Glioma has been considered as one of the most prevalent and common malignancy of the nervous system; however, the underlying mechanisms that are responsible for the occurrence and development of glioma still remain largely unknown. Amounting evidence highlights the critical regulatory function of miRNAs in carcinogenesis. Here, we showed that the expression of miR-150-3p was significantly decreased in glioma tissues and cell lines. Suppressed expression of miR-150-3p was associated with the lymph node metastasis of the glioma patients. Overexpression of miR-150-3p significantly inhibited the proliferation of glioma cells. Molecular study uncovered that the transcription factor specificity protein 1 (SP1) was identified as one of the targets of miR-150-3p. Highly expressed miR-150-3p in glioma cells significantly decreased both the mRNA and protein levels of SP1. Consistently, the abundance of phosphatase and tension homolog deleted on chromosome ten (PTEN), a negative downstream target of SP1, was increased with the ectopic miR-150-3p. Collectively, these results suggested that miR-150-3p suppressed the growth of glioma cells partially via regulating SP1 and possibly PTEN.

Introduction

Glioma is the most common and malignant brain tumor, which accounts for approximately 80% of the brain carcinogenesis [1,2]. Surgical resection, radiotherapy, and chemotherapy have been the standard treatments for glioma. However, due to the high aggressive proliferation and invasion rate, as well as the resistance to necrosis, the medial survival of the glioma patients is only approximately 12 months and the 5-year survival rate of these patients remains less than 3% [3,4]. Therefore, it is quite urgent to identify novel targets and investigate the underlying molecular mechanisms which regulate the progression of glioma.

Increasing evidence has demonstrated the aberrant expression and critical involvement of miRNAs in human cancers [5-10]. MiRNAs were characterized as small (18-24 nts), single-stranded, non-coding RNA, which bind to the 3’-UTR of the target genes and inhibit the gene expression [11-13]. Due to the basic function of miRNAs in regulating gene expression, miRNAs are involved in a broad range of physiological processes, including cell proliferation, differentiation, stress condition, and tumorigenesis [13]. Notably, miRNA-mediated resistance to chemotherapy has been observed in a variety of cancers [14-19]. These studies indicated the critical roles of miRNAs in the initiation and progression of cancers. To search for miRNAs that were involved in the tumorigenesis of glioma, our previous work [20] screened the miRNAs that were aberrantly expressed in glioma tissues compared with normal brain tissues. The result showed that the expression of miR-150-3p was significantly decreased in glioma tissues (Supplementary Table S1), however, the potential function of miR-150-3p in glioma still remains unknown.

Specificity protein 1 (SP1) is a ubiquitously expressed transcription factor that recognizes the GC-boxes in the downstream target genes [21]. Overexpression of SP1 has been found in a variety of cancers and was
correlated with the worse prognosis of the cancer patients [22-24]. Recent studies reported that the expression and activity of SP1 were regulated by miRNAs and affected the cancer progression [25-29]. Amongst these miRNAs, miR-411 down-regulated the expression of SP1 and inhibited the growth of breast cancer cells [26]. Additionally, miR-326 reversed chemoresistance in lung cancer by targeting SP1 [30]. It was also reported that miR-31-5p inhibited the proliferation, migration of HepG2 hepatocellular carcinoma (HCC) via targeting SP1 [31]. These studies demonstrated that SP1 served as a good target of miRNAs and involved in the tumorigenesis of human cancers. As a transcription factor, to understand the role of SP1 in cancers, the function of the downstream targets of SP1 deserves further investigation. Amongst these targets of SP1, the phosphatase and tension homolog deleted on chromosome ten (PTEN) is a well-established tumor suppressor, which is down-regulated or mutated in cancers and contributes to the initiation and development of cancers [32-34]. Recent study demonstrated that SP1 bound to the promoter of PTEN and negatively regulated the expression of PTEN [33]. This finding uncovered the novel molecular mechanism of SP1 in tumorigenesis via regulating PTEN.

In the present study, we detected the expression of miR-150-3p in glioma tissues and cell lines. The effect of miR-150-3p on the growth of glioma cells was investigated by overexpressing miR-150-3p. The downstream targets of miR-150-3p were predicted by the bioinformatics analysis and SP1 was predicted as one of the targets of miR-150-3p. The down-regulation of SP1 by miR-150-3p increased the expression of PTEN. Inverse correlation between the expression of miR-150-3p and SP1 was also observed in glioma tissues. These results uncovered the novel functional mechanism of miR-150-3p in glioma.

