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Original Article

The up-regulation of LRIG1 expression inhibits the proliferation, apoptosis and invasion of glioma cells

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Abstract: Objective: To illustrate the role of LRIG1 in regulating the Notch signaling pathway and glioma cell proliferation, apoptosis and invasion. Methods: The glioma cells (U373) were divided into control group, NC group and LRIG1 group. After transfection, the CCK-8 assay, Transwell assay, and Flow cytometry were used to explore the biological function of LRIG1 in glioma cells. At the end, Western blot was used to detect the expression of LRIG1, Notch1, Hes1, Bcl-2, and Bax. Results: The LRIG1 expression in U373 cells was remarkably lower than that in normal glial cells (*P*=0.019). The LRIG1 expression in the LRIG1 group was successfully increased when compared with that in the control group (P=0.004). The cell viability of the LRIG1 group was significantly lower than that of the NC group and control group at 24 h, 48 h, and 72 h (P=0.040, 0.025; P=0.041, 0.041; P=0.035, 0.035) respectively. Increased LRIG1 expression level in glioma cells strongly inhibits cell migration in transwell experiment. Flow cytometry results indicated that the apoptosis rate of the LRIG1 group was critically higher than that of the NC group and control group (P=0.003; P=0.003). According to results of Western blot, the expression levels of Notch1, Hes1, Hes5, and Jagged1 in LRIG1 group were dramatically higher than that in NC group and control group (P=0.006, 0.013; P=0.025, 0.026; P=0.001, 0.004; P=0.025, 0.027; P=0.029, 0.021) reespectively. While Bax expression in LRIG1 group was lower than that of NC group and control group (P=0.018, 0.021). Conclusion: The up-regulation of LRIG1 can inhibit the proliferation and migration of glioma cells and promote apoptosis by regulating the Notch signaling pathway.

Keywords: LRIG1, notch signal, glioma, cell proliferation, apoptosis, cell invasion

Introduction

Glioma is one of the most common malignant tumors that threaten people's lives. Gliomas account for about 80% of all cerebral tumors, and once diagnosed, over 90% of the patients have less than 5 years survival [1]. Despite the improvement of the treatment and diagnosis of glioma in recent years, its incidence and mortality remain high. The pathogenesis of Glioma is related to various factors, including biology, genetics, chemistry, physics, environment, ionizing radiation, and nitroso compounds. Its etiology has not been fully clarified yet [2, 3]. The current treatment methods include surgery, radiotherapy, and chemotherapy, but the efficacy is usually unfavorable. More molecular-level studies are urgently needed in order to improve disease treatment efficacy [4].

Leucine-rich Repeat and Immunoglobulin Domain-containing Protein-1 (LRIG1), a member of the LRIG gene family, is located at the site 3p14.3 of the human chromosome. Transcription products of LRIG1 gene are abundantly expressed in normal tissues, such as the brain, heart, kidney, skeletal muscle, stomach, and testis [5]. It has been proved in previous studies that LRIG1 gene is a tumor suppressor gene, and its down-regulation is connected with the poor prognosis of tumor. However, the specific regulatory mechanism of LRIG1 has yet been clarified [6]. Notch signaling pathway is a highly conservative signal transduction system

that can regulate cell proliferation, apoptosis, differentiation, migration, and invasion [7]. It has been found that TNF signaling [8] can induce cell apoptosis by activating Notch1. At the same time, there is an abnormal expression of Notch signaling pathway during the development of nerve neoplasms. However, it still needs further research to show whether LRIG1 regulates Notch signaling pathway in gliomas. Therefore, this study explored and analyzed the effects of LRIG1 on proliferation, apoptosis, and invasion of glioma cells by adjusting Notch signaling pathway.

Material and methods

Reagents and instruments

The glioma cell line U373 and normal glial HA-1800 cells were both purchased from Shanghai Cell Bank of Chinese Academy of Sciences. The sources of the remaining materials are as follows: DMEM medium and Trypsin (Hyclone Co., USA); Fetal bovine serum (Invitrogen USA); CCK-8 kit (Shanghai Beyotime Biological Co., Ltd.); DMSO (Sigma, USA); Annexin V-FITC/PI (Jiangsu Keygen Biotech Corp., Ltd.); The monoclonal antibody of LRIG1 mouse (Abcam Co., USA.); Horseradish goat anti-rat IgG (Shanghai Beyotime Biological Co., Ltd.); The overexpressed LRIG1 virus and control virus (Shandong Weizhen Biological Co., Ltd.).

Cell culture and construction of cell lines

U373 cells were conventionally cultured in DMEM medium containing 10% fetal bovine serum, and normal glial HA1800 cells were cultured with astrocyte medium (basal medium, 10% fetal bovine serum, 10% astrocyte growth additive, 1% penicillin and streptomycin). The cells at logarithmic phase were grouped into the control group (untreated), NC group (infected with control lentivirus), and the LRIG1 group (transfected with LRIG1 lentivirus, which was designed, constructed, and synthesized by Shanghai Shengbo Co., LTD., with a titer of 2.0×10^9 TU/mL). After 48 h of infection, the cells were replaced with the complete medium that containing 1 μg/ml puromycin. After 24 h incubation with puromycin, those stable living cells were obtained. Subsequently, the expression of LRIG1 in three groups of cells was analyzed by RT-PCR and Western Blot.

