ORIGINAL RESEARCH

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Golgi phosphoprotein-3 promotes invasiveness of gastric cancer cells through the mTOR signalling pathway

Abstract

Purpose: Golgi phosphoprotein-3 (*GOLPH3*) is an oncogene that is overexpressed in multiple cancers and is associated with poor prognosis. The aim of this study was to examine the impact of *GOLPH3* on the migration and metastasis of gastric cancer cells.

Methods: Following the shRNA-mediated knockdown of *GOLPH3*, we analyzed cytoskeletal reorganization and cell invasion, migration and adhesion, and determined the impact of components of the mammalian target of the rapamycin (mTOR) signalling pathway.

Results: The *GOLPH3* mRNA and protein expression were significantly lower in both SGC-7901 and MKN-28 cells as compared with poorly-differentiated BGC-823 cells. The *GOLPH3* knockdown also significantly reduced cell invasion in all three cell lines through reduced migration as compared with the non-targeting control sequence group. The *GOLPH3* knockdown also reduced F-actin in all three cell lines, and decreased cell adhesion in BGC-823 and SGC-7901 cells. Finally, p-mTOR, p70S6K, p-4EBP1 and RhoA protein levels were significantly downregulated in *shGOLPH3*-1-treated cells.

Conclusions: In conclusion, *GOLPH3* increased in poorly-differentiated gastric cancer cells, activating the mTOR-70S6K/4EBP1-RhoA signalling pathway to promote the migration and metastasis of gastric cancer cells.

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Gastric cancer is the second most common cause of cancer-related death world-wide [1]. Although gastric cancer treatment, including surgery and chemotherapy, has undergone several advances, the prognosis of patients with gastric cancer remains poor. The relative 5-year survival rate in the United State of America (USA) and Europe is 10%-20% [2]; therefore, identification of novel therapeutic targets and tumorigenic mechanisms responsible for gastric cancer is of great clinical significance.

Vesicular trafficking, particularly endocytic trafficking, plays an important role in cancer development and metastasis [3], and the signalling pathways and surface receptors responsible for endocytosis may represent novel targets. Recent studies have shown that a Golgi-associated endocytic protein, Golgi phosphoprotein-3 (GOLPH3) [4], is required for efficient anterograde vesicle formation and plays the role of oncogene in multiple cancers [5]. In patients with oral tongue cancer, overexpression of *GOLPH3* was associated with clinical stage and lower survival rate [6]. In patients with early-stage non-small-cell lung cancer, *GOLPH3* expression was an independent prognostic factor; patients with high *GOLPH3* tumor expression had significantly lower 5-year overall survival [7]. Similarly, in patients with esophageal squamous cell carcinoma, expression of *GOLPH3* was associated with clinical stage TNM classification, histological differentiation and vital status; it was also an independent prognostic factor of 5-year overall survival [8]. In addition, overexpression of *GOLPH3* was also found to be associated with gastric tumor size, grade, invasion and TNM stage as well as the poor prognosis of gastric cancer patients [9]; its association with metastasis and lymph node involvement has also been shown [10]. However, few studies have analyzed the mechanism by which *GOLPH3* is associated with poor prognosis in gastric cancer.

Although the regulatory mechanism and signalling pathways associated with *GOLPH3* in cancer are not fully known, a previous study demonstrated that the mammalian target of rapamycin (mTOR) signalling pathway was modulated by *GOLPH3* in human lung cancer cell line A549 to control cell size [11]. It also has been reported that *GOLPH3*-regulated cell migration and invasion through mTOR signalling in glioblastoma and *GOLPH3*-overexpressing xenografts were more sensitive to rapamycin, an mTORC1 inhibitor [12]. Specifically, in gastric cancer patients, *GOLPH3* expression correlated positively with p-mTOR, p-p70S6 and p-4EBP1 expression [10], but in gastric

cancer, the regulatory mechanism of *GOLPH3* and mTOR signalling pathway still remains unclear.

Because the role of *GOLPH3* in gastric cancer cells has not been analyzed, this study aimed to test the hypothesis that *GOLPH3* may enhance the cell migration and metastasis of gastric cancer cells. For these studies, we suppressed *GOLPH3* expression in three gastric cancer cell lines of different states of differentiation, including BGC-823 (poorly-differentiated), SGC-7901 (moderately-differentiated) and MKN-28 (well-differentiated) cells, and analyzed their cytoskeletal reorganization, invasion, migration and adhesion. In addition, the relationship between *GOLPH3* and the mTOR signalling pathway was explored. The results suggest that *GOLPH3* may serve as a therapeutic target for gastric cancer in future studies.

