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Overexpression of PIP5KL1 suppresses cell proliferation and migration in human gastric cancer cells

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Abstract Phosphatidylinositol-4-phosphate 5-kinase-like 1 (PIP5KL1), the forth member of phosphatidylinositol-4-phosphate 5-kinases (PIPKs) type I, acts as a scaffold for localization and activation of PIPKs, which mediates numerous cellular processes. However, the role of PIP5KL1 in the development of human cancer is still lacking. We therefore examined the expression of PIP5KL1 in human normal and cancer tissues by tissue microarrays (TMAs). Reverse transcription-polymerase chain reaction (RT-PCR) and immunofluorescence imaging analysis were used to testify the mRNA and protein levels of PIP5KL1 in human gastric cancer cell line (BGC823). The cell proliferation was investigated with 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide (MTT) assay. Both wound healing and transwell migration assay were performed to study the cell migration. The phosphorylation of v-akt murine thymoma viral oncogene homolog 1 (AKT1) was determined by western immunoblot analysis. Immunostaining of gastric cancer tissue microarrays revealed a negative correlation between PIP5KL1 overexpression and gastric cancer in situ. Transient transfection PIP5KL1 induced a significant increase expression at both transcriptional and translational levels and consequent robust inhibition of proliferation ($P < 0.05$) and migration ($P < 0.05$) of BGC823 cells. Overexpression of PIP5KL1 markedly inhibited ($P < 0.05$) serum-induced phosphorylation of AKT1. Taken together, these studies indicate a functional negative correlation between elevated levels of PIP5KL1 and the development of human gastric cancer, suggesting that PIP5KL1 overexpression may suppress gastric cancer formation.

Keywords PIP5KL1 · Proliferation · Migration · AKT1 · Gastric cancer cell

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Introduction

Phosphoinositide (PI) signaling networks have been implicated in the regulation of numerous cellular processes, including cell survival, cell proliferation, motility, cytoskeletal regulation and intracellular vesicle trafficking. Phosphatidylinositol-4,5-bisphosphate [PI(4,5)P] is at the heart of phosphoinositide signaling, although it represents quantitatively minor phospholipids (<1%) of membrane phospholipids[1–3]. Accumulated evidence indicates that the amount and localization of these important second messengers were strictly regulated through a set of specific lipid kinases [phosphoinositide 3-kinase (PI3K), phosphatidylinositol-4-phosphate 5-kinases (PIP5K)], and phosphatases [phosphatase and tensin homologue deleted on chromosome ten (PTEN), Src-homology-2 (SH2) domain-containing inositol 5-phosphatase (SHIP)]. Accordingly, these PI-metabolizeing enzymes and their function have gained much attention because of their strongly linkage with the development of human disease, especially in the development of cancers [4, 5].

It is well known that phosphatidylinositol phosphate kinases (PIPKs) utilize phosphatidylinositol-4-phosphate [PI(4)P] or phosphatidylinositol-5-phosphate [PI(5)P] as substrates to generate the majority of PI(4,5)P2, which is currently thought the predominant mechanism of PI(4,5)P2 synthesis in mammalian cells. The PIPKs compose two

families of phosphoinositide kinase, designated type I PIPKs (also named as phosphatidylinositol 4-phosphate 5-kinases, PIP5K), and type II PIPKs (also named as phosphatidylinositol 5-phosphate 4-kinases, PIP4K). Both type I and II PIPKs are consisting of α, β, and γ isoforms [6, 7]. Recently, the forth member of PIP5K, phosphatidylinositol-4-phosphate 5-kinase-like 1 (PIP5KL1) also termed as phosphatidylinositol phosphate kinase homolog (PIPKH), was identified by searching the human genome sequence and EST data bases. As the new member of PIP5K family, it is highly expressed in the brain and testis, but only with a very low intrinsic phosphoinositide kinase activity. It acts as a scaffold for localization and activation of PIPK activity, which in turn increases PI(3,4,5)P levels [8]. It also reports that PIP5KL1 is closely associated with cell viability by using cell-based high-throughput assay, and further study identified that PIP5KL1 induces 293 T cells necrosis and apoptosis [9]. But other physiological/pathological roles of PIP5KL1, especially in the human cancer is still lacking.

Recently, several reports showed that PIPKs family participated in the regulation of tumor cell migration, adhesion and invasion [10, 11]. They demonstrated that in different subset of breast cancer cell lines, overexpression of PIP5K2β increased cell proliferation and anchorage-independent growth [10]. Similarly, another study showed that PIP5Kγ was required for growth factor stimulated cell migration in HeLa and A431 cells [11]. These findings greatly suggested a possible functional link between PIPKs and cancer development.

In this study, we presented the direct evidence that PIP5KL1 was rarely expressed in gastric tumor when compared with human normal gastric tissue using tissue microarrays (TMAs), and overexpression of PIP5KL1 at both transcriptional and translational levels inhibited the proliferation and migration of BGC823 cells. This suggested that PIP5KL1 appear to be a potential tumor suppressor in gastric tumor formation.

