PKD1 is downregulated in non-small cell lung cancer and mediates the feedback inhibition of mTORC1-S6K1 axis in response to phorbol ester

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A B S T R A C T
Protein kinase D1 (PKD1) is increasingly implicated in multiple biological and molecular events that regulate the proliferation or invasiveness in several cancers. However, little is known about the expression and functions of PKD1 in non-small cell lung cancer (NSCLC). In the present study, 34 pairs of human NSCLC and matched normal bronchiolar epitheliums were enrolled and evaluated for PKD1 expression by quantitative real-time PCR. We showed that PKD1 was downregulated in 26 of 34 cancer tissues in comparison with matched normal epitheliums. Moreover, patients with venous invasion or lymph node metastasis showed significant lower expression of PKD1. Exposure of NSCLC A549 and H520 cells to the PKD family inhibitor kb NB 142-70 (Kb), at concentrations that inhibited PKD1 activation, strikingly potentiated S6K1 phosphorylation at Thr389 and S6 phosphorylation at Ser235/236 in response to phorbol ester (PMA). Knockdown of PKD1 with siRNAs strikingly enhanced S6K1 phosphorylation whereas constitutively active PKD1 resulted in the S6K1 activity inhibition. Furthermore, the PI3K inhibitors LY294002, BKM120 and MEK inhibitors U0126, PD0325901 blocked the enhanced S6K1 activity induced by Kb. Collectively, our results identify decreased expression of the PKD1 as a marker for NSCLC and the loss of PKD1 expression increases the malignant potential of NSCLC cells. This may be due to the function of PKD1 as a negative regulator of mTORC1–S6K1. Our results suggest that re-expression or activation of PKD1 might serve as a potential therapeutic target for NSCLC treatment.

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1. Introduction
Lung cancer remains the leading cause of cancer mortality in the world, with an overall 5-year survival approximating 16% (Siegel et al., 2013). Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer patients. Despite ongoing efforts to find effective treatments, current therapies offer very limited survival benefits. Novel therapeutic strategies are urgently required to treat this aggressive disease. Protein kinases constitute a large family of regulatory enzymes which mediate most of the signal transduction in cancer cells. Several mutations or dysregulations of these enzymes have been implicated in cancers rendering these proteins a very attractive target for therapeutic strategies (Blume-Jensen and Hunter, 2001).

Protein kinase D (PKD), a novel member of serine/threonine kinases belongs to the calcium/calmodulin-dependent protein kinase (CaMK) superfamily, is involved in multiple functions in both normal and diseased states (Valverde et al., 1994; Johannes et al., 1994; Rozengurt et al., 2005). The PKD family consists of three members, PKD1, PKD2 and PKD3, with distinct structural features and enzymological properties (Wang, 2006; Rykx et al., 2003; Fu and Rubin, 2011). PKD1 is the founding and most extensively characterized member of the family. In unstimulated cells, PKD1 is present in the cytoplasm with low kinase activity maintained by autoinhibition mediated by the N-terminal domain, a region containing a repeat of cysteinerich zinc finger-like motifs and a pleckstrin homology (PH) domain (Iglesias and Rozengurt, 1998; Waldron and Rozengurt, 2003). In response to cellular stimuli including phorbol esters, growth factors and G protein-coupled receptor (GPCR) agonists, PKD1 is converted into a form with high catalytic activity and recruited to multiple cellular compartments such as the plasma membrane, the nucleus or the Golgi, which allow them to influence different signaling pathways (Van Lint...
et al., 2002; Rykx et al., 2003). A growing body of evidence shows that PKD1 is implicated in multiple biological processes including membrane trafficking, signal transduction, cell adhesion, migration, survival, proliferation, differentiation and apoptosis (Liljedahl et al., 2001; Prigozhina and Waterman-Storer, 2004; Manning et al., 2002; Guha et al., 2010). Given its involvement in many cellular functions, it is not surprising that PKD1 plays an important role in cancer. Recent studies revealed altered PKD1 expression levels in diverse cancer types, such as prostate cancer, breast cancer, gastric cancer and pancreatic cancer (Jaggi et al., 2003; Eiseler et al., 2009; Du et al., 2009; Kim et al., 2008; Guha et al., 2002). PKD plays a significant role in mediating growth signaling in colorectal cancer (Wei et al., 2014) while it may represent opposite effect in breast cancer cells (Karam et al., 2012; Eiseler et al., 2009). However, little is known about the expression and function of PKD1 in lung cancer.

