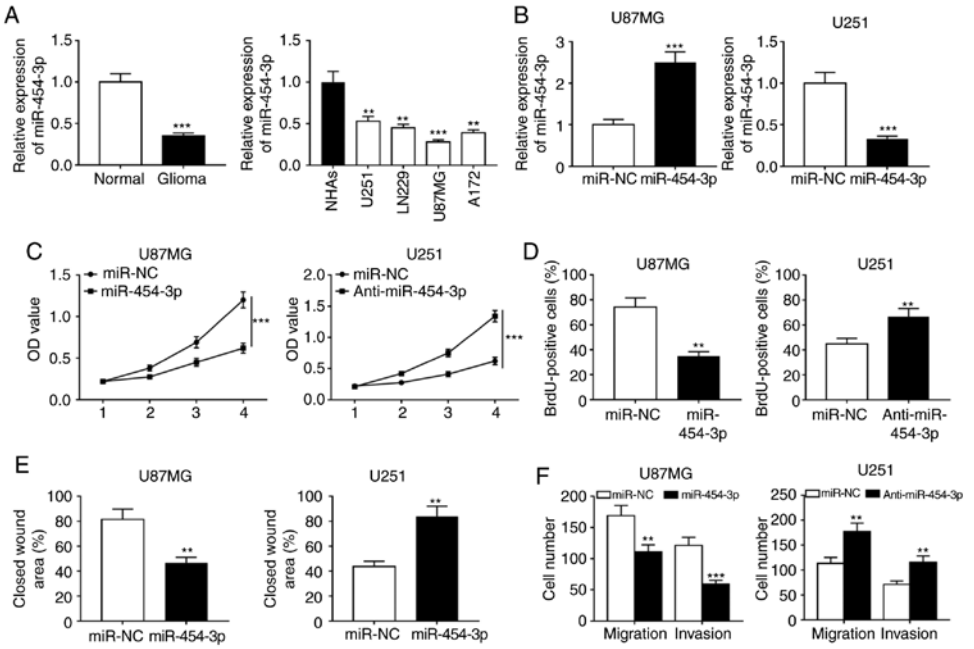

Figure 4. miR-454-3p significantly inhibits the malignant biological behavior of glioma cells. (A) Expression of miR-454-3p in glioma and normal tissues and in glioma cell lines [U251, LN229, U87MG (glioblastoma of unknown origin) and A172] and NHAs were detected via RT-qPCR. **P<0.01 and ***P<0.001, compared with normal tissue or NHAs. (B) Transfection efficiency was also detected via RT-qPCR. ***P<0.001 vs. miR-NC group. (C and D) Cell proliferation was detected using Cell Counting Kit-8 and BrdU assays. **P<0.01 and ***P<0.001 vs. the miR-NC group. (E) The motility of glioma cells was detected by performing a wound healing assay. **P<0.01 vs. the miR-NC group. (F) The migration and invasion abilities of glioma cells was detected following Transwell assays. **P<0.01 and ***P<0.001 vs. the miR-NC group. NHAs, normal human astrocytes; miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR.

