ORIGINAL ARTICLE

Lysophosphatidic Acid Stimulates Activation of Focal Adhesion Kinase and Paxillin and Promotes Cell Motility, via LPA1–3, in Human Pancreatic Cancer

Yan Liao · Ganggang Mu · Lingli Zhang · Wei Zhou · Jun Zhang · Honggang Yu

Abstract

Background Pancreatic cancer is highly metastatic and with poor prognosis. In previous studies, lysophosphatidic acid (LPA) was shown to be a critical component of ascites which promoted the invasion and metastasis of pancreatic cancer. Two focal adhesion proteins, focal adhesion kinase (FAK) and paxillin, were crucially involved in cell migration, cytoskeleton reorganization, and the dynamics of focal adhesion.

Objectives This study examined the involvement of LPA1–3 in LPA-induced activation of FAK and paxillin, and in cell motility, in pancreatic cancer PANC-1 cells.

Methods Reverse transcriptase polymerase chain reaction analysis was used to examine mRNA expression of LPA receptors in PANC-1. Cellular protein expression of FAK and paxillin was analyzed by western blotting. The subcellular location of FAK and paxillin was visualized by immunofluorescence. Cell migration was measured by use of a transwell migration chamber.

Results Three LPA receptors (LPA1, LPA2, and LPA3) were significantly expressed in PANC-1 cells. Treatment with LPA induced both time and dose-dependent tyrosine phosphorylation of FAK and paxillin. LPA also affected translocation of FAK and paxillin from cytoplasm to focal adhesions at the cell periphery and enhanced cell motility of PANC-1. Pretreatment with 3-(4-(4-((1-(2-chlorophenyl)eth-oxy)carbonyl amino)-3-methyl-5-isoxazolyl)benzylsulfa-nyl)propanoic acid (Ki16425), an antagonist of LPA1 and LPA3, before LPA attenuated the LPA-induced tyrosine phosphorylation and redistribution of FAK and paxillin and abrogated LPA-induced cellular migration activity.

Conclusions These results suggest LPA induces activation of FAK and paxillin via LPA1–3, which may contribute to the increased cell motility in human pancreatic cancer PANC-1 cells. Thus, an understanding of the regulation by LPA of cell motility in pancreatic cancer could identify novel targets for therapy.

Keywords Lysophosphatidic acid · LPA receptor · Focal adhesion kinase · Paxillin · Pancreatic cancer · Cell migration

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Introduction

Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer-related death in men and the fifth leading cause in women, with five-year survival of only 3–5 % [1]. Pancreatic cancer is a highly metastatic disease characterized by widespread peritoneal dissemination and ascites formation [2]. Extensive invasion and metastasis is the principal reason for the poor prognosis. Thus, clarifying mechanisms of pancreatic cancer metastasis is important in the development of new therapy.

Lysophosphatidic acid (LPA), a naturally occurring bioactive phospholipid, is produced by activated platelets and inflammatory cells [3]. LPA evokes multiple cellular responses via its G protein-coupled receptors. Three predominant high-affinity cell-surface LPA receptors, LPA1 (Edg2), LPA2 (Edg4), and LPA3 (Edg7), are members of the endothelial differentiation gene (Edg) family of orphan receptors. Different LPA receptors initiate multiple distinct signaling pathways and mediate diverse cellular effects of LPA.

LPA has recently been implicated in the etiology of many cancers because of its involvement in a wide range of cellular effects, including cell proliferation, cell survival, angiogenesis, and cell migration [3–7]. LPA has also been shown to promote the invasion and metastasis of pancreatic cancer. LPA is a critical component of ascites in pancreatic cancer, and LPA1 may mediate the motility of human pancreatic cancer cells [2, 8]. Administration of 3-(4-(4-((1-(2-chlorophenyl)ethoxy)carbonylamino)-3-methyl-5-isoxazolyl)benzylsulfanyl)propanoic acid methyl ester (Ki16198), an effective antagonist of LPA1 and LPA3, inhibited LPA-induced cell migration and invasion in pancreatic cancer cells both in vitro and in vivo [9], and LPA2 mediated inhibition of migration of pancreatic cancer cells in response to LPA [2]. These results suggest that LPA is crucially involved in the invasion and metastasis of pancreatic cancer.

Focal adhesion kinase (FAK), a nonreceptor tyrosine kinase, is crucially involved in cell migration, assembly of focal adhesions, and actin cytoskeleton remodeling [10–12]. FAK phosphorylation is crucial to turnover of focal adhesions and cell migration. Paxillin is another important focal adhesion protein. Tyrosine phosphorylation of paxillin located at the periphery and at focal adhesions indicates that phosphorylation of multiple tyrosines in paxillin was necessary for temporospatial regulation of focal adhesion formation and actin cytoskeletal organization [13].

LPA induces tyrosine phosphorylation and translocation of FAK and paxillin to the focal adhesions at the periphery, a drastic increase of actin bundles, and focal adhesion assembly in colon cancer cells and ovarian cancer cells [10, 14, 15]. This evidence links LPA with FAK and paxillin activation and cell motility.