Methods

Cell culture

Human gastric cancer cell lines BGC-823, SGC-7901 and MKN-28 were purchased from Tongpai Biological Technology (Shanghai, China). Cells were maintained in a humidified environment with 5% CO₂ at 37°C in RPMI-1640 medium (HyClone Minimum Essential Medium, Fisher Scientific, Atlanta, GA, USA) containing 100 U/mL penicillin, 100 g/mL streptomycin and 10% calf serum.

qRT-PCR analysis

Total RNA was extracted with a MiniBEST Universal RNA Extraction Kit (Takara, Japan) and was stored at -80°C. The cDNA was isolated using a PrimeScript® RT Reagent Kit with gDNA Eraser (Takara, Japan) according to manufacturer's protocol. The qRT-PCR analysis was performed using a SYBR Premix Ex Taq (Thermo-Fischer, Takara, Japan) and a Bio Rad CFX-96 Connect System (BioRad, Hercules, CA, USA) under the following conditions: 95°C for 3 min; 40 cycles at 95°C for 10 s, 58°C for 15 s, and 72°C for 20 s; and 80 cycles of 95°C for 1 min, 65°C for 1 min, and 65°C–95°C (at increments of 0.5°C per cycle). Primer sequences were as follows: *GOLPH3*-F, 5'-CTCCAGAAACGGTCCA -GAAC-3'; *GOLPH3*-R, 5'-CCACCAGGTTTCTTAGCTAATCG-3'; β -actin-F, 5'-AGCCTCGCCTTTGCCGA-3'; β -actin-R, 5'-CTGGTGCCTGGGGCG-3'. The 2- $\Delta\Delta$ Ct method was used to calculate the relative mRNA expression.

Western blot analysis

Cells were lysed in 100 μ L of RIPA lysis buffer (Solarbio, Beijing, China) followed by centrifugation at 4°C for 15 min at 12000 $\times$ g. The supernatant was harvested and stored at -80°C until further use. For SDS-PAGE, 50 μ g of total protein was separated and transferred onto a PVDF membrane. After the membranes were incubated in blocking buffer (BD Biosciences, San Jose, CA, USA) for 1 h, they were incubated with the following primary antibodies at 4°C overnight (Proteintech Group, Chicago, IL, USA): anti- β -actin antibody (1:1000) and anti-GOLPH3 antibody (1:1000). The membrane was incubated with secondary antibody (goat anti-rabbit IgG HRP, 1:1000, Beyotime Biotechnology, Jiangsu Province, China) at room temperature for 2 h. The bands were visualized with ECL reagent (Millipore, Billerica, MA, USA). The protein expression of *GOLPH3* was normalized with β -actin.

shRNA transfection

The following pGPU6/GFP/Neo-shRNA and non-targeting control sequence shRNA (shNC) primers were purchased from Genepharma (Genepharma, Shanghai, China): pGpU6/GFP/Neo-(*GOLPH3*)1 sense, 5'-CACCGGCTGTATGTTAATTGAATTTCA-AGAGAATTCAATTAACATACAG-CCTTTTTTG-3' and antisense, 5'-GATCCAAAAAAGGCTGTATGTTAATTGAATTTCTCTTGAAATTCATTAACA TACAGCC-3'; pGpU6/GFP/Neo-(*GOLPH3*)2 sense, 5'-CACCGTCCAGAACTGGA-TTGAATTTCAAGAGAATTCAATCCAGTTC TGGACTTTTTTG-3' and antisense, 5'-GATCCAAAAAAGTCCAGAACTGGATTGAATTTCTCTTGAAATTCATCCA GTTCTGGAC-3'; pGpU6/GFP/Neo-(*GOLPH3*) 3 sense, 5'-CACCGCCTCATCAAG-AAAGTACATTCAAGAGATGTACTTTCTTGATGAGGCTTTTTTG-3' and antisense, 5'-GATCCAAAAAAGCCTCATCAAGAAAGTACATCTCTTGAATGTA CTTTCTTGATGAGGC-3'; and pGPU6/GFP/Neo-shNC sense, 5'-CACCGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTTCGAGAAC-3' and antisense, 5'-GATCCAAAAAATTTCTCCGAACGTGTCACGTTCTCTTGAAACGTGACACGTTTCGAGAAC-3'.