### Materials and methods

#### Tissues and cell lines

Sixty glioma tissues were collected from the glioma patients by surgical reaction at The Second Xiangya Hospital of Central South University between January 2014 and October 2015. Thirty normal brain tissues were obtained from patients who underwent internal decompression surgery after traumatic brain injury. The tissues were immediately frozen in liquid nitrogen before miRNA extraction. The basic information of the patients including age, gender, lymph node metastasis, cancer stage and grade were summarized as Table 1. Written informed consent was obtained from all patients. The present study was approved by the Ethics Committee of The Second Xiangya Hospital of Central South University.

Human glioma cell lines including Uppsala 87 Malignant Glioma (U87-MG), U251, A172, SWO-38, and Suzhou Human Glioma-44 (SHG-44) were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). These cells were cultured with Dulbecco’s modified Eagle’s medium (DMEM, Hyclone, UT, U.S.A.) supplemented with 10% FBS (Gibco, CA, U.S.A.). The normal human astrocytes cell NHA was purchased from Lonza (Basel, Switzerland) and cultured with the AGM™ BulletKit™ (Lonza, Basel, Switzerland), which contains basic medium, insulin, ascorbic acid, l-glutamine, rhEGF, GA-1000, and 10% FBS. All the cells were maintained at 37°C in a humidified atmosphere with 5% CO₂.

| Clinical parameters               | n |
|-----------------------------------|---|
| **Age**                           |   |
| <60                               | 20|
| ≥60                               | 40|
| **Gender**                        |   |
| Male                              | 29|
| Female                            | 31|
| **Cancer stage**                  |   |
| I and II                          | 22|
| III and IV                        | 38|
| **Lymph node metastasis**         |   |
| Positive                          | 39|
| Negative                          | 21|
| **Tumor grade**                   |   |
| G1–G2                             | 18|
| G3                                | 42|

Table 1 Clinical characteristics of the glioma patients
Oligonucleotides and cell transfection
The miR-150-3p mimics, mimics control miRNA, miR-150-3p antagonim, and antagonist negative control miRNA were chemically synthesized by Ribobio (Guangzhou, Guangdong, China). Cells were cultured with DMEM for 36 h and the transfection was performed with the Lipofectamine 2000 (Thermo Fisher Scientific, MA, U.S.A.) according to the manufacturer’s instructions. After transfection for 48 h, the expression level of miR-150-3p was determined by the real-time quantitative PCR (RT-qPCR) analysis.

MiRNA isolation and quantitative real-time PCR
MiRNA extraction from the tissues or cell lines was performed with the miRcute miRNA isolation kit (DP501, TianGen Biotechnology, Beijing, China). MiRNA was reverse transcribed with the miRcute miRNA First-Strand cDNA Synthesis Kit (KR201, TianGen Biotechnology, Beijing, China) according to the manufacturer’s protocol. The qPCR reaction was performed with SsoFast™ EvaGreen® Supermix kit (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.) on ABI Prism 7300 system (Applied Biosystems). The PCR conditions were set as follows: 95°C for 10 min and 40 cycles at 95°C for 15 s, 57°C for 1 min. The relative expression of miR-150-3p was normalized to the expression of U6 RNA with the 2^−ΔΔC_T method.

Cell viability assay
Glioma cells transfected with miR-150-3p mimics or control miRNA were cultured in the 96-well plate. The cell viability was measured with cell counting kit-8 (CCK-8) at 0, 24, 48, 72, and 96 h after transfection. Ten microliters of CCK-8 reagent was added into the cells and incubated at 37°C for 2 h. The absorbance of each well was determined at 450 nm with the microplate reader. The experiment was performed in triplicate.

Colonies formation assay
Glioma cells with overexpressed miR-150-3p or control miRNA were seeded into the six-well plate with a density of 500 cells/well. Cells were cultured for 2 weeks with fresh medium containing 10% FBS. To observe the formation of colonies, cell culture medium was discarded and the cells were washed with PBS for three times. And then cells were fixed with 100% methanol for 30 min at room temperature (RT) and stained with 0.1% Crystal Violet for 20 min. The colonies were observed with the microscope and the number of colonies was recorded.