However, the molecular mechanism of LPA-induced cell migration in pancreatic cancer remains poorly understood. In this study we investigated the possible involvement of LPA1–3 specific inhibitor 3-(4-(4-((1-(2-chlorophenyl)ethoxy)carbonylamino)-3-methyl-5-isoxazolyl)benzylsulfanyl)propanoic acid (Ki16425) in LPA-induced responses in PANC-1. Our results demonstrated that treatment of PANC-1 cells with LPA caused tyrosine phosphorylation and activation of FAK and paxillin, leading to increased cell migration. Furthermore, LPA

modulated these events via LPA1–3, because Ki16425 reduced LPA-induced effects.

Methods

Reagents

1-*O*-Oleoyl-2-OMe-lysophosphatidic acid and fatty acid-free bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St Louis, MO, USA). Ki16425 was from Cayman Chemical (Ann Arbor, Michigan, USA). Goat polyclonal anti-actin antibody, rabbit polyclonal anti-FAK antibody, and rabbit polyclonal anti-p-FAK (Tyr397) antibody were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal anti-paxillin antibody, rabbit polyclonal anti-paxillin (pY31) antibody, and rabbit polyclonal anti-paxillin (pY118) antibody were from Invitrogen (Camarillo, CA, USA). Peroxidase-conjugated immunopure rabbit anti-goat IgG, goat anti-mouse IgG, and goat anti-rabbit IgG were purchased from Pierce, USA. Goat anti-rabbit IgG-FITC and goat anti-mouse IgG-TR were from Santa Cruz Biotechnology. TRIzol reagent was purchased from Invitrogen Life Technologies. The RevertAid First Strand cDNA Synthesis Kit was purchased from Fermentas Life Sciences.

Cell Culture

The human pancreatic carcinoma cell line PANC-1 was purchased from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10 % (*v/v*) fetal bovine serum (FBS; Gibco), 10 U/ml penicillin, and 10 U/ml streptomycin in a humidified atmosphere containing 95 % air and 5 % CO_2 at 37 °C.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Analysis of LPA Receptors

Total cellular RNA was extracted by use of TRIzol reagent, and the RevertAid First Strand cDNA Synthesis Kit was used for reverse transcription synthesis of cDNAs. Polymerase chain reaction (PCR) amplification was performed with 35 cycles of 45 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C. The oligonucleotide primer pairs used were: LPA1, sense 5′ GGGCTGGAACTGTATCTG 3′ and antisense 5′ CATCATGGTATCCCGATT 3′ (expected size of the product, 211 bp); LPA2, sense 5′ TGGCTCAACCCAAC CAAC 3′ and antisense 5′ CCTCATTACCCAGTCA TACCG 3′ (expected size of the product, 335 bp); LPA3,

sense 5′ TGCTTCCCTCACCAACTT 3′ and antisense 5′ CCGCAGGTACACCACAAC 3′ (expected size of the product, 283 bp); β-actin, sense 5′ ACCCACACTGTG CCCATCTA 3′ and antisense 5′ CGGAACCGCTC ATTGCC 3′ (expected size of the product, 289 bp). The PCR products were resolved by electrophoresis in an agarose gel with Gelview staining.

Western Blotting Analysis

Protein expression levels were analyzed by Western blotting. Briefly, cells were washed twice with phosphate-buffered saline (PBS) and lysed in ice-cold RIPA lysis buffer (20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.5 % Nonidet P-40, 1 mM EDTA, 50 μg/ml leupeptin, 30 μg/ml aprotinin, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride (PMSF)). Cell lysate (20 μg) was separated on a sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), and then transferred to a nitrocellulose membrane (Pall, NY, USA) by use of a wet transfer system (Bio-Rad, Hercules, CA, USA). Nonspecific binding sites were then blocked for 2 h at room temperature in Tris-buffered saline (TBS, pH 7.4) containing 0.1 % Tween-20 and 10 % BSA. Indicated primary antibodies were diluted (1:1,000) in TBS containing 0.1 % Tween-20 and 5 % BSA and incubated with the membranes overnight at 4 °C. The appropriate peroxidase-conjugated secondary antibodies were used at 1:2,000. Positive antibody reactions were detected by enhanced chemiluminescence.

Immunofluorescence Staining

Cells were plated on 20-mm circular microscope coverslips. After the indicated stimulation, cells were fixed in 4 % paraformaldehyde for 15 min and permeabilized with 0.1 % Triton X-100 for 15 min. Nonspecific binding sites were then blocked for 1 h at room temperature in PBS (pH 7.4) containing 0.1 % Tween-20 and 5 % BSA. Primary antibodies (1:200) in PBS containing 0.1 % Tween-20 and 1 % BSA were added overnight at 4 °C in a moist chamber. Cells were then incubated with appropriate FITC-conjugated anti-rabbit or TR-conjugated anti-mouse secondary antibodies (1:200) for 1 h at room temperature in a dark moist chamber. 4′,6-Diamidino-2-phenylindole (DAPI; 10 μg/ml) was used for 30 s to detect the cell nucleus. Images were obtained by fluorescence microscopy (Olympus, Hamburg, Germany). Representative typical images of cells were from at least three independent experiments.

