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GOLPH3 regulates the migration and invasion of glioma cells though RhoA

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ABSTRACT

Golgi phosphoprotein 3 (GOLPH3) has been reported to be involved in the development of several human cancers. However, the biological significance of GOLPH3 in glioma progression remains largely unknown. In this study, we report, for the first time, that downregulation of GOLPH3 led to clear reductions in glioma cell migration and invasion. In addition, downregulation of GOLPH3 inhibited the expression of the small GTPase RhoA as well as cytoskeletal reorganization, which are both required for glioma cell migration. Furthermore, we found that the observed reductions in glioma cell migration and RhoA level could be rescued by RhoA overexpression. Taken together, these results show that GOLPH3 contributes to the motility of glioma cells by regulating the expression of RhoA.

1. Introduction

Glioblastoma multiform (GBM) is the most common adult primary malignant intracranial neoplasm in humans [1]. Even when multimodal treatment approaches are applied, the median patient survival time remains less than 1 year [2,3]. One of the major reasons for the failure of traditional treatments is the highly invasive nature of glioma cells [4]. Curing this intractable disease will thus require a deeper understanding of the detailed mechanisms underlying this invasive behavior.

Several studies have shown that amplification of human chromosome 5p13 is important for tumor formation, development and metastasis [5,6]. Several genes found in this area are considered oncogenes, including GOLPH3 [7]. The product of GOLPH3 is GOLPH3 (Golgi phosphoprotein 3, also called GPP34, GMx33, and MIDAS). This protein, located in the trans-Golgi network, has been identified as a Golgi protein that plays vital roles in the Golgi secretory pathway and protein glycosylation [8–10]. Recently, Scott et al. identified GOLPH3 as a novel oncogene by genome-wide array-based comparative genome hybridization and tumor tissue microarrays [7]. Gain- and loss-of-function studies determined that GOLPH3 can promote cell transformation and tumor growth by constitutively activating mammalian target of rapamycin (mTOR) signaling and conferring increased sensitivity to rapamycin in melanoma cells. GOLPH3 is also upregulated in breast cancer, where it promotes proliferation and tumorigenicity via inhibition of the FOXO1 transcription factor [11]. Later studies investigated the clinical significance of GOLPH3 in prostate cancer, oral tongue cancer and glioma and found that GOLPH3 is not only upregulated in these cancers but is also indicative of poor prognosis and more aggressive tumors [12–14]. These results further demonstrate that GOLPH3 plays important roles in the progression of many tumors. However, its roles in cell migration and invasion, the critical characteristics of malignant glioma, remain unclear. In this study, we investigated the effect of GOLPH3 on the motility of malignant glioma cells and found that GOLPH3 regulates glioma cell migration through modulation of RhoA expression.

2. Materials and methods

2.1. Cell culture

Human U251 and U87 glioma cell lines were purchased from Shanghai Cell Bank, Type Culture Collection Committee, Chinese Academy of Sciences. The cells were cultured in DMEM/F-12 media (Gibco) supplemented with 10% fetal bovine serum (FBS, Evergreen Biological Engineering Co., Hangzhou, China) in a humidified incubator with 5% CO_2 at 37 °C.

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2.2. Antibodies

The following antibodies were used in this study: rabbit monoclonal anti-GOLPH3 (Abcam); rabbit monoclonal anti-MMP-14, mouse polyclonal anti-RhoA/B/C and mouse monoclonal anti-β-actin (Millipore); and rabbit polyclonal anti-GFP (Santa Cruz).

2.3. Transient transfection of GOLPH3 siRNAs and RhoA plasmid

Transfection of GOLPH3 siRNAs and the RhoA plasmid was performed using the Lipofectamine™ 2000 transfection reagent (Invitrogen) following the manufacturer's instructions. Three sets of siRNA duplexes (Shanghai GenePharma Co.) targeting human GOLPH3 are listed below:

siRNA1: 5′-GUUAAGAAAUGUACGGGAATT-3′;

siRNA2: 5′-CAAGAAAGGUAAUCUGUAATT-3′;

siRNA3: 5′-GAAUUAGCAUUGAGAGGAATT-3′. GFP-tagged wild-type RhoA constructs were gifts from the Institute of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

2.4. Wound healing assay

Twenty-four hours after GOLPH3 siRNA transfection, a lesion was created using a plastic pipette tip, and the cells were washed twice with PBS to remove the debris. The monolayer was then maintained in serum-free DMEM and cultured for 24 h. At the designated time, five randomly selected fields at the lesion border were acquired under an inverted microscope (Olympus, IX71). The number of cells across the wound was counted.