For the transfection, the cells were seeded into 6-well plates at a density of 5 $\times$ 10⁵ cells/well. After 24 h, the cells were transfected with Lipofectamine 2000 (Invitrogen) and Opti-MEM according to manufacturer's instructions. At

4-6 h after transfection, the medium was refreshed. For cells with a transfection efficiency that was higher than 80% under a fluorescence microscope at 24 h, the target plasmid was used in following experiment.

Immunofluorescence analysis

The BGC-823, SGC-7901 and MKN-28 cells were transfected with *GOLPH3* shRNA and immunofluorescence staining was undertaken to detect the microfilaments. After the cells (5 $\times$ 10⁵) were plated on coverslips and reached 50% confluence, they were fixed in phosphate-buffered saline (PBS) containing 4% formaldehyde at room temperature for 10 min and then incubated with 0.5% Triton X-100 for 20 min. The cells were blocked in 1% bovine serum albumen (BSA) for 30 min, incubated with 200 μ L of TRITC-conjugated Phalloidin solution (Yeasen, Shanghai, China) at 37°C in the dark for 1 h, then counterstained with 200 μ L of 100nM DAPI for 30 s. Cells were mounted using Fluoromount-GTM (Southern Biotech, Birmingham, AL, USA) and imaged using a confocal microscopy (LSM710, ZEISS, Carl Zeiss, Oberkochen, Germany). The red fluorescence was detected in three randomly selected fields with Image Pro Plus.

Transwell invasion assays

The transwell chamber was coated with Matrigel (diluted 1:4); 50 μ L of the diluted Matrigel solution was added to the transwell chamber and incubated at 37°C for 4 h. The BGC-823 cells were seeded into 6-well plates (5 $\times$ 10⁵ cells/well). When the cells had adhered to the wall, sh*GOLPH3*-treated cells were transfected with Lipofectamine RNAiMAX Reagent (Invitrogen). Cell suspensions in 0.2% BSA were prepared at a cell density of 1 $\times$ 10⁶ cells/mL, and 200 μ L of the cell suspension was added to the transwell chamber. In addition, 600 μ L of FBS was added to the lower chamber. After 24 h, cells were washed twice in PBS and fixed in methanol for 30 min. The chambers were air-dried, and the cells were stained with 0.1% crystal violet for 20 min. The non-migrated cells were removed with a cotton swab, and the chamber was washed three times with PBS. Cells were observed under a light microscope at a magnification of 400, and five fields were randomly selected for cell counting.

Wound healing migration assay

Approximately 5 $\times$ 10⁵ cells were added to each 6-well plate and cultured overnight. On the second day, a pipette tip was

used to scratch lines into the cell monolayers and the damaged cells were removed by washing. After addition of serum-free medium, the cells were photographed at 0 h and 24 h.

Adhesion assay

After 10 $\mu\text{g/mL}$ fibronectin was added to 96-well plates (70 $\mu\text{L/well}$) followed by an overnight incubation at 4°C, the cells were blocked in 1% BSA for 1 h at 37°C.

Cells in logarithmic growth phase were re-suspended in serum free medium and seeded into fibronectin-pre-coated 96-well plates (5000 cells/well) with three wells in each group. After incubation at 37°C for 1 h, cells that were not adhered to the wall were removed by washing. The cells were fixed in 5% glutaraldehyde at 4°C for 30 min, followed by staining with 0.1% crystal violet at room temperature for 30 min. After washing in PBS, 100 μL of cells were incubated with 10% acetic acid for 5-10 min, and the absorbance was measured at 595 nm to determine the cell count.

Statistical analysis

Data are presented as mean $\pm$ standard deviations (mean $\pm$ SD). One-sample t-tests were performed to identify the difference in relative endogenous *GOLPH3* mRNA expression in the SGC-7901 and MKN-28 cells as compared with BGC-823 cells; they were also used to identify differences in relative *GOLPH3* mRNA expression level after *shGOLPH3* knockdown compared with non-targeting control sequence group in each experiment (setting the mean=1 of relative mRNA expression for sequence. Two-sample t-tests were used to compare the relative mRNA expression between SGC-7901 and MKN-28 cells or between sequence and *shGOLPH3*-1-treated BGC-823 cells in immunostaining assays, transwell migration assays, wound healing assays, adhesion assays and western blot analyses. One-way ANOVA with *post-hoc* Bonferroni pair-wise comparisons were used to compare relative protein expression among the three cell lines as well as the relative *GOLPH3* mRNA and protein expression among untreated and *shGOLPH3*-treated BGC-823 cells. All statistical

FIGURE 1. Endogenous *GOLPH3* mRNA and protein expression in gastric cancer cell lines of varying differentiation states.

