

Knockdown of PLK1 inhibits invasion and promotes apoptosis in glioma cells through regulating autophagy

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Abstract. – **OBJECTIVE:** Polo like kinase 1 (PLK1), an oncogene, is a ubiquitously expressed serine/threonine protein kinase. We aimed at investigating the role of PLK1 in glioma.

PATIENTS AND METHODS: Clinical glioma specimens were obtained from Zhejiang Hospital (Hangzhou, Zhejiang, China). The mRNA and protein levels of PLK1 in glioma tissues and different glioma cells were analyzed by Real-time PCR and Western blot, respectively. The expression of PLK1 protein in glioma tissues was also determined by immunohistochemistry staining. Then, the effect of PLK1 on cell proliferation and apoptosis of U251 and U87 cells was analyzed by using CCK-8 assay and Annexin V/PI staining, respectively. Furthermore, the migration and invasion of glioma cells were examined by transwell assay. Finally, the protein levels of autophagy indicators LC3-II, ATG5 and p-p70 S6 in U251 and U87 cells were detected by Western blot, and the expression of E-cadherin, vimentin and MMP9 and apoptosis associated indicators Bax, cleaved caspase-3 and Bcl-2 in U251 cells were also determined using Western blot.

RESULTS: PLK1 was upregulated in glioma tissues and cells. Knockdown of PLK1 significantly inhibited cell proliferation, migration, invasion, and induced apoptosis of U87 and U251 glioma cells. Furthermore, the data demonstrated that knockdown of PLK1 significantly elevated expression of cleaved caspase-3, BIM, BAX, and E-cadherin, and reduced expression of MMP9, ATG5 and LC3-II in U251 and U87 cells. Additionally, we found that knockdown of PLK1 can inhibit autophagy in glioma cells.

CONCLUSIONS: Knockdown of PLK1 can inhibit the glioma development by suppressing the autophagy and enhancing the apoptosis of glioma cells. PLK1 may be a potential therapeutic target in gliomas.

Key Words:

PLK1, Glioma, Autophagy, Proliferation, Migration.

Introduction

Malignant gliomas, one of the most serious tumors in the central nervous system, account for 50% of the human adult brain tumors^{1,2}. This cancer has been classified into grades I-IV by the World Health Organization (WHO) following increasing levels of malignancy³. Glioblastoma multiform (GBM, WHO grade IV) is the most death related and widespread malignant glioma⁴. Although various strategies including novel surgical treatments, chemotherapy and effective radiation for the treatment of GBM, the 5 years survival rate of patients remains low⁵. The metastasis of malignant gliomas has become one of the main difficulties for surgical resection and radiotherapy⁶. The following factors including abnormal proliferation, rapid diffuse infiltration, and apoptosis resistance, afford the invasiveness characterization of glioma^{6,7}. Thus, investigating the molecular mechanisms of glioma tumorigenesis and progression, will provide novel treatment for gliomas.

Polo like kinase 1 (PLK1) is a serine/threonine-protein kinase⁸. PLK1 belongs to a family of serine/threonine kinases (PLK1-5) involved in cell cycle regulation⁹⁻¹¹. The members of polo like kinase (PLK) family contain a polo-box domain that determines their kinase activity and subcellular localization. As we known, PLK1 is the best-described PLK protein, which controls several steps of the cell cycle and is essential for the G2/M transition and cell division^{10,12}. Additionally, it is a crucial component of the DNA damage response pathway^{13,14}. PLK1 is ubiquitously expressed and drives cellular proliferation by promoting mitosis and cytokinesis, which make it recognized as an oncogene¹². Up to now, PLK1 has been found

overexpressed in many human cancers including hepatocellular carcinoma, bladder cancer, pancreatic cancer, colorectal cancer and so on¹⁵⁻²⁰. It has been reported²¹ that high PLK1 expression correlates with poor prognosis in many cancer including glioma. In addition, previous researches²¹⁻²³ showed that overexpression of PLK1 in many cancers was linked to higher tumor grades, which make PLK1 served as a prognosis marker of many cancers including glioma. Nevertheless, the mechanism of PLK1 involved in development of gliomas remains unclear and needs to be further elucidated.

We explored the roles of PLK1 in the tumorigenicity of glioma. The data showed that PLK1 was overexpressed in human gliomas and glioma cells. Knockdown of PLK1 may suppress proliferation, invasion of glioma cell lines, and trigger cell apoptosis perhaps through regulating autophagy. During the autophagy, the intracellular constituents are degraded under stress or nutrient deprivation²⁴. Precious investigations have proved that autophagy enables survival of tumor cells and plays a key role in drug resistance of tumors^{25,26}. Autophagy can also promote tumor growth in low oxygen and nutrient conditions^{27,28}. Several works^{29,30} indicate that inhibition of autophagy inhibits tumor growth, induces tumor cell apoptosis and overcomes drug resistance. We showed that knockdown of PLK1 inhibited autophagy in glioma cells establishing PLK1 as a promising target in gliomas.

Patients and Methods

Clinical Samples

34 clinical glioma specimens were obtained from Zhejiang Hospital (Hangzhou, Zhejiang, China) from January 2014 to December 2016. 15 specimens were from male and 19 specimens were from female patients. Specimens were collected immediately after surgical resection and stored at -80°C for further analysis. The study was approved by the Ethics Committee of our hospital. Informed and written consents were obtained from all patients or their advisors conforming to the Ethics Committee guidelines.

