European Review for Medical and Pharmacological Sciences 2019; 23: 4243-4253

Long non-coding RNA H19 regulates the development of gliomas through the Wnt/β-catenin signaling pathway

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Abstract. – OBJECTIVE: We investigated the effects of long non-coding RNA (lncRNA) H19 on glioma cell proliferation, invasion, migration, and apoptosis and the underlying mechanisms.

PATIENTS AND METHODS: H19 expression in glioma tissues, para-carcinoma tissues, and glioma cell lines was analyzed by Real-time polymerase chain reaction (RT-PCR). After transfecting U251 and U87MG cells with siRNA-H19, cell proliferation was detected by the cell counting kit-8 (CCK8) assay. Invasion and migration were detected by a transwell assay; cell cycle distribution and apoptosis were measured by flow cytometry analysis; Dvl2, GSK-3β, cyclin D1, and β-catenin expressions were detected by RT-PCR and Western blotting.

RESULTS: H19 expression in glioma tissues was higher than that in para-carcinoma tissues and associated with poor prognosis in glioma patients. Cell proliferation, invasion, and migration significantly decreased, the percentage of glioma cells in G0/G1 significantly increased, the percentage of glioma cells in the S phase significantly decreased, and apoptosis significantly increased in U251 and U87MG cells transfected with siRNA-H19 compared to those in the siRNA-NC group. Downregulation of H19 decreased DVL2, cyclin D1, and β-catenin expression and increased GSK-3β expression. The inhibitory effects of downregulation of H19 on glioma cell proliferation, invasion, and migration were reversed by SKL2001 via the activation of the Wnt/β-catenin signal pathway, which was further enhanced by inhibition of the Wnt/β-catenin signal pathway by XAV939.

CONCLUSIONS: H19 was overexpressed in glioma tissues and glioma cell lines. Downregulation of H19 inhibited cell proliferation, invasion, and migration, arrested cell cycle progression in the G0/G1 phase, and induced cell apoptosis by restraining activation of the Wnt/β-catenin signaling pathway in glioma cells. Therefore, H19 is a potential therapeutic target for glioma therapy.

Key Words:
lncRNA H19, Glioma, Proliferation, Invasion, Migration, Apoptosis, Wnt/β-catenin.

Introduction

Gliomas are highly malignant tumors which are prone to metastasis and that exhibit poor responses to radiotherapy and chemotherapy, with poor outcomes. Although treatment regimens for gliomas have been improved, the long-term survival rates of patients remain low[1]. Identifying molecular targets for the diagnosis and treatment of gliomas is important. Long non-coding RNAs (lncRNAs) are a subtype of non-coding RNAs more than 200 nucleotides (nt) in length. Compared to coding RNAs, lncRNAs show stronger tissue and cellular specificity. Differential expressions of lncRNAs in different cells, tissues, and development stages are closely associated with the onset and development of many diseases[2]. LncRNAs play important regulatory roles in development, differentiation, cellular proliferation and apoptosis, and metabolism. Bioinformatics analysis has predicted that there are numerous lncRNAs in the human genome, making lncRNAs the largest class of regulatory factors of gene expression, which regulate one-third of genes in humans[3]. Studies have shown that lncRNA H19 plays an important role in colorectal cancer, breast cancer, bladder cancer, and melanomas[4-6]. In gliomas, H19 can regulate glioma proliferation, invasion, and metastasis through its effects on miR-675 and miR-140. However, the specific mechanisms are not completely understood[7,8]. Therefore, we performed reverse transcription polymerase chain reaction (RT-PCR) to quantify H19 expression

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levels in glioma tissues, tumor-adjacent normal tissues, and glioma cell lines. Additionally, cell proliferation, invasion, migration, apoptosis, and expression status of molecules associated with the Wnt/β-catenin signaling pathway after transfection of siRNA-H19 in U251 and U87MG cells were examined to determine the effects and mechanisms of action of H19 in gliomas.

Patients and Methods

Tissue Specimens

Sixty glioma patients hospitalized in our hospital from July to December 2015 were selected. The average age of patients was 55.8 ± 10.3 years and there were 38 males and 22 females. Of these patients, 15 were in grade II tumors (GBM), 20 were in grade III tumors and 25 were in grade IV tumors. Glioma tissues and tumor-adjacent normal tissues were excised during surgery. All tissue specimens were divided into equal sizes and stored in liquid nitrogen. No glioma patients underwent surgery, biological therapy, radiotherapy, or chemotherapy before surgery. This study was approved by our Hospital Ethics Committee and all patients signed an informed consent form.

Primary Reagents

The CCK-8 reagent kit was obtained from (Beyotime Institute of Biotechnology, Jiangsu, China). The RNA reverse transcription kit was obtained from TaKaRa (Otsu, Shiga, Japan). Lipofectamine 2000 was obtained from Invitrogen (Carlsbad, CA, USA). Transwell chambers were obtained from Chemicon (Tokyo, Japan). Matrigel was obtained from BD Biosciences (Franklin Lakes, NJ, USA). Rabbit anti-human Dvl2, GSK-3β, p-GSK-3β, β-catenin, and cyclin D1 antibodies were obtained from Sigma Aldrich (St. Louis, MO, USA). Mouse anti-human GAPDH monoclonal antibody was obtained from Boster Biological Technological Co., Ltd. (Wuhan, China). Horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG and goat anti-mouse IgG were obtained from Cell Signaling Technology (Danvers, MA, USA). XAV939 and SKL2001 were obtained from Millipore (Billerica, MA, USA).

Cell Culture and Transfection

A172, U373, U251, and U87MG glioma cells lines and the normal astrocyte cell line NHA were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were grown in DMEM/F12 medium (Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) in a 37°C, 5% CO_2 constant humidity incubator. Cells in log-phase were used for experiments. Additionally, 1 μM XAV939 (Millipore Co, Ltd, Billerica, MA, USA) was added into the Wnt inhibitor group and 20 μM SKL2001 (Millipore Co, Ltd, Billerica, MA, USA) was added to the Wnt agonist group. The transfection reagent Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), serum-free DMEM (Carlsbad, CA, USA) and siRNA-H19 or siRNA-NC were mixed and incubated for 30 min. They were then added into cells with complete medium containing 15% fetal bovine serum (FBS). The H19 interfering sequences were 5′-CUUUCUGUCACAUUGACCACACCUG-3′ (sense) and 5′-UCUGAUUGCAGCAUCUUCUUGAUUC-3′ (antisense). Each group contained 5 replicate wells.

Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from tissues and cells using the TRIzol reagent kit (TaKaRa, Dalian, China) following the manufacturer's instructions. Standard fluorescence quantitative PCR was used for quantitation. β-actin was used as an internal reference and samples were tested at absorbance wavelengths of 260 and 280 nm. The qRT-PCR mix was formulated based on the manufacturer's instructions in the SYBR Green reagent kit (TaKaRa, Dalian, China). The Prime Script™ RT reagent kit (TaKaRa, Dalian, China) was used to quantify RNA and a reverse transcription kit (TaKaRa, Dalian, China) was used to synthesize cDNA. β-actin and U6 were used as an internal control of Dvl2, GSK-3β, cyclinD1, β-catenin or H19, respectively. Expression levels were quantified using the methods of $2^{-\cdots CT}$. The primer sequences were listed in Table I.

Cell Counting Kit-8 (CCK-8) Assay

Cells in the log-phase from various groups were subcultured at a density of 2×10^3 cells in a 96-well plate and cultured routinely. Cells from various groups were cultured for 24, 48, 72, and 96 h before CCK-8 assay kit (MeiLun, Dalian, Liaoning, China) was applied and 10 μl CCK-8 solution was added and incubated for 10 min. The optical density (OD) was determined at a wavelength of 450 nm using an iBIO-RADMark microplate reader (Bio-Rad, Hercules, CA, USA).

Table I. Sequences of primers for RT-PCR.

Genes	Primer sequences
Dvl2	Forward: 5'- AGGATACCACCCTTCCGTTG -3'
	Reverse: 5'- GGCGCCAAGTACTTTTTCAA -3'
GSK-3β	Forward: 5'- GACGCTCCCTGTGATTTATGTC -3'
	Reverse: 5'- GTTAGTCGGGCAGTTGGTGTAT -3'
Cyclin D1	Forward: 5'- GAGTAGTGCGAAGCATAGGTCT -3'
	Reverse: 5'- CTAGCACGAGTAGTCGAGCGC -3'
β-catenin	Forward: 5'- CGGTACATGCATGACTGAGAC -3'
	Reverse: 5'- GTCACGTGGTACGACGTCAGAT -3'
H19	Forward: 5'- ATCGGTGCCTCAGCGTTCGG -3'
	Reverse: 5'- CTGTCCTCGCCGTCACACCG -3'
β-actin	Forward: 5'- ACGAGACCIACCTTCAACTCCATC -3'
	Forward: 5'- TAGAAGCATTTGCGGTGGACGA -3'
U6	Forward: 5'- CGCTTCGGCAGCACATATACT -3'
	Forward: 5'- CGCTTCACGAATTTGCGTGTC-3'

Migration and Invasion Assay

Fifty microliters of Matrigel were used to coat the basement membrane in the upper transwell chamber (pore size 8 μm) and air-dried at room temperature. Fifty microliters of serum-free culture medium containing bovine serum albumin were added to each well for hydration for 5 min at 37°C. Serum-free DMEM medium (Carlsbad, CA, USA) was used to resuspend the cells, which were then starved for 12 h. The cell density was readjusted to 1×10^5/ml. Two hundred microliters of cell suspension were added to the upper chamber of the Transwell chamber, which was then placed in 24-well plates containing 600 μL of culture medium containing 15% FBS and culture for 48 h. The cells were fixed in methanol for 15 min and stained with crystal violet (JissKang Biotech, Qingdao, Shandong, China). Ten random fields of view under a DYS-810 microscope (DianYing, Pudong, Shanghai, China) were selected; the number of cells that penetrated the lower layer of the microporous membrane was enumerated, and the average value was determined. For invasion analysis, the chambers were coated with Matrigel and the subsequent steps were similar to those of the migration analysis.

Flow Cytometry (FCM)

Cells in log-phase were sub-cultured at a density of 3×10^5 cells/well in a 6-well plate. Cells were collected at 24 h after transfection. The cell cycle and cell apoptosis were analyzed by flow cytometry using a FlowSight flow cytometer (Merck Millipore, Billerica, MA, USA). For cell cycle examination, an EZCell™ Cell Cycle Analysis kit (AmyJet Scientific, Wuhan, Hubei, China) was utilized. For cell apoptotic rates determination, we used a Servicebio Annexin V/PI cell apoptosis analysis kit (Servicebio, Wuhan, Hubei, China).

Western Blotting

Cells transfected for 24 h were collected and total protein was extracted using a total protein extraction kit (NiuPu, Haidian, Beijing, China). The BCA reagent kit (RongBio, Minhang, Shanghai, China) was used to quantify protein concentration. For sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), 40 μg of sample proteins were added to each well and electrophoresis was carried out at 110 V. Transfer of proteins onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) was carried out at 250 mA. The membrane was blocked for 1 h in 5% skim milk at 37°C. The anti-human Dvl2, GSK-3β, cyclin D1, β-catenin, and β-actin (Abcam, Cambridge, MA, USA) were added to the membrane and incubated at 4°C overnight. The membrane was washed three times with Tris-buffered saline containing Tween 20 (TBST) for 10 min each before secondary antibody was added and incubated at 37°C for 1 h. The membrane was washed three times with TBST for 10 min each before electrochemiluminescence (ECL) development. The proteins were detected with a QuantumCX5 infrared scanner (VILBER, Fengtai, Beijing, China) using an ECL Western Blotting Substrate kit (YRBio, Changsha, Hunan, China).

Statistical Analysis

SPSS 20.0 software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for statistical analysis. Data are expressed as the mean ± standard deviation. The methods of

one-way ANOVA and Student's *t*-test were used to analyze these data, and multiple comparison between the groups was performed by SNK method. Survival curve was assessed by the Kaplan-Meier method, and differences were analyzed by the log-rank test. A difference of $p < 0.05$ indicated statistical significance.

Results

H19 Expression Levels in Gliomas and Associated with Poor Prognosis

H19 expression in glioma tissues was significantly higher than that in tumor-adjacent normal tissues (Figure 1A) and H19 expression in gliomas with greatest tumor diameter ≥ 5 cm was increased compared to glioblastoma with greatest tumor diameter < 5 cm (Figure 1B). We further analyzed H19 expression levels in glioma patients at different tumor stages. The results showed that H19 expression levels in grade III and IV gliomas were significantly higher than those in grade II gliomas and H19 expression levels in grade IV gliomas were significantly higher than those in grade III gliomas (Figure 1C). Meanwhile, the high expression of H19 was associated with poor survival rates compared to low expression of H19 (Figure 1D).