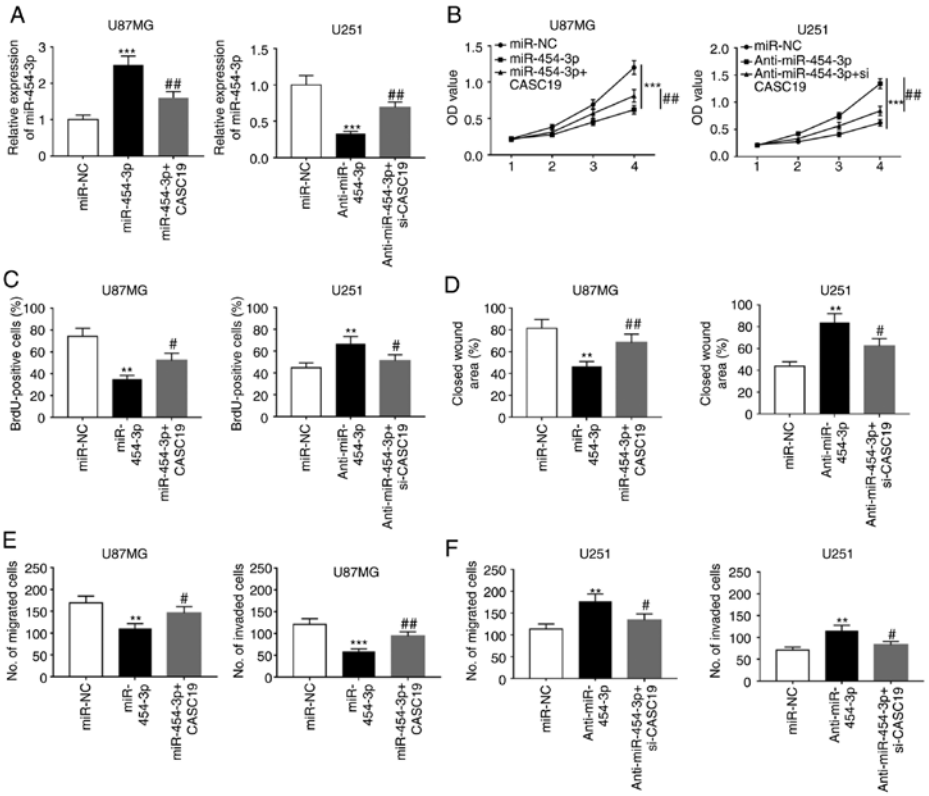

Figure 5. CASC19/miR-454-3p axis regulates the malignant phenotype of glioma cells. (A) miR-454-3p and CASC19 overexpression plasmids were transfected into U87MG cells and anti-miR-454-3p and CASC19 siRNA were co-transfected into U251 cells. Expression of miR-454-3p in glioma cells was detected via RT-qPCR. ***P<0.001 vs. the miR-NC group; $^{\#\#}$P<0.01 vs. the miR-454-3p or anti-miR-454-3p alone group. (B and C) Proliferation ability of the glioma cells was detected by Cell Counting Kit-8 and BrdU assays. **P<0.01 and ***P<0.001 vs. the miR-NC group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the miR-454-3p or anti-miR-454-3p alone group. (D) Motility of glioma cells was detected by performing a wound healing assay. **P<0.01 vs. the miR-NC group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the miR-454-3p or anti-miR-454-3p alone group. (E and F) The invasion and migration of glioma cells were detected via Transwell assays. **P<0.01 and ***P<0.001 vs. the miR-NC group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the miR-454-3p or anti-miR-454-3p alone group. CASC19, cancer susceptibility 19; miR, microRNA; siRNA or si, small interfering RNA; RT-qPCR, reverse transcription-quantitative PCR.