Cell Culture

The cell lines 293FT and the five glioblastoma cell lines were obtained from the Cell Bank of the Shanghai Biology Institute, Chinese Academy of Science (Shanghai, China). U87 and U373 cells

were measured in Eagle's Minimum Essential Medium (EMEM; Thermo Fisher, Waltham, MA, USA), the other cells were cultured in Dulbecco's modified Eagle's Medium (DMEM; Thermo Fisher, Waltham, MA, USA). The mediums contained 10% fetal bovine serum (FBS; Thermo Fisher, Waltham, MA, USA) and 1% antibiotic (penicillin/streptomycin; Gibco, Waltham, MA, USA). All the cells were incubated in 5% CO₂ at 37°C.

RNA Isolation, Complementary DNA (cDNA) Synthesis, and Real-time Quantitative Polymerase Chain Reaction Detecting System (RT-qPCR)

Total RNA was extracted from glioma tissues or glioma cells employing TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following manufacturer's instructions. Then, the complementary DNA (cDNA) was synthesized with Reverse Transcription M-MLV (TaKaRa, Dalian, China). The primers were listed as below: PLK1, sense, 5'-TTC GTG TTC GTG GTG TTG GA-3', antisense, 5'-CTC GTC ATT AAG CAG CTC GT-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sense, 5'-GCA GTG GCA AAG TGG AGA TT-3', antisense, 5'-TGA AGT CGC AGG AGA CAA CC-3', and the GAPDH was used as an internal control. The Real-time PCR analysis was performed by Applied Biosystems 7500 Real-time PCR System (Applied Biosystems, Danvers, MA, USA) using the SYBR1 Green PCR kits (TaKaRa, Otsu, Shiga, Japan). The relative mRNA levels were analyzed using the 2- $\Delta\Delta C_t$ method as described previously³¹.

Vector Construction and Virus Infection

The sequences of human PLK1 small interfering RNA (siRNA) and negative control were 5'-GGA UCA AGA AGA AUG AAU ACA-3' (siPLK1#1), 5'-CGA GGU GCU GAG CAA GAA AGG-3' (siPLK1#2), and 5'-CGG AUC AGC UCG CGC UAU CAU CGC A-3' (siNC). The plasmids encoding short hairpin RNA (shRNA) were synthesized by Shanghai GenePharma (Shanghai, China) by using annealing buffer (Beyotime, Shanghai, China). The target sequences were inserted into PLKO.1 lentiviral vector. The plasmids carrying shPLK1 or the negative control shNC and packaging vectors were co-transfected into HEK293FT by employing lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) to produce lentiviruses Lv-shPLK1#1 (shPLK1#1), Lv-shPLK1#2 (shPLK1#2) and Lv-shNC (shNC). These lentiviruses were prepared for further experiments. After 72 h tran-

sfection, the lentiviruses particles were prepared to infect U251 and U87 cells.

Cell Proliferation

Cell proliferation was determined using Cell Counting Kit-8 (CCK-8) (Keygentec, Nanjing, China) as described previously³². U251 and U87 cells were planted in 96-well plates about 2×10^3 cells per well cultured for 16 h. After the cells were infected with lentiviruses for 0, 1, 2 and 3 days, the CCK-8 solution was added to each well for another 2 h. The absorption at OD450 nm was measured by scanning with a microplate reader (Bio-Rad, Hercules, CA, USA). Each experiment was repeated three times.

Cell Apoptosis Analyzed by Flow Cytometry

The detection of apoptotic cells was carried out by employing Annexin V-FITC/propidium iodide (PI)-labeled apoptosis detection kit³³ (Beyotime, Shanghai, China). Briefly, 2×10^6 cells/mL cells were planted in 10 cm dishes and cultured overnight in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS). These cells were transfected with lentiviruses, shPLK1#1, shPLK1#2 and shNC (control shRNA) for 48 hours. The transfected cells were harvested and centrifuged for 5 min at $4^\circ\text{C} \times 250g$. Then, the cells were resuspended in cold binding buffer (10 mM HEPES buffer pH 7.4, 5 mM KCl, 150 mM NaCl, 1.8 mM CaCl_2 , and 1 mM MgCl_2). These cells were firstly stained in the dark with fluorescein isothiocyanate (FITC)-labeled 25 ng/mL Annexin V for 30 min. Next, the above samples were stained with 50 ng/mL PI for 5 min. These samples were analyzed with a Becton Dickinson flow cytometer (Franklin Lakes, NJ, USA). The data were analyzed with Cell Quest software. In this research, the apoptotic cells were the population of Annexin V-positive and PI-negative cells. Both Annexin V- and PI-positive cells were scored as necrotic or late apoptotic.

In Vitro Migration and Invasion Assays

The glioma cells (U87 and LN-229) transfected stably with shRNA were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 0.1% FBS for 12 hours. Then, 1×10^5 cells were seeded in the upper chambers of transwell plates (8 μm pore size, Corning, Shanghai, China), and DMEM with 10% FBS was applied to the lower chambers. After incubation overnight, migrated cells in the lower chambers were fixed with paraformaldehyde and stained with crystal violet to visualize

nuclei, but non-migrated cells in the upper chambers were removed. The number of migrating cells in six fields was counted under ($\times 200$) magnification. The results were repeated three times.

