European Review for Medical and Pharmacological Sciences 2019; 23: 7475-7481

Long noncoding RNA CASC15 is upregulated in glioma and facilitates cell proliferation and metastasis via targeting miR-130b-3p

Y. XIE, Y. CHENG

Department of Neurosurgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

Abstract. – OBJECTIVE: Recent studies have discovered a class of long non-coding RNAs (lncRNAs) which are dysregulated in various tumors and linked to carcinogenesis. This paper aimed to uncover the molecular functions of lncRNA CASC15 in glioma tumorigenesis.

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was performed to detect CASC15 expression in 50 glioma samples and 4 glioma cells. Besides, proliferation assay, transwell assay and wound healing assay were performed to explore the role of CASC15 in glioma progression *in*. Furthermore, the interaction between C and miR-130b-3p in glioma was studied by forming the Dual-Luciferase reporter assa addition, tumor formation and metastasis as were performed *in vivo*.

RESULTS: CASC15 expres remark ably upregulated in glioma ples mpared with that in adjacent sam Cell p eration, invasion and migration oma ed via knockdown of SC Mor , miR-130b-3p was u ulated nockdown of CASC15 in vitro. es, miR-13 was a direct target of C in glioma. forma tion and meta is v hibited aft CASC15 was knock wn *in vivo*.

CONC ONS: These lts suggest that CASC1 uld repress meta is and prolifer ation glioma sponging miR-130b-3p *in vitro in viv* ich may offer a new therapeutic i for gli patients.

Key Words:
non-co A, CASC15, Glioma, MiR-130b-3p.

Introduction

ma is one of the most malignant primary intracranial tumors worldwide[1]. Despite therapeutic treatment developments over the last decades, the prognosis remains poor in glioma patients with a median survi f 15 mont Th ore, it is extremely u discover pot therapeutic targets fo oma.

Long non-codin As (lncRNAs) are a sub f non-codi As and participate i ny cellular proces s of tumorigenesis. instance, cRNA PCAT-1 modulates 3-miR-215- T-1-CRKL axis and makes a function umorigenesis of hepatocellula ino LncRNA-CCHE1 expression is posi ated to the malignancy of colorectal carcinoma *via* regulation of the ERK/ pathway[5]. LncRNA SNHG1 could inhi differentiation of Treg cells thereby impeding the immune escape of breast cancer[6]. Activated by ZEB1, lncRNA HCCL5 accelerates cell viability, cell migration, epithelial-mesenchymal transition and the malignancy of hepatocellular carcinoma[7].

In this study, we first found out that the expression level of CASC15 was remarkably upregulated in glioma samples. Moreover, further experiments revealed that CASC15 promoted cell proliferation, invasion and migration of glioma *in vitro* and *in vivo*. Furthermore, we discovered that CASC15 played its function in glioma by sponging miR-130b-3p.

Patients and Methods

Clinical Samples

Human tissues were obtained from 50 glioma patients who underwent surgery at The Second Affiliated Hospital of Chongqing Medical University. All tissues were kept at -80°C. Informed consent was obtained by every glioma patient before the surgery. This study was approved by the Ethics Committee of The Second Affiliated Hospital of Chongqing Medical University.

Corresponding Author: Yuan Cheng, MD; e-mail: 345735915@qq.com

Cell Culture

The glioma cell lines (U87, SHG44, T98, and U373) was obtained from the Chinese Type Culture Collection, Chinese Academy of Sciences (Shanghai, China). The normal human astrocyte 1800 cell line was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The culture medium Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) and 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) were used to incubate the cells.