Endogenous expression of *GOLPH3* mRNA and protein level were detected by qRT-PCR (A) and western blot (B) in human gastric cancer cell lines BGC-823, SGC-7901 and MKN-28 cells. Compared with well differentiated MKN-28 cells, expression of *GOLPH3* mRNA and protein level are highly expressed in BGC-823 (poorly-differentiated) cells. Three samples were analyzed in each group and values (mean $\pm$ SD) shown in parentheses represent the relative protein abundance, which has been normalized with corresponding β -actin and expressed as folds of BGC-823.

* $p < 0.05$ different from the BGC-823 group.

†significantly different as compared with SGC-7901 cells.

assessments were two-tailed and considered significantly different at $p < 0.05$. All statistical analyses were carried out with IBM SPSS statistical software version 22 for Windows (IBM, Armonk, NY, USA).

Results

GOLPH3 expression in gastric cancer cell lines

To investigate the role of GOLPH3 in gastric cancer cell differentiation and malignancy, we selected three gastric cancer cell lines of varying differentiation states: BGC-823 (poorly-differentiated), SGC7901 (moderately-differentiated)

and MKN-28 (well-differentiated). We first examined endogenous expression of *GOLPH3* mRNA and protein expression level by qRT-PCR and western blot analyses, respectively. As shown in Figure 1, the relative level of *GOLPH3* mRNA and protein expression was significantly lower in both SGC-7901 and MKN-28 cells as compared with BGC-823 cells (mRNA expression: 0.204 ± 0.039 , 0.076 ± 0.009 vs. 1 ± 0 , respectively; protein expression: 1.029 ± 0.055 , 0.606 ± 0.038 vs. 1.432 ± 0.054 , respectively). Moreover, MKN-28 cells also had lower relative *GOLPH3* mRNA and protein expression than BGC-823 cells.

FIGURE 2. shRNA-mediated knockdown of *GOLPH3* expression in BGC-823, SGC-7901, and MKN-28 cell lines.

Cells were transfected with non-targeting control sequence, *shGOLPH3*-1, *shGOLPH3*-2 and *shGOLPH3*-3. Expression of *GOLPH3* mRNA and protein levels were validated by qRT-PCR(A) and western blot (B, C, D). Results show that *shGOLPH3*-1 has the greatest knockdown efficiency in suppressing *GOLPH3* mRNA and protein. Three samples were analyzed in each group and values (mean $\pm$ SD) shown in parentheses represent the relative protein abundance, which has been normalized with corresponding β -actin and expressed as folds of non-targeting control sequence group.

* $p < 0.05$ significantly different as compared with non-targeting control sequence group in each experiment.

Abbreviations: NC, non-targeting control sequence; *shGOLPH3*, shRNA of *GOLPH3*; mock, transfection reagent only.

FIGURE 3. Functional analysis following shRNA-mediated *GOLPH3* knockdown.

Cell migration, invasion, cytoskeleton reorganization and adhesion are evaluated by transwell assay (A), wound-healing assay (B), immunofluorescence (C) and adhesion assay (D) in knockdown *GOLPH3* in BGC-823, SGC-7901 and MNK-28 cells. Knockdown of *GOLPH3* significantly decreases cell migration, invasion and cytoskeleton reorganization in BGC-823, SGC-7901 and MNK-28 cells. Cell adhesion is only decreased in BGC-823 and SGC-7901, but not in MNK-28. Three samples were analyzed in each group, and values represent the mean $\pm$ SD

* $p < 0.05$ significantly different as compared with non-targeting control sequence group in each experiment.

Abbreviations: NC, non-targeting control sequence; shGOLPH3, shRNA of *GOLPH3*.

Effects of *GOLPH3* expression on gastric cancer cell cytoskeletal reorganization, migration, invasion and adhesion

To elucidate the role of *GOLPH3* in the malignancy of gastric cancer cell lines, we analyzed three shRNAs (shGOLPH3-1, shGOLPH3-2 and shGOLPH3-3) to knockdown *GOLPH3*. Among the three gastric cancer cell lines, shGOLPH3-1 induced the greatest knockdown efficiency in suppressing *GOLPH3* mRNA (Figure 2A) and protein (Figure 2B, 2C and 2D) expression. Thus, shGOLPH3-1 was selected for use in subsequent functional assays.

