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PTPH1 promotes tumor growth and metastasis in human glioma

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Abstract. – OBJECTIVE: Glioma is the most common form of brain tumor, accounting for over 50% of all primary tumors. Despite progress in the treatment of glioma, the prognosis is still poor. In this study, we examined protein-tyrosine phosphatase H1 (PTPH1) in human gliomas.

MATERIALS AND METHODS: Cell growth potential was measured by CCK-8 assay and colony formation. Cell cycle distribution was measured by flow cytometry. Transwell assay was used to detect the motility of tumor cells. Real-time PCR and Western blot were used to measure the mRNA and protein expression of indicated genes. Xenograft model was established to measure the role of PTPH1 *in vivo*.

RESULTS: The expression of PTPH1 was significantly higher in the tumor tissues as compared with that in the adjacent normal tissues. Knockdown of PTPH1 significantly slowed cell proliferation and reduced colony formation abilities in glioma cell lines U87 and U251. Additionally, knockdown of PTPH1 caused cell cycle arrest in the S-phase. Furthermore, depletion of PTPH1 in glioma U87 cells significantly limited tumor growth in a xenograft model. Interestingly, knockdown of PTPH1 also decreased cell migration abilities in both U87 and U251 cells. Accordingly, matrix metalloproteinase 9 (MMP9) was also decreased upon knockdown of PTPH1 in both cell lines. Moreover, we found that phosphorylated MEK (p-MEK) and phosphorylated MAPK (p-MAPK) were both decreased, whereas the total levels of MEK and MAPK remained unchanged after depletion of PTPH1 in both cell lines.

CONCLUSIONS: Our data suggest that PTPH1 may be a novel biomarker that indicates the aggressiveness of gliomas. Targeting PTPH1 might be a promising strategy for the treatment of gliomas.

Key Words:
PTPH1, Growth, Metastasis, Glioma.

Introduction

Brain tumors are one of the most lethal threats. According to the World Health Organization (WHO) classification, with the exception of grade I tumors that are considered biologically "benign" the malignancies of the other grades are significant. Grade II tumors are low-grade malignancies and are associated with prolonged survival. Grade III and IV are highly malignant gliomas that can result in death within few years or within nine to twelve months, respectively[1,2]. As the most common form of tumor in the brain, gliomas account for over 50% of all primary brain tumors. Despite progress in the treatment of glioma, the prognosis of this malignancy is still poor. Grade IV glioma (glioblastoma multiforme, GBM), the most frequent form of glioma, remains a malignancy with a dire prognosis, mainly due to its resistance to radiotherapy and chemotherapy. Surgery may save a certain percentage of patients, but is largely limited when the malignancy has infiltrated into surrounding tissues[3]. Chemotherapeutic strategies, such as Temozolomide (TMZ) may prolong the survival by up to twenty months[4], but most patients die within two years. Thus, the development of novel therapeutic strategies for the treatment of refractory glioma is imperative.

Protein-tyrosine phosphatase H1 (PTPH1) is a 120-kDa protein that belongs to the non-transmembrane PTP superfamily[5]. PTPH1 and several other members of this family were initially found in the cytosol and enriched at the plasma membrane in circulating T lymphocytes[6]. PTPH1 functionally inhibits T cell activation by dephosphorylating membrane-associated targets involved in T cell antigen receptor signaling[7]. In addition to its role in regulating the immune response, PTPH1 is also critically involved in human tumorigenesis. Genetic analysis shows that PTPH1 is mutated in colon cancer[8]. PTPH1 dephosphorylates and cooperates with p38γ mitogen-activated protein kinase (MAPK) to promote Ras oncogenesis through a complex formation[9]. Interestingly, p38γ MAPK phosphorylates

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PTPH1 in regulating Ras protein oncogenesis and stress response[10]. More importantly, the mRNA of PTPH1 is significantly increased in esophageal cancer tissues compared with that in normal mucosa[11]. PTPH1 is also overexpressed in breast cancer and cooperates with the vitamin D receptor to promote breast cancer growth[12]. PTPH1 has even been reported to increase the sensitivity of breast cancer to anti-estrogens by dephosphorylating the estrogen receptor[13]. All these data suggest that PTPH1 plays a critical role in the development and progression of human tumors.

However, the expression of PTPH1 and the role it plays in human glioma remain largely unknown. In view of previous reports, we hypothesized that PTPH1 might be involved in MAPK signaling and thereby control tumor growth and metastasis in human glioma. We tested this hypothesis by initially investigating the expression of PTPH1 in clinical glioma samples. In this study, the biological activity of PTPH1 was explored in glioma cell lines and a mouse model of glioma. Regulation of MAPK-related signals by PTPH1 was also examined.

Materials and Methods

Human Samples and Mouse Xenograft Model

This study was approved by the Ethical Committee of the hospital. Fifteen samples of human glioma tissue and the adjacent non-cancerous tissue were collected. All patients provided their full consent to participate in the study. Twelve male BALB/c nu/nu mice (six-weeks-old) were purchased from SLRC Laboratory Animal Co. (Shanghai, China) and evenly divided into two groups, Lv-shCon group and Lv-shPTPH1 group. A total of 1.0×10^6 U87 cells, either stably expressing scrambled shRNA or specific shRNA against PTPH1, were injected subcutaneously into the abdomen of each mouse accordingly. Mice were fed with ad libitum access to water and food in a sterile laboratory environment. The tumor size was measured twice a week and at the end of the inoculation period, all mice were handled according to the protocol approved by the Committee on the Ethics of Animal Experiments of the hospital. All tumors were dissected, and sizes and weights were measured and recorded.