Cell Transfection

For transfection, lentivirus expressing short-hairpin RNA (shRNA) targeting CASC15 was compounded and then cloned to pLenti-EF1a-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA). CASC15 shRNA (CASC15/shRNA) and empty vector were packaged in 293T cells and for transfection in T98 glioma cells. The entire transfection process was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

RNA Extraction and Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was utilized to isolate total RNA from tissues and cells. SYBR green (Roche, Basel, Switzerland) was conducted to measure the relative expression levels normalized to β-actin. The relative expression was calculated by performing the $2^{-\Delta\Delta CT}$ method. The primers were the following: CASC15 forward: 5′-CATGGAAAACCC-3′ and reverse: 5′-GAGGACCTGAGCTC-AGCC-3′; β-actin forward: 5′-GATGGAA-AGAGGCT-3′ and reverse: 5′-TGG-ACTTA-GGAAATGC-3′. The thermal cycle was as follows: 2 sec at 95°C, 5 sec for 40 cycles at 95°C, 35 sec at 60°C.

Cell Proliferation Assay

For the proliferation (Dojindo Molecular Technologies, Kumamoto, Japan), cell proliferation of treated cells in 96-well plates was monitored by Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan) every 24 h. A spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to measure the absorbance at 450 nm.

Wound Healing Assay

The cells, transferred into 6-well plates, were cultured in DMEM medium overnight. After scratched with a plastic tip, cells were cultured in serum-free DMEM. 48 h later, wound closure was viewed. Each assay was repeated three times independently.

Transwell Assay

The treated cells were transformed to the top of Matrigel-coated invasion chambers (24-well insert, 8-μm pore size; BD Biosciences, Franklin Lakes, NJ, USA) with 200 μL of serum-free DMEM. The bottom chamber was added with DMEM and FBS as a chemoattractant. After being incubated for 48 h, non-invaded cells were removed from the upper part of the insert by cotton swab. The lower cells were fixed in 4% formaldehyde, cells in the lower chamber surface were stained with 0.1% crystal violet. The microscope was used to manually count invading cells in three randomly chosen fields and pictures were taken.

Dual-Luciferase Reporter Assay

For the Luciferase assay, the 3′-UTR of CASC15 was cloned into the pGL3 vector (Promega, Madison, WI, USA), which was identified as wild-type (WT) 3′-UTR. Quick-change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) was used for site-directed mutagenesis of the miR-101-3p binding site in CASC15 3′-UTR, which was named as mutant (MUT) 3′-UTR. Cells were transfected with WT-3′-UTR or MUT-3′-UTR and miR-ctrl or miR-101-3p for 48 h. Then, the Luciferase assay was conducted on the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA).

Tumor Formation Assay

Transfected T98 cells (6×10^5/mL) were replaced into NOD/SCID mice (6 weeks old) subcutaneously. Tumor diameters were detected every 5 days after inoculation. Tumor volume was calculated as the formula (volume= length × width^2 × 1/2). Tumors were extracted after 4 weeks.

Tumor Metastasis Assay

Transfected T98 cells (6×10^5/mL) were injected into the tail vein of NOD/SCID mice (6 weeks old). The mice were sacrificed, and the lung was extracted after 4 weeks. Then, the number of metastatic nodules in the lung was counted.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA) was

utilized to conduct the statistical analysis. The two-tailed Student's *t*-test was performed to analyze the significance. When $p<0.05$, the data were considered statistically significant.

Results

Expression Level of CASC15 in Tissues and Cells of Glioma

First, Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was conducted for detecting CASC15 expression in 50 patients' tissues and 4 glioma cells. As a result, CASC15 was significantly downregulated in tumor tissue samples than that in the adjacent tissues (Figure 1A). Besides, CASC15 level was markedly lower in glioma cells than that in the normal human astrocyte 1800 cell line (Figure 1B).

CASC15 Knockdown Repressed Cell Growth Ability in Glioma Cells

According to CASC15 expression in glioma cells, we chose T98 glioma cells for knockdown of CASC15. The CASC15 shRNA (CASC15 R NA) and the scramble vector (NC) were s sized and transduced into T98 cells. The e CASC15 expression was determined by RT-q (Figure 2A). CCK-8 assay results suggested t cell growth ability of glioma c inhibite after CASC15 was knocked d (F 2B).

CASC15 Knockdown Repressed Cell Migrated and Invaded Ability in Glioma Cells

The wound healing assay results show t the migrated length of glioma cell w ed by knockdown of CASC15 (Fig 2C). Be sides, transwell assay results s d that the number of invaded glioma cells reduced after CASC15 was knocked wn (F 2D).