Detection of mRNA expression of LRIG1 and Notch1 by RT-PCR

The cells in the above three groups were cultured to the logarithmic growth stage and digested by trypsin. The cell density was adjusted, and the cells were inoculated into 6-well plates for further culture for 48 h. The cells' total RNA was extracted with the Trizol reagent. After the concentration and purity of total RNA were determined, it was synthesized by reverse transcription into cDNA (reaction system 20 μL). Then the cDNA was synthesized by using cDNA synthesis master mix kit (reaction system 20 μL). The reaction conditions were as follows: 30 s of pre-denaturation by 95°C, 5 s by 95°C, 30 s by 60°C, with a total of 40 rounds. The relative mRNA expression of LRIG1 and Notch1 was calculated by $2^{-\Delta\Delta Ct}$ with GADPH as the internal reference gene. The forward and reverse primer sequences of LRIG1 are 5'-GGTGACCtGGCCTTATGTGATA-3' and 5'-CACCACCATCCTGCACCTCC-3'. The forward and reverse primers of Notch1 are 5'-CTCCCCGTTCCAGCAGTCTC-3' and 5'-CAGCCACTCGCATTGACCat-3'; and the forward and reverse primers of GADPH are 5'-AccTGCCAAATATGatGAC ATC-3' and 5'-GTATCCAGTGCAGGGTCC-3'.

Detection of cell proliferation by CCK-8

The above three groups of cells were cultured to the logarithmic growth stage and digested with trypsin. The cell density was adjusted to 2×10^5 per mL, and 100 μL cells were inoculated on 96-well-plate for culturing of 24 h, 48 h, and 72 h. After 20 μl of CCK-8 solution was added for continuous incubation for 2 h, the absorbance value at 450 nm of the cells in each group was detected by a microplate reader. Each cell was set with 5 duplicate pores.

Detection of cell migration by Transwell

The matrix gel was diluted with serum-free DMEM and transferred to the Transwell upper chamber for coagulation. The device was cleaned with serum-free medium twice before use. The above three groups of cells were digested by trypsin and resuspended in a serum-free medium. The cell density was adjusted to 2×10^5 per mL, the cells were inoculated in the upper chamber of Transwell, and a 500 μL DMEM medium that containing 10% serum was inoculated in the lower chamber. The device was incubated at 37°C and 5% CO_2 for 24 h, fixed, and stained with crystal violet.

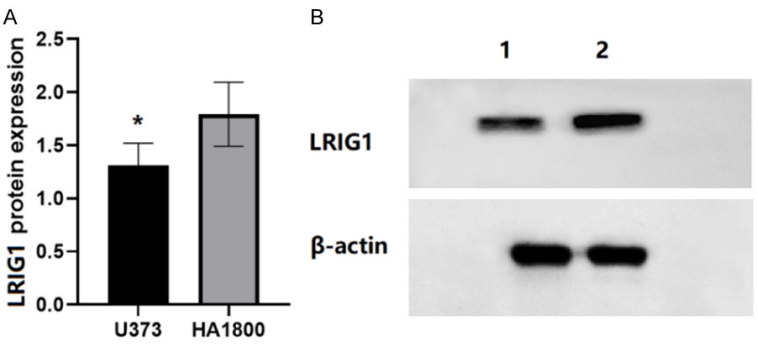

Figure 1. The protein expression of LRIG1 in normal glial cells (HA1800) was significantly lower than that in glial cancer cells (U373). A. Quantified protein level of LRIG1 (n=3, #P<0.05); B. The representative western blot images of LRIG1.

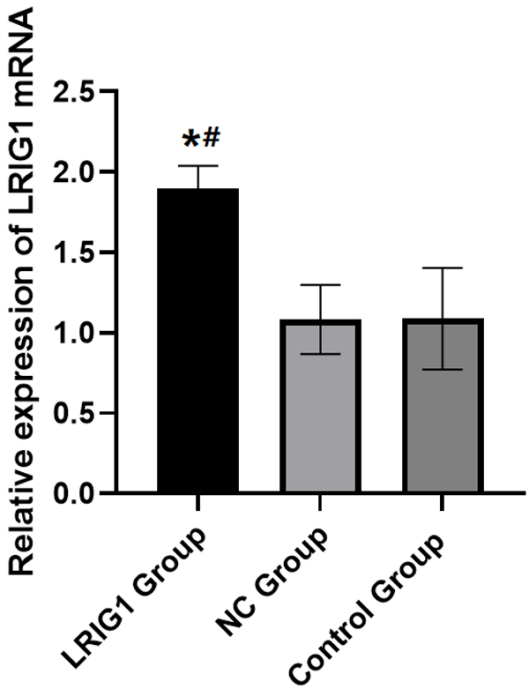

Figure 2. The relative mRNA expression of LRIG1 in U373 cells. (n=3, Compare with NC Group, tested by SNK-Q, *P<0.05; Compare with Control Group, tested by SNK-Q, #P<0.05).

Then the number of cells was calculated under the microscope.

Flow cytometry

The cells were cultured for 48 h after transfection. Firstly, the cells were digested with 0.25% EDTA-free trypsin and rinsed with PBS three times. Then the cells were collected by 5 min centrifugation at the speed of 1000 r/min. Secondly, the cells were resuspended with 200 μL Annexin V-FITC binding solution. After Annexin V-FITC (5 μL) was added, the mixture was incubated for 15 min in dark. Thirdly, after twice rinse with PBST, 5 μL PI staining solution was added to stain the cell followed by the instruction of kit. Finally, the cells were rinsed twice with PBS and re-suspended in binding buffer for testing.

Detection of protein expression by western blot

The cells were cultured for 48 h after transfection. Firstly, the three groups of cells were collected, fully lysed with lysate, and reacted on ice for 30 min. After being centrifuged at 12000 r/min for 10 min, the cell supernatant (extracted total protein) was collected. The concentration of protein was measured by the BCA method. Secondly, 50 μg of total protein was added with 5× loading buffer and boiled under 100°C for 5 min for denaturation. After 10% SDS-PAFE electrophoresis, the cells were transferred to the PVDF membrane and blocked with 5% BSA at 37°C for 1 h. Subsequently, the primary antibodies (LRIG1, Notch1, Hes1, Hes5, Jagged1, Bcl-2 and Bax) were added to the membranes, incubated at 4°C overnight. After incubation, the membrane was washed with TBST 3 times. The membrane was added with HRP-labeled secondary antibody, incubated for 2 h at room temperature, and rinsed with TBST. Then, the ECL chromogen regent was dripped into the PVDF membrane for film development.