H19 Expression Levels in Glioma Cell Lines and Down-Expression of H19 Inhibited Glioma Cell Proliferation, Invasion and Migration

H19 expression levels in A172, U373, U251, and U87MG glioma cell lines were significantly higher than those in the NHA normal human astrocyte cell line (Figure 2A). We further examined the function of H19 in gliomas by using siRNA interference technology to transfect siRNA-NC and siRNA-H19 into U251 and U87MG cells. The results showed that after siRNA-H19 transfection, H19 expression levels in U251 and U87MG cells were significantly reduced compared to

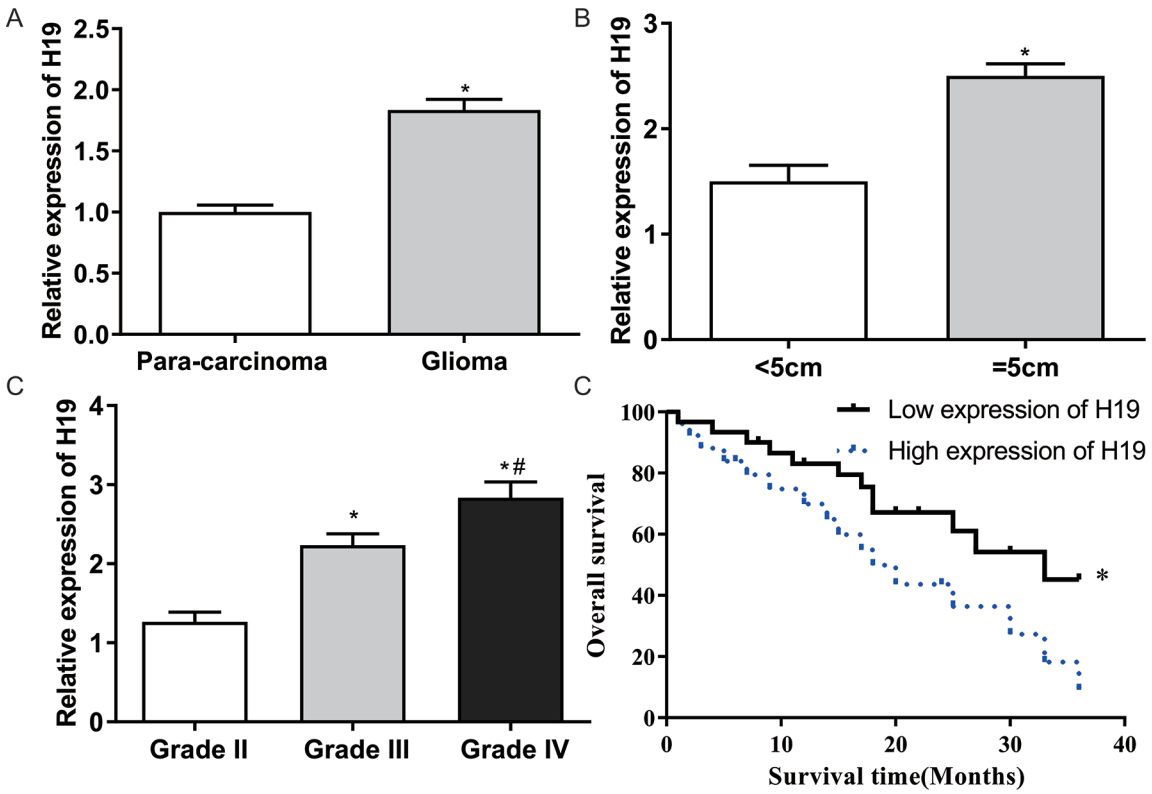

Figure 1. H19 expression levels in glioma patients and associated with poor prognosis. ***(A)*** H19 expression levels in glioma tissues and tumor-adjacent normal tissues were detected by qRT-PCR. ***(B)*** H19 expression levels in glioma patients with different tumor diameter were detected by qRT-PCR. ***(C)*** H19 expression levels in glioma patients with different grades. ***(D)*** H19 expression levels were associated with poor prognosis in glioma patients. All data are shown as mean ± SD based on at least three independent experiments, *$p < 0.05$.