To examine the invasive and metastatic roles of *GOLPH3* in gastric cancers, a series of functional analyses were performed in shGOLPH3-1-transfected BGC-823,

SGC-7901 and MKN-28 cells. We first investigated the invasive roles of *GOLPH3* in gastric cancers using a transwell assay. As shown in Figure 3A, cells with *GOLPH3* knockdown showed significantly reduced invasive ability as compared with the non-targeting control sequence group. The effect of *GOLPH3* on gastric cancer cell migration was further validated using a wound healing assay. As shown in Figure 3B, all three gastric cancer cell lines displayed reduced migration following *GOLPH3* knockdown as compared to the non-targeting control group. To investigate the effect of *GOLPH3* in cytoskeletal reorganization, immunostained F-actin using TRITC-phalloidin, which was then quantified by fluorescence intensity. As shown in Figure 3C, *GOLPH3* knockdown significantly reduced F-actin in all three gastric

FIGURE 4. qRT-PCR analysis of *mTOR*, *p70S6K*, *RhoA*, and *4EBP1* mRNA expression in gastric cancer cell lines.

Expression of *mTOR*, *p70S6K*, *RhoA* and *4EBP1* was detected by qRT-PCR in BGC-823 (A), SGC-7901 (B), and MKN-28 (C) cells transfected with shGOLPH3-1 as compared with the non-targeting control sequence group. As compared with non-targeting control sequence group, knockdown *GOLPH3* decreased the mRNA expression of *mTOR*, *p70S6K*, *RhoA*, and *4EBP1* in three cell lines, except p-mTOR in SGC-7901 cells. Three samples were analyzed in each group, and values represent the mean $\pm$ SD

* $p < 0.05$ significantly different as compared with non-targeting control sequence group in each experiment.

* Abbreviations: NC, non-targeting control sequence; shGOLPH3, shRNA of *GOLPH3*.

cancer cell lines as compared with the non-targeting control sequence group. We next examined the role of *GOLPH3* in cell adhesion. As illustrated in Figure 3D, *GOLPH3* knockdown reduced cell adhesion in BGC-823 and SGC-7901, but this was not observed in MKN-28.

Identification of the signalling mediators of *GOLPH3*

To investigate the possible regulators of the *GOLPH3* signalling pathway, the mRNA of p-mTOR, p70S6K, p-4EBP1 and *RhoA* and protein activation in non-targeting sequence and shGOLPH3-treated cells were analyzed by qRT-PCR and western blot analyses, respectively. As shown in Figure 4, the mRNA levels of the signalling mediators were significantly decreased in *GOLPH3* knockdown of all

three gastric cancer cell lines, with the exception of p-mTOR in SGC-7901 cells. Similarly, mTOR, p70S6K and 4EBP1 activation and *RhoA* protein level were significantly downregulated in shGOLPH3-1-treated BGC-823 (Figure 5A,5B), SGC-7901(Figure 5A,C) and MNK-28 (Figure 5A, 5D).

Discussion

Since few studies have analyzed the effects of *GOLPH3* in gastric cancer, the present study aimed to examine the relationship of *GOLPH3* with cell migration and metastasis of three gastric cancer cells lines of varying differentiation states. In all three cell lines, *GOLPH3* knockdown significantly reduced cell migration, invasion and F-actin

FIGURE 5. mTOR, p70S6K, 4EBP1 protein activation and the level of RhoA in shGOLPH3-1-treated cells.

mTOR, p70S6K, 4EBP1 activation and RhoA protein levels were analyzed by western blot in three cell lines. Results show that mTOR, p70S6K, and p-4EBP1 activation and the level of RhoA are decreased in shGOLPH3 group BGC-823 (A,B), SGC-7901 (A,C) and MKN-28 (A,D) as compared with the non-targeting control sequence group. Three samples were analyzed in each group, and values represent the mean ± SD

* $p < 0.05$ significantly different as compared with non-targeting control sequence group in each experiment.