CASC15 Inhibited oma T origenesis Via Sponging MiR-

We used DI Li E Pre ed v.2 (http://caroli his.athen on.gr/diana_tools/ x.php?r=l ev2%2Findex-pred d) t dict potential target microRNAs of CASC R-130b-3p was one of the ed microR ported to suppress t igenesis of many tumors. The binding ar- f CASC15 b miR-130b-3p was shown in re 3A. Mor er, miR-130b-3p was upregu d in the C 15/shRNA group compared wit NC p (Figure 3B). Furthermore, the res e Luciferase assay showed that the Luciferase activity was significantly re- through the co-transfection of CASC15- d miR-130b-3p, while no significant changes in the Luciferase activity were observed through the co-transfection of CASC15-MUT and miR-130b-3p (Figure 3C).

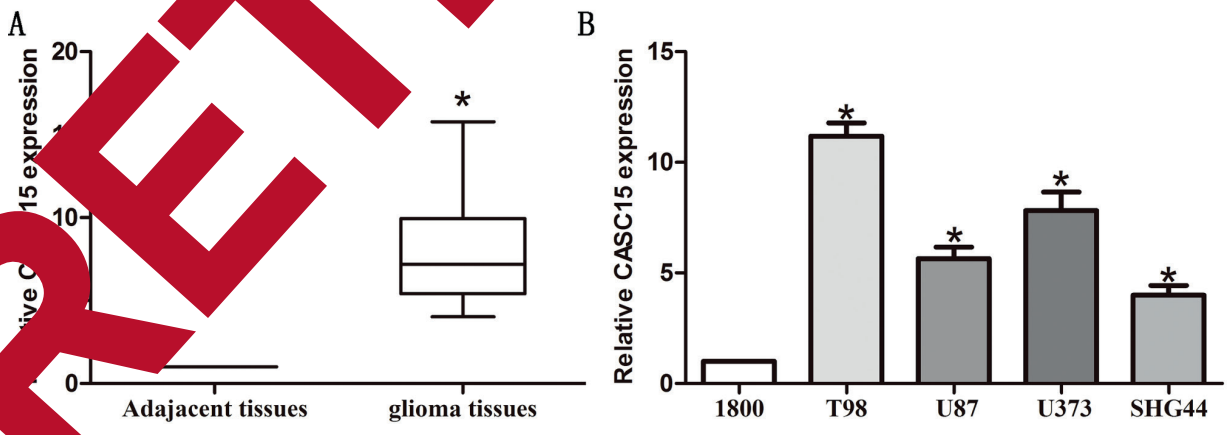
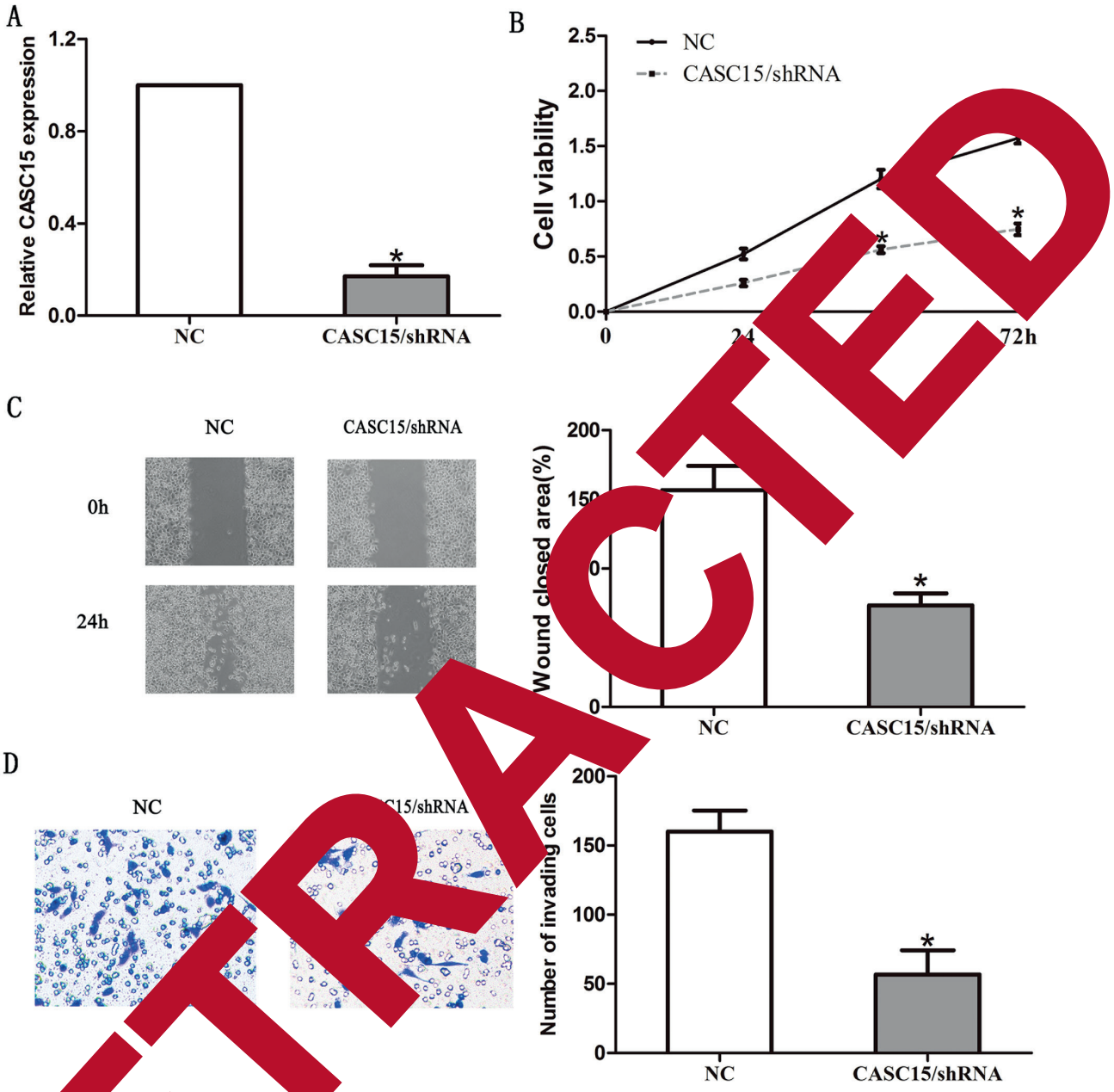