Cell Migration Assay

Chemotactic directional migration was examined by use of a 24-well transwell chamber with 8-μm pores (Corning

Costar, Cambridge, MA, USA). Cells (5×10^4/200 μl) were resuspended in serum-free DMEM and incubated with or without Ki16425 (10 μM) for 30 min at 37 °C. They were then seeded in the upper chamber and left to migrate toward the lower chamber containing 600 μl DMEM in the presence or absence of LPA (10 μM) as chemoattractants for a total of 6 h in a humidified incubator (37 °C; 5 % CO_2). Adherent cells on the filter membrane were fixed in methanol for 10 min and stained using Giemsa dye, before cells on the upper surface of the filter membrane were removed by scraping. Cells migrating to the lower side of the membrane were visualized under the microscope and quantified by counting the number of cells in three randomly chosen visual fields under microscopy at 400× magnification. Data were expressed as relative migration (numbers of migration cells/field) from at least three independent experiments.

Statistics and Data Analysis

All data were expressed as mean ± standard deviation (SD). Differences between groups were calculated by one-way ANOVA. $P < 0.05$ was considered statistically significant. All experiments were repeated at least three times. Calculations were performed by use of SPSS computer software version 13.0 (SPSS, Chicago, IL, USA).

Results

Expression of LPA Receptors in PANC-1

In this study, we evaluated expression of mRNA of three high-affinity LPA receptors in PANC-1 cells by RT-PCR analysis. Consistent with previous studies [2, 8, 16, 17], mRNAs of LPA1, LPA2, and LPA3 were all expressed in PANC-1 (Fig. 1). There were, however, some difference for LPA3. Fumikazu Okajima [2, 8] suggested that LPA1 and LPA2 were major LPA receptor subtypes compared with low-expressed LPA3 in PANC-1 whereas other studies [16, 17] and our results suggested that the LPA3 mRNA was clearly expressed in PANC-1.

Effects of LPA on Tyrosine Phosphorylation of FAK and Paxillin

FAK and paxillin are two important scaffolding molecules associated with integrins and actin stress fibers. Recent studies have suggested that FAK and/or paxillin phosphorylation mediates migratory response to LPA [18]. FAK autophosphorylation on tyrosine residue 397 recruits Src, which, in turn, phosphorylates other tyrosine sites on FAK, which is needed for maximum FAK-associated
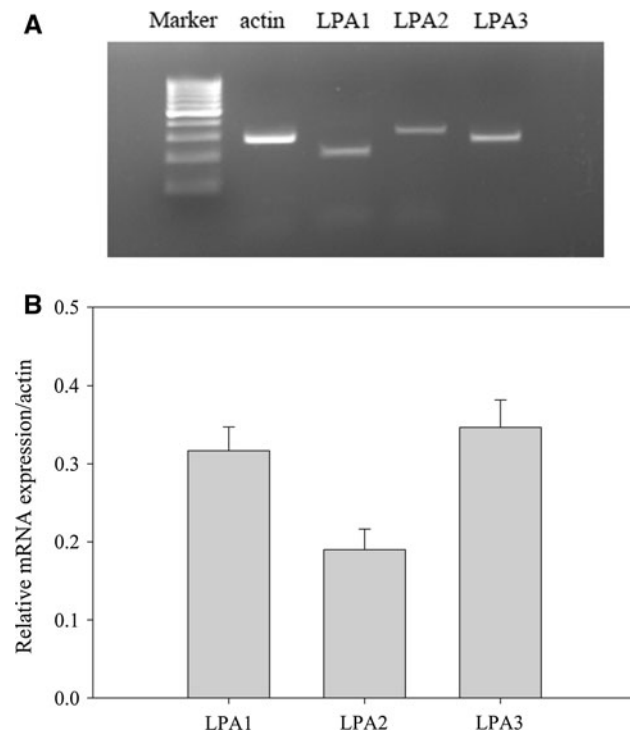
Fig. 1 Expression of LPA receptors in PANC-1. **a** Expression of mRNA of LPA receptors in PANC-1 cells. **b** Relative expression of mRNA of LPA receptors (mean ± SD; semi-quantitative). Results were normalized to β-actin

activity [19]. Paxillin, another important focal adhesion protein, contains six phosphorylation tyrosine residues; tyrosine residues (Tyr) 31 and 118 are reported to be predominant targets of phosphorylation by kinases and to create binding sites for the SH2 domain of adaptor proteins [13, 20]. Integrin-mediated phosphorylation of paxillin at Tyr31 and Tyr118 was involved in cell motility and epithelial mesenchymal transition (EMT) [21]. Tyrosine phosphorylated paxillin, located at the cell periphery and focal adhesions, was necessary for proper function of paxillin and regulated actin cytoskeleton organization and focal adhesion formation [13].