Materials and methods

Cell culture and plasmid transfection

A human gastric cancer cell line (BGC823) were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Gibco, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained at 37°C in a 5% CO_2 humidified incubator. Human PIP5KL1 cDNA (GenBank # NM_173492) was subcloned into the pcDNA3.1 and pEGFPC1 eukaryotic expression vectors. Transfection of the recombinant vector was performed by Lipofectamine (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Briefly, before transfection, cells were washed and cultured in RPMI 1640. The recombinant vectors mixed with lipofectamine were added. After 6 h of transfection, the medium was supplemented with serum and antibiotics, and then incubated for the desired time period.

Polyclonal anti-PIP5KL1 antibody preparation

Polyclonal antibodies against PIP5KL1 were generated by immunization of New Zealand rabbits with PIP5KL1 protein which was over-expressed in *E. coli*. BL21 (DE3) and purified by affinity chromatography. After immunizations, the antibody was purified by protein G (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Antibodies were validated by immunohistochemistry and immunofluorescence.

Tissue microarrays (TMAs) and immunohistochemical analysis

TMAs were obtained from Beijing Friendship Hospital (Beijing, China). These arrays contained 32 samples of human normal gastric tissue and 81 samples of gastric cancer. All samples in all arrays were provided in duplicate as 0.6-mm diameter tissue cores. The TMAs sections were deparaffinised, rehydrated and endogenous peroxidase activity within the rehydrated tissue was blocked with a solution of 3% hydrogen peroxide in methanol for 10 min at room temperature. Antigen retrieval was performed by boiling in 10 mM sodium citrate buffer (pH 6.0) for 15 min. The slides were blocked in PBS plus 10% normal goat serum for 30 min and then incubated with a rabbit anti-PIP5KL1 polyclonal antibody at a dilution of 1:50 for 2 h at 37°C. For negative control, the primary antibody was replaced by phosphate-buffered saline. The Polymer Hepler (Santa Cruz, CA) was incubated for 20 min at room temperature, followed by incubation with polyperoxidase-anti-mouse/rabbit IgG (Santa Cruz, CA) for 30 min. After rinsing in PBS, all sections were visualized with 0.05% 3,3′-diaminobenzidine (DAB). The sections were then counterstained with hematoxylin. The immunohistochemically stained tissue sections were reviewed and scored based on the intensity and the number of cells staining. The final scores of the sections were recorded as 0 (negative), 1 (weak), 2 (medium) and 3 (strong). Sections having a final staining score of 1 or higher were considered to be positive.

RNA extraction and RT-PCR

Total RNA was prepared using Trizol reagent (Life Technologies, Inc., Gaithersburg, MD, USA) according to

the manufacturer's instruction. Four micrograms of total RNA were used to synthesize the first strand of cDNA using cDNA Synthesis Kit (Fermentas, Burlington, ON, Canada). The primers sequences of PIP5KL1 were used as follows: sense 5′-AGGCTATCAGGAGGCTGTAATC-3′and antisense 5′-TTCAGGGCAAGACCATCAAC-3′. β-Actin was used to ascertain the equal amount of cDNA in each reaction. A total of 30 cycles were performed with each cycle consisting of 30 s at 94°C, 35 s at 65°C, and 40 s at 72°C with an initial denaturation of 5 min at 95°C and a final extension of 7 min at 72°C. The reaction products were separated on 1% agarose gel and analyzed by an imaging system.

Immunofluorescence imaging analysis

Cells seeded in chamber slides were transfected with plasmid DNA for 24 h. After fixation with freshly prepared 4% paraformaldehyde in PBS for 15 min, cells were subsequently permeabilized with 0.1% Triton X-100 and blocked with PBS containing 2% bovine serum albumin for 1 h. For immunostaining, cells were incubated with anti-PIP5KL1 antibody for 1 h at room temperature, and then incubated with TRITC-conjugated anti-rabbit IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 30 min. The nuclei of cells were stained with 4,6-diamidino-2-phenylindole (DAPI) and then examined under a TCS-SP2 confocal microscope (Leica Microsystems, Bensheim, Germany).

MTT assay

The 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) assay was performed as previously described with the following modifications [12]. Briefly, cells were seeded into 96-well plate with 0.6×10^4 cells/well and maintained in 200 μl of RPMI-1640 medium containing 10% FBS. At the indicated time points, cells were washed with PBS and 10 μl MTT (5 mg/ml) was added to each well and further incubated for 4 h. The MTT solution was carefully removed by aspirating, and the formazan product was dissolved in 150 ml DMSO. Absorbance was measured at 570 nm on a Shimadzu Graphicord UV-240 spectrophotometer (Shimadzu, Kyoto, Japan). All MTT assays were repeated four times.

Wound healing assay

Cells were cultured in a six-well culture plate in RPMI 1640 supplemented with 10% FBS. After reaching confluence, a scratch wound was carried out by creating a linear cell-free region using p200 pipette tip. Remove the debris and smooth edge of the scratch by washing the cells twice with phosphate-buffered saline (PBS) and re-fed with RPMI 1640 medium containing 0.1% BSA. Then place the dishes in the incubator for 48 h. The progress of cell migration into the scratch was photographed at 0 and 48 h using an inverted microscope. The images are further analyzed quantitatively by using National Institutes of Health ImageJ software.