The grayscale value of each strip was calculated by Quantity One. The relative expression of each target protein was quantified by using beta-actin as the internal reference. The experiment was carried out for three times. The dilutions of primary antibodies used in western blot are list below: LRIG1 antibodies (Abcam, 1:3000), Notch1 antibodies (Abcam, 1:1500), Hes1 antibodies (Abcam, 1:800), Hes5 antibodies (Abcam, 1:4000), Jagged1 antibodies (Abcam, 1:1000), Bcl-2 antibodies (Abcam, 1:1000), Bax antibodies (Abcam, 1:5000).

Notch1 inhibition assay

In LRIG1 high-expression group, the Notch1 inhibitor, 10 μ0ol DAPT N-(3,5-difluorophenacetyl)-

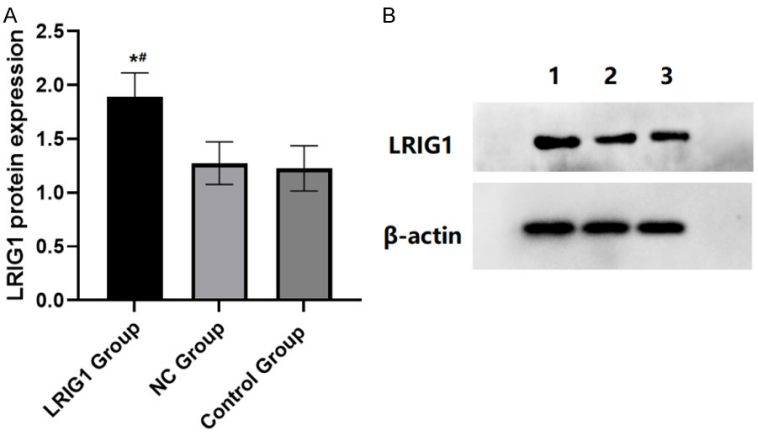

Figure 3. The protein expression level of LRIG1 in U373 cells. A. Quantified LRIG1 protein expression in LRIG1 group, NC group and control group (n=3, compare with NC Group, tested by SNK-Q, *P<0.05; compare with Control Group, tested by SNK-Q, #P<0.05); B. The representative western blot images of LRIG1.

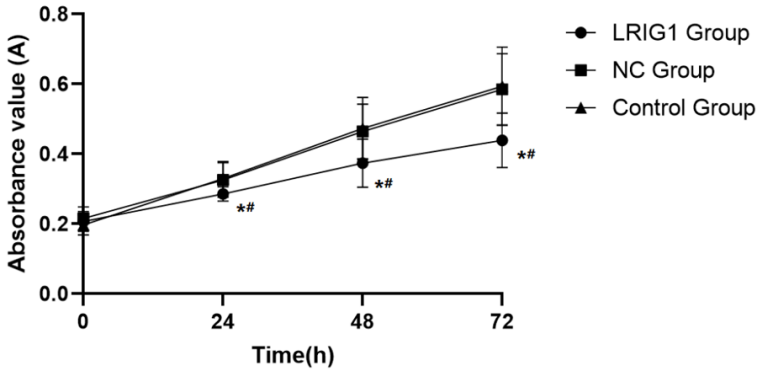

Figure 4. Cell viability of U373 cells in different treatment groups after being cultured for 24, 48, and 72 hours (n=3, compare with NC Group, tested by SNK-Q, *P<0.05; compare with Control Group, tested by SNK-Q, #P<0.05).

L-alanyl-(S)-phenylglycinet-butylester was added into the culture medium. Thereafter, cells were collected for following experiment: 1) detection of mRNA expression LRIG1 and Notch1 in cells by qPCR; 2) cell apoptosis and migration assay after the inhibition of Notch1 signal was confirmed.

Statistical analysis

SPSS25.0 was used for data analyses. The measurement data were expressed as ($\bar{x}\pm s$), and comparisons among multiple groups were conducted using one-way analysis of variance, and the pairwise comparisons between the mean of each group was tested by SNK-Q test. The difference was statistically significant at *P<0.05.*

Results

LRIG1 expression level was downregulated in U373 cells

As shown in **Figure 1B**, the LRIG1 expression level in U373 cells was obviously lower than that in HA1800 cells (*P*= 0.019).

Detection of relative mRNA expression of LRIG1 in each group of cells by RT-PCR

The relative expression of LRIG1 mRNA in the LRIG1 group was remarkably higher than that in the NC group and control group (P=0.001, P= 0.004), while there was no significant difference in the relative mRNA expression of LRIG1 between the NC group and control group (P=0.968) (**Figure 2**).

LRIG1 protein expression in each group

LRIG1 protein expression in LRIG1 group was remarkably higher than that in NC group and control group (P=0.002, P=0.001), but there was no obvious difference between NC group and control group (P=0.725) (**Figure 3**).

The influence of LRIG1 overexpression on cell proliferation

The cell viability of LRIG1 group after 24 h, 48 h, 72 h of culture was apparently lower than that in NC group and control group (P=0.040, 0.025; P=0.041, 0.041; P=0.035, 0.035), and there was no significant difference in cell viability between NC group and control group at 0, 24, 48, and 72 h, respectively (P=0.362; P=0.837; P=0.829; P=0.898) (**Figure 4**).