2.5. Transwell invasion and migration assays

Cell invasion and migration assays were performed using a transwell system (Corning, NY) according to the manufacturer's protocol. To assess invasion ability, filters were precoated with matrigel (BD Biosciences, Franklin Lakes, NJ). Approximately 5×10^4 cells in serum-free media were added to the top chamber, and the bottom chamber was filled with DMEM containing 10% FBS. After 24 h of incubation, the cells on the upper surface were gently removed with a cotton swab, and the membrane was fixed in 4% methanol for 30 min and stained with a 0.1% crystal violet solution for 30 min. The cells that migrated to the lower side of the membrane were captured, and the cell number was counted. The same experimental design was used for migration experiments except that the filters were not precoated with matrigel.

2.6. Gelatin zymography assay

MMP2 released into the conditioned media was measured by gelatin zymography. Cells transfected with the siRNAs were incubated in serum-free media for 24 h, and the conditioned media was harvested, centrifuged, and resuspended in SDS loading buffer without β-mercaptoethanol. All samples were subjected to 10% SDS–PAGE with 0.2% gelatin (Sigma). After electrophoresis, the gels were washed and incubated in reaction buffer for 24 h at 37 °C. The gels were stained with 0.25% Coomassie brilliant blue R-250 and destained. MMP2 proteolytic activity in the gel was visualized as clear white bands at 64 kDa against a blue background.

2.7. Semi-quantitative reverse transcription PCR

Total tumor cell RNA was isolated, and cDNA synthesis was carried out using reverse transcription reagents (TaKaRa RNA PCR Kit (AMV) v. 3.0, Dalian, China) according to the manufacturer's protocol. All PCR primers were synthesized by Sangon Biotech (Shanghai) and based human sequences. Primer sequences, hybridization temperatures, numbers of cycles and PCR conditions are summarized in Table 1.

2.8. Cell elongation analysis

The U251 cell elongation index was measured as previously described [15]. Briefly, the elongation index of a cell was measured as the ratio of the process length to the cell width and required two lines to be drawn across the cell. One line, drawn from the center of the nucleus to the furthest visible microtubule tip, was measured as the cell length. The second line drawn across the cell passed through the center of the nucleus at a 90° angle to the first line and was considered the cell width. The lengths were measured in arbitrary units using ImageJ software. Sixty to one hundred cells were measured in each experimental condition.

2.9. GST pull-down assay

A glutathione S-transferase-rhotekin binding domain (GST-RBD) pull-down assay was used to detect RhoA activity (RhoA-GTP). The GST-rhotekin plasmid was a gift from Yongjin Li (School of Medical Science and Laboratory Medicine, Jiangsu University). Cells were lysed and incubated overnight at 4 °C with glutathione-Sepharose beads bound to 4 μg of GST-RBD. The proteins precipitated on the agarose beads were washed, boiled, subjected to SDS–PAGE and immunoblotted with the corresponding antibodies.

2.10. Western blot analysis

At the designated time points, cells were lysed, and equal amounts of protein lysates were subjected to 12% SDS–PAGE, transferred to PVDF membranes (Millipore), and probed with primary and secondary antibodies. Bound antibodies were detected with the Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific, Inc.) and exposed to X-ray film. Analysis of band densities was performed using ImageJ software. All fold changes in band densities were determined relative to the negative control (NC) group.

2.11. Immunofluorescence staining

Cells grown on coverslips were fixed, blocked and permeabilized. Cells were incubated with the GOLPH3 antibody overnight at 4 °C and then incubated with the secondary antibody at room temperature for 1 h. The cells were then stained with FITC-conjugated phalloidin (5 μg/ml) for 60 min. Actin filaments (F-actin) were visualized and photographed with an Olympus IX71 digital inverted microscope. More than 200 cells from three independent experiments were analyzed for each condition. The average number of dot or fan-like protrusions around the cell was counted as an index for lamellipodia formation.

2.12. Statistical analysis

Data are presented as the mean ± SEM. Statistical significance was determined using Student's *t*-test, with $P < 0.05$ considered significant.

3. Results

3.1. Downregulation of GOLPH3 inhibited glioma cell migration

To examine the role of GOLPH3 in glioma cell migration and invasion, we used an RNA interference approach to downregulate GOLPH3 in U251 and U87 cells. Forty-eight hours after transfection,

Table 1
Primer sequences, hybridization temperature and number of cycles for primer used in semi-quantitative RT–PCR.

Gene name	Primer direction	Sequences (5′–3′)	Number of cycle	Hybridization temperature
GOLPH3	Forward Reverse	TGTAAGTCAGATGCTCCAACAGG-3′ TCACCCATTTGTCAAGAACGG	27	57
MMP-2	Forward Reverse	CAGGATCATTGGCTACACACC CCATACTTCACACGGACCACT	29	58
MMP-14	Forward Reverse	CCTGCGTCCATCAACACT GCCCATGAATGACCCTCT	29	52
β-Actin	Forward Reverse	CTGGGACGACATGGAGAAAA AAGGAAGGCTGGAAGAGTGC	23	61

the downregulation efficacy of the three GOLPH3 siRNAs was determined by Western blot. As shown in Fig. 1A and B, compared with the negative control (NC) group, the GOLPH3 protein level was reduced by 60% in the GOLPH3 siRNA-transfected cells, indicating the high downregulation efficacy of the GOLPH3 siRNAs.