Western Blotting

For Western blotting analysis, total protein was extracted and quantified using the Bradford method. About 60 μg total protein of each sample were separated by 12% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% milk in phosphate-buffered saline (PBS) containing 0.05% tween, membranes were incubated with primary antibody overnight at 4°C . Next, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies and finally detected with enhanced chemiluminescence (ECL) substrate kit (Tanon, Shanghai, China). The HRP-conjugated secondary antibodies and primary antibodies against β -actin and PLK1, MMP9, LC3, and Bcl-2 were purchased from Invitrogen (Carlsbad, CA, USA), and the antibodies against BAX, cleaved caspase-3, p-P70 S6, e-cadherin and vimentin were from Cell Signaling Technology (Danvers, MA, USA).

Immunohistochemistry Staining (IHC)

The immunohistochemistry procedures were used as described previously³⁴. The sections were subjected to dewaxing in xylene and dehydration in graded ethanol. The activity of endogenous peroxidase was blocked by 3% H_2O_2 for 10 min. The sections were then heated at 100°C in 0.1 M citrate buffer (pH 6.0) for 30 min to retrieve the antigens. These tissues were incubated with anti-PLK1 for overnight at 4°C . After washing with phosphate-buffered saline (PBS), tissues were incubated with second antibodies, horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (Envision kit, Dako, Glostrup, Denmark) following the manufacturer's instructions. At last, sections were stained with hematoxylin. These stained tissues were photographed with a microscope from Carl Zeiss (Axio Observer A1, Jena, Germany). As described previously³⁵, the staining results were scored by 2 investigators blinded to the clinical data.

Statistical Analysis

All data were described as the mean $\pm$ standard deviation (SD). Statistical differences were

analyzed by using the two-tailed unpaired Student's *t*-tests. Comparison between groups was done using One-way ANOVA test followed by Least Significant Difference (LSD). Differences were significant when statistical significance was $*p<0.05$, $\&p<0.01$ or $\#p<0.001$.

Results

PLK1 Was Overexpressed in Human Glioma Tissues

In order to analyze the expression of PLK1 in human glioma clinical tissues, 34 glioma tissues and their adjacent normal ones were collected. The total RNA of each specimen was extracted with TRIzol and the expression of gene was detected by Real-time PCR. The results revealed that the expression of PLK1 was considerably up-regulated in glioma tissues compared with adjacent normal ones (Figure 1A). Western blots were also conducted to investigate protein levels of PLK1

in glioma tissues. It found that PLK1 was also significantly increased at protein levels in glioma tissues compared with the control tissues (Figure 1B). Furthermore, the expression of PLK1 was observed in glioma specimens determined by immunohistochemistry staining (Figure 1C). Our results confirmed that PLK1, as a possible oncogene, was overexpressed in human glioma tissues.

PLK1 Overexpression in Human Glioma Cells

To explore the role of PLK1 in tumorigenesis of glioma, the mRNA and protein levels of PLK1 were measured by using the Real-time PCR and Western blot in the glioma cells. The results showed that PLK1 was significantly increased in U251 and U87 cells rather than in other three-glioma cells (Figure 2A-B). To evaluate the relationship between PLK1 and tumorigenesis of glioma, U251 and U87 cells were selected for the highest expression of PLK1 among the five cell lines. Lentiviral vectors carrying shRNA of PLK1