The influence of LRIG1 overexpression on cell migration

According to Transwell experiment, the quantity of migrated cells in the LRIG1 group was obviously less than that in the NC group and control

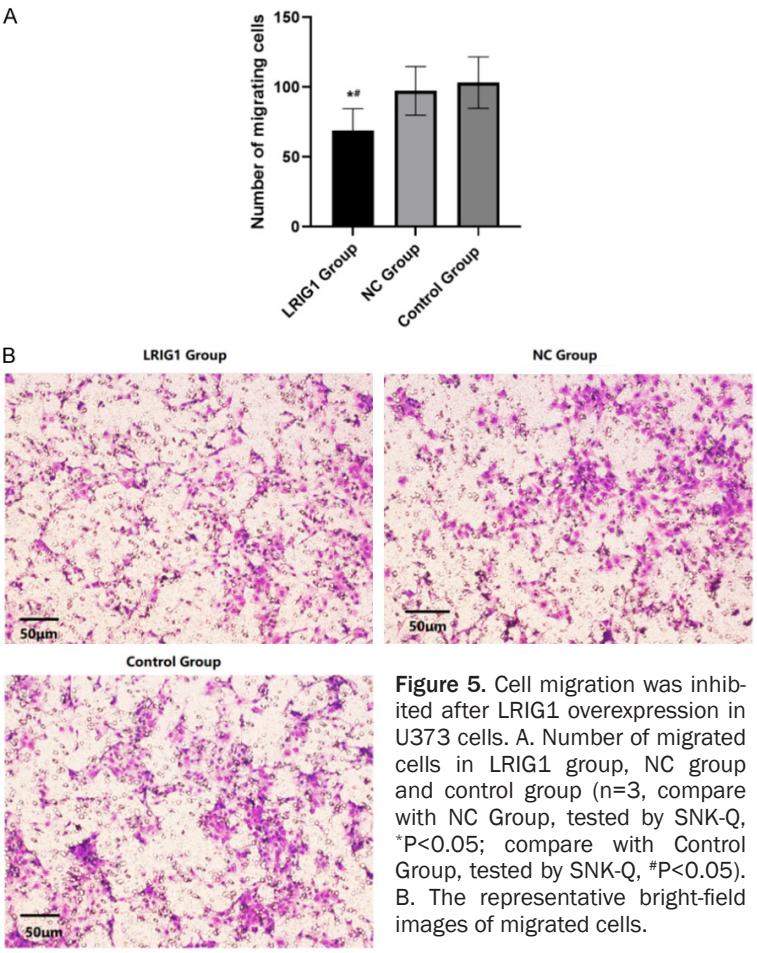

Figure 5. Cell migration was inhibited after LRIG1 overexpression in U373 cells. A. Number of migrated cells in LRIG1 group, NC group and control group (n=3, compare with NC Group, tested by SNK-Q, *P<0.05; compare with Control Group, tested by SNK-Q, #P<0.05). B. The representative bright-field images of migrated cells.

group (P=0.027; P=0.013); While there was no remarkable difference in the number of migrated cells between NC group and control group (P=0.618) (**Figure 5**).

The influence of LRIG1 overexpression on cell apoptosis

It was revealed by the results of Flow cytometry that the LRIG1 group had dramatically higher apoptotic rate than the NC group and the control group (P=0.003; P=0.003), while there was no statistical difference between the NC group and control group (P=0.731) (**Figure 6**).

Influence of LRIG1 overexpression on Notch1 pathway proteins

Western blot results indicated that the expressions of Notch1, Hes1, Hes5, Jagged1 and Bax in LRIG1 group were obviously higher than those in NC group and control group (P=0.006, 0.013; P=0.025, 0.026; P=0.001, 0.004; P=0.025, 0.027; P=0.029, 0.021), the expression of Bax in LRIG1 group was lower than that in NC group and control group (P=0.018, 0.021), and the expression of Notch1 pathway related molecules between NC group and control group was not showing statistical difference (P=0.765; P=0.627; P=0.968; P=0.787; P=0.585; P=0.908) (**Figure 7**).

Inhibition of Notch1 signaling pathway restored the viability and migration potential of tumor cells

First, the Notch1 signaling pathway was successfully inhibited by using its inhibitor DAPT. As shown in **Figure 8**, the downstream items of the Notch1 signal pathway were decreased in DAPT groups when compared with those makers in the LRIG1 group. However, the expression level of LRIG1 was not affected by adding DAPT. This might indicate that LRIG1 is an upstream gene of Notch1 signaling pathway. And the cell viability and migration ability were not different from those of the control group after the cell was exposed to the inhibitor.

Discussion

Glioma is the most common intracranial malignant tumor in the central nervous system. It has a high incidence, recurrence, and mortality rate with rapid invasion and high heterogeneity. Study shows that [9] glioma accounts for about 80% of cerebral tumors, and over 90% of patients survive less than 5 years after diagnosis. The predisposing factors of glioma may be biological, genetic, or environmental factors [10]. At present, the dominant treatment for glioma is surgical treatment, but the glioma cells proliferate rapidly and grow infiltrating. Malignant glioma cells can quickly infiltrate surrounding tissues, making the boundary unclear and difficult to remove completely, which is the fundamental reason for high recurrence [11]. Since glioma is one of the most chanllenging