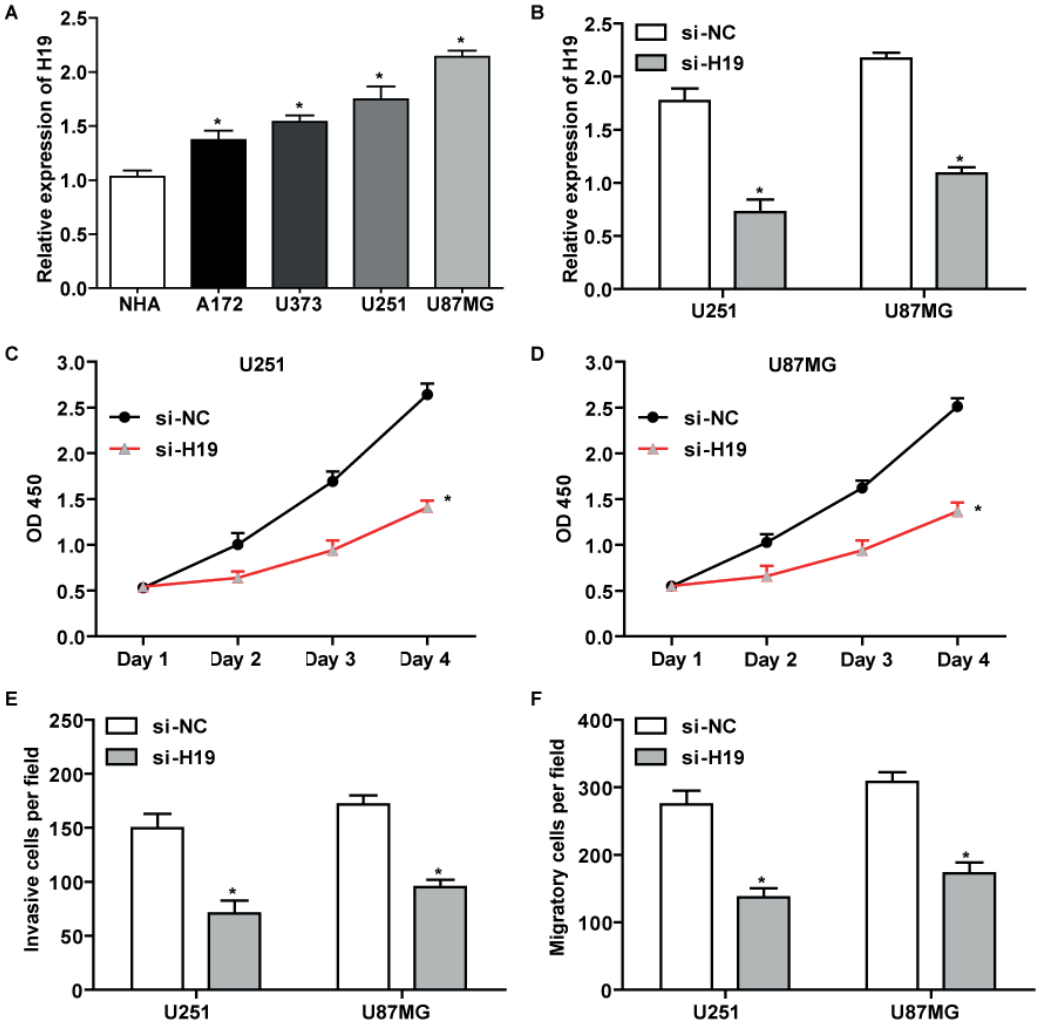

Figure 2. H19 expression levels in glioma cell lines and down-expression of H19 inhibited glioma cell proliferation, invasion and migration. ***(A)*** H19 expression levels in A172, U373, U251, and U87MG glioma cell lines were detected by qRT-PCR. ***(B)*** H19 expression levels in U251 and U87MG cells after siRNA-H19 transfection were detected by qRT-PCR. ***(C)*** After transfecting siRNA-H19 into U251 cells, the cells proliferation activity was detected by CCK8 assay. ***(D)*** After transfecting siRNA-H19 into U87MG cells, the cell proliferation activity was detected by CCK8 assay. ***(E)*** After siRNA-H19 transfection of U251 and U87MG cells, the cell invasive abilities were detected by transwell assays. ***(F)*** After siRNA-H19 transfection of U251 and U87MG cells, the cell migratory abilities were detected by transwell assays. All data are shown as mean ± SD based on at least three independent experiments, * $p < 0.05$.

those cells transfected with siRNA-NC (Figure 2B). After transfecting siRNA-H19 into U251 and U87MG cells, the cells were cultured for 4 days. The results showed that after siRNA-H19 transfection, cellular proliferation of U251 and U87MG cells was significantly reduced compared to that of cells transfected with siRNA-NC (Figure 2C and D). We further analyzed the effects of H19 on glioma cell invasion and migration. After siRNA-H19 transfection of U251 and U87MG cells, the number of invaded and migrated cells was significantly reduced compared to that of cells transfected with siRNA-NC (Figure 2E and F). All data revealed that down-expression of H19 inhibited glioma cell proliferation, invasion and migration.

Down-Expression of H19 Arrested Glioma Cell Cycle and Induced Glioma Cell Apoptosis

Flow cytometry was employed for quantitative cell cycle analysis after siRNA-H19 transfection of U251 and U87MG cells. The results showed that in U251 and U87MG cells transfected with