Transwell migration assay

The activity of invasion and migration of cells was performed using 24-well transwell chamber with 8.0-μm pore polycarbonate filter inserts (Costar, Cambridge, MA, USA). Cells (1×10^4 cells/well) suspended in serum-free RPMI 1640 containing 0.1% BSA were overlaid in the upper chamber of each transwell. In each lower chamber, 600 μl of RPMI 1640 supplemented with 10% FBS was added. Then the inserts were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. The cells that had not penetrated the filters were removed using cotton swabs. The migrated cells attached to the bottom side were fixed in 100% methanol for 10 min and stained in 0.2% crystal violet for 20 min, rinsed in PBS and examined under a bright-field microscope with 10× magnification. The value of migratory activity was expressed as the average number of migrated cells per microscopic field over the 5 fields in each assay from four independent experiments.

Western immunoblot analysis

After 24 h of transfection, cells were serum deprived for 24 h and treated with 10% FBS for 0–30 min. Cells were washed twice with ice-cold PBS, and then harvested and lyzed in buffer (10 mM HEPEs, pH 7.9, 1.5 mM MgCl, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 5 mg/ml leupeptin, 5 mg/ml aprotinin, 1 mM PMSF) for 30 min on ice. Lysates were clarified by centrifugation at 18,000*g* for 10 min at 4°C. The protein concentrations in supernatants of the lysates were determined using the BCA protein assay reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA). Equal amounts of protein (20 μg) were separated on 12% SDS–PAGE gels, electroblotted onto PVDF membranes (Pall Corporation, East Hills, NY, USA). Proteins on the membranes were immunoblotted with an antibody against total (1:2,000; Cell Signaling Technology, Inc., Danvers, MA, USA) or phospho-AKT1 (Ser473) (1:500; Cell Signaling Technology, Inc., Danvers, MA, USA). Immunoreactive proteins were visualized by a chemiluminescence system (Amersham Life Science Inc., Arlington Heights, IL, USA) and quantified by scanning densitometry (model GS 670; Bio-Rad, Hercules, CA, USA). Data on phosphorylated AKT1 was normalized to total AKT1.

Statistical analysis

Statistical analyses were performed using the statistical software SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± SEM. Student's *t*-test was used for statistical comparison. A correlation between PIP5KL1 immunostaining levels and gastric cancer was obtained using the Pearson's χ^2 test or the Fisher's exact test. Cell proliferation, migration and wound-healing assay were tested using one-way ANOVA. Statistical significance was defined as $P < 0.05$ in all analyses.

Results

The expression of PIP5KL1 in human normal gastric tissue and gastric cancer

To determine if the expression of PIP5KL1 indeed correlates with the development of human gastric cancer, we compared the PIP5KL1 immunoreactivity between human normal gastric tissue and gastric cancer using tissue microarrays. Representative immuno-stained samples are presented in Fig. 1. As summarized in Table 1, in the normal gastric tissue, 7 of 32 cases (22%) had negative immunoreactivity for PIP5KL1, whereas 25 cases (78%) showed PIP5KL1 positivity (Fig. 1c, d). In comparison, 65% were negative staining (Fig. 1a, b), and 35% were positive staining in gastric cancer. χ^2 test analysis of the data indicated a statistically significant difference in PIP5KL1 staining in normal versus cancer tissues ($P < 0.001$). Furthermore, by Fisher's exact test, it showed that there are significant difference for PIP5KL1 immunoreactivity between different grade of differentiation (well/moderation/poor), with the strongest correlation between PIP5KL1 staining and the differentiation of human gastric cancer cell ($P = 0.00008$). It is noteworthy that this P value is much lower, since the number of cancer is too small. Further study is required to confirm this result by increasing the number of samples. Together, these studies revealed a negative correlation between high levels of PIP5KL1 and human gastric cancer.

The overexpression and subcellular localization of PIP5KL1

To examine the biological effect induced by PIP5KL1, we transiently transfected the pcDNA3.1-PIP5KL1 recombinant vector into human gastric cancer BGC823 cells. An empty vector was transfected as a negative control. The expression of PIP5KL1 was identified by RT-PCR and immunofluorescence. The data of RT-PCR showed that the levels of PIP5KL1 mRNA was greatly increased ($P < 0.05$) in PIP5KL1 transfectants (Fig. 2). This result was also verified by immunofluorescence. As shown in Fig. 3, when transfected BGC823 cells were incubated with anti-PIP5KL1 antibody followed by a TRITC-conjugated secondary antibody, the fluorescence intensity which represented PIP5KL1 expression level in PIP5KL1