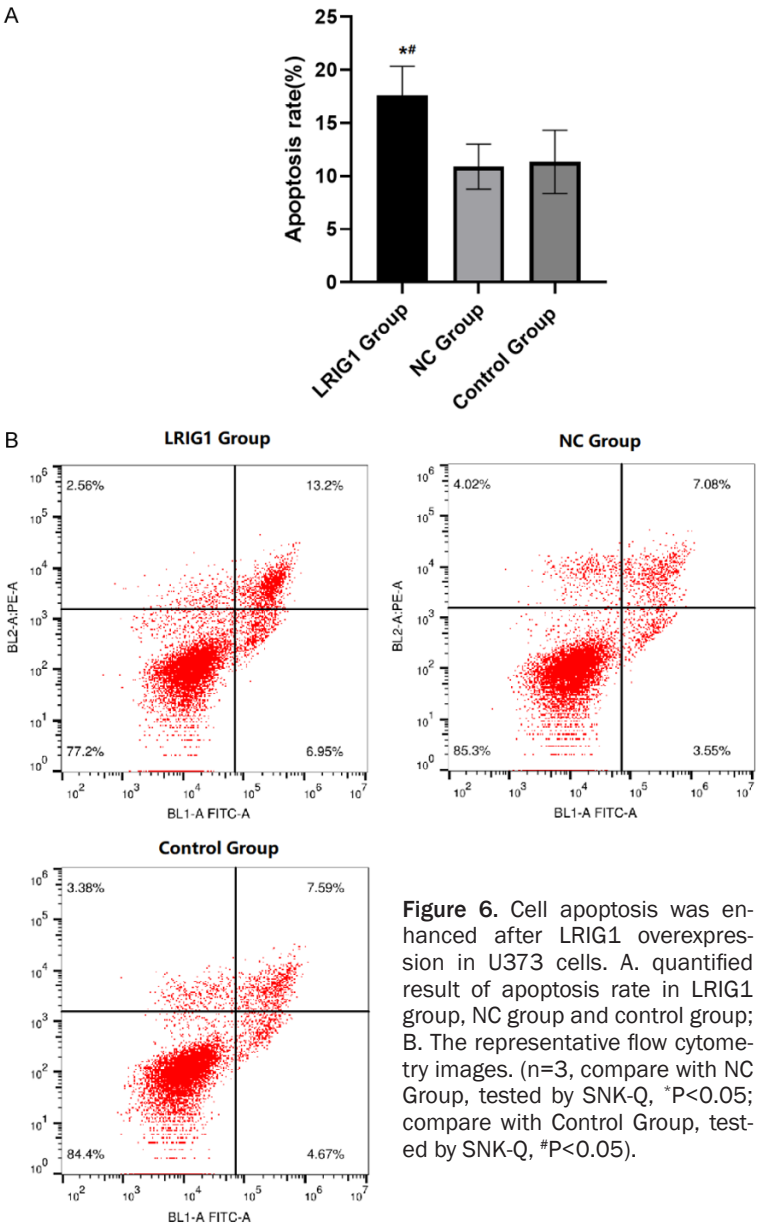

Figure 6. Cell apoptosis was enhanced after LRIG1 overexpression in U373 cells. A. quantified result of apoptosis rate in LRIG1 group, NC group and control group; B. The representative flow cytometry images. (n=3, compare with NC Group, tested by SNK-Q, $^{*}P<0.05$; compare with Control Group, tested by SNK-Q, $^{\#}P<0.05$).

diseases in neurosurgery, elucidating its pathogenesis and exploring new and effective therapeutic targets are both hot and difficult issues [12]. LRIG is a gene that has attracted much attention in recent years. LRIG family members include LRIG1, LRIG2 and LRIG3, among which LRIG1 is the most well-studied. It has been confirmed that LRIG1 is widely expressed in normal tissues of human body but down-regulated or lost in most tumors. This suggests that LRIG1 may play a tumor suppressive role in regulation of the growth, proliferation, invasion and metastasis of tumor cells [13]. The mouse homologous gene of LRIG1 is *LRIG1*, which is located at 3p14.3 of human chromosome and is a frequently deleted region in human malignant tumors such as breast and pulmonary tumors [14]. LRIG1 expression was lost or down-regulated in most tumors, while up-regulated in some tumors. Hakan et al. [15] found that LRIG1 was highly expressed in prostate cancer, lung cancer, astrocytoma and leukemia, and is much complex in breast and colorectal cancer. In astrocytomas, patients with perinuclear expression of LRIG1 protein had a better prognosis and higher survival rate, while those with the cytoplasmic expression of LRIG1 protein had a poorer prognosis. This indicates that except that the LRIG1 expression level is a crucial factor in cancer metabolism, subcellular localization of LRIG1 also shows significant biological significance. It is speculated that LRIG1 may also exert a dual role of oncogene and tumor suppressor genes due to the different subcellular localization of protein in partial tumors. In terms of the mechanism of glioma, Mao F et al. [16] studied the role of LRIG1 in gliomas by PCR and immunohistochemistry, and found that the high expression of LRIG1 in cells changed the expression pattern of cell cycle regulatory protein cyclinD1, resulting in inhibiting the cell proliferation. He et al. [17] revealed that EGFR and its downstream signaling pathway Akt/mTORC1 were both down-regulated in U87 cells with up-regulated LRIG1 expression; while LRIG1 gene knockout and restoration of Akt activation through RNAi can lead to a enhanced proliferation of U87 cells, suggesting that LRIG1 can cause cell growth inhibition and apoptosis by inhibiting EGFR and Akt/mTORC1. XieXiao et al. [18] showed that EGFR and downstream of PI3K/Akt and MAPK/ERK signaling pathways were activated in GL15 glioma cells with low