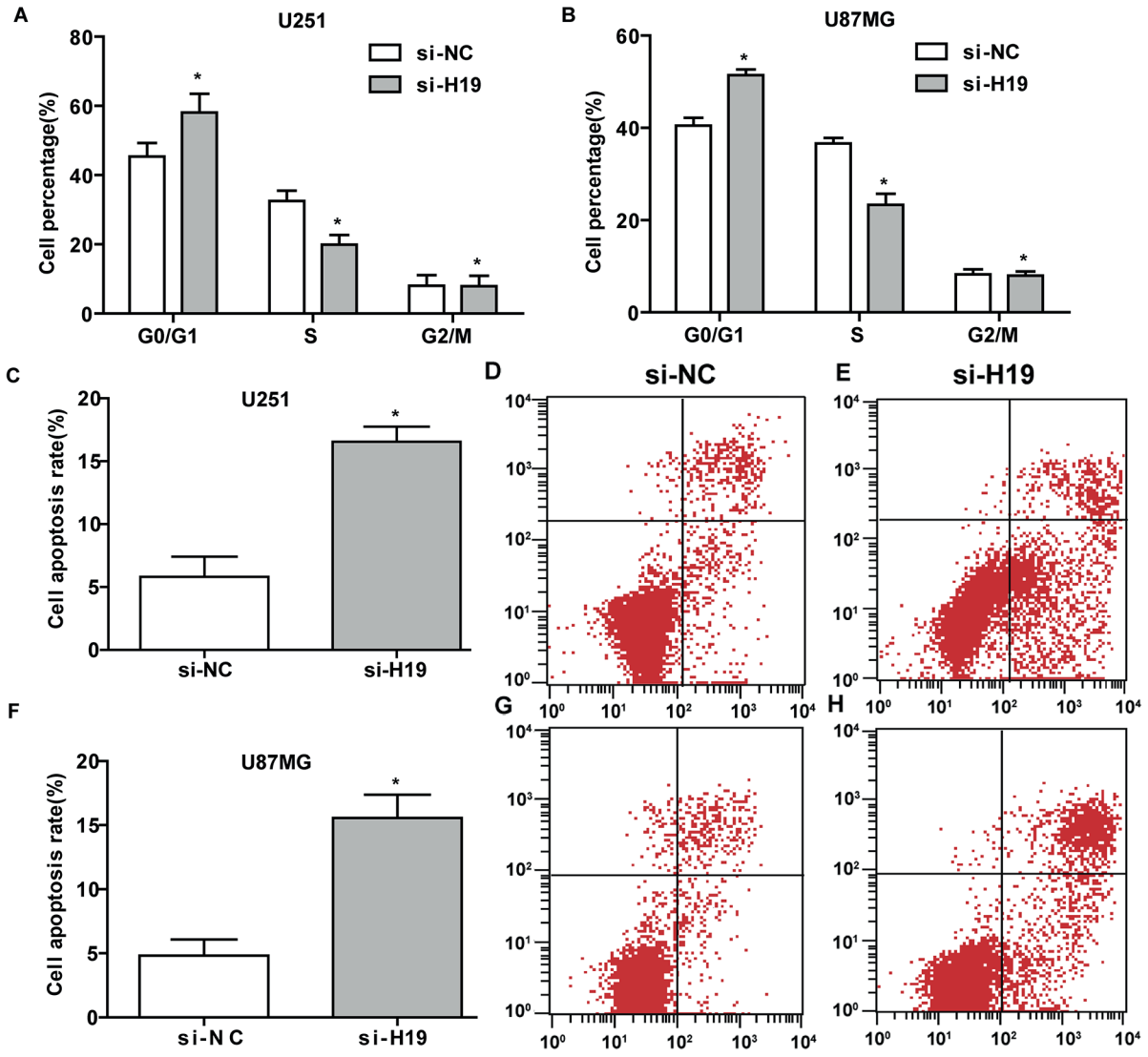

Figure 3. Down-expression of H19 arrested glioma cell cycle and induced glioma cell apoptosis. ***(A)*** After siRNA-H19 transfection of U251 cells, the cell cycle distribution was detected by Flow cytometry analysis. ***(B)*** After siRNA-H19 transfection of U87MG cells, the cell cycle distribution was detected by Flow cytometry analysis. ***(C-E)*** After siRNA-H19 transfection of U251 cells, the cell apoptosis rate was detected by Flow cytometry analysis. ***(F-H)*** After siRNA-H19 transfection of U87MG cells, the cell apoptosis rate was detected by Flow cytometry analysis. All data are shown as mean ± SD based on at least three independent experiments, *$p < 0.05$.

siRNA-H19, the proportion of cells in the G_0/G_1 phase was significantly higher than that in cells transfected with siRNA-NC. The proportion of cells in the S phase was significantly lower than that in the siRNA-NC group, and there were no significant differences in the proportion of cells in the M phase between both groups (Figure 3A and B). After siRNA-H19 was transfected into U251 and U87MG cells, the cells exhibited significant cell cycle arrest. We further employed flow cytometry to analyze the effects of H19 on glioma cell apoptosis. The results showed that after siRNA-H19 transfection, the apoptosis rates in U251 and U87MG cells were significantly increased compared to those in cells transfected with siRNA-NC (Figure 3C-H). This suggests that low expression of H19 can cause cell cycle arrest, thereby inducing cellular apoptosis.

Down-Expression of H19 Restrained Activation of the Wnt/β-Catenin Signaling Pathway in Glioma Cells

The Wnt/β-catenin signaling pathway plays an important role in gliomas. To further analyze

the effects of H19 on the Wnt/β-catenin signaling pathway, RT-PCR and Western blotting were performed to determine the mRNA and protein expression levels of Dv12, GSK-3β, cyclin-D1, and β-catenin. The results showed that after siRNA-H19 transfection into U251 and U87MG cells, the mRNA expression levels of Dv12, cyclin-D1, and β-catenin were significantly lower than in cells transfected with siRNA-NC. GSK-3β mRNA expression levels were significantly increased compared to those in cells transfected with siRNA-NC (Figure 4A and B). Meanwhile, the protein expression levels of Dv12, cyclin-D1, and β-catenin in U251 and U87MG cells after siRNA-H19 transfection were significantly lower than in cells transfected with siRNA-NC. GSK-3β protein expression levels were significantly increased compared to those in cells transfected with siRNA-NC (Figure 4C-F). This suggests that down-expression of H19 restrained activation of the Wnt/β-catenin signaling pathway in glioma cells.