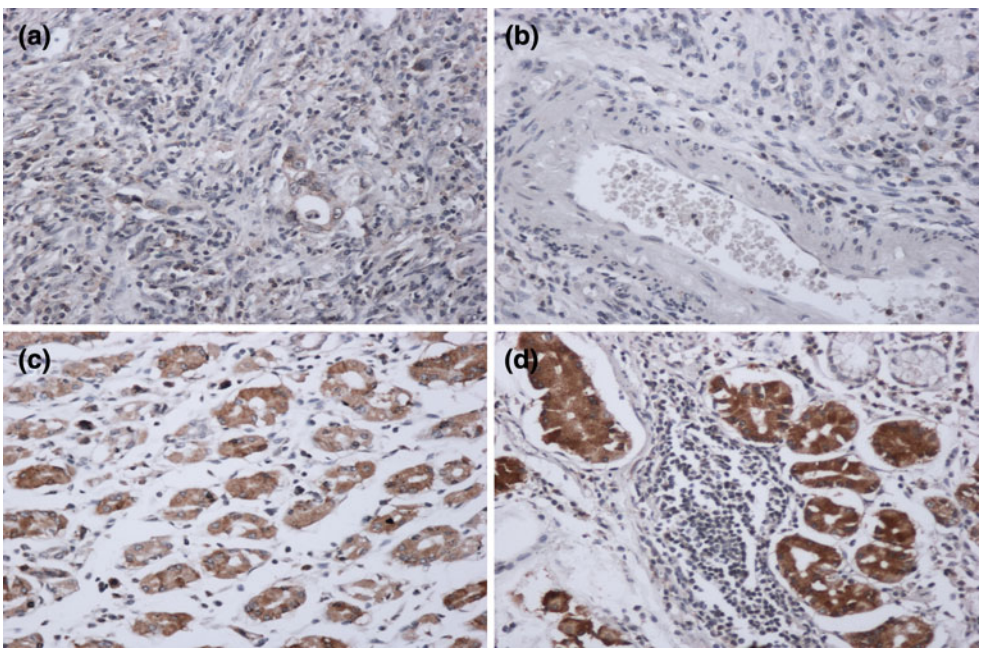

Fig. 1 Expression of PIP5KL1 in human gastric tissue by immunohistochemical staining. Representative immunostaining of TMAs illustrating human gastric cancer and normal gastric tissue employing the anti-PIP5KL1 polyclonal antibody. **a**, **b** Negative immunostaining in gastric cancer. **c**, **d** Positive immunostaining in normal gastric tissue. (Original magnification ×200)

Table 1 Immunohistochemical staining for PIP5KL1 in the samples from tissue microarrays

Samples and variables	No. of negative samples (%)	No. of positive samples (%)	Statistical significance
Normal gastric tissue	7 (22.0)	25 (78.0)	$P < 0.001$[a]
Gastric cancer	52 (65.0)	29 (35.0)	
Differentiation			
Well	4 (30.8)	9 (69.2)	$P < 0.001$[b]
Moderate	14 (50.0)	14 (50.0)	
Poor	34 (85.0)	6 (15.0)	
*P*trend[c]		0.00008	

[a] Gastric tumor tissues versus normal gastric tissues (P value from χ^2 test)

[b] Differentiation grade, well versus moderate versus poor (P value from χ^2 test)

[c] Test for trend of number of samples were two-side and based on likelihood ratio test assuming a multiplicative model

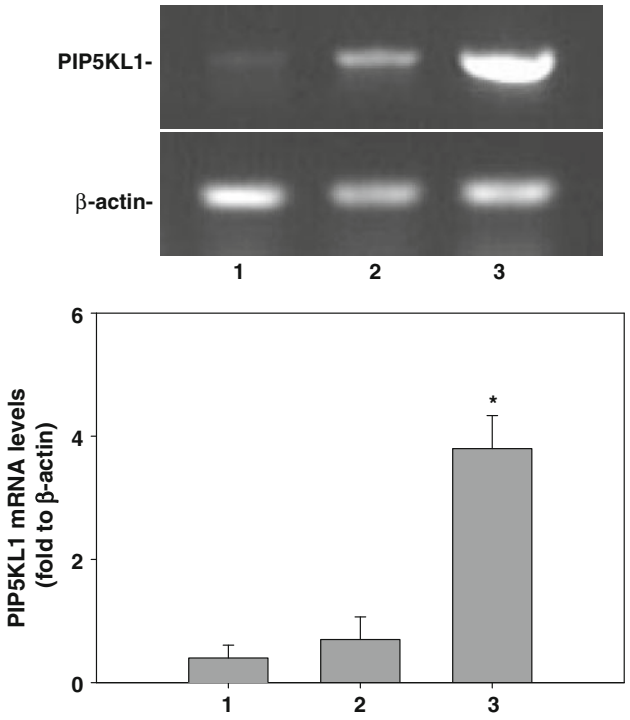

Fig. 2 Expression of PIP5KL1 mRNA in BGC823 cells by RT-PCR. Cells were transfected with pcDNA3.1 or pcDNA3.1-PIP5KL1. After 24 h of transfection, total RNA was isolated and then analyzed by RT-PCR. Quantified data are expressed as means ± SEM from three independent experiments. * Differ ($P < 0.05$) from the BGC823 control and BCG823 pcDNA3.1. *Lane 1*: BGC823 cells; *Lane 2*: BGC823pcDNA3.1 cells and *Lane 3*: BGC823pcDNA3.1-PIP5KL1 cells

transfectants (Fig. 3b) displayed a significant promotion when compared with the negative controls (Fig. 3a). In addition, to determine the subcellular localization of PIP5KL1, we expressed GFP-PIP5KL1 fusion proteins in mammalian cells. BGC823 cells transfected with pEGFPC1-PIP5KL1 showed clear GFP signal in the perinuclear area (Fig. 3d). Cells transfected with pEGFPC1 displayed diffuse GFP signal throughout the cell (Fig. 3c). This result also was confirmed by above-mentioned immunofluorescence staining, which an intense fluorescence was observed around the perinuclear area (Fig. 3b). This suggested that PIP5KL1 proteins are mostly localized to the perinuclear area.