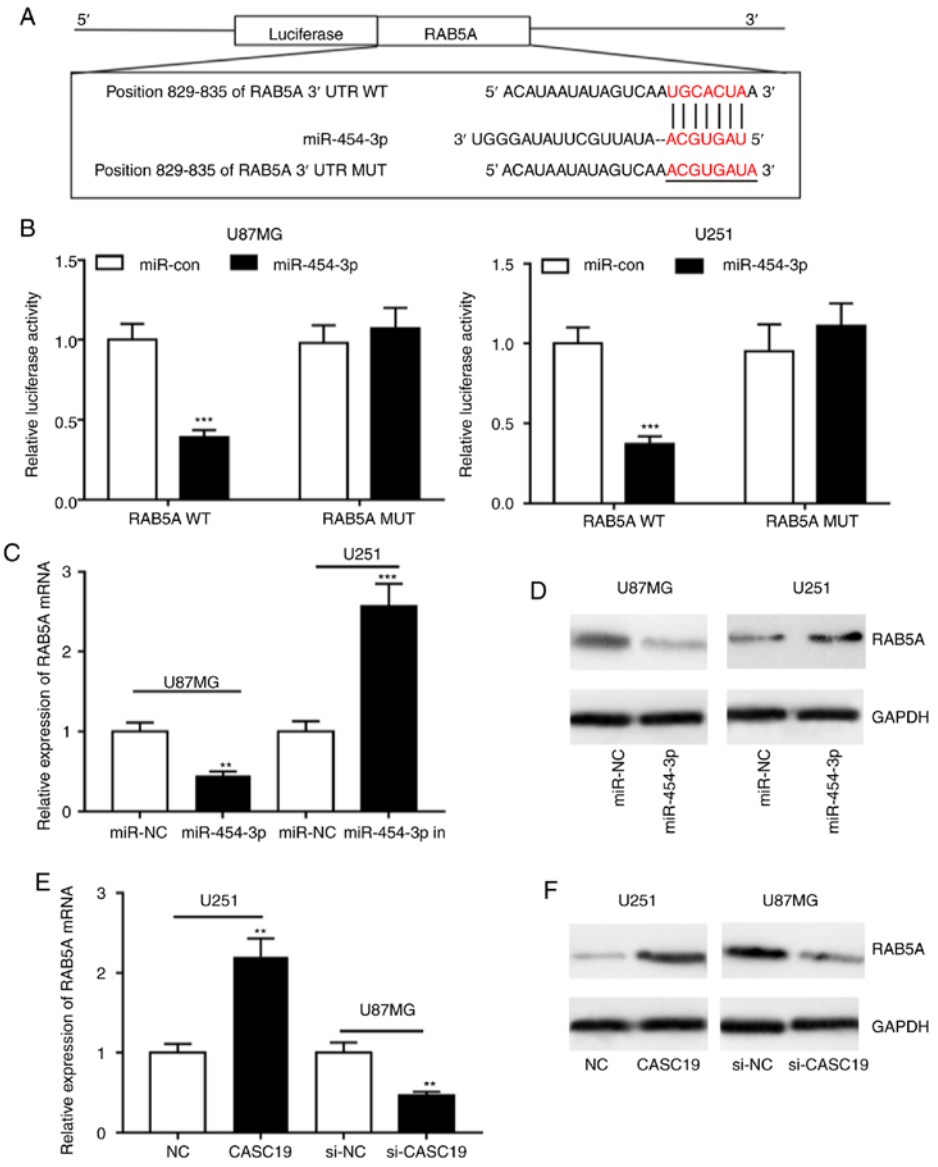

Figure 6. CASC19/miR-454-3p axis regulates the expression of RAB5A in gliomas. (A) The binding site between miR-454-3p and RAB5A was predicted using the TargetScan database (http://www.targetscan.org/vert_72/). (B) Interaction between miR-454-3p and RAB5A was verified using a luciferase reporter gene assay. ***P<0.001 vs. the miR-con group. (C and D) After upregulating or downregulating miR-454-3p (in), RT-qPCR and western blotting were performed to detect RAB5A mRNA and protein expression, respectively. **P<0.01 and ***P<0.001 vs. the miR-con group. (E and F) After CASC19 overexpression or knockdown (si-CASC190, the expression of RAB5A mRNA and protein were detected via RT-qPCR and western blotting, respectively. **P<0.01 vs. the NC or si-NC group. CASC19, cancer susceptibility 19; miR, microRNA; RAB5A, ras-related protein in brain 5; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; siRNA or si, small interfering RNA.

metastases and frequent postoperative recurrence (22). In recent years, long non-coding RNAs (lncRNAs) have been reported to be dysregulated in glioma, participating in the regulation of malignant tumor cell phenotypes and serving a role as a cancer-promoting factor or tumor suppressor (23). A previous study demonstrated the clinical significance of lncRNAs in a variety of human malignancies, including gliomas (24). For instance, lncRNA forkhead box D1 antisense RNA 1 (FOXD1-AS1) was found to enhance cell proliferation and metastasis and was found to be significantly associated with increased glioma grade, meaning its high expression may be used as an indicator in glioma diagnosis (25). Furthermore, lncRNA small nucleolar RNA host gene 20 (SNHG20) was found to promote glioma development by activating the PI3K/Akt/mTOR signaling pathway (26). The results of the present study demonstrated that lncRNA cancer susceptibility 19 (CASC19) expression is upregulated in glioma tissues and cell lines, and its high expression is positively associated with glioma tumor diameter and pathological grade. Additionally, its high expression was found to be significantly associated with tumor MRI signal heterogeneity and peritumoral edema, which indicates an unfavorable prognosis. Furthermore, functional experiments suggested that CASC19 promoted the proliferation, migration and invasion of glioma cells. To the best of our knowledge, the present study identified for the first time that CASC19 is an oncogenic lncRNA in glioma.