Glioma cell motility was then investigated by a wound healing assay. We found that 24 h after scratching, NC cells had healed the wound to a greater extent than the GOLPH3-downregulated U251 cells (Fig. 1C and D). We then conducted a transwell chamber-based three-dimensional cell migration assay to investigate the impact of GOLPH3 downregulation on directional motility. Consistent with the aforementioned results, the GOLPH3 knockdown U251 cells exhibited an attenuated ability to migrate from the top to the bottom chamber 48 h after transfection (Fig. 1E and F). Similarly, U87 cells showed a suppressed migratory ability after GOLPH3 downregulation (Fig. 1E and F). These results demonstrate that downregulation of GOLPH3 inhibits glioma cell migration.

3.2. Downregulation of GOLPH3 suppressed glioma cell invasion

Migration and invasion are widely considered to be two closely interrelated processes. As downregulation of GOLPH3 attenuated the migration of human glioma cells, we next tested the function of GOLPH3 in cell invasion using matrigel-precoated transwell chambers. As shown in Fig. 2A and B, in both U251 and U87 cell lines, the number of invasive cells decreased markedly after GOLPH3 knockdown.

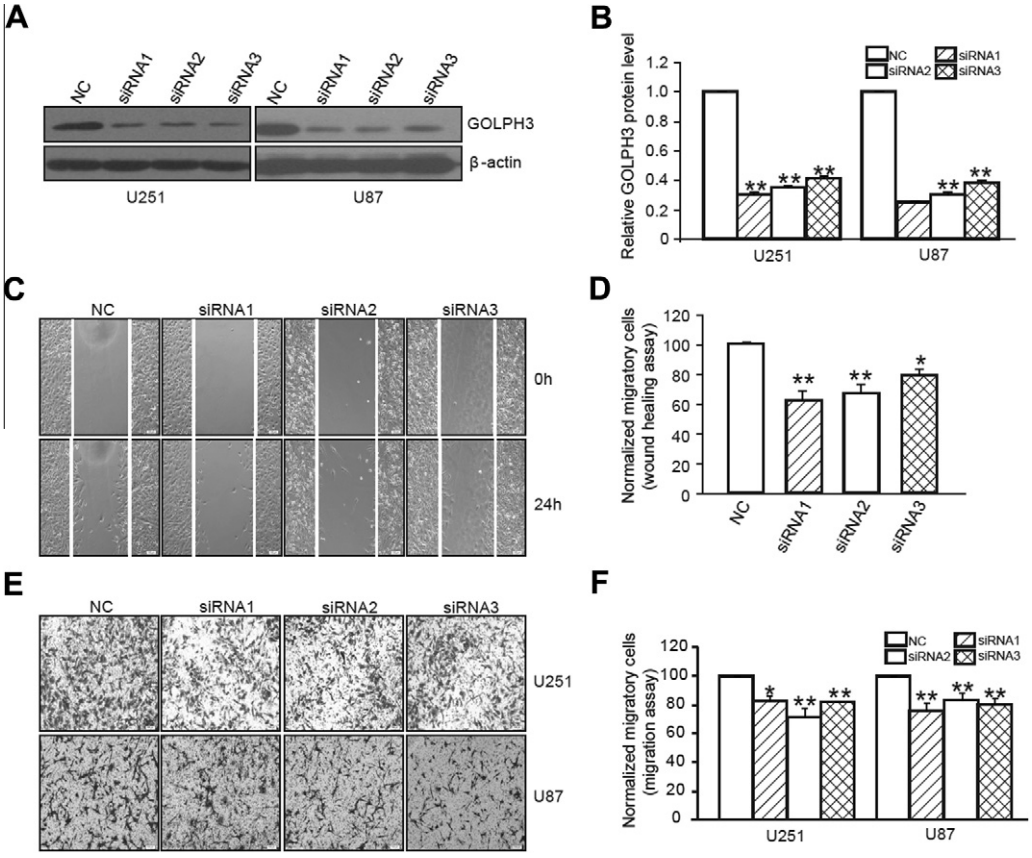

Fig. 1. GOLPH3 downregulation inhibited glioma cell migration. (A) The downregulation efficacy of GOLPH3 siRNAs in U251 and U87 glioma cells. Cell lysates from control (NC) or GOLPH3 siRNA-transfected cells were subjected to Western blot with an anti-GOLPH3 antibody. β-actin was used as a loading control. (B) Quantitative evaluation of GOLPH3 levels. (C) Effect of GOLPH3 downregulation on U251 cell migration as examined by a wound healing assay. Representative digital pictures were taken at 0 and 24 h. (D) Quantitative analysis of migratory cell numbers at the indicated time points. The numbers of migratory cells were normalized to that of the NC group, which was set at 100. (E) Effect of GOLPH3 downregulation on cell migration as examined by a transwell migration assay. (F) Quantitative analysis of the numbers of cells that migrated through the filter. The results are normalized to the NC group and presented as the mean ± SEM from three independent experiments. Scale bar, 100 μm. $^{*}P < 0.05$; $^{**}P < 0.01$.