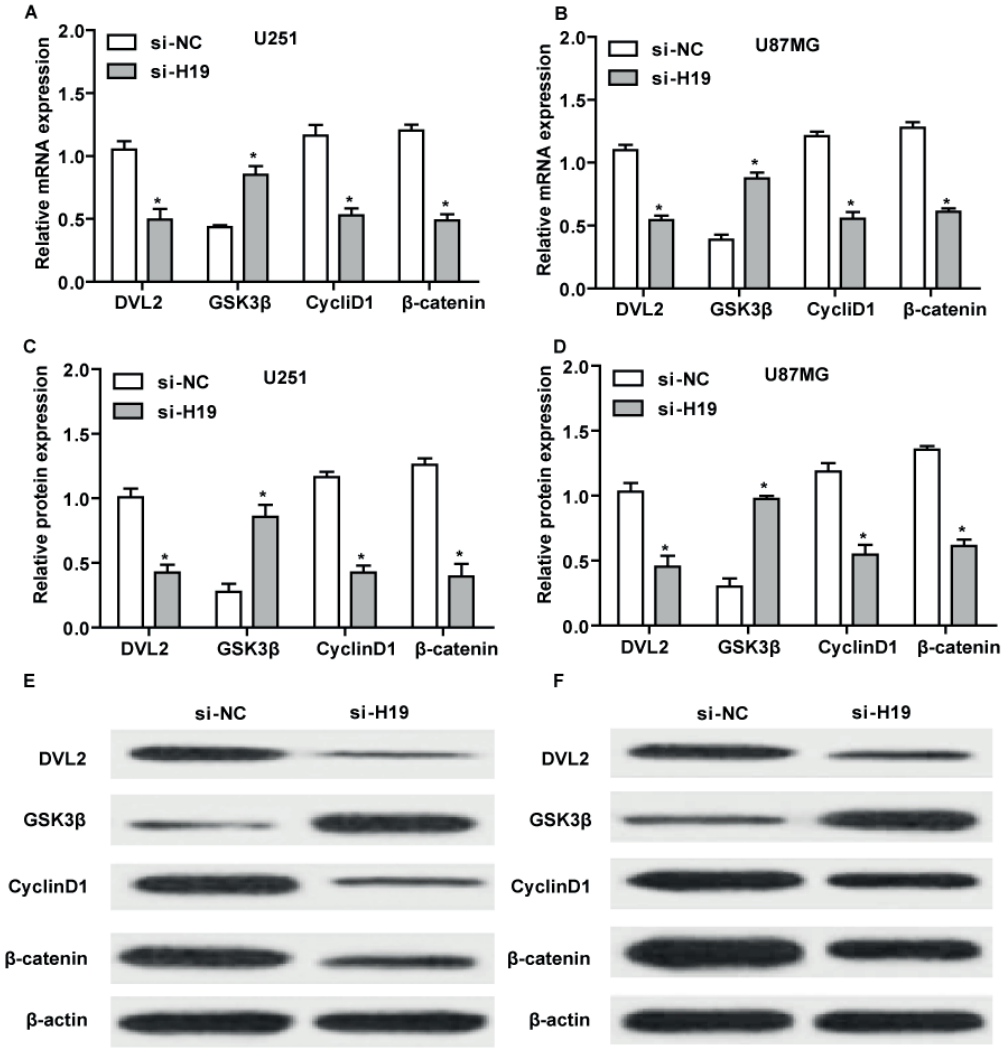

Figure 4. Down-expression of H19 restrained activation of the Wnt/β-catenin signaling pathway in glioma cells. ***(A)***After siRNA-H19 transfection of U251 cells, The Dvl2, GSK-3β, cyclinD1 and β-catenin mRNA expression were detected by PCR. ***(B)*** After siRNA-H19 transfection of U87MG cells, The Dvl2, GSK-3β, cyclinD1 and β-catenin mRNA expression were detected by PCR. (***C-E)*** After siRNA-H19 transfection of U251 cells, the Dvl2, GSK-3β, cyclinD1 and β-catenin protein expression were detected by Western blot. ***(D-F)*** After siRNA-H19 transfection of U87MG cells, The Dvl2, GSK-3β, cyclinD1 and β-catenin protein expression were detected by Western blot. All data are shown as mean ± SD based on at least three independent experiments, * $p < 0.05$.

Effects of Activating or Blocking the Wnt/β-Catenin Signaling Pathway on Cell Invasion and Migration in U251 and U87MG Cells After siRNA-H19 Transfection

To further reverse validate that downregulated H19 inhibits glioma growth through the Wnt/β-catenin signaling pathway, SKL2001 and XAV939 were used to activate and inhibit the Wnt/β-catenin signaling pathway, respectively. The results showed that after transfection of siRNA-H19 and addition of SKL2001, there were no significant differences in cell proliferation (Figure 5A and B), invasion (Figure 5C) and migration (Figure 5D) in U251 and U87MG cells compared to cells transfected with siRNA-NC alone. After transfection of siRNA-H19 and addition of XAV939, cell proliferation, invasion and migration in U251 and U87MG cells were significantly lower than those in the siRNA-NC transfection only group and siRNA-H19 transfection only group (Figure 5A-D). This suggests that downregulation of H19 inhibits activation of the Wnt/β-catenin signaling pathway to inhibit glioma cell proliferation, invasion, and migration. Further, it arrests cells at the G_0/G_1 phase and promotes glioma cell apoptosis.

Discussion

Gliomas are the most common tumors of the central nervous system, accounting for 80% of brain

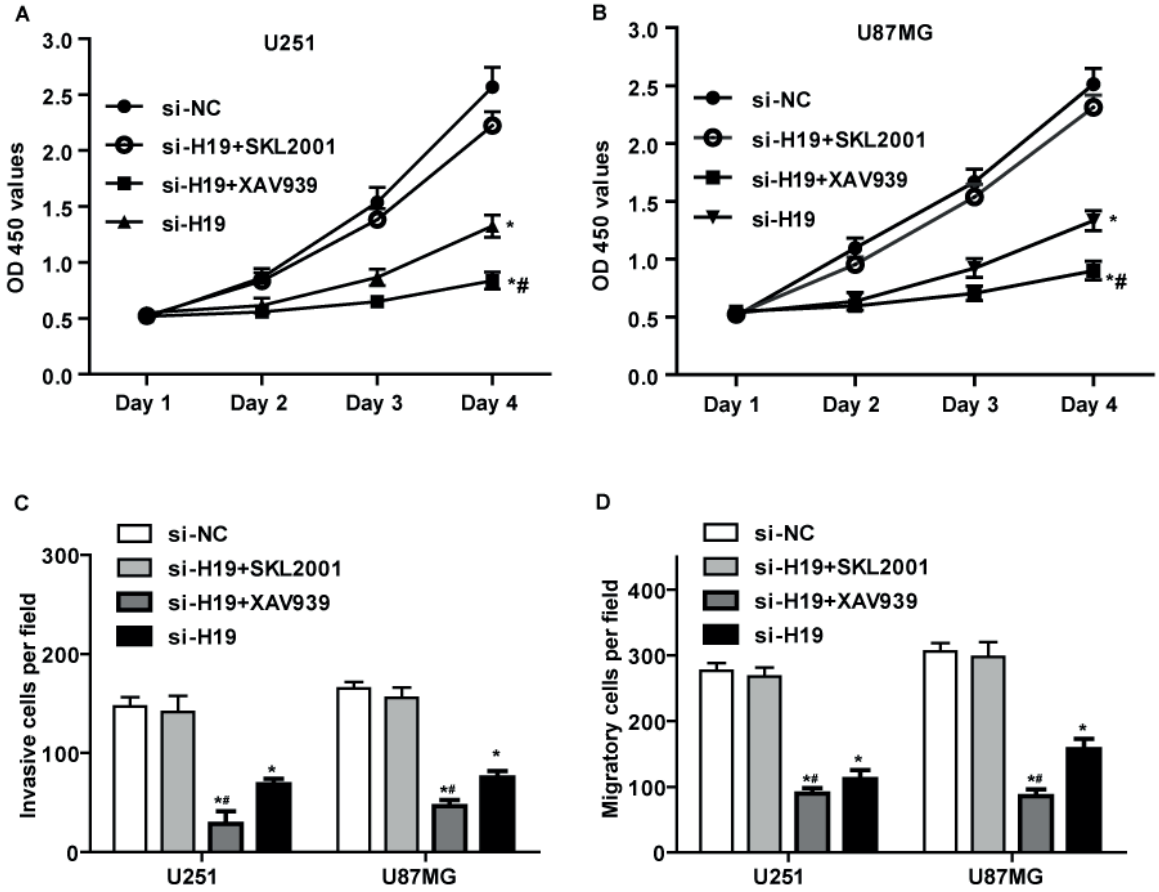

Figure 5. Effects of activating or blocking the Wnt/β-catenin signaling pathway on cell invasion and migration in U251 and U87MG cells after siRNA-H19 transfection. ***(A)*** After siRNA-H19 transfection or together treated with SKL2001 and XAV939 in U251 cells, the cells proliferation activity was detected by CCK8 assay. ***(B)*** After siRNA-H19 transfection or together treated with SKL2001 and XAV939 in U87MG cells, the cell proliferation activity was detected by CCK8 assay. ***(C)*** After siRNA-H19 transfection or together treated with SKL2001 and XAV939 in U251 and U87MG cells, the cell invasive abilities were detected by Transwell assays. ***(D)*** After siRNA-H19 transfection or together treated with SKL2001 and XAV939 in U251 and U87MG cells, the cell migratory abilities were detected by transwell assays. All data are shown as mean ± SD based on at least three independent experiments, * $p < 0.05$.