Next, we examined the effects of LPA on tyrosine phosphorylation of FAK at Tyr397 and paxillin at Tyr31 and Tyr118 in pancreatic cancer cell line PANC-1. As depicted in Fig. 2, LPA treatment significantly enhanced Tyr397 phosphorylation of FAK and Tyr31 and Tyr118 phosphorylation of paxillin in a dose and time-dependent manner. A maximum response was observed for 10 μM LPA (Fig. 2a, b), which was used in all subsequent experiments. In addition, the optimum time was 45 min for tyrosine phosphorylation of both FAK and paxillin (Fig. 2c, d). These results suggested that FAK and paxillin phosphorylation specifically mediated LPA-induced signal transduction in a dose and time-dependent manner.

Effects of Ki16425 on FAK and Paxillin Phosphorylation in Response to LPA

Ki16425 is the selective pharmacological inhibitor of LPA1–3, showing preference for LPA1 and LPA3 over LPA2. To further characterize the signaling cascades leading to FAK and paxillin phosphorylation in response to LPA, Ki16425 was used to examine these effects. We observed that pretreatment with Ki16425 (10 μM) for 30 min before LPA treatment (10 μM, 45 min) attenuated LPA-induced tyrosine phosphorylation of FAK and paxillin. As illustrated in Fig. 3, Ki16425 reduced LPA-stimulated tyrosine phosphorylation of FAK and paxillin. Furthermore, the basal level of FAK tyrosine phosphorylation was also attenuated by Ki16425. These results suggested that LPA mediated the tyrosine phosphorylation of FAK and paxillin through LPA1–3.

Effects of Ki16425 on LPA-Activated Focal Adhesion Localization of FAK and Paxillin

Cell migration begins with protrusion of the plasma membrane at the leading edge. The protrusion is driven by polymerization of actin filaments and is stabilized by formation of focal adhesions [15]. LPA induced rearrangement of the actin cytoskeleton and focal adhesion structures [8]. FAK and paxillin are two major focal adhesion proteins located at the focal contacts.

As shown in Fig. 4, immunofluorescent staining revealed that in the absence of LPA, FAK and paxillin were predominantly distributed diffusely in the cytoplasm. These quiescent, serum-starved cells had very few focal adhesions and the amount of FAK and paxillin staining at the focal adhesions was extremely small. After LPA stimulation, FAK and paxillin were recruited and translocated to the focal adhesions. The intensity and number of focal adhesions containing FAK and paxillin were higher than for the untreated cells. Ki16425 significantly reduced LPA-induced formation of focal adhesions, inhibited membrane localization of FAK and paxillin, and reduced the number and the size of the membrane protrusions in PANC-1. These findings suggested involvement LPA in the modulation of cancer cells, by activation of FAK and paxillin and by inhibition of the effects of LPA through Ki16425.

Effects of Ki16425 on LPA-Stimulated Cell Motility

Several lines of evidence suggest a significant migration response to LPA of pancreatic cancer cells [2, 8, 17]. Furthermore, LPA1 stimulated, whereas LPA2 inhibited, migration of pancreatic cancer cells in response to LPA
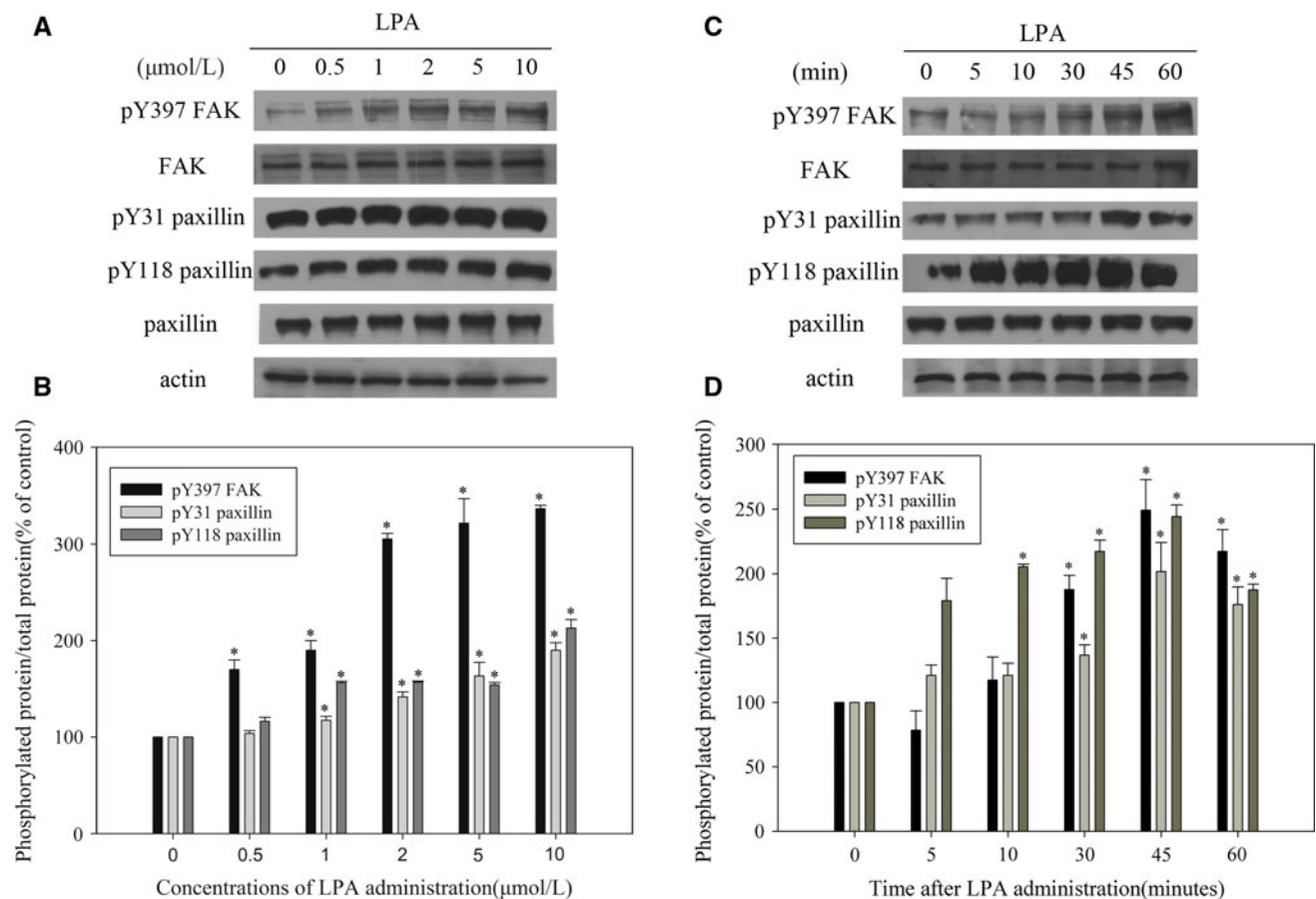
Fig. 2 Effects of LPA on tyrosine phosphorylation of FAK and paxillin in a dose (a, b) and time (c, d)-dependent manner. *$P < 0.05$ compared with the control