Cell Culture and Lentivirus Infection

Human glioma cell lines U87 and U251 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in the recommended media supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA). The shRNA sequence targeting the human PTPH1 gene was designed[9] and chemically synthesized by GenePharma Co. (Shanghai, China). For lentivirus infection, cells were evenly seeded into six-well plates at a concentration of 5×10^4 cells per well and infected with the lentivirus, with either scrambled shRNA or PTPH1-specific shRNA, at a multiplicity of infection (MOI) of 100. After 96h of co-incubation, fluorescence microscopy was performed to examine the infection efficiency by calculating the percentage of GFP positive cells.

RNA Isolation and Real-time PCR

RNA was isolated from both human glioma cell lines and tissues using Trizol reagent (TaKaRa, Dalian, China). Total RNA (1 μg) was reverse transcribed into cDNA with the PrimeScript RT Master Mix Perfect Real-Time (TaKaRa). Afterwards, real-time polymerase chain reaction (RT-PCR) was performed using the SYBR Premix Ex Taq Kit (TaKaRa) with the ABI PRISM 7900 Real-Time System. The primers used were as follows: PTPH1 [Forward: 5′-ATGACCTCCCGGTTACGTGCGTTGGGT-3′ and Reverse: 5′-GCTGGCGTCCTCGGTG-GAGCCCCCTTTG-3′], GAPDH [Forward: 5′-GTGGAACATCCGCAAAGAC-3′ and Reverse: 5′-AAAGGGTGTAACGCAACTA-3′].

Western Blot Analysis and Antibodies

Total cellular proteins were extracted from cultured U87 and U251 cells using RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplied with protease inhibitor (PI, Beyotime Biotechnology, Nanjing, China) following the manufacturer's instructions. Protein concentration was determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA), with bovine serum albumin (BSA) as a standard, at 570 nm. The protein samples (50 μg per sample) were loaded into each lane on a10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, and after electrophoresis, the protein was transferred onto a nitrocellulose membrane (Millipore, Billerica, MA, USA). After the membrane had been blocked

with 5% fat-free milk for 1h at room temperature, it was incubated with primary antibodies overnight at 4°C. Next, secondary antibodies (1:1000 dilution) were added and incubated for an additional 1h at 37°C. Finally, the signals were detected using an ECL reagent (Thermo Fisher Scientific, Waltham, MA, USA). Primary antibodies against PTPH1, -actin, CDK2, Cyclin B1, MMP9, MEK, and phosphorylated antibody against MEK were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against MAPK was obtained from Abcam (Cambridge, MA, USA).

Cell Proliferation Assay

The U87 and U251 cells were diluted into single cell suspensions and seeded into 96-well plates (1×10^6 cells/well) with 100 μL DMEM media supplied with 1% FBS, and replaced by 10% FBS medium after 24h incubation. Afterwards, 10 μL of CCK8 (Dojindo, Kumamoto, Japan) solution was added to each well on the1st, 2nd, 3rd, 4th, and 5th day during the experimental period. Air bubbles were strictly avoided during the process. The plates were then incubated for another 1-4 h at 37°C. The absorbance of each well was read and compared at 450 nm. The CCK-8 Kit was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Colony Formation Assay

U87 and U251 cells, either with PTPH1 knockdown or control, were seeded in a 6-well culture plate in triplicate at a concentration of 100 cells per well. After incubation for 12 d at 37°C, cells were washed twice with PBS and stained with crystal violet. The number of colonies containing 50 cells or more was counted under a Nikon microscope. The percent of colony formation efficiency was determined as (number of colonies/number of cells inoculated) × 100.

Cell Cycle Assay

For cell cycle analysis, U87 and U251 cells, untreated (control), treated with scrambled shRNA (Lv-shCon), or specific shRNA against PTPH1 (Lv-shPTPH1), were seeded in triplicate wells. After the incubation period, cells were trypsinized and fixed in 70% pre-chilled ethanol for 30 min at 4°C. Cells were then resuspended in 200 μL PBS containing propidium iodide (50 μg/mL) and RNase A (0.75 mg/mL). Cell cycle assay was performed using a BD LSR II flow cytometer. Flowing Software 2.5.1 (Turku Centre for Biotechnology, University of Turku, Finland) was used for quantifying the percentage of U87 and U251 cells in G0/G1, S, and G2/M phases.

Transwell Assays

U87 or U251 cells (1×10^4) in 100 μL DMEM medium without FBS were seeded on a polycarbonate membrane inserted into a Transwell apparatus (Corning, Cambridge, MA, USA). In the lower chamber, 600 μL DMEM with 10% FBS was supplied as the chemoattractant. After the cells had been incubated for 6 h at 37°C, the membrane was washed with PBS twice, and cells on the upper surface were removed with a cotton swab. Cells adhering to the lower surface were fixed with methanol, stained with crystal violet solution for 5 min, and counted under a Nikon microscope in five predetermined fields (× 200). All assays were repeated independently at least three times.

Statistical Analysis

Experiments were performed in triplicate and repeated at least three separate times. Mean and standard deviation (SD) were obtained to establish statistical comparisons. The results were calculated as the mean ± the standard derivation (SD). Student's *t*-test was used to evaluate the significance of each group. Values with $p < 0.05$ were considered statistically significant.

Results

PTPH1 is Overexpressed in Clinical Glioma Tissues

We investigated the expression of PTPH1 in glioma using qRT-PCR analysis. Compared to the adjacent non-cancerous tissues, glioma tissues from 15 patients showed a higher expression of PTPH1, approximately 3.5-fold in average (Figure 1). These data revealed that PTPH1 is upregulated in human glioma.