Overexpression of PIP5KL1 inhibits the proliferation of BGC823 cell in vitro

To examine the effect of PIP5KL1 on the BGC823 cell proliferation, cells were transfected with pcDNA3.1 and pcDNA3.1-PIP5KL1, respectively. After transfection, the difference in cell growth inhibition rate between PIP5KL1 transfected cells from other groups was not statistically significant in the first 3 days. The significant growth inhibition in PIP5KL1 transfected BGC823 cells was detected from the fourth day and that was markedly lower ($P < 0.01$) than that in pcDNA3.1 transfected BGC823 cells and that in control BGC823 cells (Fig. 4).

Overexpression of PIP5KL1 inhibits the migration of BGC823 cell in vitro

Wound healing assay was performed to assess whether PIP5KL1 overexpression is associated with the BGC823 cell migration. We found that PIP5KL1 overexpressing cells (BCG823 pcDNA3.1-PIP5KL1) displayed a significant decrease in cell migration ability compared with the vector control and BGC823 cell control, respectively (Fig. 5). It is noteworthy that this wound healing assay is a combination of migration and proliferation based on recent review by Yarrow et al. [13]. To exclude the possibility that PIP5KL1 inhibited-cell proliferation plays a significant role in the cell migration, we further performed the chamber transwell assay. Consistent with above results, the migratory ability of the PIP5KL1 overexpressing cells was significantly suppressed in comparison with the vehicle-transfected

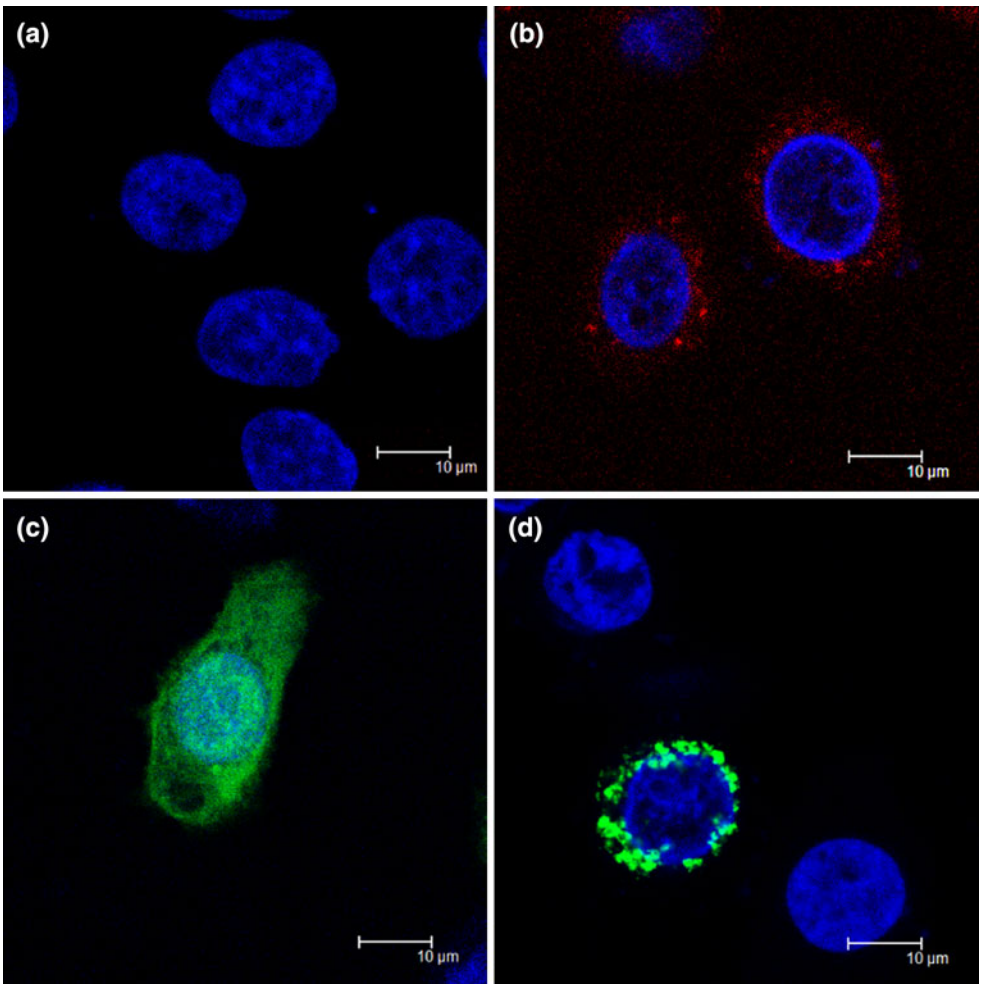

Fig. 3 Expression and subcellular localization of PIP5KL1 in BGC823 cells identified by immunofluorescent staining. Cells were transiently transfected with pcDNA3.1 (**a**), pEGFPC1 (**c**) empty plasmid, pcDNA3.1-PIP5KL1 (**b**) and pEGFPC1-PIP5KL1 (**d**) recombinant plasmids. GFP and GFP-PIP5KL1 expression was directly observed by fluorescence microscope, and PIP5KL1 expression was detected by immunofluorescence staining using anti-PIP5KL1 antibody

BGC823 cells and BGC823 control cells (Fig. 6). This indicates that a rise in PIP5KL1 expression is negatively correlated with the migration of BGC823 cells.