[2, 8]. We examined whether Ki16425 can affect LPA-mediated the motility of the pancreatic cancer cell line (Fig. 5). When LPA were loaded into the lower chamber of the transwell apparatus, a large number of PANC-1 cells migrated toward the lower chamber, suggesting that LPA stimulated directional migration (chemotaxis) of pancreatic cancer cells. The migration response to LPA was specifically inhibited by pre-incubation with Ki16425 (10 μM) for 30 min. These results supported the idea that LPA enhanced the migratory activity of pancreatic cancer cells via LPA1–3.

Discussion

LPA is a bioactive phospholipid mediator produced by activated platelets, inflammatory cells, adipocytes, peritoneal mesothelial cells, fibroblasts, and cancer cells. LPA interacts with cells through specific transmembrane receptors on the cell surface to induce biological effects. LPA induces smooth muscle cell contraction, platelet aggregation, neurite retraction and cell rounding, cell

proliferation, protection from apoptosis, modulation of chemotaxis, and transcellular migration [22, 23].

Evidence suggests LPA is a lipid mediator that functions as a mitogen and motility factor for many cancer cell types. LPA induces proliferation of colon, gastric, and prostate cancer cells [4, 24–26], enhances cell migration and invasion in colon, gastric, ovarian, breast, and fibrosarcoma cancer cells and melanoma cells [3, 5, 24, 25, 27–33], and protects colon cancer and melanoma cells against apoptosis [34, 35]. LPA also stimulates cancer colony dispersal into single migratory cells [27, 36], an important early process in the invasion and metastasis of cancer.

LPA is expressed at a high level in malignant ascites of pancreatic cancer [8], implying that LPA may be involved in the carcinogenesis of pancreatic cancer. Indeed, LPA and malignant ascites stimulated pancreatic tumor cell migration [2, 8, 17, 37–39]. The effect was mediated through multiple signaling pathways, including Gα13, Gi/ERK, GTPases, and K-ras [17, 37, 39]. There is also evidence LPA1 is involved in the migratory activity of pancreatic cancer. A significant level of LPA1 mRNA was expressed in pancreatic cancer cells with high migration
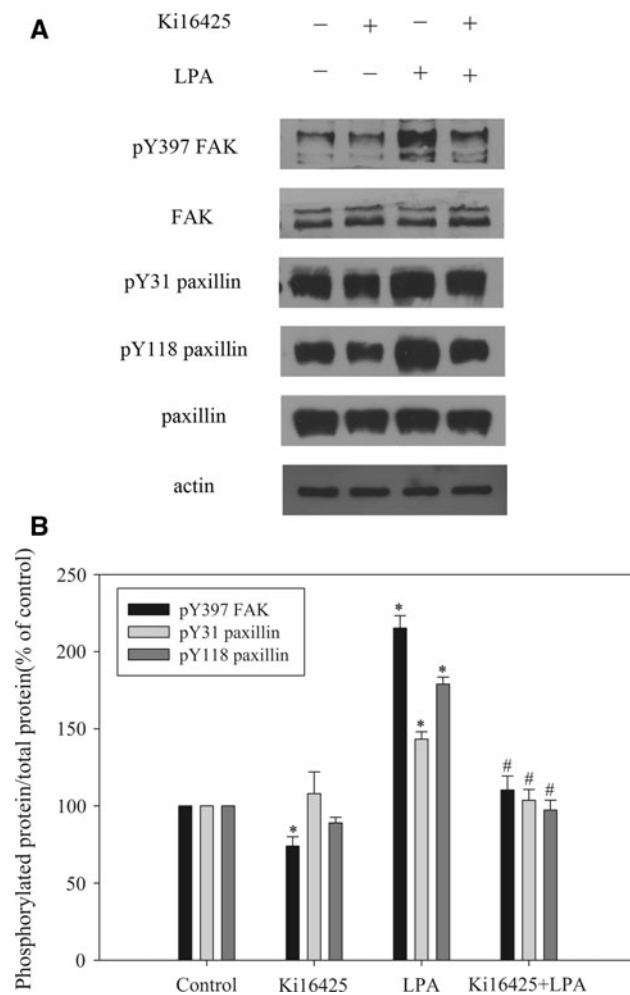
Fig. 3 Effects of Ki16425 on FAK and paxillin tyrosine phosphorylation in response to LPA. *$P < 0.05$ compared with the control, #$P < 0.05$ compared with the LPA administration group