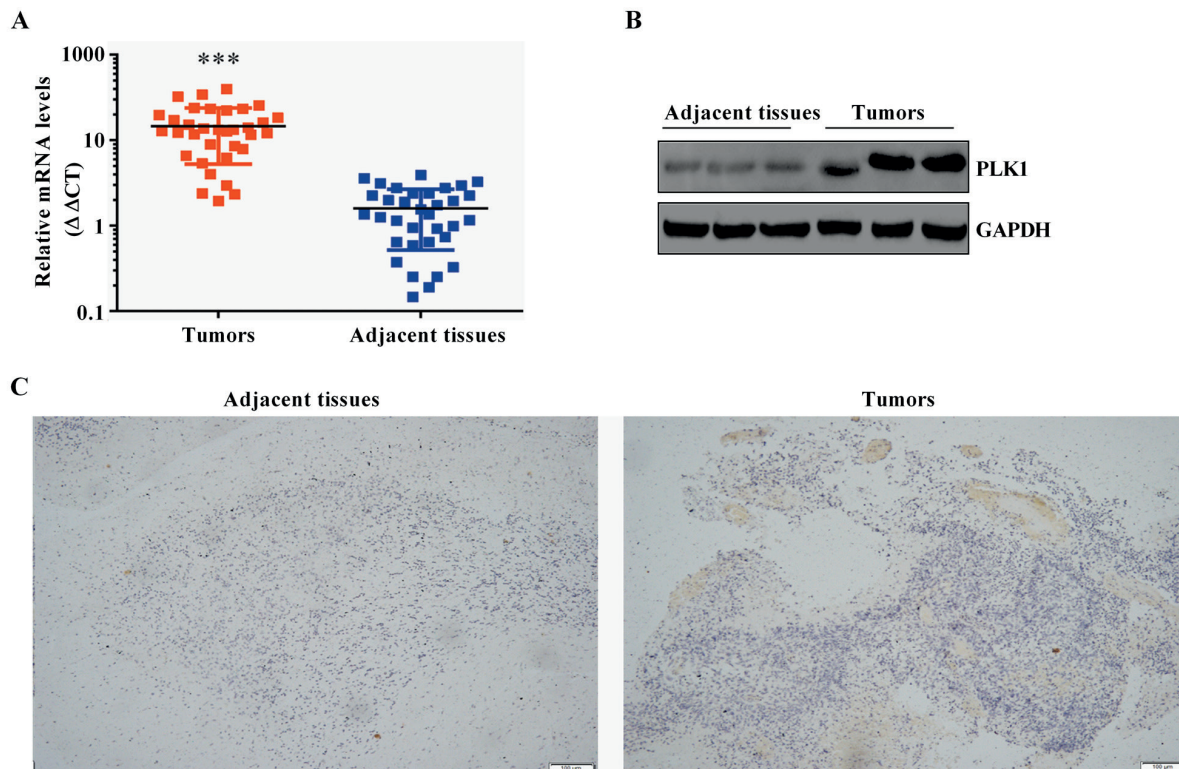


Figure 1. The expression of PLK1 in human glioma tissues. **A**, The mRNA levels of PLK1 were significantly increased in glioma tissues (n=34) compared with adjacent normal brain tissues analyzed by Real-time PCR. **B**, Protein levels of PLK1 were also significantly increased in glioma tissues compared with adjacent normal tissues analyzed by Western blots. **C**, The expression of PLK1 was determined by immunohistochemistry staining in glioma tissues. Scale bars, 100 μm; the data were presented as mean $\pm$ standard deviation; *** $p<0.001$ analyzed by two-tailed Student's *t*-test.

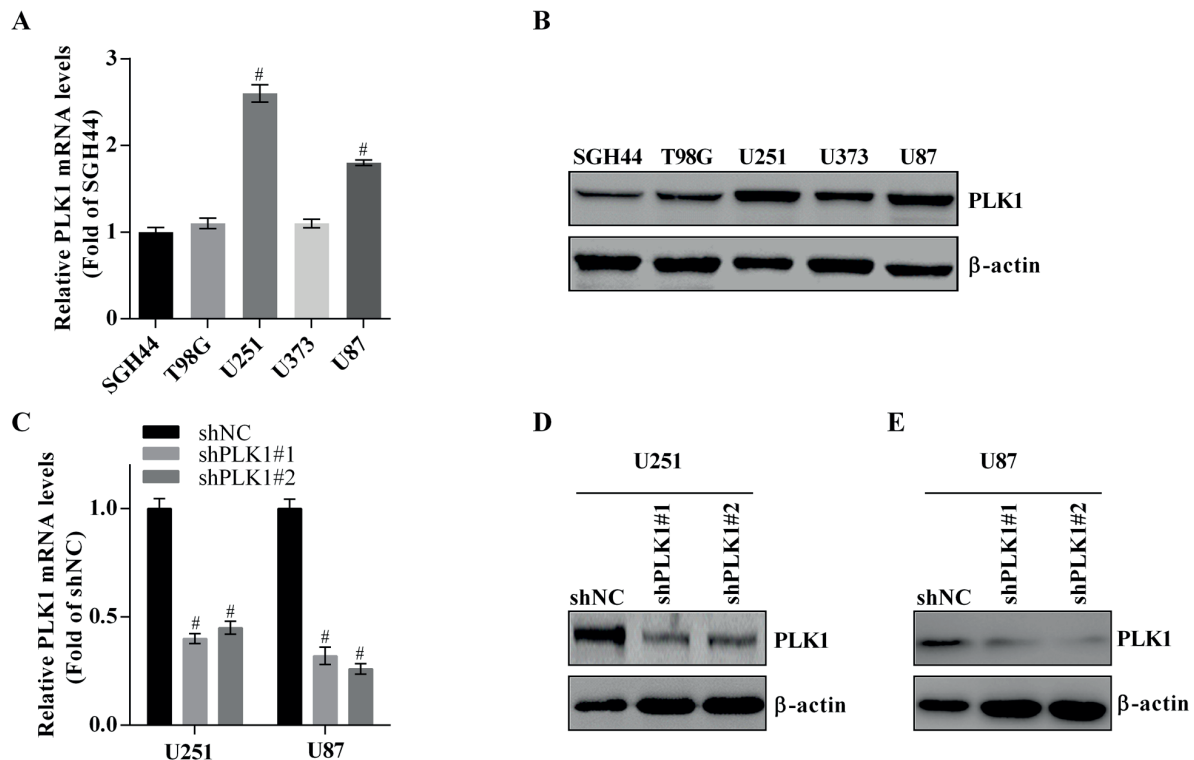


Figure 2. The expression of PLK1 is varying with glioma cells. **A**, PLK1 expression levels in five glioblastoma cell lines were analyzed by Real-time PCR; the data were presented as mean $\pm$ standard deviation, $\#p < 0.001$, vs. the SGH44 group. **B**, Western blot assays examined the protein levels of PLK1 in five glioblastoma cells and β -actin was used as the internal control. **C**, Real-time PCR showed PLK1 mRNA expression was knockdown by shPLK1 (Lv-shPLK1) in glioblastoma cells; the data were presented as mean $\pm$ standard deviation, $\#p < 0.001$, vs. the shNC group. **D-E**, Knockdown of PLK1 notably inhibited protein levels of PLK1 in U251 and U87 cells analyzed by Western blots, and β -actin served as the internal control. ShNC is the Lv-shRNA used as negative control and shPLK1 is the Lv-shRNA used for expressing shPLK1.

was employed to suppress the expression of PLK1 in U251 and U87 cells. The data showed that the mRNA levels of PLK1 in glioma cells were significantly decreased by shPLK1#1 and shPLK1#2, indicating that PLK1 can be knocked down by lentiviruses, shPLK1#1 and shPLK1#2 efficiently (Figure 2C). The relative levels of PLK1 transfected with shPLK1#1 were reduced by 64.15% in U251 cells and 67.28% in U87 cells, while shPLK1#2 was able to efficiently inhibit PLK1 expression by 55.63% and 72.48% in U251 and U87 cells, respectively. Next, the results demonstrated that the PLK1 protein levels were significantly down-regulated in shPLK1 groups, compared with the control groups (Figure 2D-E).