Lentivirus-Mediated PTPH1 Knockdown in Both U87 and U251 Cells

Next, the role of PTPH1 in human glioma was explored. To this end, we constructed U87 and U251 cell lines that stably expressed the specific shRNA against PTPH1 to knock down the expression levels of PTPH1. Figure 2A shows that more than 95% of the cells were infected with the corresponding lentivirus in both cell lines. Additionally, qRT-PCR and Western blot assays

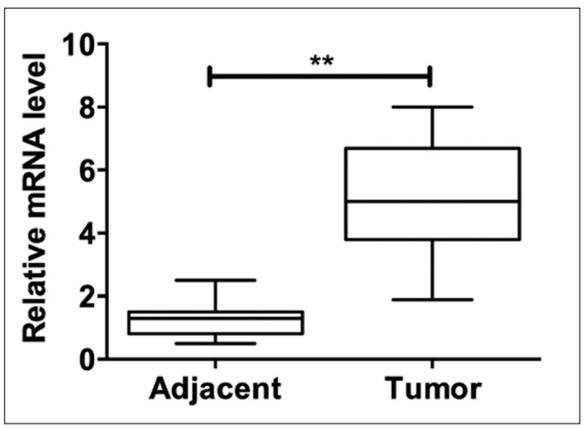

Figure 1. PTPH1 overexpression in human glioma. qRT-PCR analysis of 15 clinical glioma cases as well as their adjacent non-cancerous tissues. Relative mRNA level of PTPH1 in the tumor tissues was notably higher than that in the adjacent tissues. $^{**}p < 0.01$, tumor *vs*. adjacent.

suggest that compared to the untreated control group, scrambled shRNA-infected cells showed no significant difference in both mRNA and protein levels of PTPH1.In contrast, lower PTPH1 levels were observed in PTPH1-specific shRNA-treated U87 and U251 cells (Figure 2B and 2C). These data confirmed that the high infection efficiency of lentivirus and powerful shRNA interference efficiently knocked down the expression of PTPH1.

Knockdown of PTPH1 Inhibits Cell Proliferation in Both U87 and U251 Cells

Next, we investigated the effects of PTPH1 on cell viability and proliferation using the MTT assay and the colony formation assay. There was no notable difference between the three groups in the first two days. However, on the third day, the