Figure 1. Expression levels of CASC15 were upregulated in glioma tissues and cell lines. ***A,*** CASC15 expression was significantly upregulated in the glioma tissues compared with the adjacent tissues. ***B,*** Expression levels of CASC15 relative to β-actin were determined in the human glioma cell lines and normal human astrocyte 1800 cell line by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.



Figu Knock of CASC15 inhibited glioma cell proliferation, migration and invasion. ***A,*** CASC15 expression in glioma ansdu ith scramble vector (NC) or CASC15 shRNA (CASC15/shRNA) was detected by RT-qPCR. β-actin was used a control CK-8 assay showed that knockdown of CASC15 significantly repressed cell proliferation in T98 glio ***C,*** Wou aling assay showed that the migrated length of cells in the CASC15/shRNA group was markedly ed c d y e NC group in T98 glioma cells (magnification: 40×). ***D,*** The transwell assay showed that the number vaded ls was decreased in the CASC15/shRNA group compared with the NC group (magnification: 40×). The lts repres e average of three independent experiments (mean ± standard error of the mean). *p<0.05.

ASC15 Knockdown Inhibited Tumor mation and Metastasis In Vivo

ability of CASC15 in tumor formation and metastasis was detected *in vivo*. The tumor size in the CASC15/shRNA group was smaller compared with the NC group (Figure 4A). The number of metastatic nodules in the lung from the CASC15/shRNA group was significantly reduced compared to the NC group (Figure 4B). Moreover, the expression level of CASC15 and miR-130b-3p in dissected tumor tissues was detected by RT-qPCR. The results showed that

CASC15 of nude mice was lower-expressed in the CASC15/shRNA group compared with the NC group (Figure 4C), while miR-130b-3p of nude mice was higher-expressed in the CASC15/shRNA group compared with the NC group (Figure 4D).