The mTOR (mammalian target of rapamycin) has emerged as one of the most important intracellular signaling enzyme regulating cell growth, survival and motility in cancer cells (Hay, 2005). mTOR exists in two multiprotein complexes: mTOR complexes 1 and 2 (mTORC1 and mTORC2). mTORC1 activation controls protein synthesis by phosphorylating two translational regulatory proteins: p70 ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1). The activation of S6K and inactivation of 4EBP1 promotes translation initiation for protein synthesis (Barbet et al., 1996; Brunn et al., 1997; Dorrello et al., 2006; Wullschleger et al., 2006). It was recently reported that mTOR/S6K1 pathway was abnormally activated and correlated with poor survival in NSCLC patients (Liu et al., 2011; Yoshizawa et al., 2010; Zhang et al., 2013). Also mTOR has been identified as a downstream target of both the PI3K (Scott et al., 1998; Nave et al., 1999) and Ras (Bailiff et al., 2005; Ma et al., 2005; Roux et al., 2004; J ohannessen et al., 2005) signaling pathways. Recently, we and others found that PKD1 activation mediates negative feedback of PI3K/Akt signaling in response to phorbol esters or G protein-coupled receptor (GPCR) agonists (Lee et al., 2011; Ni et al., 2013). However, the role of PKD1 regulates mTOR signaling is still unknown.

In this study, we show for the first time that the expression level of PKD1 is decreased in NSCLC. Inhibition of PKD1 enhances the activation of S6K1 and S6 in response to PMA stimulation. Loss of PKD1 expression increases the proliferation of NSCLC cells.

2. Materials and methods

2.1. Tissue samples and cell culture

Thirty-four pairs of primary NSCLC and corresponding normal bronchiolar epitheliums were obtained from patients in the Shandong Provincial Hospital Affiliated to Shandong University from 2012 to 2013 with informed consent. All tissue samples were from untreated patients undergoing surgery and all clinicopathologic information (age, gender, pathology, differentiation, invasion depth, lymph node metastasis) was available. The study was approved by the Hospitals’ Ethical Review Committee. All samples were snap frozen in liquid nitrogen and stored at −80 °C until the extraction of RNA.

Human non small cell lung cancer cell lines (A549 and H520) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 100 units/mL penicillin and 1% streptomycin and maintained at 37 °C with 5% CO2.

2.2. Real-time PCR – based detection of PKD1 mRNA

Total RNA was obtained from NSCLC tissues and normal bronchiolar epitheliums using TRIzol reagent (Invitrogen), according to the manufacturer’s instructions. RNA concentration was determined spectrophotometrically and integrity was checked by gel electrophoresis. RNA quality was confirmed in an Agilent 2100 Bioanalyzer (Agilent Technologies).

PKD1 mRNA was quantified by SYBR Green – quantitative real-time PCR and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). PCR primers used were as follows: PRKD1, 5′-GCCAGAGCCATACCCG-3′ (forward) and 5′-CTCCACCACACCTCCTCC-3′ (reverse), and glyceraldehyde-3-phosphate dehydrogenase, 5′-TCGACCATCTTATGCCTAG-3′ (forward) and 5′-CCACTTCTTGCTGTAGC-3′ (reverse). All PCRs were done in triplicates.

2.3. Western blotting

Cells treated with inhibitors and/or agonists were lysed with cold lysis buffer (50 mM Tris–HCl pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA and 1% Protease Inhibitor Cocktail) for 30 min on ice. Equivalent amounts of total protein extract were separated on 8% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. The transfer was carried out at 100 V, 0.4 A for 4 h using a Bio-Rad transfer apparatus. The transfer buffer consisted of 200 mM glycine, 5 mM Tris, 0.01% SDS, and 20% CH3OH. Then membranes were blocked using 5% nonfat dried milk in PBS (pH 7.2) and then incubated with the desired antibodies diluted in PBS containing 3% nonfat dried milk at 4 °C overnight. After washing with PBS 3 times, the membranes were incubated with their corresponding secondary antibodies at room temperature for 1 h. The membranes were washed again, and the antigen–antibody reaction was visualized by Amersham ECL detection system (GE Healthcare). The phosphospecific polyclonal antibodies used detect the phosphorylated state of S6K1 at Thr389, S6 at Ser421/422, Akt at Ser473, ERK1/2 at Thr202 and Tyr204, PKD1 at Ser916.