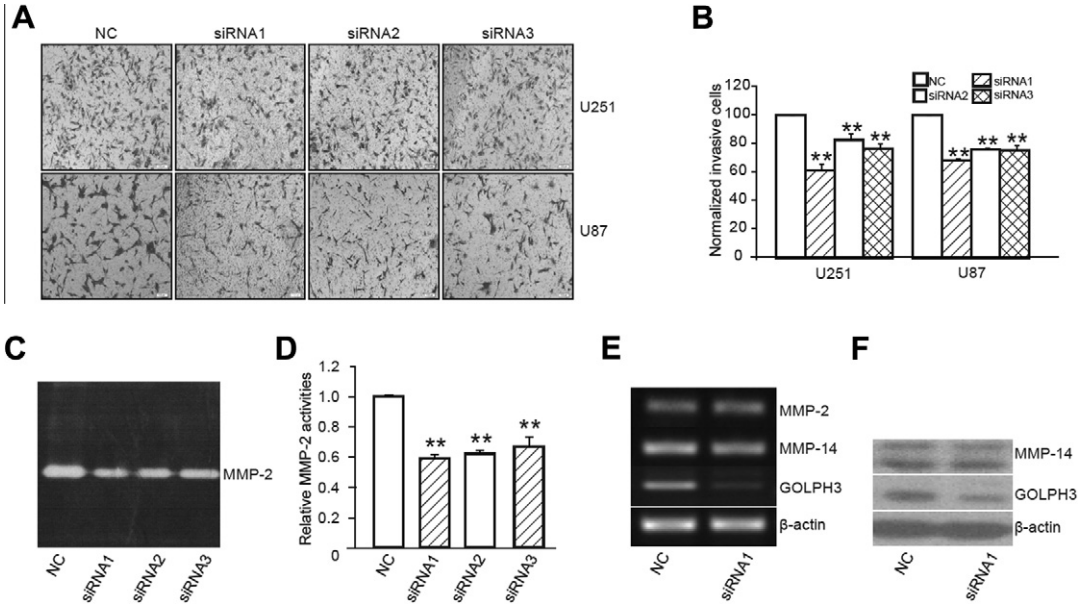

Fig. 2. GOLPH3 downregulation suppressed glioma cell invasion. (A) Effect of GOLPH3 downregulation on cell invasion ability as examined by a transwell invasion assay. Scale bar, 100 μm. (B) Quantitative analysis of the numbers of cells invading through the filter. The numbers of invading cells were normalized to that of the NC group. (C) Gelatin zymography assay showing that downregulation of GOLPH3 suppressed the excretion of MMP2. (D) Quantitative analysis of the results of the gelatin zymography analysis. The results for the siRNA-transfected groups were normalized to that of the NC group. (E) and (F) The effect of GOLPH3 downregulation on the expression of MMP2 and MMP14 as evaluated by RT–PCR and Western blot, respectively. The results are expressed as the mean ± SEM from three independent experiments. $^{**}P < 0.01$.

MMPs, especially MMP2, are known to be crucial for cell invasion in many tumors, including glioblastoma [16,17]. Using a gelatin zymography assay, we found that downregulation of GOLPH3 induced a clear reduction in the secretion of MMP2 (Fig. 2C and D), indicating that GOLPH3 genuinely affected the invasive ability of glioma cells. Because MMP2 is regulated by MMP14 [18], we examined the possibility that GOLPH3 downregulation affected the expression of MMP2 and MMP14. Unexpectedly, downregulation of GOLPH3 altered neither the mRNA levels of MMP-2 or MMP-14 nor the protein levels of MMP-14 (Fig. 2E and F). Taken