Discussion

Evidence has proved that lncRNAs participate in the regulation of glioma development. For example, lncRNA HOXD-AS2 regulates cell cycle of glioma and is a potential therapeutic target for glioma[8]. LncRNA MEG3 inhibits glioma proliferation and cell invasion *via* regulating the PI3K/AKT/mTOR pathway[9]. LncRNA ATB promotes tumor metastasis in glioma by suppressing miR-2043p[10].

Cancer susceptibility candidate 15 (CASC15), located on chromosome 6p22.3, is first discovered in silico. Recent studies have indicated that CASC15 plays an important role in tumorigenesis in several cancers. For example, CASC15 was reported[11] to promote cell proliferation in gastric cancer and be a risk factor for gastric cancer prognosis. CASC15 was correlated with poor prognosis of hepatocellular carcinoma, enhanced cell metastasis and hepatocarcinogenesis in hepatocellular carcinoma[12]. In addition, upregulated CASC15 promoted metastasis of melanoma[13]. We showed that the expression of CASC15 was upregulated in both glioma tissues and cells. Furthermore, after CASC15 was knocked down, the cell growth activity, migrated and invaded ability were suppressed in glioma. These data indicated that CASC15 functioned as an oncogene and promoted tumorigenesis of glioma.

The bioinformatic analysis was used to predict the potential target microRNAs of CASC15. MiR-130b-3p was one of predicted RNAs, takes part in regulating numerous biological processes of many carcinomas. For example, miR-130b-3p is downregulated in breast cancer and inhibits cell invasion and migration