Knockdown of PLK1 Suppresses Proliferation and Enhances Apoptosis of Glioblastoma Cells

To explore the role of PLK1 in the proliferation of glioblastoma cells, CCK-8 assay was performed to assess the growth of U251 and U87 cells.

shPLK1#1 (lentivirus) infection significantly reduced the proliferation of U251 cells by 27.3% at 48 h and 35.3% at 72 h, and decreased the proliferation of U87 cells by 33.3% at 48 h and 39.5% at 72 h, respectively (Figure 3A-B). Similar results were obtained from the shPLK1#2-transfected cells. Furthermore, the effects of PLK1 knockdown on apoptosis of glioma cells were analyzed. Our results showed that downregulation of PLK1 markedly induced and increased apoptosis by approximately 2.8 folds and 3.3 folds in U251 and U87 cells, respectively, compared with shNC-transfected cells (Figure 3C-D). Collectively, all of the results indicated that PLK1 played an important role in the growth of glioma cells and knockdown of PLK1 reduced cell proliferation and promoted apoptosis of glioma cells.

Downregulation of PLK1 Suppresses Migration and Migration of Glioma Cells

Our studies indicated that silencing PLK1 inhibited the proliferation and induced apoptosis

of U251 and U87 cells. We subsequently investigated the migration and invasion of U251 and U87 cells after infected with lentivirus shPLK1. To investigate the role of PLK1 in migration, shPLK1- and shNC-infected cells were seeded in a Boyden chamber. The results showed that both shPLK1-transfected U251 (Figure 4A) and shPLK1-transfected U87 glioma cells (Figure 4C) exhibited weaker migratory ability. Statistical analysis indicated that the difference between shPLK1#1 or shPLK1#2 group compared with shNC group was significantly ($p < 0.01$), the cancer cells that passed through the filters were reduced

more than 58% when infected with shPLK1 (Figures 4B and 4D).

The effects of PLK1 on cell invasion were analyzed by employing a matrigel-coated transwell chamber. After incubation for 24 h, the number of migrated cancer cells was calculated as follows: 162 ± 14 cells in shNC-infected U251; 153 ± 11 cells in shNC-infected U87 (Figure 4). However, knockdown of PLK1 strongly blocked invasive ability, shPLK1#1-infected U251, 53 ± 4 cells; shPLK1#2-infected U251, 59 ± 6 cells; shPLK1#1-infected U87, 51 ± 9 cells; shPLK1#2-infected U87, 54 ± 8 cells, respectively.