Abbreviations: NC, non-targeting control sequence; shGOLPH3, shRNA of *GOLPH3*.

levels. Furthermore, p-mTOR, p70S6K, p-4EBP1 and RhoA protein levels were significantly downregulated in shGOLPH3-1-treated cells, suggesting that the effects of *GOLPH3* expression in gastric cancer cells may be mediated through the mTOR signalling pathway.

Patients with high *GOLPH3*-expressing tumors have poor prognosis in different cancers [6-9], suggesting that *GOLPH3* may have a role in tumor progression. Previous studies demonstrated that overexpression of *GOLPH3* was observed in human gastric cancer tissue and correlated with overall survival rate and prognosis of gastric cancer [9,10]. Overexpression of *GOLPH3* was accompanied by p-mTOR, p-p70S6 and p-4EBP1 expression in gastric cancer tissue

[10]. Taken together, these data suggests that *GOLPH3* promotes tumor progression by increasing cell migration and invasion through the mTOR signalling pathway in human gastric cancer. Study results show that mTOR can regulate p70S6K and 4EBP1 activation [13,14], and regulate cell migration through RhoA and actin reorganization [15,16]. Also, mTOR and RhoA activation [2,11] and F-actin reorganization [17] have been found to be modulated by *GOLPH3*. The *GOLPH3* has been reported to be involved in the motility of glioma cells by regulating the expression of RhoA [18]; however, the biological significance of *GOLPH3* in gastric cancer progression remains largely unknown. In the present study, we used an *in vitro* cell model to determine

that GOLPH3 regulated cell migration and invasion through the mTOR-p70S6K and 4EBP1/RhoA signalling pathways in human gastric cancer cells. In summary, GOLPH3/mTOR/RhoA signalling may play an important role in gastric cancer tumorigenesis and progression.

Our data revealed the function of GOLPH3 in gastric cancer, but the regulatory mechanism of GOLPH3 is still not fully understood. It has been reported that ATF3, one of the members of the ATF/CREB transcription factor, enhances breast cancer metastasis [19], and regulates *GOLPH3* expression by inhibiting miR-590 in breast cancer [20]. In gastric cancer, it has not been reported whether *GOLPH3* can be regulated by ATF3; therefore, the regulatory mechanism of GOLPH3 needs further investigation.

In the present study, suppression of *GOLPH3* expression reduced cytoskeletal reorganization in gastric cancer cells, which may have been responsible for suppression of invasion and migration. Consistent with the present study, downregulation of *GOLPH3* in glioma cells decreased cytoskeletal reorganization as well as RhoA expression [2]. Given that GOLPH3 interacts with MYO18A, connecting the Golgi to F-actin [21,22], its knockdown likely disturbed the cytoskeletal connections required for reorganization. Further studies are needed to determine whether the impact of *GOLPH3* expression on cytoskeletal reorganization was mediated by RhoA in gastric cancer cells, as well as to analyze the clinical significance of targeting GOLPH3 in suppressing gastric cancer metastasis.

In addition to cell migration and invasion, GOLPH3 activity also correlated positively with cell proliferation and tumor growth in esophageal [23] and breast cancer [20,24]. Furthermore, *GOLPH3* expression was associated with microvascular density in early-stage non-small-cell lung carcinoma patients [7] and in angiogenesis of hepatocellular carcinoma [25]. Taken together, results of these studies suggest that GOLPH3 has a significant role as a possible regulator of gastric cancer progression and metastasis. Further analyses will examine the role of GOLPH3 in gastric cancer cell proliferation and angiogenesis.

In the present study, the relative level of GOLPH3 expression was significantly lower in both SGC-7901 and MKN-28 cells as compared with the BGC-823 cells. This suggests that *GOLPH3* mRNA and protein expression levels decreased with increasing differentiation. This is consistent with the results of other studies in which *GOLPH3* expression was significantly associated with differentiation in

non-small-cell lung carcinoma samples [26,27]. Given that lower differentiation status may be related to tumor progression, our further studies will examine the precise relationship between *GOLPH3* expression and gastric cancer differentiation, which may have clinical applications in determining tumor progression.

In conclusion, our results support the relationship between GOLPH3 and gastric cancer, which we have shown promotes the migration and invasion of gastric cancer cells. In addition, *GOLPH3* expression may increase in poorly-differentiated gastric cancer cells, activating the mTOR-70S6K/4EBP1-RhoA-signalling pathway to promote the migration of gastric cancer cells. Further studies are necessary to explore the prognostic value and clinical impact of targeting GOLPH3 in gastric cancer patients.

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