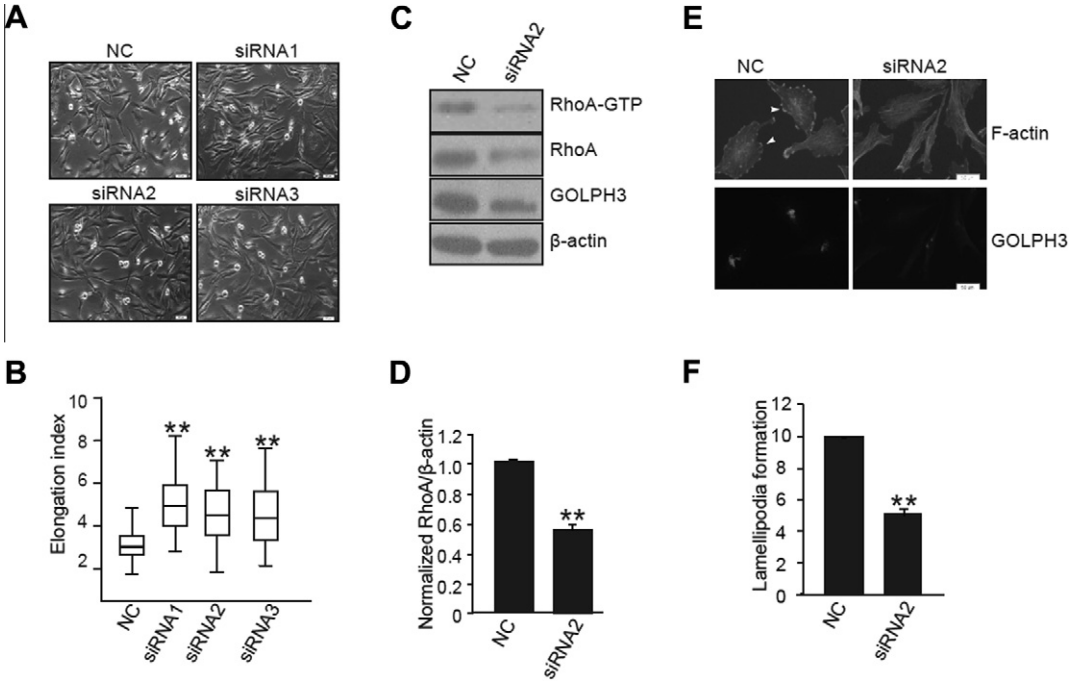

Fig. 3. GOLPH3 downregulation inhibited RhoA expression and lamellipodia formation. (A) Effect of GOLPH3 downregulation on U251 cell morphology. (B) Quantitative analysis of cell elongation upon GOLPH3 downregulation. The results are presented as the mean ± SEM from three independent experiments; 60 cells were analyzed per condition. Scale bar, 50 μm. $^{**}P < 0.01$. (C) The total levels and activity of RhoA were inhibited after GOLPH3 downregulation. Levels of active RhoA were examined using a GST-RBD pull-down assay. GST beads containing rhotekin served as bait for capturing GTP-RhoA protein. (D) Total RhoA levels were normalized to β-actin. (E) Lamellipodia formation was visualized by F-actin staining 48 h after GOLPH3 siRNA transfection. Lamellipodia were stained with FITC-conjugated phalloidin (upper panel), and GOLPH3 was detected by immunostaining (bottom panel). Cells were photographed with an Olympus IX71 digital inverted microscope. Arrowheads indicate lamellipodia. Scale bar, 50 μm. (F) The average number of lamellipodia was counted in an arbitrarily selected 200 cells from each group. Quantitative results were normalized to that of the NC group. The results are presented as the mean ± SEM from three independent experiments. $^{**}P < 0.01$.