Western blot
After transfection for 48 h, glioma cells were harvested and lysed with the NP-40 lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris/HCl (pH 8.0), 1 mM EDTA) on ice for 30 min in the presence of protease inhibitor. The protein concentration was quantitated with the BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Twenty micrograms protein of each sample was loaded and separated by the SDS/PAGE (15% gel). The protein was then transferred on to the nitrocellulose membrane (Millipore, Billerica, MA, U.S.A.) and blocked with 5% of non-fat milk at RT for 1 h. Subsequently, membrane was incubated with primary antibodies against PTEN (ab32199, 1:2000 dilution, Abcam), GAPDH (sc-20357, 1:3000 dilution, Santa Cruz Biotechnology, Inc., Dallas, TX, U.S.A.), SP1 (#5931, 1:2000 dilution, Cell Signaling Technology, Danvers, MA, U.S.A.) for 2 h at RT, respectively. After washing with TBS and Tween-20 (TBST), the membrane was incubated with the secondary antibody conjugated with horseradish peroxidase (HRP) for 1 h at RT. The protein bands were detected with ECL (Amersham Pharmacia Biotech, Little Chalfont, U.K.) and analyzed using ImageJ Software (version 1.62; National Institute of Health, Bethesda, MD, U.S.A.).

Dual-luciferase reporter assay
The wild-type (WT) or mutant 3′-UTR of SP1 was synthesized and constructed into the pGL3 luciferase reporter vector (Promega Corporation, Madison, WI, U.S.A.). Glioma cells were cultured in the 96-well plate and co-transfected with WT or mutant pGL3-SP1-3′-UTR in the presence of miR-150-3p mimics. The pGL3 Renilla luciferase reporter plasmid was transfected as the internal control. After transfection for 48 h, cells were harvested and the luciferase activity was measured using the Dual-luciferase Reporter Assay Kit (Promega Corporation, Madison, WI, U.S.A.). The experiment was performed in triplicate.

Cell apoptosis
The percentage of cell apoptosis was determined with the Annexin V-FITC Apoptosis Detection kit (Thermo Fisher Scientific, MA, U.S.A.) according to the manufacturer’s instructions. Glioma cells were transfected with miR-150-3p
mimics or control miRNA. After transfection for 48 h, cells were harvested, washed, and resuspended to approximately $1 \times 10^6$ cells/ml with the binding buffer. And then 5 μl annexin V and 1 μl of 100 μg/ml propidium iodide (PI) were added into 100 μl of the cell suspension and incubated at RT for 15 min. Finally, 400 μl binding buffer was added and the stained cells were analyzed using the Beckman Coulter Epic XL flow cytometer.

**Statistical analysis**

The data were presented as mean ± S.D. All statistical analyses were performed with GraphPad Prism version 6 (GraphPad Prism version 6.0, Inc., California, USA). The differences between two or more groups were analyzed by Student’s t test or one-way ANOVA followed by Bonferroni’s multiple comparison tests. $P < 0.05$ was considered as significant.

**Results**

**miR-150-3p was down-regulated in glioma tissues and cell lines**

To detect the expression of miR-150-3p in glioma, RT-qPCR analysis was performed with glioma tissues and normal brain tissues. As shown in Figure 1A, the expression of miR-150-3p was significantly decreased in glioma tissues in comparison with that of the normal brain tissue. Consistently, the expression of miR-150-3p in glioma cell lines including U87-MG, U251, A172, SWO-38, and SHG-44 was measured and the data showed that the level of miR-150-3p was significantly decreased in all the above glioma cell lines compared with that of the normal astrocytes cells NHA (Figure 1B).

To further characterize the relationship between the expression of miR-150-3p and the progression of glioma, the association between the expression abundance of miR-150-3p and the lymph node metastasis of the glioma patients was analyzed. The data showed that compared with patients without lymph node metastasis, significantly decreased expression of miR-150-3p was observed in the glioma tissues from patients bearing lymph node metastasis (Figure 1C). These results indicated the down-regulation of miR-150-3p in glioma tissues and might be correlated with the metastasis of glioma.