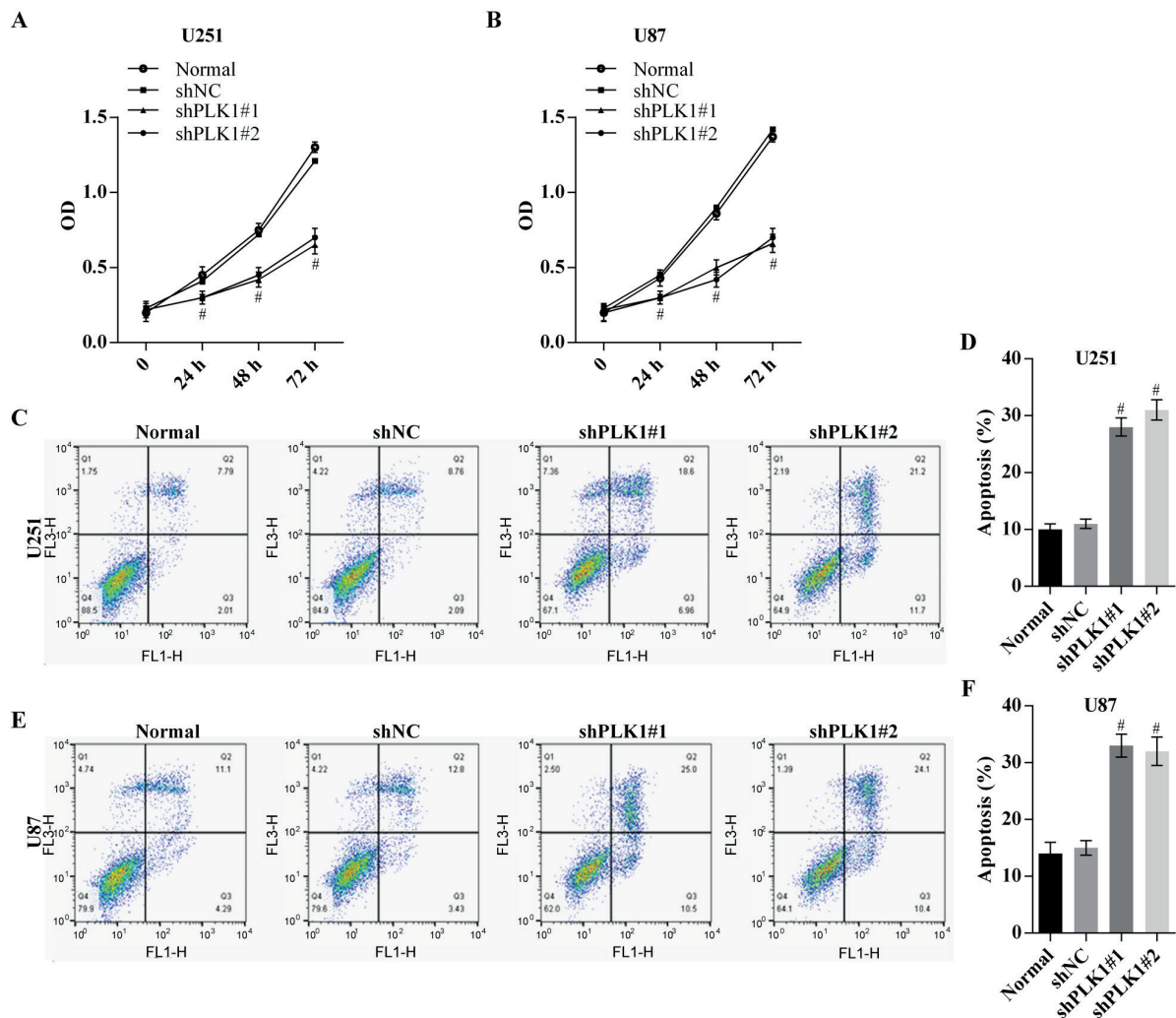


Figure 3. Knockdown of PLK1 inhibits proliferation and induces apoptosis of glioma cells. **A-B**, CCK-8 assay detected proliferation of U251 and U87 cells after infected shNC or shPLK1; U251 and U87 cells were infected with NC or shPLK1 and collected for CCK-8 analysis after 48 h incubation; the data were presented as mean $\pm$ standard deviation, $\#p < 0.001$, vs. the shNC group. **C-E**, Apoptosis of U251 and U87 cells were analyzed using the Annexin V/PI staining. **D-F**, Statistical analysis of apoptotic U251 and U87 cells above, respectively. The data were presented as mean $\pm$ standard deviation, $\#p < 0.001$, vs. the shNC group. Each experiment was repeated for 3 times.