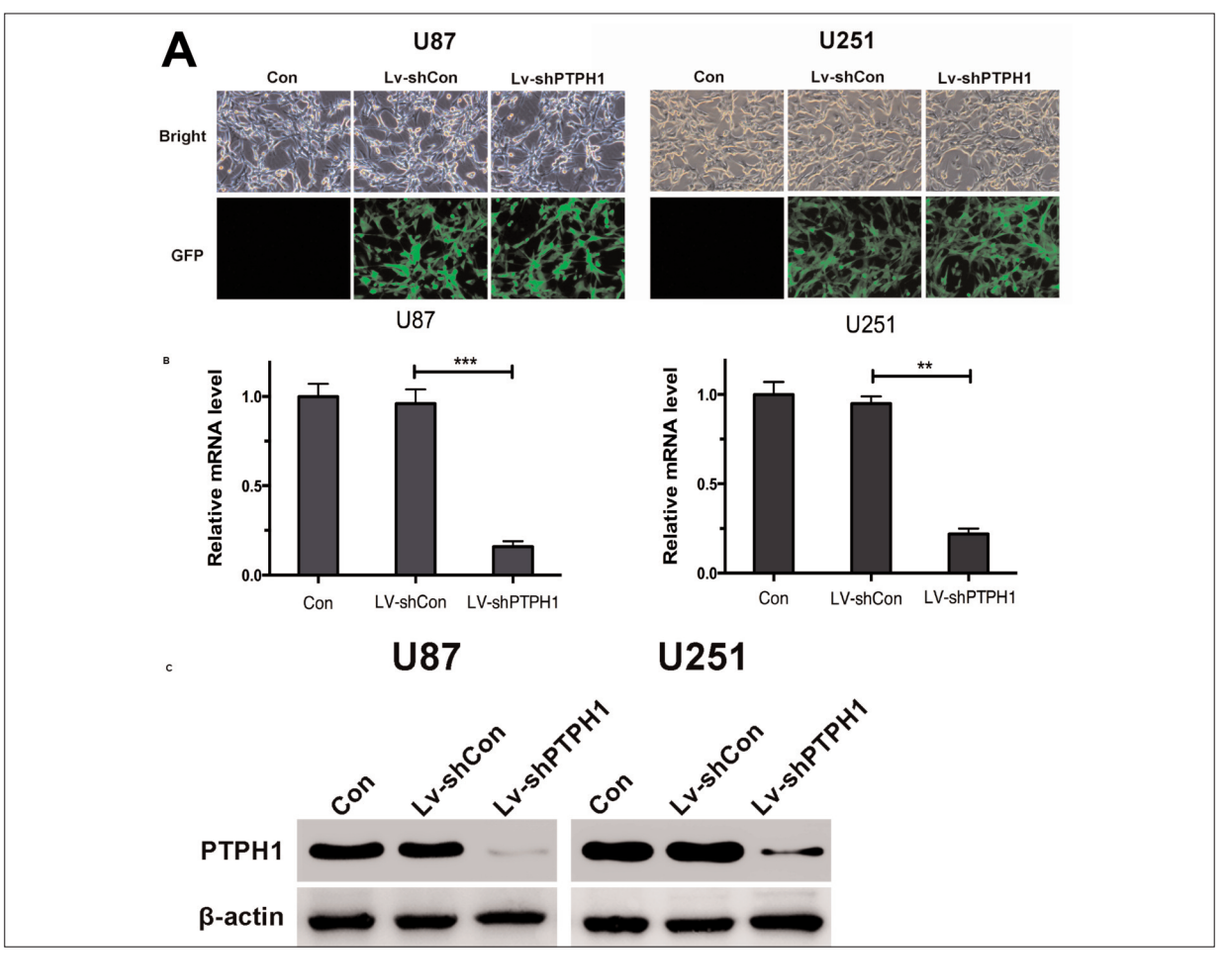

Figure 2. Lentivirus-mediated PTPH1 knockdown in both U87 and U251 cells. ***A,*** Glioma cell lines U87 and U251 were transfected with control or PTPH1 shRNA, respectively. Observation using fluorescence microscopy indicated high infection efficiency (more than 95%) for both cell lines. ***B,*** qRT-PCR analysis showed that mRNA levels of PTPH1 were remarkable decreased in U87 and U251 cells. $^{**}p < 0.01$, $^{***}p < 0.001$, LV-shPTPH1 *vs*. LV-shCon. ***C,*** Western blot analysis revealed that the expression of PTPH1 was successfully knocked down in cells treated withPTPH1-specific shRNA. β-actin was included as an internal control.

cell viability decreased by 46% and 18% in PTPH1 shRNA-treated U87 and U251 cells, respectively. The cell viability continued to decrease on subsequent days. Notably, by the fifth day, compared to the control cells, LV-shPTPH1-treated cells showed only 45% and 76% cell viabilities (Figure 3A) for U87 and U251 cells, respectively. Similar results were observed in the colony formation assay. Figure 3B and 3C showed that knockdown of PTPH1 in both cell lines inhibited the number of colonies by 73.5% and 80.7%, respectively. These results suggest that PTPH1 increased cell proliferation in both glioma cell lines.

Knockdown of PTPH1 Caused Cell Cycle Arrest in S-phase in Glioma Cell Lines

To further explore the specific effects of PTPH1 in glioma, we performed a cell cycle assay. Cells untreated or stably expressing scrambled shRNA or PTPH1-specific shRNA were starved for 24 h before processing. Figure 4A