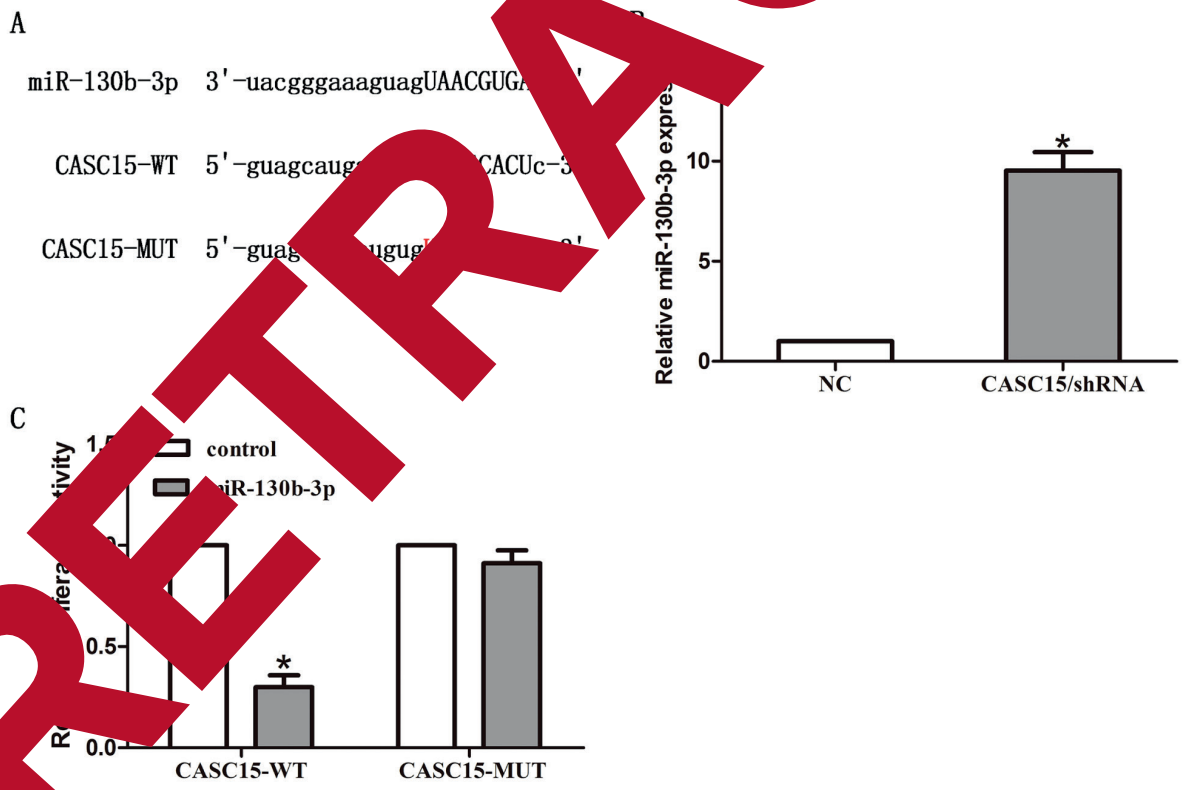


Figure 3. Interaction between miR-130b-3p and CASC15 in glioma. ***A,*** Binding sites of miR-130b-3p on CASC15. ***B,*** The miR-130b-3p expression was increased in the CASC15/shRNA group compared with the NC group. ***C,*** Co-transfection of miR-130b-3p and CASC15-WT strongly decreased the Luciferase activity, while co-transfection of miR-130b-3p and CASC15-MUT did not change the Luciferase activity either. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