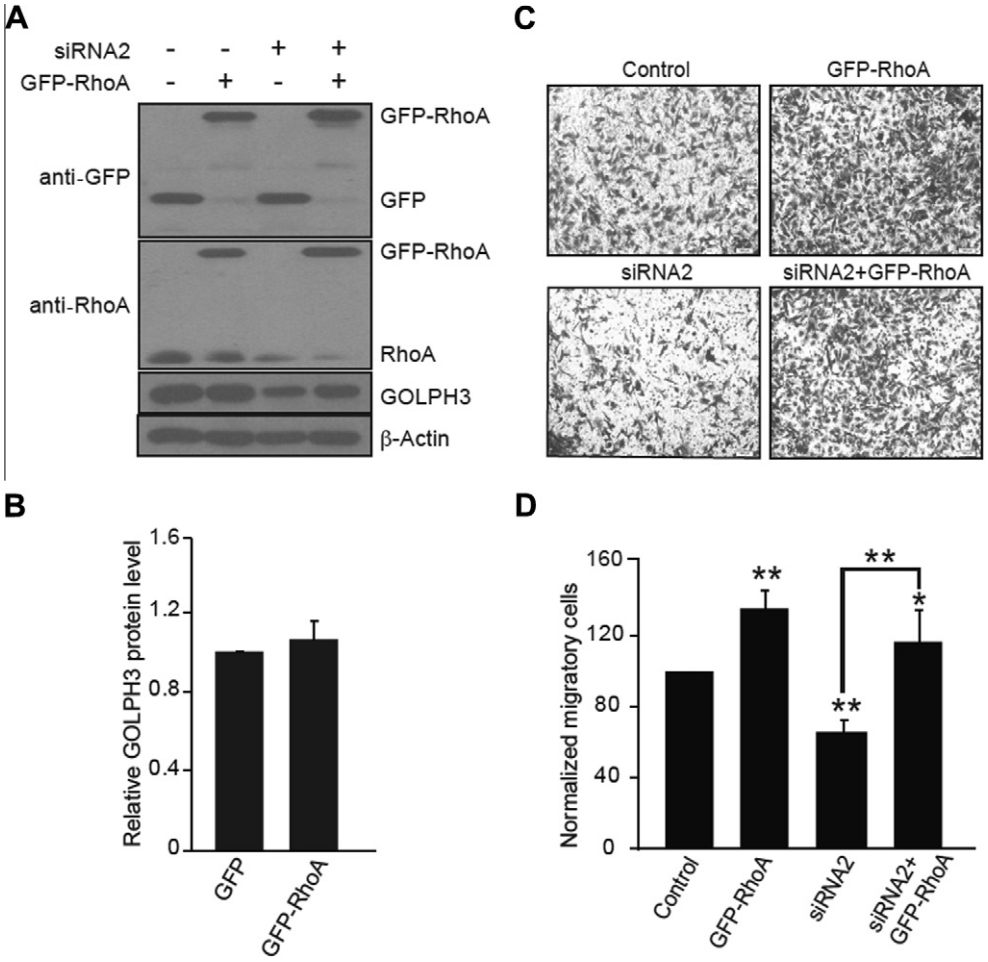

Fig. 4. The reduction in RhoA expression and the inhibition of cell migration induced by GOLPH3 knockdown are rescued by RhoA overexpression. (A) Glioma cells were co-transfected with GOLPH3 siRNA2 and GFP-RhoA. RhoA expression was evaluated by Western blot with GFP and RhoA/B/C antibodies. (B) Upregulation of RhoA did not affect GOLPH3 expression. (C) The suppression of migration induced by GOLPH3 downregulation was rescued by overexpression of RhoA in U251 cells. Scale bar, 100 μm. (D) Quantitative analysis of the numbers of cells migrating through the filter. The results are normalized to the NC group and presented as the mean ± SEM from three independent experiments. $^{**}P < 0.01$, $^{*}P < 0.05$.

together, these data reveal that GOLPH3 is involved in glioma cell migration and invasion and that this effect is not mediated through the regulation of MMP expression.

3.3. Downregulation of GOLPH3 inhibits RhoA expression

We observed that U251 cells transfected with the GOLPH3 siRNAs exhibited a distinct morphology with elongated processes (Fig. 3A). To quantitatively analyze the morphological changes, we applied the elongation index (EI), which has been widely used to quantify cell elongation [15,19]. As is shown in Fig. 3B, most cells transfected with GOLPH3 siRNAs exhibited elongated processes with EIs of 4–8, whereas NC cells generally presented EIs of <4. This finding suggests that downregulation of GOLPH3 did indeed affect cell morphology.

Previous studies reported that knockdown or inhibition of the small GTPase RhoA resulted in process elongation and suppressed the migration of glioma cells [19]. We therefore surmised that GOLPH3 downregulation might inhibit the activation or expression of RhoA, thereby inducing glioma cell elongation and suppression of migration. We measured the level of GTP-bound (active) RhoA using a GST-Rhotekin pull-down assay and found that it was significantly decreased upon GOLPH3 silencing (Fig. 3C). Surprisingly, the total level of RhoA was also reduced after GOLPH3 downregulation (Fig. 3C and D), suggesting that GOLPH3 may suppress RhoA activation by reducing the expression of RhoA.

RhoA expression and activity are important for cytoskeletal regulation in glioma cells, including F-actin stress fiber and lamellipodia formation [19,20]. We therefore examined whether GOLPH3 downregulation affects F-actin reorganization using FITC-conjugated phalloidin staining. Dot or fan-like protrusions were detected at the cell periphery in NC cells. These structures, also called lamellipodia, represent a form of concentrated F-actin. Upon GOLPH3 downregulation, the cells became longer, and the numbers of dot or fan-like protrusions at the cell margins were markedly reduced (Fig. 3E and F), consistent with our observation that RhoA expression was reduced in cells transfected with GOLPH3 siRNA.

3.4. Overexpression of RhoA rescued the inhibitory effect of GOLPH3 knockdown on cell migration

We next examined the possibility that RhoA is involved in the inhibitory effect of GOLPH3 knockdown on migration. As is shown in Fig. 4A, when GOLPH3 siRNA was co-transfected with GFP-RhoA, the reduction in RhoA expression induced by GOLPH3 siRNA was compensated by exogenous RhoA. In contrast, upregulation of RhoA did not affect the expression of GOLPH3, suggesting that

RhoA lies downstream of GOLPH3 (Fig. 4B). We also found that the inhibition of glioma cell migration induced by downregulation of GOLPH3 was restored by overexpression of RhoA (Fig. 4C and D), indicating that RhoA is downstream of GOLPH3.