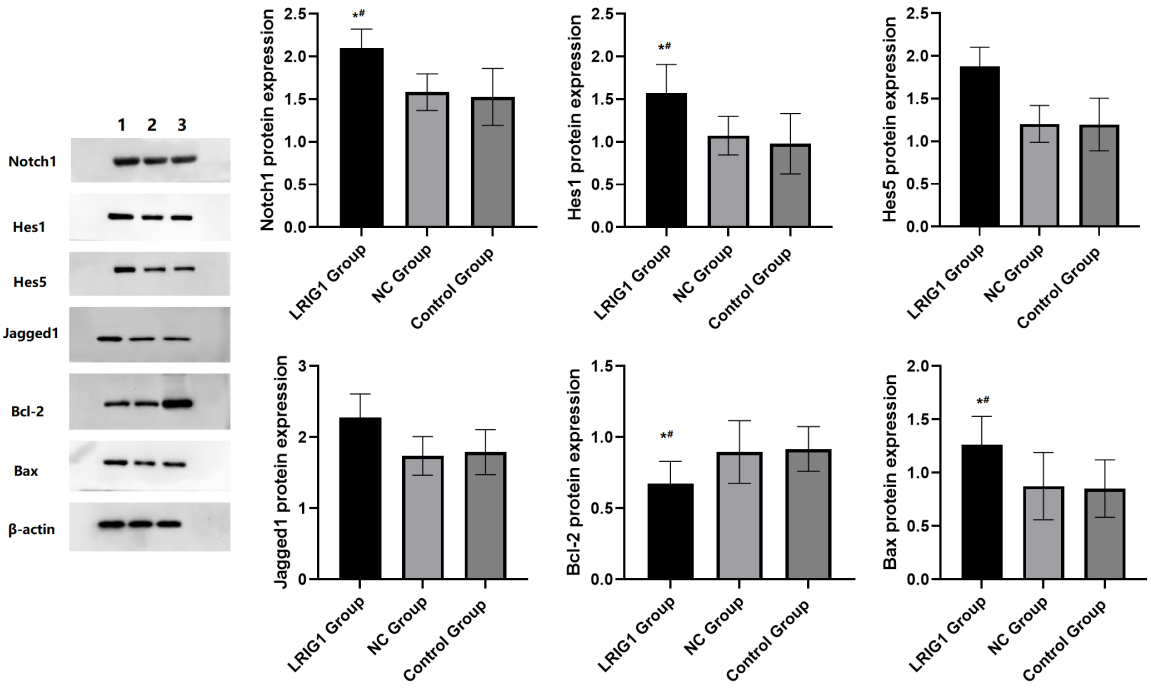

Figure 7. Expression of Notch1 pathway related molecules in U373 cells of different treatment group. (n=3, compare with NC Group, tested by SNK-Q, *P<0.05; compare with Control Group, tested by SNK-Q, #P<0.05).

expression of the LRIG1 gene. They also found the activities of MMPs, which are closely related to cell invasion, were significantly enhanced. Taken together, invasion ability of GL15 cells increases through the activation of EGFR/Akt/c-Myc. This shows that LRIG1 negatively regulates the proliferation, migration and invasion of glioma cells by inhibiting the activation of EGFR and its downstream signal pathway, promoting cell apoptosis and inhibiting oncogenes.

In the treatment of glioma, Xiao Qungen et al. [19] used liposome-mediated gene transfection technology to increase the expression of LRIG1 and found that the expression of EGFR in cells, as well as the anti-apoptotic protein Bcl-2, decreased remarkably, while the apoptotic related proteins Bax and caspase-3 increased. LRIG1 binds to EGFR through the LRR region outside the cell, and the intracellular segment forms a complex with EGFR through the E3 ubiquitin ligase c-Cbl to make EGFR ubiquitinated, endocytosed, and finally degraded, inhibiting the EGFR signaling pathway. In this study, the LRIG1 lentivirus was used to infect brain glioma U373 cells, and the results showed that the expression of LRIG1 protein after infection with LRIG1 lentivirus was significantly increased. As the upregulation of LRIG1, cell proliferation and migration were significantly inhibited, while the rate of cell apoptosis was significantly increased. Those observations were consistent to the results reported by many research groups [20-22]. Notch signaling pathway is widely found in vertebrates and invertebrates. As a conserved signaling pathway in evolution, it is involved in the genesis and development of multiple genes [23, 24]. Notch activates ligand and regulates cell apoptosis and determines the selection of cell fate. Dysregulation of Notch receptor, ligand and downstream components has been found in a variety of nervous system tumors [25, 26]. Besides, Notch signaling pathway exerts a key role in tumor vascular development, directly affecting the selection of tip cells, vascular stability, vascular smooth muscle cell differentiation and arteriovenous differentiation. A variety of tumor model studies have found that interference with DLL4/Notch signal will produce many non-functional blood vessels, which results in local hypoxia and reduces blood perfusion in tumor tissues, thereby inhibiting tumor growth. On the contrary, LRIG1 can affect tumor neovascularization by inhibiting epidermal growth factor receptor (EGFR) signal transduc-