Overexpression of PIP5KL1 inhibited serum-induced AKT1 phosphorylation in BGC823 cells

We subsequently examined the potential effect of PIP5KL1 on the activation of AKT1, which closely associated with the cell proliferation and migration. The activation of AKT1 was measured by Akt phosphorylation on Ser473. After transfection with pcDNA3.1 or pcDNA3.1-PIP5KL1 in BGC823 cells and following treatment with 10% FBS for 0–30 min, the activation of AKT1 was significantly inhibited in PIP5KL1 overexpressing transfectants (Fig. 7). This inhibitory effect may be involved in the suppression of BGC823 cell proliferation and migration by PIP5KL1 overexpression.

Discussion

PIP5KL1, also named as PIPKH, was identified as the forth member of PIP5K. It is only with a very low intrinsic phosphoinositide kinase activity and acts as a scaffold for localization and activation of PIPKs. In the present study, we observed that PIP5KL1 was rarely expressed in gastric cancer when compared with normal tissue, and overexpression of PIP5KL1 inhibited cell proliferation ($P < 0.05$) and migration ($P < 0.05$) in BGC823 cells. To the best of our knowledge, this is the first time providing the most updated and intriguing findings about the role of PIP5KL1 in gastric tumor development.

Tissue microarrays (TMAs) is a high-throughput technology for the analysis of molecular markers in oncology by using commonly available laboratory assays such as immunohistochemistry and fluorescent in situ hybridization [14, 15]. Previous data from TMAs showed that in human

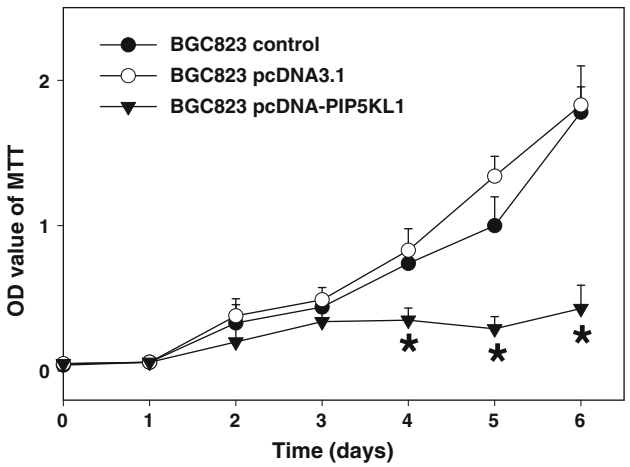

Fig. 4 Effects of PIP5KL1 overexpression on BGC823 cell proliferation by MTT assay. Cells were seeded into 96-well plate and maintained in RPMI 1640 containing 10% FBS. At the indicated time points, cell number was determined with MTT assay, and the graph showed the results of three independent experiments. * Differ ($P < 0.05$) from the BGC823 control and BCG823 pcDNA3.1

gastric, breast and kidney cancers, PIP5KL1 was expressed at very low, even undetectable levels when compared with their corresponding normal tissues. This action was observed notably in gastric tissue (unpublished data), which initiated this present study. In current report, we further confirmed the difference expression of PIP5KL1 in human gastric cancer (32 samples) and normal tissue (81 samples) via TMAs. This suggested that down-regulated expression of PIP5KL1 may correlate with human gastric cancer development. It is extremely significant that our finding is the first providing validation for the importance of PIP5KL1 in gastric cancer. But the precise mechanism is poorly defined.

Given that proliferation and migration play essential roles in the tumor formation process, we performed transient transfection in cultured BGC823 cells that have high expression of PIP5KL1 to investigate the effect on proliferation and migration. The transfection induced a significant increase in PIP5KL1 protein levels and consequent robust inhibition of proliferation and migration of these cells, further demonstrating that PIP5KL1 has a potential gastric tumor suppressive function. It is well known that the PIP kinases utilize PI(4)P or PI(5)P as substrates to generate the majority of PI(4,5)P, which in turn participate in the regulation of different cellular processes, including vesicular trafficking, cell motility/cytoskeletal assembly,