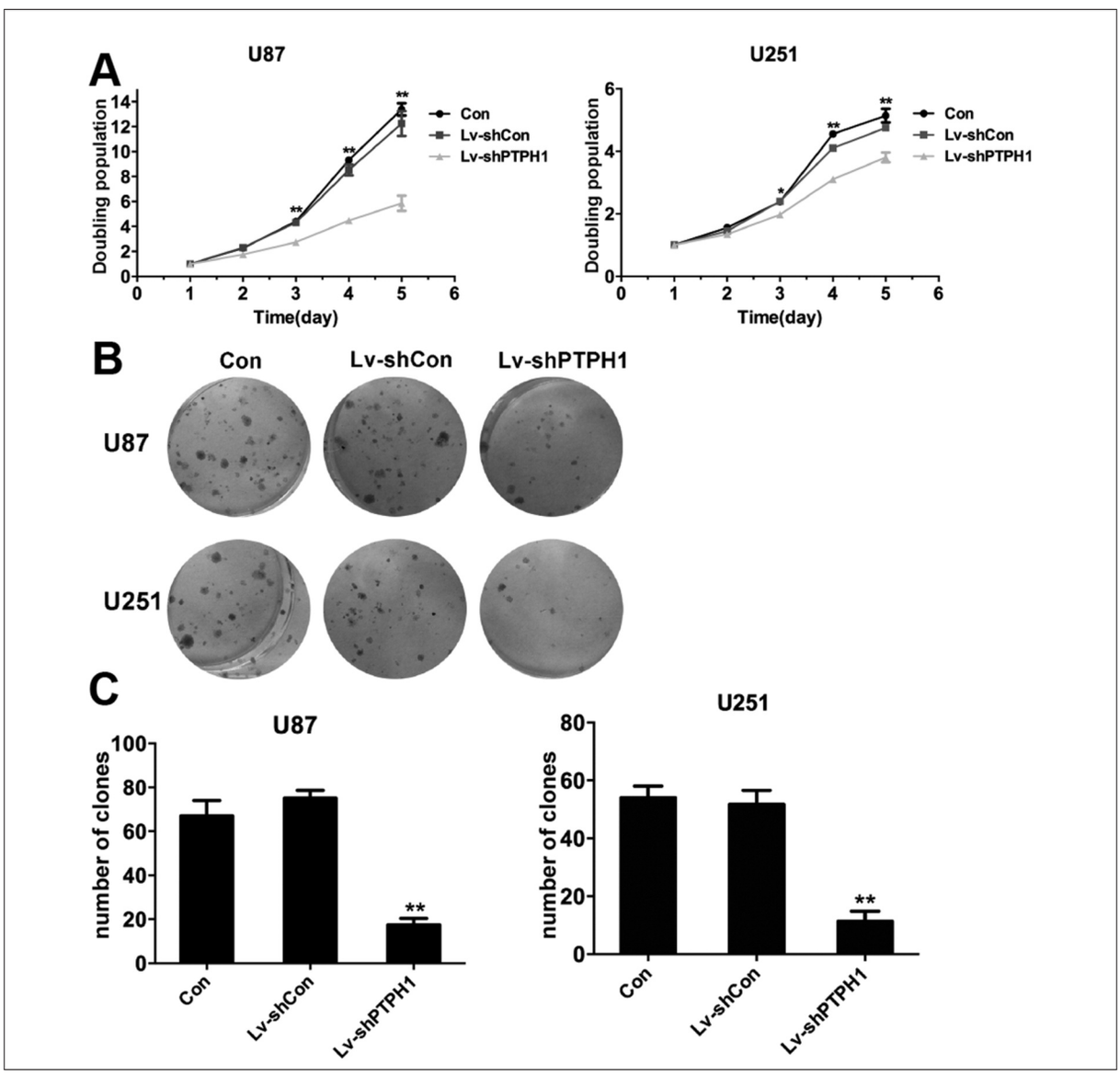

Figure 3. Knockdown of PTPH1 inhibited cell proliferation in both U87 and U251 cells. ***A,*** MTT assay revealed that the proliferation rate was significantly inhibited on the 3rd day for U87 and U251 cells after PTPH1 was knocked down, and the inhibition increased on subsequent days. ***B,*** Colony formation assay showed PTPH1 shRNA infection blocked the formation of colonies in U87 and U251 cell lines. ***C,*** Quantification of colonies indicated that knockdown of PTPH1 inhibited cell proliferation for both cell lines. *$p < 0.05$, **$p < 0.01$, LV-shPTPH1 *vs.* con.

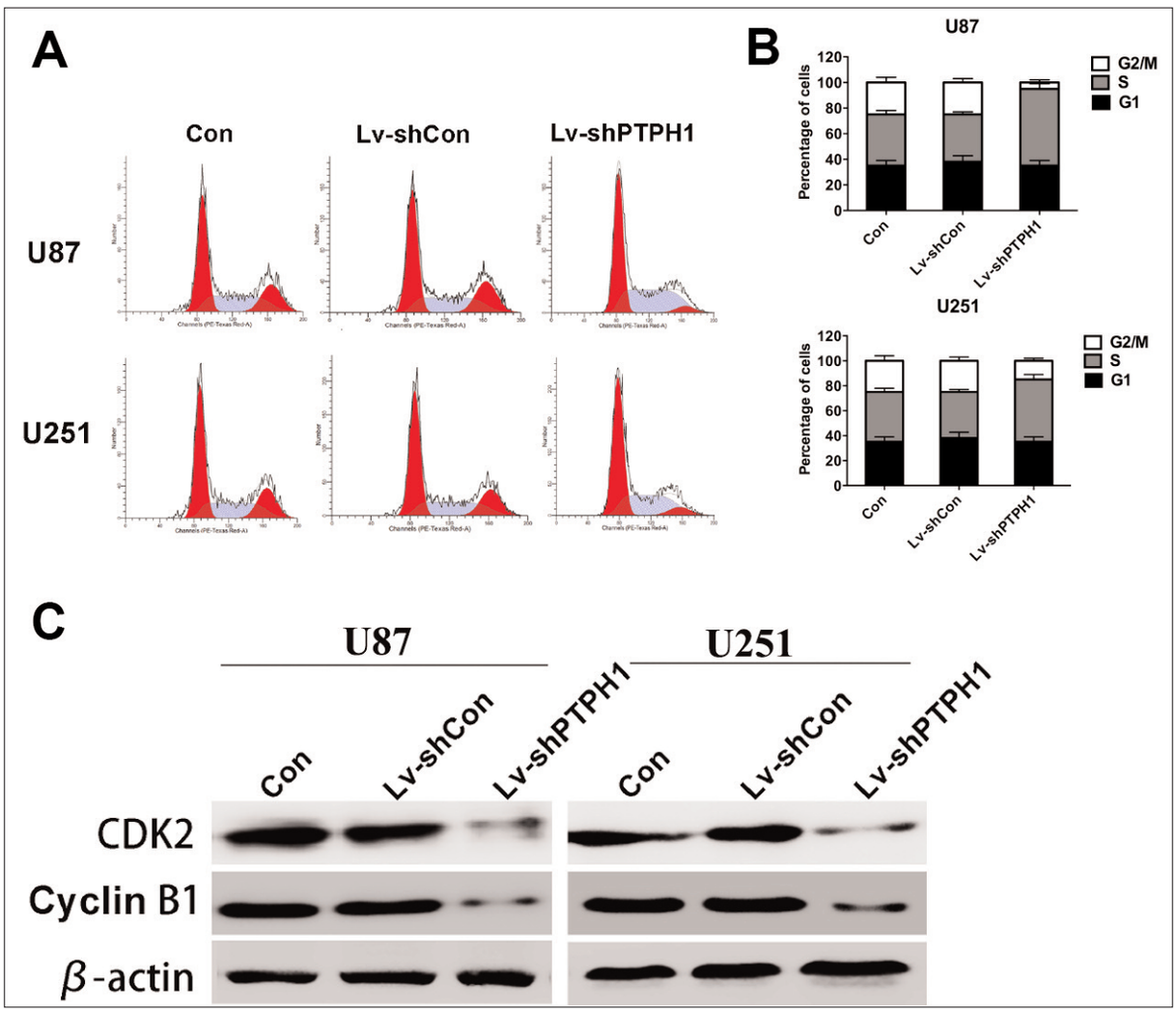

Figure 4. Knockdown of PTPH1 caused cell cycle arrest in S phase in glioma cell lines. ***A,*** Cell cycle analysis revealed that knockdown of PTPH1 retained most of the cells in S phase in U87 and U251 cell lines. ***B,*** Cell distributions in U87 cells (upper panel) and U251 cells (lower panel) after distinct treatments. It was shown that more than 18% U87 cells in G2/M phase were shifted into S phase, whereas U251 cells in S phase were only increased by 9% in the LV-shPTPH1-treated groups. ***C,*** Western blot assay revealed that with the knockdown of PTPH1 expression, the protein levels of CDK2 and cyclin B1 were also significantly decreased in U87 and U251 cells.