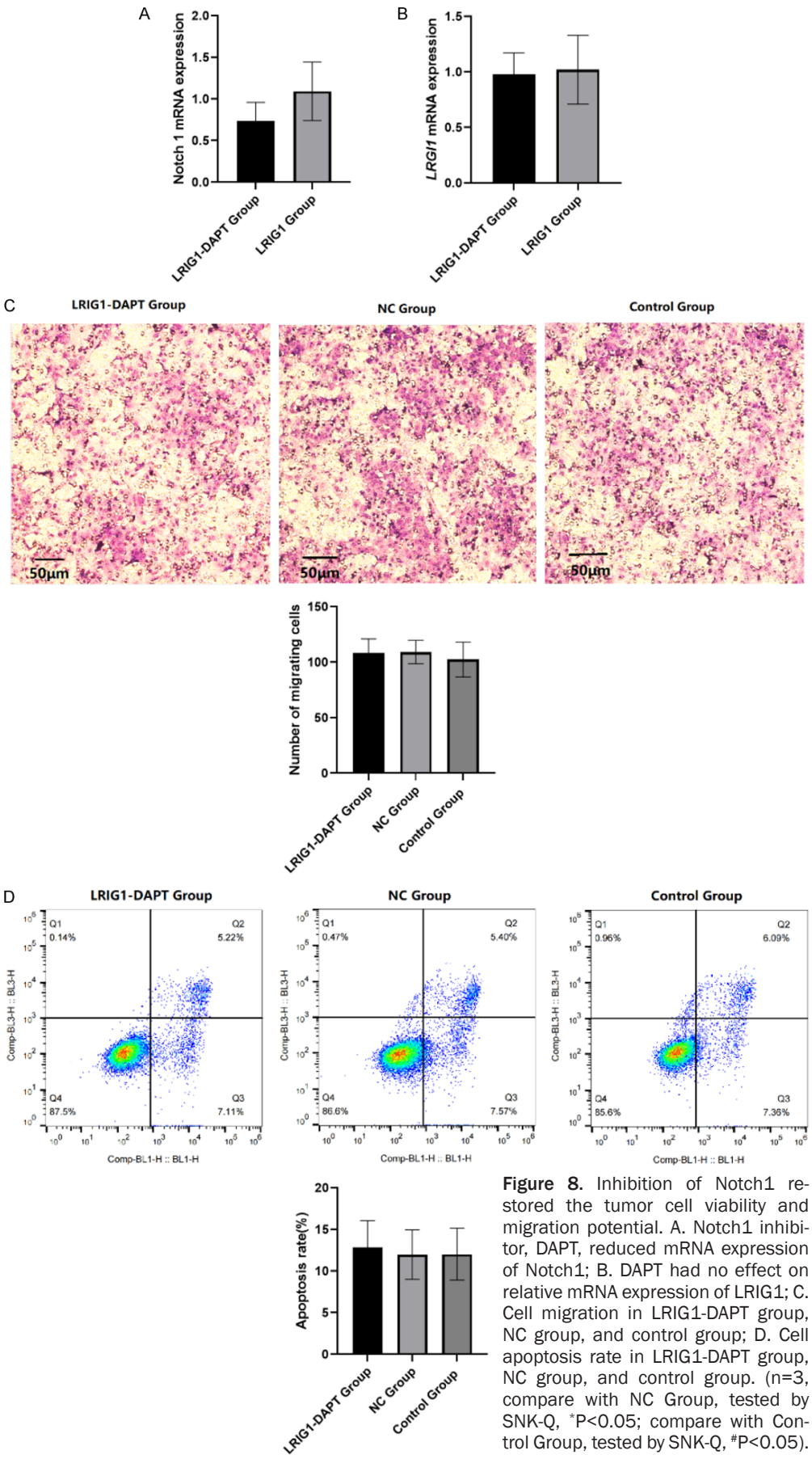

Figure 8. Inhibition of Notch1 restored the tumor cell viability and migration potential. A. Notch1 inhibitor, DAPT, reduced mRNA expression of Notch1; B. DAPT had no effect on relative mRNA expression of LRIG1; C. Cell migration in LRIG1-DAPT group, NC group, and control group; D. Cell apoptosis rate in LRIG1-DAPT group, NC group, and control group. (n=3, compare with NC Group, tested by SNK-Q, *P<0.05; compare with Control Group, tested by SNK-Q, #P<0.05).

tion, thus exerting corresponding tumor inhibition effect. Therefore, we speculated that LRIG1 could regulate the malignant biological behaviors of glioma cells through Notch signaling pathway. This research results demonstrated that the expression of Notch1, Hes1, Hes5, Jagged1, Bax in LRIG1 group was obviously higher than that of NC group and control group, while Bax expression of LRIG1 group was remarkably lower than that of NC group and control group. Studies have confirmed that the effect of LRIG1 overexpression on glioma cells is closely related to Notch signaling regulation. It affects the activity of Notch protein by regulating the binding of Notch signal receptor and ligand, and regulates the expression of downstream target genes Hes1, Hes5, Jagged1, anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax. This may be one of the mechanisms by which LRIG1 regulates the malignant behaviors of glioma cells [27]. In this study, LRIG1 overexpression on glioma cells and Notch signaling pathway activity of glioma cells were analyzed, the results suggested that LRIG1 might be a potential therapeutic target for glioma cells. However, due to the lack of in-depth research on its mechanism of action, the specific mechanism of action needs further research and analysis. In addition, the effects of LRIG1 on radiotherapy and chemotherapy will be further observed in the follow-up studies to provide new approaches for the clinical treatment of glioma.

In conclusion, the overexpression of LRIG1 can inhibit the proliferation and migration of glioma cells and promote apoptosis by regulating Notch signaling pathway. LRIG1 may be an effective target to treat glioma, which needs to be further studied and analyzed.

Disclosure of conflict of interest

None.

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References

[1] Tang F, Wang H, Chen E, Bian E, Xu Y, Ji X, Yang Z, Hua X, Zhang Y and Zhao B. LncRNA-ATB promotes TGF-beta-induced glioma cells invasion through NF-kappaB and P38/MAPK pathway. J Cell Physiol 2019; 234: 23302-23314.

[2] Chang L, Wang J, Zhou F, Wang D, Chen R, Zhang Y and Zhang J. LncRNA RP11-84E24.3 drives tumorigenesis and epithelial-to-mesenchymal transition of glioma cells by promoting TFAP2C-mediated activation of SNAI1. J Neurooncol 2021; 151: 157-171.

[3] Liu B, Cao W and Ma H. Knockdown of lncRNA LSINCT5 suppresses growth and metastasis of human glioma cells via up-regulating miR-451. Artif Cells Nanomed Biotechnol 2019; 47: 2507-2515.

[4] Liu ZZ, Tian YF, Wu H, Ouyang SY and Kuang WL. LncRNA H19 promotes glioma angiogenesis through miR-138/HIF-1alpha/VEGF axis. Neoplasma 2020; 67: 111-118.

[5] Mu M, Niu W, Zhang X, Hu S and Niu C. LncRNA BCYRN1 inhibits glioma tumorigenesis by competitively binding with miR-619-5p to regulate CUEDC2 expression and the PTEN/AKT/p21 pathway. Oncogene 2020; 39: 6879-6892.

[6] Hu YW, Kang CM, Zhao JJ, Nie Y, Zheng L, Li HX, Li X, Wang Q and 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.

[7] Gu N, Wang X, Di Z, Xiong J, Ma Y, Yan Y, Qian Y, Zhang Q and Yu J. Silencing lncRNA FOXD2-AS1 inhibits proliferation, migration, invasion and drug resistance of drug-resistant glioma cells and promotes their apoptosis via microRNA-98-5p/CPEB4 axis. Aging (Albany NY) 2019; 11: 10266-10283.