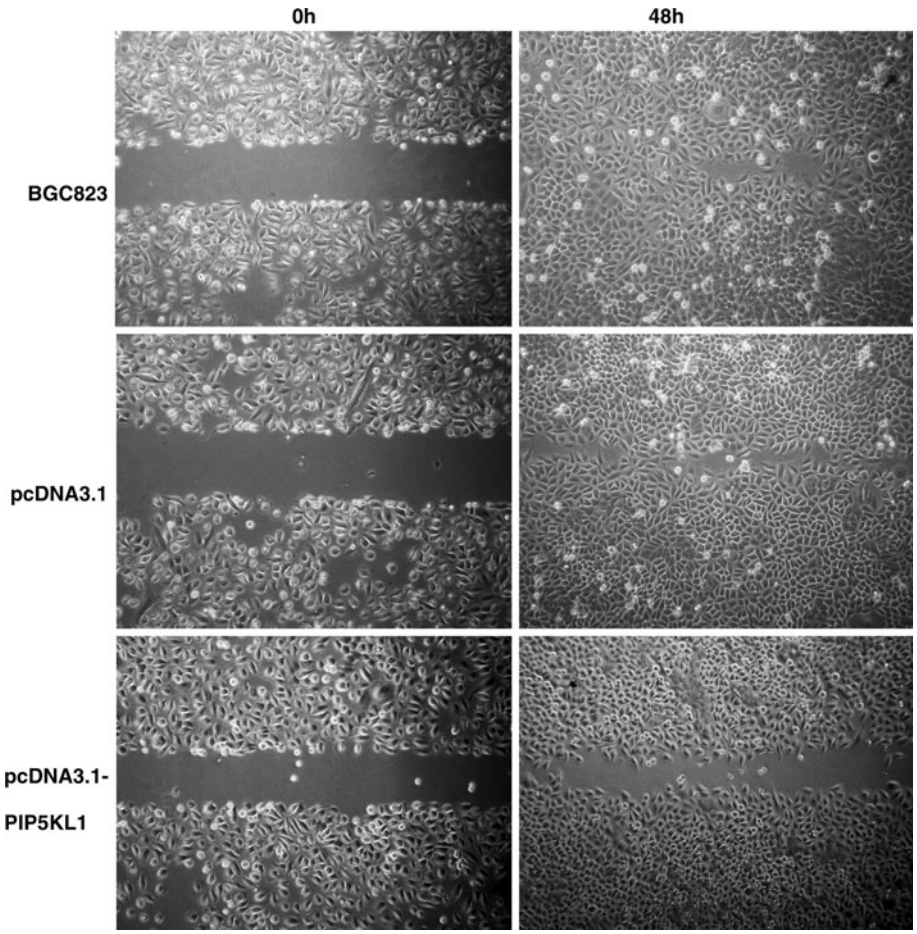

Fig. 5 Effects of PIP5KL1 overexpression on BGC823 cell migration by wound healing assay. Confluent monolayer cells were subjected to scratch wounding as described in materials and methods. Then cells were incubated in RPMI 1640 serum free media supplemented with 0.1% BSA. After 48 h migration, wound closure was photographed, and further analyzed quantitatively

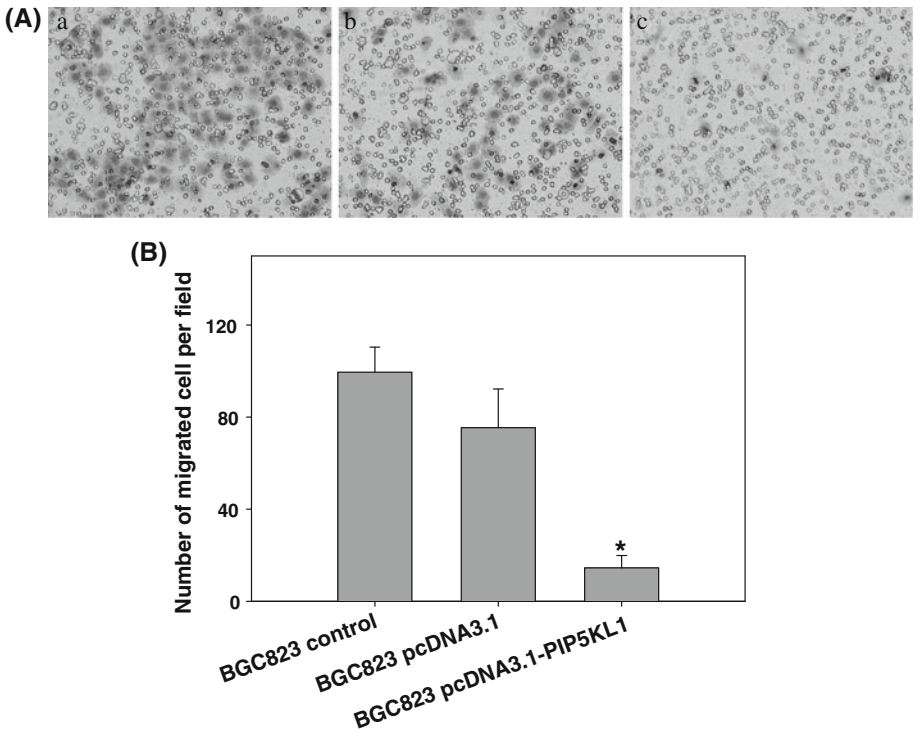

Fig. 6 Effects of PIP5KL1 overexpression on BGC823 cell migration by transwell chamber assay. Cells were overlaid in the upper chambers of 24-well tissue culture plates containing RPMI 1640 containing 0.1% BSA. After incubation for 24 h, penetrating cells were fixed and stained with 0.1% crystal violet. **A** Photographs are depicted migration of BGC823 cells (a) BGC823 pcDNA3.1 cells (b) BGC823 pcDNA3.1-PIP5KL1 cells (c). **B** Quantified data are expressed as means ± SEM from four independent experiments. *differ ($P < 0.05$) from the BGC823 control and BGC823 pcDNA3.1