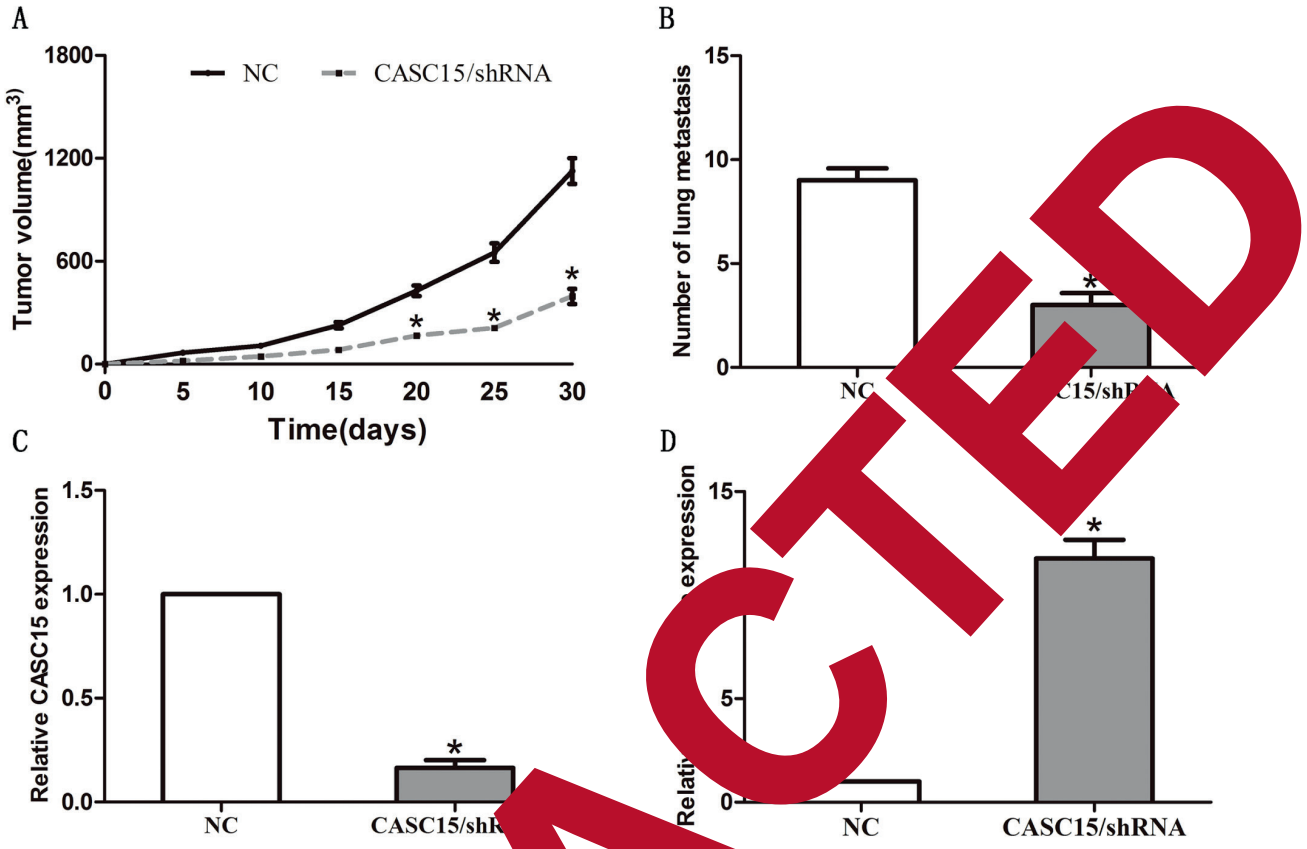


Figure 4. Knockdown of CASC15 inhibited tumor [illegible] of glioma *in vivo*. ***A,*** The tumor size in the CASC15/shRNA group was smaller compared with the [illegible]. ***B,*** The number of metastatic nodules in the lung from the CASC15/shRNA group was significantly [illegible] the NC group. ***C,*** CASC15 of dissected tumors was lower-expressed in the CASC15/shRNA group [illegible] with the NC group. ***D,*** MiR-130b-3p of dissected tumors was higher-expressed in the CASC15/shRNA group compared with the NC group. Results represent the average of three independent experiments (mean ± standard error of the mean). *p < 0.05, compared with the control cells.

in vitro[14]. MiR-130b is aberrantly expressed in prostate cancer and inhibits prostate cancer metastasis *via* targeting PTEN/PI3K/AKT[15,16]. MiR-130b inhibits cell growth and promotes cell apoptosis via regulating CYLD in gastric cancer[17]. In our work, the interaction between miR-130b-3p and CASC15 was further explored. As a result, miR-130b-3p was upregulated after CASC15 was knocked down *in vitro*. Moreover, the Luciferase assay results showed that miR-130b-3p could be directly targeted by CASC15. Moreover, a positive correlation was discovered between miR-130b-3p and CASC15 expression in glioma tissues. The results revealed that CASC15 may realize its function in glioma *via* sponging miR-130b-3p.

To further verify the function of CASC15 in glioma *in vivo*, tumor formation and metastasis assays were conducted. The results of the tumor formation assay revealed that the knockdown of CASC15 could inhibit tumor formation in nude mice; the results of the tumor metastasis assay revealed that knockdown of CASC15 could significantly suppress tumor metastasis in nude mice. Through the detection of CASC15 and miR-130b-3p expression in the extracted tumors, we found that CASC15 was downregulated and miR-130b-3p was upregulated in nude mice treated with SNHG14/shRNA.