activity to ascites but not in cells with low migration activity [8]. The effect of LPA-stimulated migration in pancreatic cancer was suppressed by inhibitory of LPA1 activity [2, 8] but not by an LPA3-selective antagonist [8]. In vivo, oral administration of Ki16198, a methylated form of Ki16425 suitable for oral administration to pancreatic cancer cell-inoculated nude mice, inhibited tumor weight and distant metastasis by reducing the level of MMP [9]. In this study, we observed that mRNAs of LPA1, LPA2, and LPA3 were all expressed in PANC-1. Furthermore, LPA significantly stimulated directional migration of PANC-1. When pre-incubated with Ki16425, the migration response to LPA was specifically inhibited. These results suggested that LPA1 may be involved in the migration of pancreatic cancer cells in response to LPA.

LPA receptors are frequently up-regulated during tumor progression. Previous studies have revealed that LPA receptors had an important effect on acquisition of malignant behavior by tumors, and different expression patterns

of LPA receptors induced different cellular response to LPA in human cancer cells. However, there is no consistent conclusion about how different LPA receptors affect the movement of cancer.

Studies suggest that overexpression of LPA1 is associated with increased tumorigenicity and metastasis of cancer cells. LPA stimulated cell migration in several carcinoma cells via LPA1, but not LPA3 [2, 8, 40], and LPA2 inhibited EGF-induced cell migration and invasion in pancreatic cancer cells [2]. In gastric cancer cells and colon cancer cells, LPA markedly increased cell migration of LPA1-expressing cells; in contrast, LPA had no significant effect on migration of cells which exclusively expressed LPA2 [25, 28]. These results indicated LPA is an active component which stimulates pancreatic cancer cell motility mediated by LPA1, but not by LPA2 or LPA3.

Recent studies have also furnished contrasting results. In hamster pancreatic cancer cells, LPA1 knockdown cells had enhanced migration and invasion activity whereas LPA3 knockdown cells had weakened motility [38]. LPA1 gene expression in hamster pancreatic ductal adenocarcinoma induced by nitroso compound was low, whereas LPA3 expression was high [38]. In rat neuroblastoma cells, expression of LPA1 markedly reduced cell motility and invasion, whereas expression of LPA2 or LPA3 increased the cell motility and invasion [41]. LPA2 stimulated the migration and invasion of hamster pancreatic cancer cells via K-ras [39]. LPA2, also, was crucially involved in carcinogenesis of intestinal-type gastric cancer, and LPA might promote progression of diffuse-type gastric cancer by transactivation of EGFR or c-Met [42]. These results implied that LPA1 negatively regulated and LPA2 and LPA3 positively regulated the carcinogenesis and motility of pancreatic cancer cells.

Despite these inconsistencies, most recent studies suggest LPA1 stimulates motility. Our studies also showed that LPA stimulated directional migration (chemotaxis) of PANC-1, and that Ki16425 inhibited the stimulating effects, suggesting LPA1–3 may participate in the migration of pancreatic cancer cells. There is insufficient evidence to indicate which type of receptor is most important in the motility of pancreatic cancer cells.