2.4. Knockdown of PKD1 levels via siRNA transfection

The Stealth siRNA duplexes were purchased from Invitrogen and designed to target the mRNA of human PKD1. Two different PKD1 siRNAs were designed: Oligo1, “GCACUAUUUGGACAUUGGAUAAG-CAA”; Oligo2, “GGGUUCUGGACAGUUUGGAUUUGU”. The siRNA was mixed with Lipofectamine RNA iMAX (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol and added to 35 mm dishes. A549 cells were then plated on top of the siRNA/Lipofectamine RNA iMAX complex at a density of 1 × 104 cells/35 mm dish. Control transfections were carried out with Stealth siRNA negative control (Invitrogen, Carlsbad, CA). Experiments were performed 48 h after transfection.

2.5. Plasmids constructs and transfection

The GFP-tagged PKD1 ca (constitutively active, S744/748E) was purchased from Genescript (Nanjing, China). The S744/748E mutant was generated by site-directed PCR mutagenesis and confirmed by DNA sequencing. GFP-tagged PKD1 ca or a control plasmid was transiently transfected into A549 cells using lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol, and cells were harvested 48 h posttransfection.

2.6. Cell proliferation assay

The cell proliferation was carried out using the WST-8 cell proliferation assay. According to protocol, cells transfected with PKD1 siRNA were planted in 96-well plates. Forty-eight hours after transfection, cells were then stimulated with 50 nM PMA. Meanwhile, each well was added with 10-μL cell counting assay kit-8 solution
and the absorbance was measured at 450 nm using a microplate reader at 12 h, 24 h and 48 h respectively.

2.7. Materials and reagents

RPMI-1640 medium was obtained from Invitrogen. PMA was purchased from Sigma–Aldrich and kb NB 142-70 was obtained from R&D Systems. LY294002, BKM120, U0126 and PD0325901 were obtained from Selleck Chemicals. All antibodies were purchased from Cell Signaling Technology. All other reagents were of the highest grade available.

3. Results

3.1. PKD1 is downregulated in NSCLC tissues in comparison with matched normal bronchiolar epitheliums

The clinicopathologic findings of 34 patients are shown in Table 1. Total RNA was isolated from NSCLC tissues and matched normal bronchiolar epitheliums and the level of PKD1 mRNA expression was determined by real-time PCR. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal standard. Of 34 matched cancer and normal tissues, PKD1 is downregulated in 26 cancer tissues compared with the matched normal tissues (Fig. 1A). The PKD1 expression was significantly lower in NSCLC tissues than in matched normal tissues (P < 0.05). Next, the correlation of T/N ratios for PKD1 expression with the clinicopathologic factors listed in Table 1 was examined. The T/N ratios in patients with venous invasion or lymph node metastasis were significantly lower than those without them (P < 0.05; Fig. 1B and C).

3.2. PKD1 significantly impacts the activity of mTORC1-S6K1 pathway

Previous studies have shown that treatment of intact cells with phorbol esters, cell-permeant DAGs induces a dramatic activation of PKD1 (Rozengurt et al., 2005). We also determined the effect of phorbol 12-myristate 13-acetate (PMA) on the phosphorylation of S6K1 at Thr389, a site targeted by mTORC1. Serum-starved A549 cells treated with 100 nM PMA for different time at 37 °C and then lysed. As shown in Fig. 2A, S6K1 activation in A549 cells increased dramatically to maximum level within 30 min and declined after 2 h of PMA stimulation. Meanwhile we examined the S6K1 phosphorylation in response to increasing concentrations of PMA for 30 min. PMA stimulation increased S6K1 phosphorylation in a concentration-dependent manner with maximal effect at 50 nM (Fig. 2B). As controls, the levels of endogenous S6K1 were not altered.

![Fig. 1](image-url)

Fig. 1. Significant decreased expression level of PKD1 in NSCLC tissue specimens. Total RNA was isolated from normal and lung cancer tissues. PKD1 expression was analyzed by quantitative real-time PCR and normalized to GAPDH expression. (A) A comparison of PKD1 expression between matched normal bronchiolar epitheliums and NSCLC tissues in 34 patients. T, tumor tissue; N, normal tissue. (B) The ratios of tumor to normal tissue for PKD1 expression were presented as relative T/N ratio of PKD1 expression. The T/N ratios were analyzed statistically in patients with venous invasion or without. v, venous invasion. *P < 0.05. n, lymph node metastasis (panel C). **P < 0.03.
Fig. 2. PMA induced time- and concentration-dependent phosphorylation of S6K1 in A549 cells. (A) A549 cells were treated with 50 nM PMA for 1, 2, 5, 10, 30 min and 1, 2, 4, 8, 16, 24 h. All cultures were then lysed with 2 × SDS-PAGE sample buffer. The samples were analyzed by SDS-PAGE and immunoblotting with antibodies that detect the phosphorylated state of S6K1 at Thr389 and total S6K1 to verify equal gel loading. (B) A549 cells were treated with increasing concentrations of PMA (0.5, 1, 2, 5, 10, 50, 100, 200, 400 nM) for 30 min. Cells were harvested and subjected immunoblotting for indicated proteins. (C, D) The expression levels of pS6K1 were quantified by densitometry analysis and normalized against total S6K1 levels. The experiments were repeated three times and results from representative experiments are shown.