[8] Huang W, Shi Y, Han B, Wang Q, Zhang B, Qi C and Liu F. LncRNA GAS5-AS1 inhibits glioma proliferation, migration, and invasion via miR-106b-5p/TUSC2 axis. Hum Cell 2020; 33: 416-426.

[9] Liu J, Cheng LG and Li HG. LncRNA SNHG20 promoted the proliferation of glioma cells via sponging miR-4486 to regulate the MDM2-p53 pathway. Eur Rev Med Pharmacol Sci 2019; 23: 5323-5331.

[10] Yu BX, Zou L, Li S and Du YL. LncRNA SAMD12-AS1 down-regulates P53 to promote malignant progression of glioma. Eur Rev Med Pharmacol Sci 2019; 23: 8456-8467.

[11] Liu J, Cheng LG and Li HG. LncRNA SNHG20 promoted the proliferation of glioma cells via sponging miR-4486 to regulate the MDM2-p53 pathway. Eur Rev Med Pharmacol Sci 2019; 23: 5323-5331.

[12] Qin WX, Shi Y, Zhu D, Li YP, Chen YH, Cui J, Cui GY, Pan JX and Ren ZY. EZH2-mediated H3K27me3 enrichment on the lncRNA MEG3 promoter regulates the growth and metastasis of glioma cells by regulating miR-21-3p. Eur Rev Med Pharmacol Sci 2020; 24: 3204-3214.

[13] Fu C, Li D, Zhang X, Liu N, Chi G and Jin X. LncRNA PVT1 facilitates tumorigenesis and progression of glioma via regulation of MiR-128-3p/GREM1 axis and BMP signaling pathway. Neurotherapeutics 2018; 15: 1139-1157.
[14] Miao W, Li N, Gu B, Yi G, Su Z and Cheng H. LncRNA DLGAP1-AS2 modulates glioma development by up-regulating YAP1 expression. J Biochem 2020; 167: 411-418.
[15] Hedman H and Henriksson R. LRIG inhibitors of growth factor signalling-double-edged swords in human cancer? Eur J Cancer 2007; 43: 676-82.
[16] Mao F, Wang B, Xiao Q, Xi G, Sun W, Zhang H, Ye F, Wan F, Guo D, Lei T and Chen X. A role for LRIG1 in the regulation of malignant glioma aggressiveness. Int J Oncol 2013; 42: 1081-1087.
[17] He XY, Liu XJ, Chen X, Bian LG, Zhao WG, Shen JK and Sun QF. Gambogic acid induces EGFR degradation and Akt/mTORC1 inhibition through AMPK dependent-LRIG1 upregulation in cultured U87 glioma cells. Biochem Biophys Res Commun 2013; 435: 397-402.
[18] Xie R, Yang H, Xiao Q, Mao F, Zhang S, Ye F, Wan F, Wang B, Lei T and Guo D. Downregulation of LRIG1 expression by RNA interference promotes the aggressive properties of glioma cells via EGFR/Akt/c-Myc activation. Oncol Rep 2013; 29: 177-184.
[19] Xiao GQ, Zhang T, Mao F, Wang Y, Xie RF, Wang BF, Guo DS and Lei T. Effects of LRIG1 overexpression on cisplatin-induced apoptosis ofglioblastoma cells and mechanism. Journal of Huazhong University of Science and Technology (Medical Edition) 2013; 41: 656-659.
[20] Li Z, Zhang J, Zheng H, Li C, Xiong J, Wang W, Bao H, Jin H and Liang P. Modulating lncRNA SNHG15/CDK6/miR-627 circuit by palbociclib, overcomes temozolomide resistance and reduces M2-polarization of glioma associated microglia in glioblastoma multiforme. J Exp Clin Cancer Res 2019; 38: 380.
[21] Liu X, Zheng J, Xue Y, Yu H, Gong W, Wang P, Li Z and Liu Y. PIWIL3/OIP5-AS1/miR-367-3p/CEBPA feedback loop regulates the biological behavior of glioma cells. Theranostics 2018; 8: 1084-1105.
[22] Gong X, Liao X and Huang M. LncRNA CASC7 inhibits the progression of glioma via regulating Wnt/beta-catenin signaling pathway. Pathol Res Pract 2019; 215: 564-570.
[23] Mazor G, Levin L, Picard D, Ahmadov U, Carén H, Borkhardt A, Reifenberger G, Leprivier G, Remke M and Rotblat B. The lncRNA TP73-AS1 is linked to aggressiveness in glioblastoma and promotes temozolomide resistance in glioblastoma cancer stem cells. Cell Death Dis 2019; 10: 246.
[24] Wang WJ, Sun K, Li FY, Hui XB, Liu D, Liu J and Wang XD. LncRNA HANR aggravates the malignant progression of glioma via targeting miRNA-335. Eur Rev Med Pharmacol Sci 2020; 24: 758-765.
[25] Zong Z, Song Y, Xue Y, Ruan X, Liu X, Yang C, Zheng J, Cao S, Li Z and Liu Y. Knockdown of LncRNA SCAMP1 suppressed malignant biological behaviours of glioma cells via modulating miR-499a-5p/LMX1A/NLRC5 pathway. J Cell Mol Med 2019; 23: 5048-5062.
[26] Xu CH, Xiao LM, Liu Y, Chen LK, Zheng SY, Zeng EM and Li DH. The lncRNA HOXA11-AS promotes glioma cell growth and metastasis by targeting miR-130a-5p/HMGB2. Eur Rev Med Pharmacol Sci 2019; 23: 241-252.
[27] Wang C, Chen Y, Wang Y, Liu X, Liu Y, Li Y, Chen H, Fan C, Wu D and Yang J. Inhibition of COX-2, mPGES-1 and CYP4A by isoliquiritigenin blocks the angiogenic Akt signaling in glioma through ceRNA effect of miR-194-5p and lncRNA NEAT1. J Exp Clin Cancer Res 2019; 38: 371.