**Overexpression of miR-150-3p inhibited the proliferation of glioma cells**

Due to the aberrant expression of miR-150-3p in glioma, to detect the effect of miR-150-3p on the growth of glioma cells, U87-MG and U251 cells harboring relatively low abundance of miR-150-3p amongst the glioma cell lines we used, were transfected with miR-150-3p mimics or control miRNA. The expression level of miR-150-3p was confirmed by RT-qPCR (Figure 2A). The results showed that overexpressed miR-150-3p significantly inhibited the viability of both U87-MG and U251 cells as detected by the CCK-8 assay (Figure 2B,C). To confirm the inhibition of miR-150-3p on the growth of glioma cells, colony formation of both U87-MG and U251 cells that transfected with
Figure 2. Overexpression of miR-150-3p inhibited the growth of glioma cells

(A) The level of miR-150-3p in both U87-MG and U251 cells that were transfected with miR-150-3p mimics or control miRNA were detected by RT-qPCR. **P<0.01, Student’s t test. Data were obtained from three biological replicates. (B, C) Effect of miR-150-3p on the viability of glioma cells was determined by the CCK-8 assay. **P<0.01, ***P<0.001, two-way ANOVA test. Data were obtained from three biological replicates. (D) Colony formation of both U87-MG and U251 cells that harbored miR-150-3p mimics was significantly decreased. ***P<0.001, Student’s t test. Experiment was performed with three biological replicates. (E) Glioma cells were transfected with miR-150-3p mimics or control vector, and the cell migration of the cells were compared. **P<0.01, Student’s t test. Experiment was performed with three biological replicates. (F) The percentage of cell apoptosis of glioma cells expressing miR-150-3p mimics or control miRNA was determined. ***P<0.001, Student’s t test. Data were obtained from three biological replicates.

highly expressed miR-150-3p was evaluated. The results indicated that compared with the control group, overexpression of miR-150-3p significantly decreased the colony formation of glioma cells (Figure 2D). Consistent with these data, cells harboring highly expressed miR-150-3p presented decreased cell migration ability in comparison with that of cells expressing control miRNA (Figure 2E). To further validate the influence of miR-150-3p on the growth of glioma cells, the cell apoptosis rate of both U87-MG and U251 cells transfected with miR-150-3p mimics or control miRNA was detected. As shown in Figure 2F, overexpression of miR-150-3p significantly enhanced the apoptosis rate of both U87-MG and U251 cells. These data demonstrated the negative regulation of miR-150-3p on the growth of glioma cells.
SP1 was a target of miR-150-3p in glioma cells
To understand the underlying mechanism by which miR-150-3p regulated the growth of glioma cells, the downstream targets of miR-150-3p were predicted by the TargetScan database. Amongst all the candidates, the 3'-UTR of SP1 was found to have the putative binding site of miR-150-3p (Figure 3A). Furthermore, the predicted binding site of miR-150-3p in the 3'-UTR of SP1 was highly conserved across different species including human, chimp, rhesus, squirrel, rabbit, pig, cow, cat, dog, and brown bat) (Figure 3B). To confirm the interaction between miR-150-3p with the 3’-UTR of SP1, luciferase reporter assay was performed by co-transfecting the plasmid containing WT or mutant 3’-UTR of SP1 in the presence of miR-150-3p mimics or control miRNA. As shown in Figure 3C,D, compared with the control cells, overexpression of miR-150-3p significantly decreased the luciferase activity of vector bearing WT but not mutant 3’-UTR of SP1 in both U87-MG and U251 cells.

To further explore the effect of miR-150-3p on the expression of SP1, glioma cells were transfected with miR-150-3p mimics or control miRNA and the mRNA level of SP1 was detected. As shown in Figure 3E, transfection of miR-150-3p significantly decreased the mRNA abundance of SP1. Consistently, the protein level of SP1 was also examined by Western blot and reduced protein expression of SP1 was observed in both U87-MG and U251 cells that bore overexpressed miR-150-3p (Figure 3F). To provide more evidence to characterize the negative regulation between miR-150-3p and SP1, the endogenous miR-150-3p was down-regulated by transfecting miR-150-3p antagonist into the glioma cells A172 and SHG-44, which harbored relatively higher expression of miR-150-3p amongst all the glioma cells we used. As presented in Figure 3G,H, both the mRNA and protein level of SP1 of glioma cells were increased with the reduction in miR-150-3p. These data demonstrated that miR-150-3p targeted SP1 and negatively regulated the expression of SP1 in glioma cells.