FAK has been shown to be tyrosine-phosphorylated and activated in response to a variety of stimuli through heterotrimeric G protein-coupled receptors. Activated FAK recruits other proteins, including paxillin, p130^{Cas}, vinculin, and talin in focal adhesions. These oligomeric protein complexes link actin stress fibers to integrins at the surface of the plasma membrane, stabilize focal adhesions and provide an anchor for cell migration. Many studies have found that FAK and paxillin are crucially involved in LPA-induced migration, cytoskeleton reorganization, and the dynamics of focal adhesions. LPA treatment stimulated
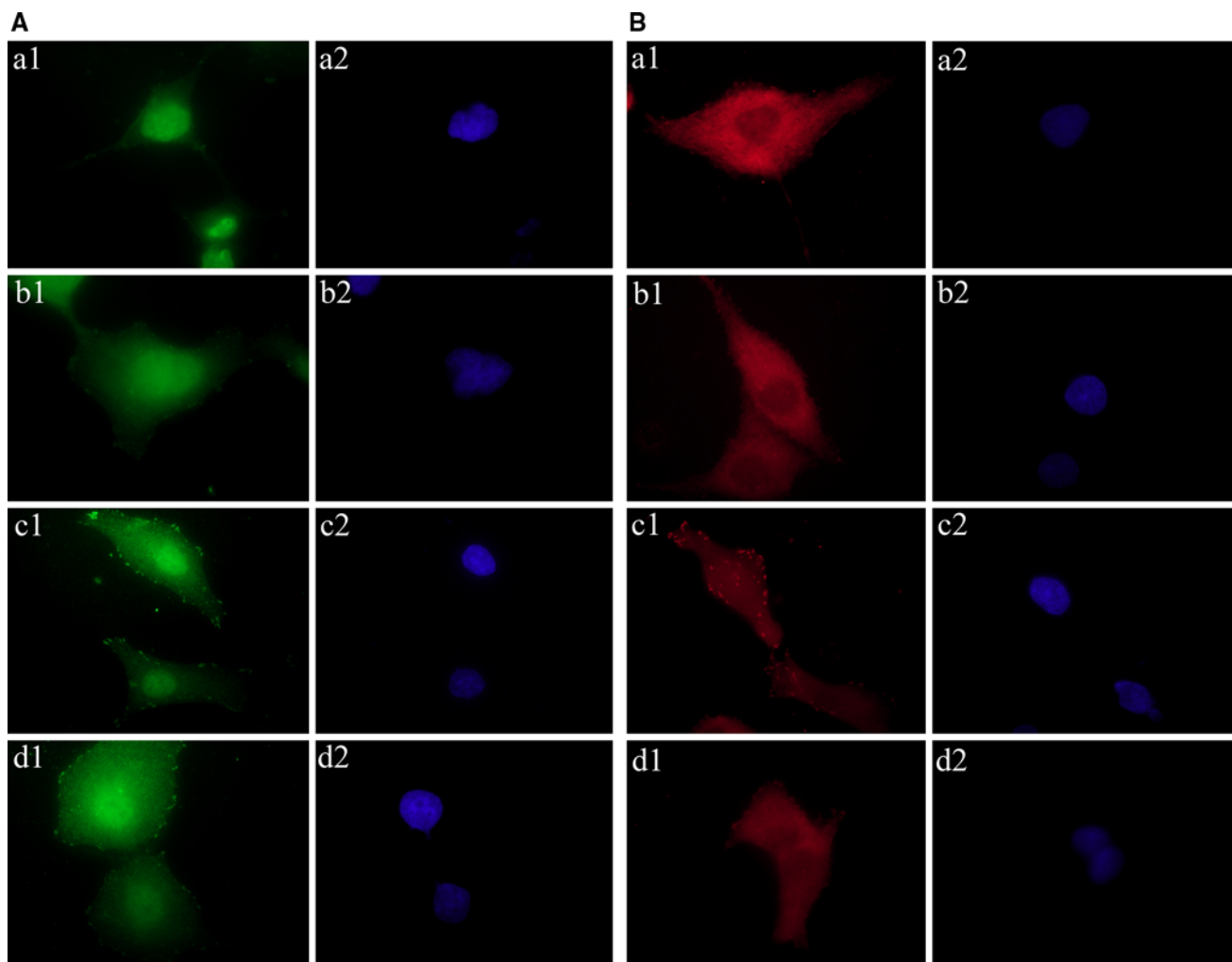
Fig. 4 Effects of Ki16425 on LPA-activated focal adhesion localization of FAK (**a**) and paxillin (**b**) (×1,000). *a1*, *a2* The cells were serum-starved overnight without any treatment. *b1*, *b2* The cells were treated with Ki16425 (10 μM, 30 min). *c1*, *c2* The cells were incubated with LPA (10 μM, 45 min). *d1*, *d2* The cells were pre-incubated with Ki16425 (10 μM, 30 min) and then treated with LPA (10 μM, 45 min). *a1*, *b1*, *c1*, *d1* FAK or paxillin staining. *a2*, *b2*, *c2*, *d2* cellular nucleus staining (*blue*)

tyrosine phosphorylation of FAK and paxillin [10, 13–15, 18, 43–51], leading to enhancement of cell motility [14, 18] and formation of stress fibers and focal adhesions [13, 14, 47, 49, 50]; for these effects the integrity of the actin cytoskeleton is essential [43, 44, 46], and the Gi/o and Rho/ROCK pathways are involved [14, 43, 44]. We found that LPA treatment significantly enhanced Tyr397 phosphorylation of FAK and Tyr31 and Tyr118 phosphorylation of paxillin in a dose and time-dependent manner. Pretreatment with Ki16425 attenuated LPA-induced tyrosine phosphorylation of FAK and paxillin. These results suggested that LPA mediated the tyrosine phosphorylation of FAK and paxillin through LPA1–3.