and 4B suggest that little difference was observed in the LV-shCon group compared with the untreated control group. However, in both LV-shPTPH1-infected U87 and U251 cells, knockdown of PTPH1 decreased the percentage of cells in the G2/M phase and increased the proportion of S phase by 18% and 9%, respectively. The cell cycle transition is controlled strictly by CDKs and their inhibitors, as well as cyclins[14]. Therefore, we also assessed the effects of PTPH1 on CDK2 and cyclin B1. Notably, as shown in Figure 4C, knockdown of PTPH1 significantly suppressed the expression of CDK2 and cyclin B1 in both cell lines. These data demonstrated that PTPH1 promoted cell division, and the effect was likely due to the increased expression of CDK2 and cyclin B1.

Knockdown of PTPH1 Suppressed Tumor Growth in a Mouse Model of Glioma

We next explored the effects of PTPH1 *in vivo* by subcutaneously injecting U87 cells with or without PTPH1 knockdown. It was shown in Figure 5A that no notable difference occurred in the first 16 d after injection between LV-shCon mice and LV-shPTPH1 ones. However, on the 20th day,

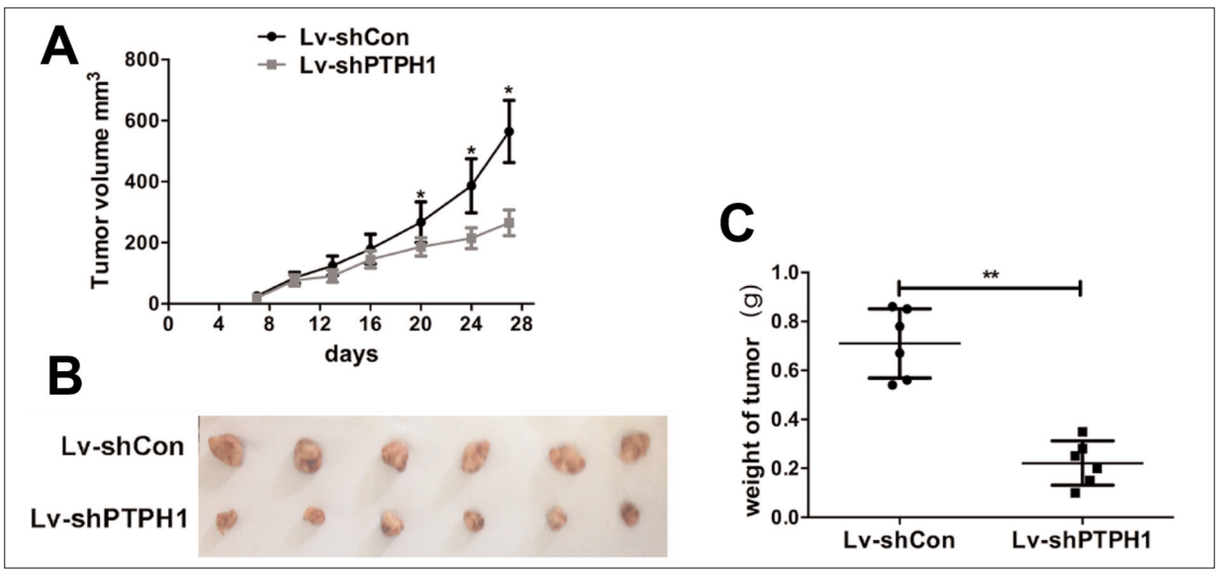

Figure 5. Knockdown of PTPH1 suppressed tumor growth in a mouse model of glioma. A nude mouse model of glioma was established, and the tumor volume of each mouse was measured twice a week. ***A,-B,*** Generally, mice in the control shRNA-treated group exhibited a larger tumor size compared with PTPH1 shRNA-treated mice on the 20th day after inoculation. The difference in tumor size between the two groups became more significant as the time was extended. *$p < 0.05$, LV-shPTPH1 *vs*. LV-shCon. ***C,*** LV-shPTPH1-treated mice showed lower tumor weights at the end of the experiment than the LV-shCon-treated mice. **$p < 0.01$, LV-shPTPH1*vs*. LV-shCon.

the rate of growth of the tumors in LV-shPTPH1-injected mice was reduced by 14.3%. The effects were more prominent at later time points, which can be observed in Figure 5B. At the end of the experiment, the mice were sacrificed and the tumors were dissected and weighted. When the expression of PTPH1 was decreased, the tumor weight was remarkably lower in LV-shPTPH1-injected mice (Figure 5C). From these data, we concluded that PTPH1 promotes tumor growth in the mouse model.

PTPH1 Promoted Cell Migration and Invasion In Vitro

Furthermore, we investigated the role of PTPH1 on cell migration *in vitro* using the transwell assay. Crystal violet staining revealed that numerous cells migrated to the underside of the chamber, and there were conspicuous differences between cells in the control group and the group treated with shRNA against PTPH1 (Figure 6A). Quantification of the cells that migrated through the membrane showed that knockdown of PTPH1 suppressed the migration of U87 and U251 cells by 60% and 53%, respectively (Figure 6B). Matrix metallopeptidase 9 (MMP-9) is a protein involved in the degradation of collagen IV and V in the basement membrane and the extracellular matrix, thus facilitating tumor progression, including invasion and metastasis[15]. Consequently, we also examined the effect of PTPH1 on the expression of MMP-9. Figure 6C shows that when PTPH1 was knocked down by lentivirus-mediated shRNA, the protein level of MMP-9 also decreased in both glioma cell lines. These data suggested that PTPH1 promoted cell migration in U87 and U251 cells, which is positively related to the activity of MMP-9.