Overexpression of miR-150-3p negatively regulated the SP1-PTEN pathway
Previous studies demonstrated that SP1 promoted the cancer cell migration and proliferation via inhibiting PTEN [33,35]. As miR-150-3p decreased the expression of SP1, to detect whether miR-150-3p regulates the expression of PTEN, glioma cells were transfected with miR-150-3p mimics or control miRNA, and the protein abundance of PTEN was examined by Western blot. As shown in Figure 4A, overexpression of miR-150-3p promoted the expression of PTEN in both U87-MG and U251 cells. To confirm the regulation of miR-150-3p on PTEN through SP1, the endogenous expression of SP1 was depleted by shRNA-SP1, the down-regulation efficiency of SP1 was confirmed by RT-qPCR and Western blot analyses (Figure 4B,C). U87-MG and U251 cells bearing depleted SP1 were transfected with miR-150-3p mimics or control miRNA and the protein expression of PTEN was detected. As shown in Figure 4D, highly expressed miR-150-3p in SP1 depleted cells failed to promote the expression of PTEN. These results suggested that miR-150-3p negatively regulated the SP1-PTEN pathway.

miR-150-3p was negatively correlated with the expression level of SP1 in glioma patients
As SP1 was identified as one of the downstream targets of miR-150-3p and aberrant expression of SP1 had been found to be associated with the development of cancer, we analyzed the expression level of SP1 in glioma tissues. The result showed that the expression of SP1 was significantly up-regulated in glioma tissues compared with that of the normal tissues (Figure 5A). Consistently, the expression level of SP1 in glioma cells including A172, U87-MG, SWO-38, U251, and SHG-44 was also notably increased in comparison with that of the normal cell NHA (Figure 5B). Furthermore, the correlation between the expression of SP1 and miR-150-3p in glioma tissues was also analyzed. The data indicated that the expression of miR-150-3p was significantly inversely correlated with the level of SP1 (Figure 5C). These results suggested the negative correlation between miR-150-3p and SP1 in glioma tissues.

Discussion
In recent years, the function of miRNAs is widely explored in tumorigenesis. In the present study, we demonstrated that miR-150-3p was down-regulated in glioma tissues and cell lines. Overexpression of miR-150-3p inhibited the growth of glioma cells. The underlying molecular mechanism found that miR-150-3p targeted and down-regulated the expression of SP1. These findings provided the possible functional mechanism of miR-150-3p in glioma.

Recent analyses of miRNA expression by RNA sequencing demonstrated that miR-150-3p was significantly down-regulated in head and neck squamous cell carcinoma and acted as an antitumor miRNA in HNSCC [31]. In HCC, miR-150-3p was identified as oxidative response miRNA and regulated the oxidative stress-related gene expression [36]. Kaplan–Meier analysis showed that miR-150-3p was significantly associated with the overall survival of
Figure 3. SP1 was a downstream target of miR-150-3p
(A) The putative binding site of miR-150-3p in the 3′-UTR of SP1. (B) The predicted binding sites of miR-150-3p in the 3′-UTR of SP1 were highly conserved amongst different species. (C,D) The effect of miR-150-3p on the luciferase intensity of WT or mutant 3′-UTR of SP1 was determined by the dual-luciferase activity. ***P < 0.001, Student’s t test. Data were obtained from three biological replicates. (E) Both U87-MG and U251 cells were transfected with miR-150-3p mimics or control miRNA, and the mRNA level of SP1 was detected. ***P < 0.001, Student’s t test. Data were obtained from three biological replicates. (F) Glioma cells transfected with control miRNA or miR-150-3p mimics were collected and the protein abundance of SP1 was determined by Western blot with anti-SP1 antibody. Experiment was performed with three biological replicates. (G,H) The endogenous miR-150-3p was down-regulated in A172 and SHG-44 cells. The mRNA and protein level of SP1 was detected by RT-qPCR and Western blot, respectively. Experiment was performed with three biological replicates.
HCC patients [36]. Consistent with these findings, in this study, decreased expression of miR-150-3p was observed in glioma tissues, which was associated with the lymph node metastasis of the glioma patients. Further functional study of miR-150-3p demonstrated that overexpression of miR-150-3p significantly inhibited the viability, migration, and colony formation of glioma cells. These results suggested the inhibitory effect of miR-150-3p on the growth of glioma cells, and the involvement of miR-150-3p in other types of cancers deserves further investigation.