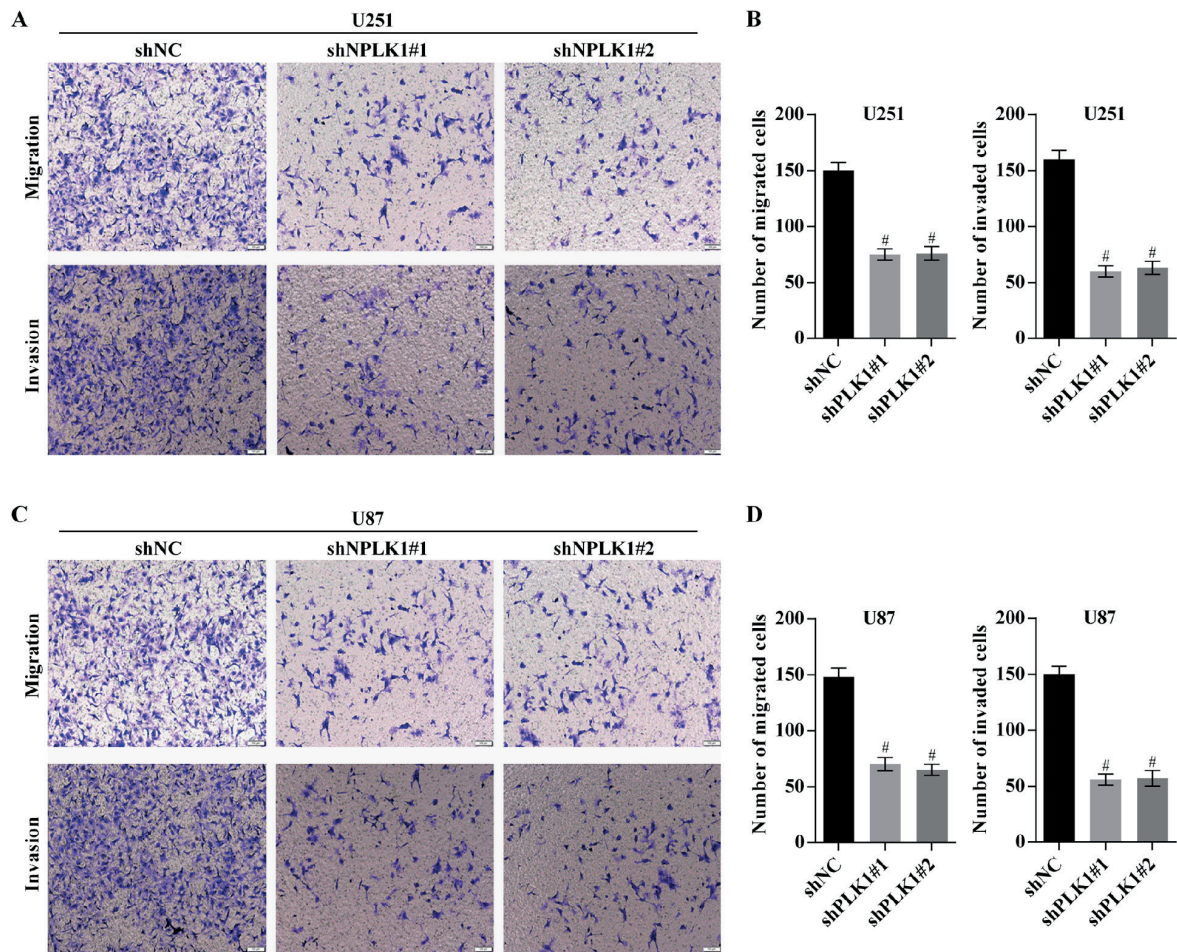


Figure 4. Silencing of PLK1 suppresses migration and invasion of glioma cells. U251 and U87 cells were infected with shNC or shPLK1. **A-B**, Migration (upper panel) and invasion (lower panel) of U251 cells were observed (A) and the number of migrated or invaded cells was calculated in (B). **C-D**, Migration (upper panel) and invasion (lower panel) of U87 cells were showed and the number of migrated or invaded cells was calculated in (D). Scale bars, 100 μm; the data were presented as mean ± standard deviation; # $p < 0.001$, vs. the shNC group.

vely (Figure 4). Thus, downregulation of PLK1 inhibited migration and invasion of glioma cells.

Knockdown of PLK1 Represses the Autophagy of Glioma Cells

We further investigated the role of PLK1 in the progression of metastasis in glioma cells. Autophagy often performed as a dynamic regulator of tumorigenesis through various molecular mechanisms, including regulation of tumor cell metabolism, promotion of tumor cell survival, modulation of cellular invasion and metastasis as well as drug resistance. Thus, we examined whether the effects of PLK1 on tumorigenesis were closely associated with autophagy. Firstly, Western blot assays were conducted to detect the protein levels of autophagy marker LC3-II when

PLK1 was knocked down in U251 and U87 cells. shPLK1 significantly reduced LC3-II levels in both U251 (Figure 5A) and U87 (Figure 5B) cells. Moreover, the protein levels of autophagy related 5 (ATG5) were also reduced when PLK1 was knocked down, while the phosphorylation of the mTOR complex-1 (mTORC1) substrate RPS6KB (p70 S6) was upregulated in U251 and U87 cells (Figures 5A-B). These data indicated that PLK1 could promote tumorigenesis of glioma through regulating autophagy of glioma cells. It has been reported that repression of autophagy could inhibit cell proliferation and induce apoptosis. We further analyzed the apoptosis and metastasis signaling-related proteins in shPLK1-infected U251 cells. The BAX and cleaved caspase-3 in apoptosis and E-cadhe-

rin in invasion were significantly elevated in shPLK1-infected U251 cells, while the anti-apoptotic protein Bcl-2 and the invasion-related factors (vimentin and MMP9) were significantly downregulated in shPLK1-infected U251 cells (Figures 5C-D). Although the role of PLK1 in regulating autophagy in glioma cells needs further research, knockdown of PLK1 successfully suppressed invasion and induced apoptosis of glioma cells through inhibiting autophagy.

Discussion

PLK1 presents the well-known member of the Polo-like kinases (PLKs), a family of serine/threonine kinases highly conserved from yeasts to mammals³⁶. PLK1 has been reported to be an essential regulator of spindle formation and chromosome segregation during mitosis³⁷. It is well known that expression of PLK1 was a new indicator of many malignant tumors including glioma²¹.