tumors, and are highly invasive. The current main treatments include surgery, radiotherapy, chemotherapy, and biological therapy. However, patients often have poor prognosis[9]. LncRNAs have recently gained attention in oncology research. A recent study[10] showed that lncRNAs may have important roles in tumor development and tumor suppression pathways. These new genes show aberrant expression in multiple tumors and the biological functions of most lncRNAs remain unknown. Aberrant expression of multiple lncRNAs has been detected in gliomas, including SNHG16, DANCR, PLAC2, and CCDC26, among others[11-14]. Jia et al[15] showed that H19 is significantly expressed in gliomas and inhibition of H19 expression and inhibited epithelial-mesenchymal transition in gliomas. This suggests that H19 plays an important role in tumor development. In this study, we quantified H19 expression levels in glioma tissues and cells. The results showed that H19 was highly expressed in glioma tissues and cells. We further quantified H19 expression levels in patients with different tumor stages and found that H19 expression was significantly increased in patients with grade IV gliomas. This suggests that H19 is intimately associated with glioma progression. Meanwhile, the high expression of H19 was associated with poor survival rates. This indicates that H19 was closely related to glioma. Cellular experiments were conducted to validate the effects of H19 on gliomas. siRNA-H19 transfection into U251 and U87MG cells showed that H19 expression levels in the cells were significantly decreased. When H19 was downregulated, the proliferation, invasion, and migration capabilities of U251 and U87MG cells were significantly decreased. Additionally, H19 downregulation significantly increased the proportion of cells in G_0/G_1 phase and decreased the proportion of cells in S phase, while simultaneously increasing cellular apoptosis. Thus, H19 downregulation can induce cell cycle arrest at the G_0/G_1 phase and induce cellular apoptosis. In various tumor studies, H19 was found to be highly expressed and able to promote tumor progression. Also, H19 inhibition can significantly suppress tumor growth and induce apoptosis[16]. This suggests that H19 plays an important regulatory role as an oncogene in tumors. However, the effector mechanisms of H19 in gliomas require further confirmation. The Wnt/β-catenin signaling pathway regulates various cellular behaviors, such as proliferation, differentiation, adhesion, and cell polarity[17]. Researches[18-20] have demonstrated that the Wnt/β-catenin signaling pathway plays a crucial role in gliomas, liver cancer, colorectal cancer, gastric cancer, and bladder cancer. In tumors, aberrant activation of the Wnt/β-catenin signaling pathway is associated with aberrant expression of β-catenin and Wnt proteins[21]. Aberrant activation of the Wnt/β-catenin signaling pathway regulates cell proliferation, invasion, migration, and apoptosis by activating downstream molecules[22]. β-catenin forms an adhesion complex by binding to E-cadherin at cell junctions, which can increase cell-cell adhesion[23]. When free β-catenin protein in the cytoplasm enters the nucleus, it can activate the transcription of downstream molecules, such as cyclin D1 to protein cellular proliferation and migration[2]. Studies have shown that WWC3, LncRNA AB073614, LncRNA MEG3, and other molecules in gliomas can regulate cell growth through the Wnt/β-catenin signaling pathway[24,25]. In this study, we employed RT-PCR and Western blotting to analyze the mRNA and protein expression levels of Dv12, GSK-3β, cyclin-D1, and β-catenin. The results showed that after siRNA-H19 transfection into U251 and U87MG cells, the mRNA and protein expression levels of Dv12, cyclin-D1, and β-catenin were significantly lower than those in cells transfected with siRNA-NC, whereas GSK-3β mRNA and protein expression levels were significantly increased compared to those in cells transfected with siRNA-NC. This shows that H19 inhibition can significantly suppress activation of the Wnt/β-catenin signaling pathway. To further validate that H19 inhibits glioma growth through the Wnt/β-catenin signaling pathway, SKL2001 was used to activate the Wnt/β-catenin signaling pathway and XAV939 was used to inhibit the Wnt/β-catenin signaling pathway. The results showed that after transfection of siRNA-H19 and addition of SKL2001, there were no significant differences in cell proliferation, invasion and migration in U251 and U87MG cells. After transfection of siRNA-H19 and addition of XAV939, cell proliferation, invasion and migration in U251 and U87MG cells were significantly lower than in the siRNA-NC transfection only group and siRNA-H19 transfection only group. Thus, SKL2001 activation of the Wnt/β-catenin signaling pathway can reverse the inhibitory effects of downregulated H19 on glioma cell proliferation, invasion and migration. Moreover, XAV399 inhibition of the Wnt/β-catenin signaling pathway can promote the inhibitory effects of H19 downregulation on glioma cell proliferation, invasion and migration.