4. Discussion

Studies have shown that GOLPH3 can promote cell transformation and tumor growth by constitutively activating mTOR signaling and conferring increased sensitivity to rapamycin in human melanoma [7]. Recently, increasing evidence has shown that GOLPH3 is highly expressed in human glioma and is related to glioma aggression and poor prognosis [14,21]. However, the specific role and mechanism of GOLPH3 in cell migration and invasion had remained unclear. In this study, we found that downregulation of GOLPH3 inhibited cell migration in U251 and U87 glioma cell lines (Fig. 1). The invasive ability of glioma cells was also suppressed upon GOLPH3 downregulation (Fig. 2A and B). This is the first report that downregulation of GOLPH3 can inhibit glioma cell migration and invasion.

Cell migration and invasion are multistep processes comprising cytoskeletal reorganization, extracellular matrix (ECM) degradation, adhesion and de-adhesion [22,23]. Normally, ECM degradation is the first step required for tumor invasiveness. This process can be mediated by MMP2 and MMP14. Using a gelatin zymography assay, we found that downregulation of GOLPH3 induced a notable reduction in MMP2 secretion (Fig. 2C and D), consistent with the effect on invasion. However, knockdown of GOLPH3 did not affect the mRNA levels of MMP2 or MMP14 or protein expression of MMP14 (Fig. 2E and F). These findings indicated that GOLPH3 may regulate glioma cell migration and invasion through other pathways.

Numerous lines of evidence have indicated that Rho family GTPases play important roles in directional cell movement [24]. RhoA, one of the Rho family small GTPases, is overexpressed in astrocytomas and positively correlated with the degree of malignancy [25]. RhoA has been demonstrated to be a critical protein for cell motility and cytoskeletal organization dynamics in several cell lines, including glioma cells [26]. Rho has two major effectors, ROCK and mDia [27,28]. Previous studies have suggested that the Rho-ROCK and Rho-mDia1 pathways are involved in the regulation of tumor cell migration by Ras and Src, respectively [29]. The Rho pathway can also participate in MMP regulation [30]. We found that GOLPH3 downregulation significantly reduced RhoA expression (Fig. 3C and D). In addition, glioma cells transfected with GOLPH3 siRNA were more slender and contained fewer dot protrusions at the cell periphery compared with the NC group (Fig. 3A and E). We hypothesized that RhoA might contribute to these alterations. RhoA is generally believed to play an essential role in actin stress fiber formation [31]. However, in some cell lines, such as colon carcinoma cells [32] and hepatocarcinoma cells [33], RhoA was shown to promote lamellipodia formation and cell motility. Furthermore, it has been reported that rapamycin can suppress lamellipodia formation and cell motility by inhibiting RhoA expression through the mTOR signaling pathway [20]. It is possible that GOLPH3 may regulate RhoA expression and cytoskeletal reorganization through a similar mechanism. Our results indicate that regulation of RhoA protein expression may represent a new mechanism in GOLPH3-mediated glioma migration and invasion.

In summary, this study provides the first evidence that GOLPH3 is involved in the regulation of RhoA expression and cell migration in glioma cells. Further explication of the GOLPH3-RhoA signaling pathway may provide new targets for glioma prognosis or therapeutics.