LPA-induced redistribution of FAK and paxillin is also important for cell migration. LPA initiated redistribution of paxillin and FAK to filament ends at the sites of focal adhesions and the cell periphery, which was associated with cell motility, actin organization, and assembly of focal adhesions [10, 13, 15, 45, 47, 50, 51]. We demonstrated that in serum-starved cells, FAK and paxillin were predominantly distributed diffusely in the cytoplasm. After LPA stimulation, they were recruited and translocated to the focal adhesions. Ki16425 significantly inhibited their membrane localization. Our results indicated that LPA1–3 are involved in the LPA-induced subcellular localization of FAK and paxillin, which may contribute to the motility of pancreatic cancer cells.

In conclusion, we provided evidence that LPA is crucial for cell motility by regulating tyrosine phosphorylation and redistribution of FAK and paxillin of pancreatic cancer cell line PANC-1. Suppression of LPA1–3 resulted in repression of the activation of FAK and paxillin and cell migratory ability, indicating the importance of LPA1–3 in regulation of cell motility of pancreatic cancer cells. This

Fig. 5 Effects of Ki16425 on LPA-stimulated cell motility. **a** Typical images of stained adherent cells (×400), *a* the cells were serum-starved overnight without any treatment (control), *b* the cells were pretreated with Ki16425 (10 μM, 30 min) before loading into the upper chamber, *c* the cells were administered LPA (10 μM, 6 h), *d* the cells were treated with LPA (10 μM, 6 h) after pretreatment with Ki16425 (10 μM, 30 min). **b** Adherent cells were quantified and relative migration (mean ± SD) is presented. *$*P < 0.05$ compared with the control, $^#P < 0.05$ compared with the LPA administration group
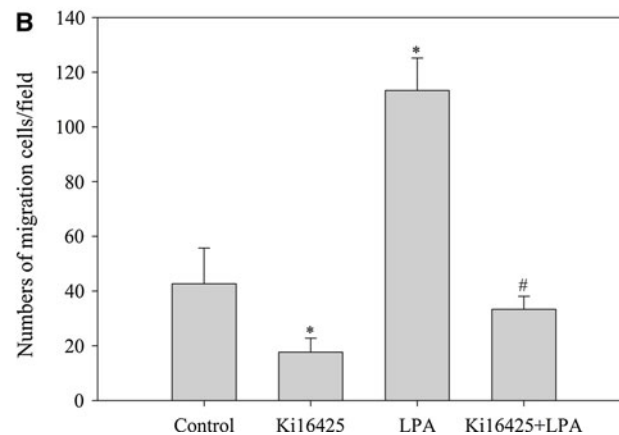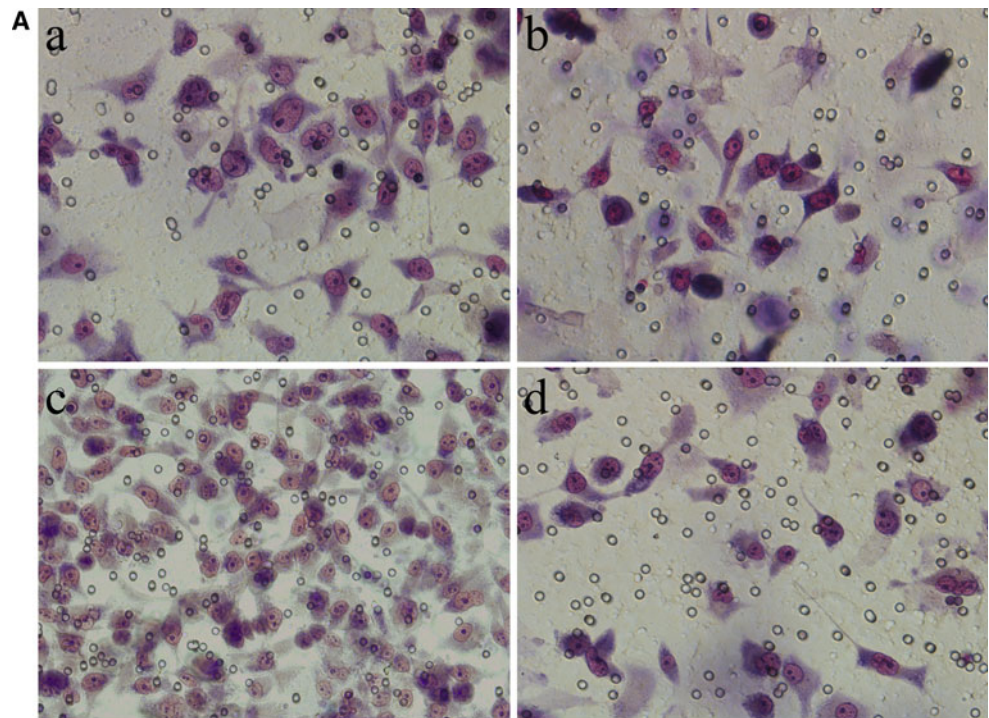
study may provide the basis for new therapy to control the metastasis of pancreatic cancer.

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