Conclusions

We revealed that H19 is highly expressed in glioma tissues and cells. Its downregulation inhibits the activation of the Wnt/β-catenin signaling pathway to inhibit glioma cell proliferation, invasion, and migration. It also arrests cells at the G_0/G_1 phase and promotes glioma cell apoptosis. Therefore, H19 is a potential therapeutic target for glioma treatment.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Liao Y, Shen L, Zhao H, Liu Q, Fu J, Guo Y, Peng R, Cheng L. LncRNA CASC2 interacts with miR-181a to modulate glioma growth and resistance to TMZ through PTEN pathway. J Cell Biochem 2017; 118: 1889-1899.
2) Zhang H, Wei DL, Wan L, Yan SF, Sun YH. Highly expressed lncRNA CCND2-AS1 promotes glioma cell proliferation through Wnt/beta-catenin signaling. Biochem Biophys Res Commun 2017; 482: 1219-1225.
3) Fang K, Liu P, Dong S, Guo Y, Cui X, Zhu X, Li X, Jiang L, Liu T, Wu Y. Magnetofection based on superparamagnetic iron oxide nanoparticle-mediated low lncRNA HOTAIR expression decreases the proliferation and invasion of glioma stem cells. Int J Oncol 2016; 49: 509-518.
4) Lv M, Zhong Z, Huang M, Tian Q, Jiang R, Chen J. lncRNA H19 regulates epithelial-mesenchymal transition and metastasis of bladder cancer by miR-29b-3p as competing endogenous RNA. Biochim Biophys Acta Mol Cell Res 2017; 1864: 1887-1899.
5) Yang W, Ning N, Jin X. The lncRNA H19 promotes cell proliferation by competitively binding to miR-200a and derepressing b-catenin expression in colorectal cancer. Biomed Res Int 2017; 2017: 2767484.
6) Zhou W. Data from: the lncRNA H19 mediates breast cancer cell plasticity during EMT and MET plasticity by differentially sponging miR-200b/c and let-7b. Sci Signal 2017; 10(483). pii: eaak9557.
7) Zhang T, Wang YR, Zeng F, Cao HY, Zhou HD, Wang YJ. LncRNA H19 is overexpressed in glioma tissue, is negatively associated with patient survival, and promotes tumor growth through its derivative miR-675. Eur Rev Med Pharmacol Sci 2016; 20: 4891-4897.
8) Zhao H, Peng R, Liu Q, Liu D, Du P, Yuan J, Peng G, Liao Y. The lncRNA H19 interacts with miR-140 to modulate glioma growth by targeting iASPP. Arch Biochem Biophys 2016; 610: 1-7.
9) Wang Q, Zhang J, Liu Y, Zhang W, Zhou J, Duan R, Pu P, Kang C, Han L. A novel cell cycle-associated lncRNA, HOXA11-AS, is transcribed from the 5-prime end of the HOXA transcript and is a biomarker of progression in glioma. Cancer Lett 2016; 373: 251-259.
10) Li J, Zhang M, An G, Ma Q. LncRNA TUG1 acts as a tumor suppressor in human glioma by promoting cell apoptosis. Exp Biol Med (Maywood) 2016; 241: 644-649.
11) Hu YW, Kang CM, Zhao JJ, Nie Y, Zheng L, Li HX, Li X, Wang Q, Qiu YR. LncRNA PLAC2 down-regulates RPL36 expression and blocks cell cycle progression in glioma through a mechanism involving STAT1. J Cell Mol Med 2018; 22: 497-510.
12) Lu YF, Cai XL, Li ZZ, Lv J, Xiang YA, Chen JJ, Chen WJ, Sun WY, Liu XM, Chen JB. LncRNA SNHG16 functions as an oncogene by sponging MiR-4518 and up-regulating PRMT5 expression in glioma. Cell Physiol Biochem 2018; 45: 1975-1985.
13) Wang S, Hui Y, Li X, Jia Q. Silencing of lncRNA-CCDC26 restrains the growth and migration of glioma cells in vitro and in vivo via targeting miR-203. Oncol Res 2018; 26: 1143-1154.
14) Xu D, Yu J, Gao G, Lu G, Zhang Y, Ma P. LncRNA DANCR functions as a competing endogenous RNA to regulate RAB1A expression by sponging miR-634 in glioma. Biosci Rep 2018; 38(1). pii: BSR20171664.
15) Jia L, Tian Y, Chen Y, Zhang G. The silencing of LncRNA-H19 decreases chemoresistance of human glioma cells to temozolomide by suppressing epithelial-mesenchymal transition via the Wnt/beta-Catenin pathway. Onco Targets Ther 2018; 11: 313-321.
16) Zhang Q, Li X, Li X, Li X, Chen Z. LncRNA H19 promotes epithelial-mesenchymal transition (EMT) by targeting miR-484 in human lung cancer cells. J Cell Biochem 2018; 119: 4447-4457.
17) Zhang L, Liu H, Mu X, Cui J, Peng Z. Dysregulation of Fra1 expression by Wnt/beta-catenin signalling promotes glioma aggressiveness through epithelial-mesenchymal transition. Biosci Rep 2017; 37(2). pii: BSR20160643. doi: 10.1042/BSR20160643. Print 2017 Apr 28.
18) Fu X, Zhu X, Qin F, Zhang Y, Lin J, Ding Y, Yang Z, Shang Y, Wang L, Zhang Q, Gao Q. Linc00210 drives Wnt/beta-catenin signaling activation and liver tumor progression through CTNNBIP1-dependent manner. Mol Cancer 2018; 17: 73.
19) Yoshida T, Sopko NA, Kates M, Liu X, Joice G, McConkey DJ, Bivalacqua TJ. Three-dimensional organoid culture reveals involvement of Wnt/beta-catenin pathway in proliferation of bladder cancer cells. Oncotarget 2018; 9: 11060-11070.
20) Zheng CH, Wang JB, Lin MQ, Zhang PY, Liu LC, Lin JX, Lu J, Chen QY, Cao LL, Lin M, Tu RH, Xie JW, Li P, Huang CM. CDK5RAP3 suppresses Wnt/beta-catenin signaling by inhibiting AKT phosphorylation in gastric cancer. J Exp Clin Cancer Res 2018; 37: 59.

21) Wang H, Sheng ZG, Dai LZ. Long non-coding RNA LINC01503 predicts worse prognosis in glioma and promotes tumorigenesis and progression through activation of Wnt/beta-catenin signaling. Eur Rev Med Pharmacol Sci 2019; 23: 1600-1609.

22) Xia S, Ji R, Zhan W. Long noncoding RNA papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) inhibits proliferation and invasion of glioma cells by suppressing the Wnt/beta-catenin signaling pathway. BMC Neurol 2017; 17: 30.

23) Kouchi M, Shibayama Y, Ogawa D, Miyake K, Nishiyama A, Tamiya T. (Pro)renin receptor is crucial for glioma development via the Wnt/beta-catenin signaling pathway. J Neurosurg 2017; 127: 819-828.

24) Wang Y, Jiang M, Yao Y, Cai Z. WWC3 inhibits glioma cell proliferation through suppressing the Wnt/beta-catenin signaling pathway. DNA Cell Biol 2018; 37: 31-37.

25) Li Y, Zhu G, Zeng W, Wang J, Li Z, Wang B, Tian B, Lu D, Zhang X, Gao G, Li L. Long noncoding RNA AB073614 promotes the malignance of glioma by activating Wnt/beta-catenin signaling through downregulating SOX7. Oncotarget 2017; 8: 65577-65587.