The regulatory mechanism of miRNAs was achieved through inhibiting the expression of downstream target genes. With the bioinformatics analysis, SP1 was predicted as one of the targets of miR-150-3p. This observation was supported by the results that miR-150-3p decreased the luciferase activity of the 3′-UTR of SP1 and negatively regulated both the mRNA and protein expression of SP1. It has been documented that SP1 was a basal transcription factor in recruiting the general transcription machinery [21]. SP1 was overexpressed in a variety of human cancers and regulated the expression of genes involved in cell proliferation, differentiation, apoptosis, and angiogenesis [21-37-39]. Besides, highly expressed SP1 was associated with the poor prognosis in cancer patients [39]. Interestingly, SP1 has been the target of miRNAs in many types of cancers [25-28]. For example, miR-760 inhibits the tumorigenesis of colon cancer via regulating SP1 [40]. Increased expression of miR-31-5p inhibited the expression of SP1 and suppressed the proliferation of HCC [41]. Additionally, miR-376a was found to inhibit the growth of glioblastoma multiforme via targeting SP1 [42]. A recent study reported that miR-377 inhibited the proliferation and invasion of glioma cells though directly targeting SP1 [43]. Combined with our results, SP1 was a promising target of different miRNAs in glioma that might function together to inhibit the carcinogenesis of glioma. As one of the downstream targets of

Figure 4. miR-150-3p regulated the SP1-PTEN pathway
(A) Glioma cells were transfected with miR-150-3p mimics or control vector and the protein level of PTEN was detected by Western blot with anti-PTEN antibody. Experiment was performed with three biological replicates. (B,C) The endogenous SP1 was down-regulated by transfecting control-shRNA or shRNA-SP1. The depletion of SP1 was confirmed by RT-qPCR and Western blot. Experiment was performed with three biological replicates. (D) MiR-150-3p or control miRNA was transfected into glioma cells with depleted SP1, and the protein abundance of PTEN was detected. Experiment was performed with three biological replicates.
Figure 5. SP1 was highly expressed in glioma tissues and inversely correlated with the expression of miR-150-3p

(A,B) The mRNA level of SP1 in glioma tissues or cell lines was examined by RT-qPCR analysis. ***P<0.001, Student’s t test. Data were obtained from three biological replicates. (C) The correlation between the expression of miR-150-3p and SP1 in glioma tissues was analyzed. *P<0.05,**P<0.01

SP1, overexpression of miR-150-3p increased the expression abundance of PTEN. These results demonstrated that miR-150-3p suppressed the glioma cell growth in part by SP1 and possibly PTEN. Due to the multiple targets of miRNAs, in addition to SP1, searching for other downstream targets of miR-150-3p and related pathways that might partially mediate the role of miR-150-3p in glioma cells is also an interesting topic to provide novel insights into the function of miR-150-3p in glioma.

In conclusion, the results of the present study uncovered the decreased expression of miR-150-3p in glioma tissues and cell lines. Highly expressed miR-150-3p suppressed the growth of glioma cells partially via targeting the SP1-PTEN signaling pathway. These findings provided novel insights into the functional mechanism of miR-150-3p in glioma.

Funding
This work was supported by the Department of Neurosurgery, The Second Xiangya Hospital of Central South University (to Y.J.).

Competing interests
The authors declare that there are no competing interests associated with the manuscript.

Author contribution
Z.T. and Y.J. designed the study and collected the tissue samples. Z.T. detected the expression of miR-150-3p in glioma tissues, performed the experiments to explore the effect of miR-150-3p on the growth of glioma cells, and detected the regulatory relationship between miR-150-3p and the SP1-PTEN pathway. J.J. predicted the targets of miR-150-3p with the bioinformatics database and performed experiments to confirm the regulation of miR-150-3p on SP1. Z.T. and Y.J. wrote the manuscript.
Abbreviations
CCK-8, cell counting kit-8; DMEM, Dulbecco’s modified Eagle’s medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GA-100, gentamycin-amphotericin B mix; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous carcinoma; PTEN, phosphatase and tension homolog deleted on chromosome ten; qPCR, quantitative PCR; rhEGF, recombinant human epidermal growth factor; RT, room temperature; RT-qPCR, real-time quantitative PCR; SHG-44, Suzhou human glioma-44; SP1, specificity protein 1; U87-MG, uppsala 87 malignant glioma; WT, wild-type.

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