Conclusions

LncRNA CASC15 is a new biomarker in the progression of glioma and could enhance glioma cell proliferation, migration and invasion by sponging miR-130b-3p.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Liu L, Zhang Y, Zhu K, Song L, Tao M, Huang P, Pan Y. Resveratrol inhibits glioma cell growth via targeting LRIG1. J BUON 2018; 23: 403-409.
2) Niu H, Li X, Yang A, Jin Z, Wang X, Wang Q, Yu C, Wei Z, Dou C. Cycloartenol exerts anti-proliferative effects on glioma U87 cells via induction of cell cycle arrest and p38 MAPK-mediated apoptosis. J BUON 2018; 23: 1840-1845.
3) Kalpathy-Cramer J, Gerstner ER, Emblem KE, Andronesi O, Rosen B. Advanced magnetic resonance imaging of the physical processes in human glioblastoma. Cancer Res 2014; 74: 4622-4637.
4) Ren Y, Shang J, Li J, Liu W, Zhang Z, Yuan J, Yang M. The long noncoding RNA PCAT-1 links the microRNA miR-215 to oncogene CRKL-mediated signaling in hepatocellular carcinoma. J Biol Chem 2017; 292: 17939-17949.
5) Gaballah HH, Gaber RA, Elrashidy MA, Elshahat DA, Hablus MA, Ebeid AM. Expression of long non-coding RNA CCHE1 in colorectal carcinoma: correlations with clinicopathological featu... ERK/COX-2 pathway. Mol Biol Rep 2018; ... s11033-018-4521-0.
6) Pei X, Wang X, Li H. LncRNA SNHG1 regu... the differentiation of Treg cells and affects the ... mune escape of breast cancer ... lating mi... 448/IDO. Int J Biol Macrom... 24-30.
7) Peng L, Jiang B, Yuan X, ... Peng ... Huang Y, Zhang C, Zhang Y, Lin Z, ... Kao W, D... Zhang Y, Meng M, Pan X, L... C, ... Bi ... Super-enhancer-a... ...ate... ...ncoding ... HCCL5 is activ... ...by ZEB... ...promotes the malignancy o... ...tocellular ca... ...a. Cancer Res 2019; 7...
8) Qi Y, Wang ... Wu F, ... Jiang T, Qiang B, Yuan J, Han W... ...g X. Long n... ...ing RNA HOXD-AS2 regulates cell cycle to promote glioma progression. J Cell Biochem 2018; 10.1002/jcb.28117.
9) Xu DH, Chi GN, Zhao CH, Li DY. Long noncoding RNA MEG3 inhibits proliferation and migration but induces autophagy by regulation of ... PI3K/AKT/mTOR pathway in glioma... Biochem 2018; 10.1002/jcb.28026...
10) Bian EB, Chen EF, Xu YD, Yang Z... ...g F, Ma CC, Wang HL, Zhao B. Exosomal lncRI... ...activates astrocytes that promote gli... ...a cell i... ... Int J Oncol 2019; 54: 713-721.
11) Yao XM, Tang JH, Zhu ... ...g Y. High expres... LncRNA CASC15 is... ...factor ... ...astric ca... prognosis and pro... ...he pr... ...ation of gastric cancer. Eur Re... Me... ...col Sci... ...; 21: 5661-5667.
12) He T, Zhan... ...ong Y, Hua... ...T, Zhou D, Zhou X... ...hang L, Lu ... ...J, Wang W. Long ... ...cod... ...NA CASC15 ... ...upregulated in hepato... ...ellular ca... ...ma and facilitates hepatocarcinogenesis. Int... ...ol 2017; 51: 1722-1730.
13) ..., Liu M, Marzese ... ...Wang H, Chong K, Kawas ..., Donovan NC, Kiyohara E, Hsu S, Nelson N, Izraely S, Sagi-Assif O... ...tz IP, Ma XJ, Luo Y, Hoon D. The CASC15 long ... ...ergenic noncoding RNA locus is involved in m... ...oma progression and phenotype ...ching. J... ...t Dermatol 2015; 135: 2464-2474.
14) ... ...uan R, Bao Q, Wu J, Yuan H, Ma C. MiR-... ...3p inhibits cell invasion and migration by targeting the Notch ligand Delta-like 1 in breast ...inoma. Gene 2017; 609: 80-87.
15) ...lho-Carvalho J, Graca I, Gomez A, Oliveira J, Henrique R, Esteller M, Jeronimo C. Downregulation of miR-130b~301b cluster is mediated by aberrant promoter methylation and impairs cellular senescence in prostate cancer. J Hematol Oncol 2017; 10: 43.
16) Chen Q, Zhao X, Zhang H, Yuan H, Zhu M, Sun Q, Lai X, Wang Y, Huang J, Yan J, Yu J. MiR-130b suppresses prostate cancer metastasis through down-regulation of MMP2. Mol Carcinog 2015; 54: 1292-1300.
17) Sun B, Li L, Ma W, Wang S, Huang C. MiR-130b inhibits proliferation and induces apoptosis of gastric cancer cells via CYLD. Tumour Biol 2016; 37: 7981-7987.