Knockdown of PTPH1 Decreased the Phosphorylation Level of MEK and MAPK

The MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) is a chain of proteins in cells that communicate a signal from a receptor on the surface of the cell to the DNA in the nucleus. We investigated the linkage of PTPH1 to this signal pathway. As shown in Figure 7, when PTPH1 was knocked down in both U87 and U251 cells, the phosphorylation levels of MEK and MAPK were corresponding decreased, while the total protein levels of MEK and MAPK remained unchanged. These data suggested that PTPH1 was positively related to this signal transfer pathway in cells.

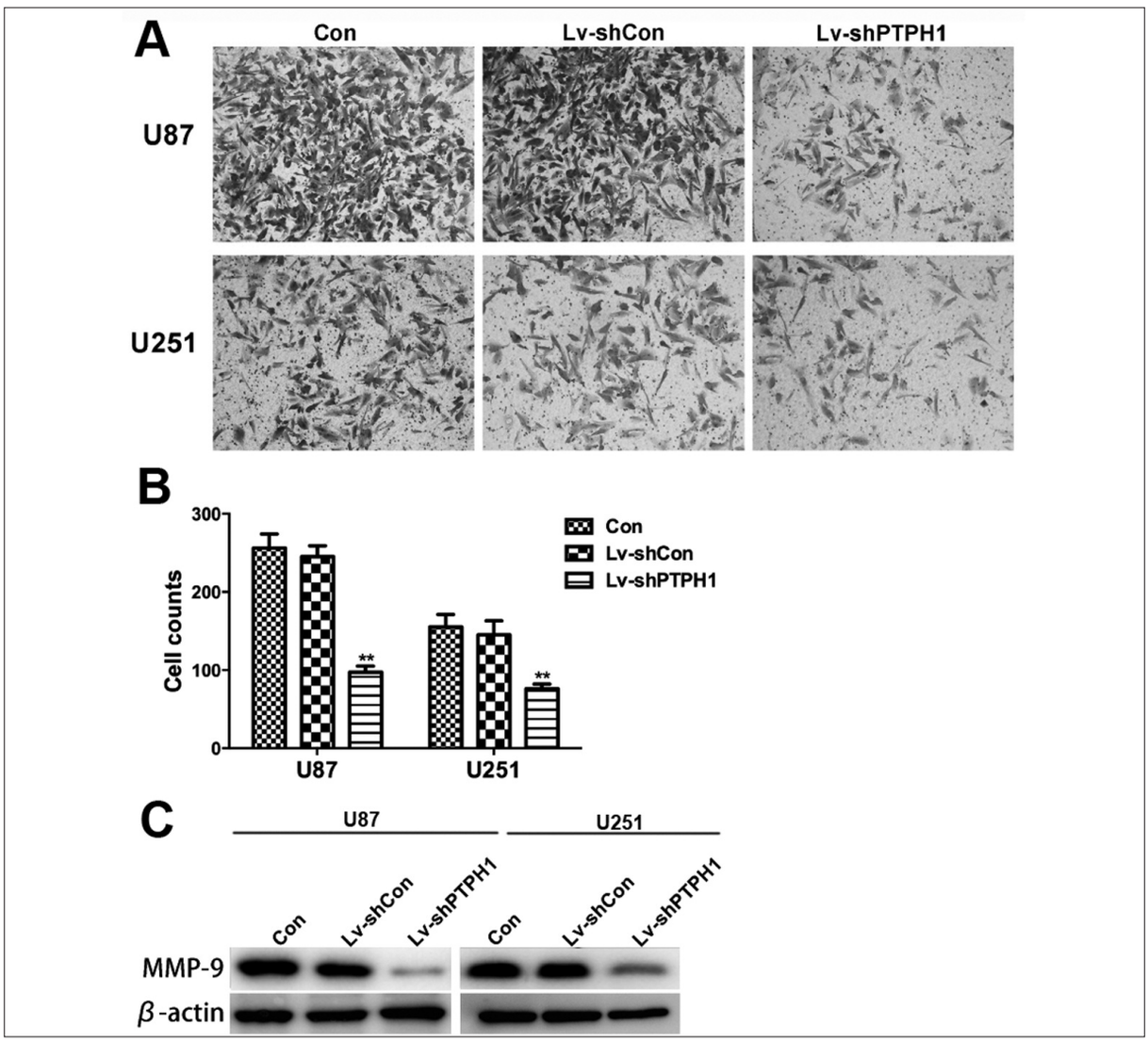

Figure 6. PTPH1 promoted cell migration and invasion in vitro. ***A-B,*** Transwell assay revealed that in both U87 and U251 cells, the cell migration ability was suppressed when PTPH1 was knocked down. $^{**}p < 0.01$, LV-shPTPH1 *vs.* control. ***C,*** Western blot assay showed that knockdown of PTPH1 decreased the expression of the MMP-9 protein. β-actin was included as an internal control, which remained unchanged between the three groups.

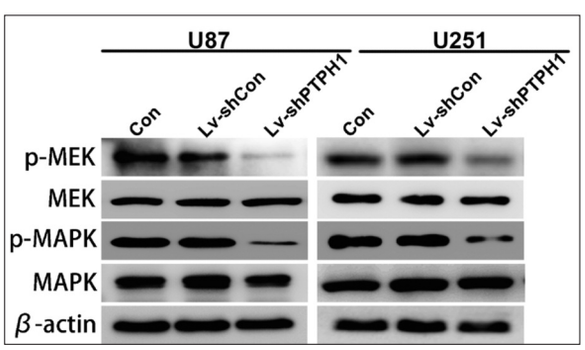

Figure 7. Knockdown of PTPH1 decreased the phosphorylation level of MEK and MAPK. Western blot assay revealed that in LV-shPTPH1-treated U87 and U251 cells, the phosphorylation levels of MEK and MAPK were highly suppressed while the total MEK and MAPK remained unchanged. GAPDH is included as an internal control.