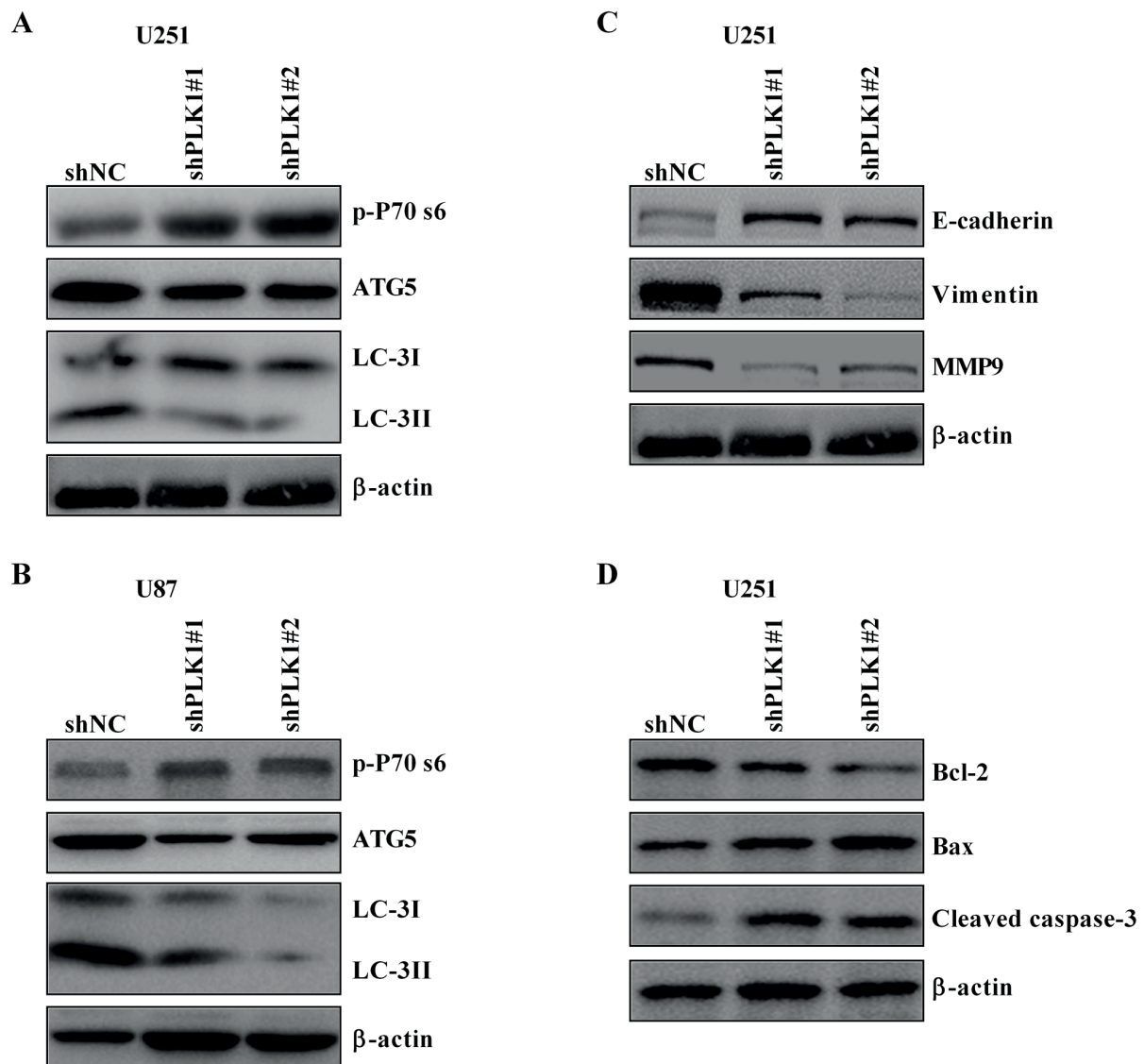


Figure 5. PLK1 regulates the development of gliomas by modulating autophagy. **A-B**, The protein levels of autophagy indicators LC3-II, ATG5 and p-p70 S6 were detected by Western blot in U251 and U87 cells and β-actin served as the internal control. **C**, Western blot analysis found that downregulation of PLK1 significant increases the expression of E-cadherin and decreased vimentin and MMP9 expression in U251 cells. **D**, Western blot analysis identified that PLK1 increased the expression of BAX and cleaved caspase-3, and PLK1 reduced the expression of anti-apoptotic protein Bcl-2 in U251 cells.

It has been reported²¹ that high PLK1 mRNA expression might result in poor prognostic for glioma patients. To date, the exact functions and mechanisms of PLK1 in tumorigenesis of human gliomas remain unclear. Our data showed that the mRNA and protein levels of PLK1 were upregulated in glioma clinical samples compared with corresponding normal brain tissues, consistently with previous reports^{21,38}. It is well known that gliomas are a class of malignant tumors for their low survival rate of five years and rapid proliferation as well as invasion, resulting in a dramatically poor prognosis. We confirmed that knockdown of PLK1 remarkably inhibited growth and induced apoptosis of glioma cells (Figure 3). Meanwhile, the migration and invasion of glioma cells were inhibited by shPLK1 (Figure 4). These data were the functions of PLK1 also found in other tumor cells¹⁸. The above results suggest that the elevated expression of PLK1 will afford to the fast proliferation, invasiveness and migration in gliomas.

We investigated the mechanistic basis for PLK1 involve in gliomas. There is increasing evidence³⁹ that autophagy plays a significant role in tumorigenesis and therapy of gliomas. Firstly, we detected autophagy in glioma cells. Our data showed that the protein levels of LC3-II in U251 and U87 cells were decreased significantly by shPLK1. These results showed knockdown of PLK1 can inhibit autophagy in glioma cells. A recently investigation⁴⁰ showed PLK1 inhibits mTOR complex1 (mTORC1) and promotes autophagy in HeLa cells. In this work, knockdown of PLK1 not only upregulated the phosphorylation of the mTORC1 substrate RPS6KB (p-P70 S6), but also inhibited the expression of autophagy related gene 5 (ATG5) in glioma cells. Our results suggest that PLK1, as an oncogene, regulates the autophagy through affecting both mTORC1 and ATG5 protein levels. However, we did not find PLK1 interacts with ATG5 directly analyzed by co-immunoprecipitation, and the data have not been shown here. Although the exact mechanisms that PLK1-mediated autophagy in gliomas needs to be further elucidated, PLK1 promotes autophagy perhaps affording to tumorigenesis of glioma.

Knockdown of PLK1 efficiently inhibited migration, invasion and induced apoptosis of glioblastoma cells. Further, we observed that the expression of BAX and cleaved caspase-3, associated with apoptosis, and the expression of E-cadherin, associated with invasion, may be increased by shPLK1. However, the expression of Bcl-2, vimentin and MMP9 was downregulated

by shPLK1 in glioblastoma cells. These results indicated that the above genes participate in PLK1-mediated glioma progression. Apoptosis is an important process coordinating cell growth and death. Autophagy is a process involved in stress or starvation by controlling the degradation of damaged proteins and cytoplasmic organelles, and inhibition of autophagy would induce and accelerate apoptosis of tumor cells³⁹. Combined with previous researches, we speculated that knockdown of PLK1 may suppress invasion and induces apoptosis of glioma cells through regulating autophagy.

Conclusions

We showed that PLK1 plays critical roles in cell proliferation, migration and invasion of gliomas. Importantly, knockdown of PLK1 is able to inhibit proliferation, migration, and invasion and to accelerate apoptosis of glioma cells through inhibiting autophagy. Therefore, these data indicate that PLK1 may be considered as a therapeutic target of glioma.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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