Acknowledgments

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References

[1] H.C. Onyegbutulem, H.O. PI, M. Reimann, et al., Metabolic syndrome in Africa: an emerging perspective, Horm. Metab. Res. 41 (2009) 75–78.
[2] H. Ohgaki, P. Kleihues, Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas, J. Neuropathol. Exp. Neurol. 64 (2005) 479–489.
[3] E.C. Holland, Gliomagenesis: genetic alterations and mouse models, Nat. Rev. Genet. 2 (2001) 120–129.
[4] H.K. Manji, J.A. Quiroz, J. Sporn, et al., Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression, Biol. Psychiatry 53 (2003) 707–742.
[5] S. Yokoi, K. Yasui, F. Saito-Ohara, et al., A novel target gene, SKP2, within the 5p13 amplicon that is frequently detected in small cell lung cancers, Am. J. Pathol. 161 (2002) 207–216.
[6] W.C. Hung, W.L. Tseng, J. Shiea, et al., Skp2 overexpression increases the expression of MMP-2 and MMP-9 and invasion of lung cancer cells, Cancer Lett. 288 (2010) 156–161.
[7] K.L. Scott, O. Kabbarah, M.C. Liang, et al., GOLPH3 modulates mTOR signalling and rapamycin sensitivity in cancer, Nature 459 (2009) 1085–1090.
[8] H.C. Dippold, M.M. Ng, S.E. Farber-Katz, et al., GOLPH3 bridges phosphatidylinositol-4-phosphate and actomyosin to stretch and shape the Golgi to promote budding, Cell 139 (2009) 337–351.
[9] K.R. Schmitz, J. Liu, S. Li, et al., Golgi localization of glycosyltransferases requires a Vps74p oligomer, Dev. Cell 14 (2008) 523–534.
[10] L. Tu, W.C. Tai, L. Chen, et al., Signal-mediated dynamic retention of glycosyltransferases in the Golgi, Science 321 (2008) 404–407.
[11] Z. Zeng, H. Lin, X. Zhao, et al., Overexpression of GOLPH3 promotes proliferation and tumorigenicity in breast cancer via suppression of the FOXO1 transcription factor, Clin. Cancer Res. 18 (2012) 4059–4069.
[12] X. Hua, L. Yu, W. Pan, et al., Increased expression of Golgi phosphoprotein-3 is associated with tumor aggressiveness and poor prognosis of prostate cancer, Diagn. Pathol. 7 (2012) 127.
[13] H. Li, L. Guo, S.W. Chen, et al., GOLPH3 overexpression correlates with tumor progression and poor prognosis in patients with clinically N0 oral tongue cancer, J. Transl. Med. 10 (2012) 168.
[14] J. Zhou, T. Xu, R. Qin, et al., Overexpression of Golgi phosphoprotein-3 (GOLPH3) in glioblastoma multiforme is associated with worse prognosis, J. Neurooncol. 110 (2012) 195–203.
[15] M.P. Iwanicki, T. Vomastek, R.W. Tilghman, et al., FAK, PDZ-RhoGEF and ROCKII cooperate to regulate adhesion movement and trailing-edge retraction in fibroblasts, J. Cell Sci. 121 (2008) 895–905.
[16] M. Egeblad, Z. Werb, New functions for the matrix metalloproteinases in cancer progression, Nat. Rev. Cancer. 2 (2002) 161–174.
[17] P. Vihinen, R. Ala-aho, V.M. Kahari, Matrix metalloproteinases as therapeutic targets in cancer, Curr. Cancer Drug Targets 5 (2005) 203–220.
[18] H. Sato, T. Takino, Y. Okada, et al., A matrix metalloproteinase expressed on the surface of invasive tumour cells, Nature 370 (1994) 61–65.
[19] G. Wan, H.P. Too, A specific isoform of glial cell line-derived neurotrophic factor family receptor alpha 1 regulates RhoA expression and glioma cell migration, J. Neurochem. 115 (2010) 759–770.
[20] L. Liu, Y. Luo, L. Chen, et al., Rapamycin inhibits cytoskeleton reorganization and cell motility by suppressing RhoA expression and activity, J. Biol. Chem. 285 (2010) 38362–38373.
[21] X.Y. Li, W. Liu, S.F. Chen, et al., Expression of the Golgi phosphoprotein-3 gene in human gliomas: a pilot study, J. Neurooncol. 105 (2011) 159–163.
[22] A.J. Ridley, M.A. Schwartz, K. Burridge, et al., Cell migration: integrating signals from front to back, Science 302 (2003) 1704–1709.
[23] M. Nakada, S. Nakada, T. Demuth, et al., Molecular targets of glioma invasion, Cell. Mol. Life Sci. 64 (2007) 458–478.
[24] D. Yamazaki, S. Kurisu, T. Takenawa, Regulation of cancer cell motility through actin reorganization, Cancer Sci. 96 (2005) 379–386.
[25] B. Yan, H.H. Chour, B.K. Peh, et al., RhoA protein expression correlates positively with degree of malignancy in astrocytomas, Neurosci. Lett. 407 (2006) 124–126.
[26] J. Esparza, C. Vilardell, J. Calvo, et al., Fibronectin upregulates gelatinase B (MMP-9) and induces coordinated expression of gelatinase A (MMP-2) and its activator MT1-MMP (MMP-14) by human T lymphocyte cell lines. A process repressed through RAS/MAP kinase signaling pathways, Blood 94 (1999) 2754–2766.
[27] T. Matsui, M. Amano, T. Yamamoto, et al., Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho, EMBO J. 15 (1996) 2208–2216.

[28] N. Watanabe, P. Madaule, T. Reid, et al., P140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin, EMBO J. 16 (1997) 3044–3056.
[29] S. Narumiya, M. Tanji, T. Ishizaki, Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion, Cancer Metastasis Rev. 28 (2009) 65–76.
[30] K. Schram, R. Ganguly, E.K. No, et al., Regulation of MT1-MMP and MMP-2 by leptin in cardiac fibroblasts involves Rho/ROCK-dependent actin cytoskeletal reorganization and leads to enhanced cell migration, Endocrinology 152 (2011) 2037–2047.
[31] S.J. Heasman, A.J. Ridley, Mammalian Rho GTPases: new insights into their functions from in vivo studies, Nat. Rev. Mol. Cell Biol. 9 (2008) 690–701.
[32] K.L. O'Connor, B.K. Nguyen, A.M. Mercurio, RhoA function in lamellae formation and migration is regulated by the alpha6beta4 integrin and cAMP metabolism, J. Cell Biol. 148 (2000) 253–258.
[33] T. Genda, M. Sakamoto, T. Ichida, et al., Cell motility mediated by rho and Rho-associated protein kinase plays a critical role in intrahepatic metastasis of human hepatocellular carcinoma, Hepatology 30 (1999) 1027–1036.