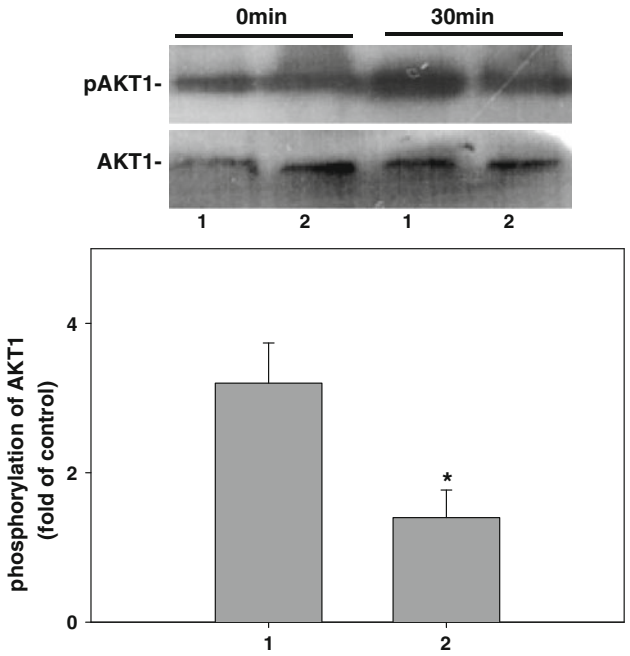

Fig. 7 Effects of PIP5KL1 overexpression on serum-induced AKT1 phosphorylation in BGC823 cells by Western immunoblot. Cells were transfected with pcDNA3.1 or pcDNA3.1-PIP5KL1 for 24 h. After serum starvation, cells were treated with 10% serum for 0–30 min. Proteins (20 μg/lane) are subjected to Western immunoblot analysis. Data normalized to total AKT1 are expressed as means ± SEM fold of the controls (time 0) from three independent experiments. Asterisks indicate significant ($P < 0.05$) differences from the BGC823 pcDNA3.1 control. 1, pcDNA3.1; 2, pcDNA3.1-PIP5KL1

regulation of cell migration, and nuclear signaling pathways [16, 17]. Especially for the effect on the cell migration, recent evidence suggests that specific isoforms of type I PIPKs are involved in different aspects of migration in different cell types, including cortical actin organization [18], membrane ruffle formation [19] and the assembly/disassembly of focal adhesions [20]. Furthermore, these different isoforms and their specific subcellular distribution can make them have distinct roles in cell migration progress. For example, study in mouse embryonic fibroblasts (MEFs) showed that PIPKIα acted positive effect on cell motility, and depletion of PIPKIα in MEFs blocked cell migration [21]. Similarly, another isoform PIPKIγ might be involved in the focal adhesion assembly and disassembly. It can either enhance or attenuate cell migration via regulation of integrin-mediated adhesions, particularly for the interaction with the cytoskeletal protein talin [20, 22]. But for PIP5KL1, it appeared to play negative effect on the regulation of BGC823 cell migration form our data. And we also observed that PIPKL1 is mostly localized to the perinuclear of BGC823 cells, which is consistent with the distribution of PIPKIβ [23]. However, PIPKIα is mainly present in the cytoplasm and nucleus [18, 24] and PIPKIγ localizes to the cytoplasm membrane [20, 25]. Except for this varied sub-cellular distribution of different PIPKs isoforms, various signaling cascades also play major roles in the regulation cell migration via PIPKs.

It is well demonstrated that phosphoinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog 1

(AKT1) pathway plays an important roles in promoting cell proliferation and migration in numerous cell systems, including of tumor cells [26–28]. Activated PI3K converts PI(4,5)P, which is mostly generated by PIPKs, to PI(3,4,5)P. The later can recruit the serine-threonine kinase AKT/protein kinase B (PKB) to the plasma membrane and further activate it. Activated AKT affects the activity or abundance of a number of downstream targets which control cell proliferation, migration and cell survival [26, 29]. But little is known the correlation between PIPKs and AKT1. Only one study reported that overexpression of PIPKIα has no effect on the PI(3,4,5)P synthesis and AKT1 activation [30]. In our present data, overexpression PIP5KL1 significantly inhibited serum-induced phosphorylation of AKT1, which probably accounts for their inhibitory effect on cell proliferation and migration. But the precise mechanisms underlying this action are till unclear. We postulated that PIP5KL1 overexpression may change the phosphoinositide kinase activity of other PIPKs isoforms, which lead to alteration of PI(3,4,5)P generation and finally impact on the recruitment and activation of AKT1. So further study still required to elucidate this mechanism.

In summary, our results show that PIP5KL1 have an important role in gastric tumor formation and progression. More importantly, PIP5KL1 acts a potential tumor suppressive function in gastric tumor, suggesting that this kinase could constitute a novel therapeutic target in cancer therapy. Elucidation of the role of this kinase in tumor growth should lead to appropriate application of this kinase in cancer treatment in the future.

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