As one of the ncRNA families, miRNAs are involved in a variety of tumor cell physiological and pathological processes (27). miRNAs bind directly to target mRNA, thereby degrading mRNA or inhibiting the translation

progression (28). It has been reported that miR-454-3p exerts anticancer effects in various human tumors, including bladder cancer and glioma. In terms of mechanism, miR-454-3p suppresses the migration and invasion of bladder cancer cells by targeting zinc finger E-box-binding homeobox 2 (ZEB2) (29). miR-454-3p was found to be downregulated in glioma tissues and to negatively regulate nuclear factor of activated T-cells, cytoplasmic 2 (NFATc2) to inhibit glioma progression (30). Consistent with previous studies, the present study demonstrated that expression of miR-454-3p is significantly decreased in cancerous tissues and cell lines. However, transfection of miR-454-3p mimics markedly inhibited the proliferation, migration and invasion of glioma cells. Conversely, the inhibition of miR-454-3p significantly promoted the aforementioned biological behaviors of glioma cells. These data confirmed that miR-454-3p exerted a tumor suppressive effect in glioma.

In recent years, it has been demonstrated that lncRNAs can act as a sponge of competitive endogenous (ce)RNAs, thereby modulating target gene expression (4). For instance, lncRNA deleted in lymphocytic leukemia 1 (DLEU2) was found to promote glioma proliferation and metastasis by sponging miR-186-5p and upregulating pyruvate dehydrogenase lipoamide kinase isozyme 3 (PDK3) (31). LINC00243 was found to enhance the proliferation of non-small cell lung cancer cells by modulating the miR-507/PDK4 axis (32). Given that CASC19 and miR-454-3p exert opposite biological effects in gliomas, the present study hypothesizes that there may be a regulatory relationship between the two. Interestingly, a binding site between CASC19 and miR-454-3p was elucidated via bioinformatics analysis. This was subsequently confirmed by a luciferase reporter assay. The results of the RIP assay also demonstrated that CASC19 could adsorb miR-454-3p. Additionally, CASC19 depletion caused an increase in miR-454-3p expression. Furthermore, the results revealed that the miR-454-3p mimics reversed the promoting effects of CASC19 overexpression on glioma cell proliferation and metastasis. CASC19 knockdown induced the inhibition of glioma cell proliferation, migration and invasion. This was partially attenuated by miR-454-3p inhibitors. Therefore, it was concluded that CASC19 may be involved in the regulation of glioma cell proliferation, apoptosis, migration and invasion by adsorbing miR-454-3p expression.

RAB5A, a member of the small GTPase Rab subfamily, is an important regulator of vesicles moving from the plasma membrane to early endosomes (33). Recently, it has been reported that RAB5A serves as an oncogene and is upregulated in various types of cancer, including oral cancer, cervical cancer, liver cancer and glioma (18,20,34-36). ERK/matrix metallopeptidase (MMP) is one of the most typical downstream signaling pathways activated by RAB5A (34). In this process, RAB5A overexpression upregulates MMP-2 expression, but also upregulates the phosphorylation of ERK, inducing cyclin D1, cyclin A and cyclin E expression to promote tumor progression (34). In addition, RAB5A is regulated by multiple miRNAs, such as miR-101 and miR-494 (20,37). Using TargetScan, it was demonstrated that miR-454-3p and RAB5A had a potential binding site. It was confirmed using a luciferase reporter experiment that miR-454-3p could bind to the 3'-UTR of RAB5A. Further assessment revealed that miR-454-3p induced RAB5A downregulation in glioma cells. However, miR-454-3p downregulation caused an upregulation of RAB5A. Further, CASC19 overexpression promoted RAB5A expression in glioma cells and CASC19 knockdown induced a decrease in RAB5A expression. These data indicated that CASC19 promoted the progression of glioma cells by sponging miR-454-3p and upregulating RAB5A expression.

Taken together, the present study revealed an increased expression of CASC19 in glioma tissues and cells. This was also associated with the malignant clinical features of patients with glioma. Additionally, the results demonstrated that CASC19 enhanced glioma cell proliferation, migration and invasion through the miR-454-3p/RAB5A axis, providing potential therapeutic targets. However, the present study was limited to *in vitro* studies and *in vivo* studies are required to confirm the conclusions drawn. Furthermore, survival analysis based on CASC19 expression should be assessed to further evaluate its role as a biomarker. Most of the patients were survivors and the number of patients involved was relatively small, thus it was difficult to obtain a significant result. A larger number of clinical samples should be utilized and after long-term follow-up.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LZ and JW conceived and designed the experiments. YJW, QSY, HC, JTW and WBW performed the experiments. JTW performed the statistical analysis. YJW, WBW and LZ wrote the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Ethics Review Board of Xiangyang Central Hospital Affiliated to Hubei University of Arts and Science, Xiangyang, Hubei, China. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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