Discussion

Glioma, particularly GBM (grade IV glioma), ranks as one of the deadliest tumors, and is currently refractory to present therapeutic strategies[16,17]. According to population-based investigations, the overall median survival for patients suffering from GBM is about 15 months, which represents a poor prognosis[18]. The existing evidence suggests that glioma develops and progresses through a stepwise accumulation of mutations and aberrant expressions of genes, such as p53, epidermal growth factor receptor (EGFR), and cyclin-dependent kinases (CDKs)[19-21]. Hence, in-depth knowledge of the molecular pathogenesis of glioma is necessary for developing new therapies for this malignancy.

In the present study, we demonstrated for the first time that the expression of PTPH1 was significantly increased in clinical glioma tissues. Overexpression of PTPH1 was previously observed in esophageal cancer and breast cancer[11,12]. The high expression level of PTPH1 in human tumors suggests its wide involvement in human tumors. Furthermore, our data showed that knockdown of PTPH1 by lentivirus-delivered shRNA significantly slowed glioma cancer cell proliferation and colony formation *in vitro*. Tumor growth was also inhibited *in vivo* in a mouse model of glioma. Interestingly, we also found that knockdown of PTPH1 was associated with significant cell cycle arrest in the S-phase. CDK2 and cyclin B1, which are critical cell cycle regulators[22-24], were suppressed after knockdown of PTPH1 in both U87 cells and U251 cells. Hence, the induction of cell cycle arrest might be one of the mechanisms by which PTPH1 exerts pro-oncogenic effects in glioma cells. Moreover, it was observed that knockdown of PTPH1 significantly decreased cell migration abilities in both cell lines. Also, MMP-9 was inhibited upon depletion of PTPH1. MMP-9 degrades type IV and V collagens and thereby promotes tumor invasionprocesses[15]. The suppression of MMP-9 after knockdown of PTPH1 supported the suggestion that PTPH1 was associated with glioma invasion. Altogether, it could be concluded that PTPH1 stimulated tumor growth and invasion in glioma.

One interesting finding is that phosphorylation levels of MEK and MAPK were remarkably decreased after knockdown of PTPH1 in U87 cells and U251 cells. However, the total protein levels of MEK and MAPK remained unchanged. The mammalian MAPK signaling pathway consists of p38, extracellular signal regulated kinase (ERK), and c-Jun N-terminal kinase (JNK). Each of these kinases has several isoforms: p38 (MAPK14), p38 (MAPK11), p38γ (MAPK12), and p38 (MAPK13); ERK1 to ERK8; and JNK1 to JNK3 (MAPK8 to10). Each MAPK signaling cascade consists of at least three layers: a MAPK kinase kinase (also known as MAP3K or MEKK), a MAPK kinase (also known as MAP2K, or MEK), and a MAPK[25]. Upon activation, phosphorylated MAPK regulates the activities of several transcription factors. MAPK can phosphorylate c-myc and MNK, which, in turn, phosphorylate CREB. MAPK also regulates the transcription of the c-Fos gene. By altering the levels and activities of transcription factors, MAPK activity leads to the altered transcription of genes that are important for cellular processes such as cell proliferation, angiogenesis, survival, and differentiation[26,27].

PTPH1 belongs to the non-transmembrane PTP superfamily[5]. PTPH1 and several other members of this family were initially found in the cytosol and enriched at the plasma membrane in circulating T lymphocytes[6]. PTPH1 functionally inhibits T cell activation by dephosphorylating membrane-associated targets involved in T cell antigen receptor signaling[7]. A unique feature of the glutamic acid-containing loop (E-loop) of the phosphatase domain defined the substrate specificity of PTPN3 toward fully activated p38γ (MAPK12). The solution structure revealed the formation of an active-state complex between p38γ and the phosphatase domain of PTPN3. The PDZ domain of PTPN3 stabilized the active-state complex through an interaction with the PDZ-binding motif of p38γ. This interaction alleviated autoinhibition of PTPN3, enabling efficient tyrosine dephosphorylation of p38γ[28].

The elevated levels of phosphorylated MEK and MAPK, instead of their total protein levels, might suggest that PTPH1 regulates MAPK signal activation and thereby promotes tumor growth in glioma. In fact, regulatory mechanisms of MAPK by PTPH1 in other types of tumors, such as breast cancer, have been reported[9,10]. In these reports, PTPH1 dephosphorylates and cooperates with p38³ MAPK to promote Ras oncogenesis[9]. Interestingly, p38γ MAPK signals also phosphorylate PTPH1 in regulating Ras protein oncogenesis and stress response. Our data, to-

gether with these previous reports, collectively suggest that PTPH1 exerted its biological activities possibly by a mechanism dependent on MAPK signaling. However, further work is required to elucidate the exact mechanism.

Conclusions

We defined PTPH1 as a key mediator of tumor growth and metastasis in glioma. Knockdown of PTPH1 significantly inhibited tumor growth *in vitro* and *in vivo*. Cancer cell migration was also suppressed *in vitro*, which was associated with the aberrant expression of MMP9. All these data suggest that a molecular target against PTPH1 might be one potential strategy to treat refractory glioma.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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