Next we treated the cells with increasing concentrations of the selective PKD family inhibitor kb NB 142-70 (Kb) for 1 h then challenged with 50 nM PMA. Prior exposure of A549 cells to Kb strikingly potentiated S6K1 phosphorylation at Thr389 and the downstream S6 phosphorylation at Ser235/236 in a dose-dependent manner and the maximal potentiation was obtained at a concentration of 5 μM (Fig. 3A). Cell lysates were also used to determine PKD1 activation by Western blotting using antibodies that specifically detect the autophosphorylation site Ser916 of PKD1. The increase of S6K1 and S6 phosphorylation occurred at Kb concentration that inhibited PKD1 activation in the same cells (Fig. 3B). As shown in Fig. 4A and B, enhancement of S6K1 activation by addition of Kb reached the maximum at 1 h after PMA stimulation and persisted until 4 h in both A549 and H520 cells. PKD1 autophosphorylation on Ser916 suppressed by Kb was verified at all times examined.

We next determined whether knockdown of PKD1 expression using siRNAs enhances PMA-induced S6K1 activation in lung cancer cells. A549 cells were transfected with two different siRNAs targeting distinct regions of PKD1, and the PKD1 protein levels were significantly reduced compared with cells transfected with negative control siRNAs (Fig. 4C and D). In contrast, knockdown of PKD1 protein by either siPKD1-1 or siPKD1-2 dramatically enhanced the S6K1 phosphorylation at Thr389 in response to PMA exposure. Meanwhile, the increased phosphorylation of Akt at Ser473 and ERK at Thr202 and Tyr204 was also observed in line with S6K1 activation.

Based on the fact that selective inhibition of PKD1 potentiated S6K1 activation, we hypothesized that active PKD1 could impair the phosphorylation of S6K1. We then transiently transfected PKD1 constitutively active S744/748E mutant plasmid in A549 cells which were not stimulated with PMA and examined phosphorylation on S6K1, S6, Akt and ERK. As shown in Fig. 4E, expression of the constitutively active PKD1 resulted in the attenuation of S6K1, S6, Akt and ERK phosphorylation.

3.3. Inhibitors of PI3K and ERK prevent the potentiation of S6K1 induced by suppression of PKD1 activity

We next explored the mechanism of the feedback inhibition of S6K1 activation mediated by PKD1. Recently, it was reported that PKD1 is required for phosphorylation of C-terminal SH2 domain of PI3K p85α subunit (Lee et al., 2011). In addition, our previous results demonstrate that PKD1 activation mediates feedback inhibition of PI3K/Akt signaling (Ni et al., 2013). In line with the preceding results we got in PKD1 siRNAs transfected cells, PMA also strikingly induced the phosphorylation of Akt at Ser473 and ERK at Thr202 and Tyr204 in A549 cells prior exposed to Kb (Figs. 4A, 5A and B). Treatment of A549 cells with PI3K inhibitor LY294002 or BKM120 completely abolished the increase of the S6K1 and its downstream S6 phosphorylation exposed to Kb and subsequently challenged with PMA for 1 h and 2 h (Fig. 5A).

Moreover, the phosphorylation of TSC2/tuberin and/or raptor by ERK and ERK-activated 90-kDa ribosomal S6 kinases (RSK) also leads to mTORC1 stimulation (Roux et al., 2004; Ma et al., 2005; Jiang et al., 2009; Carriere et al., 2011). In order to determine
Fig. 4. Prolonged activation of S6K1, Akt and ERK induced by Kb and PKD1 siRNA transfection in PMA-stimulated lung cancer cells. A549 cells (panel A) and H520 cells (panel B) were incubated in the absence (−) or presence 5 μM Kb for 1 h prior to stimulation of cells with 50 nM PMA for the indicated times. (C, D) A549 cells transfected with negative control siRNA and different sequence PKD1 siRNA: siPKD1-1 or siPKD1-2. Then, the cultures were stimulated with 50 nM PMA for 10, 30 min and 1 h. In all cases, the samples were analyzed by SDS-PAGE and immunoblotting with antibodies that detect the phosphorylated state of S6K1 at Thr389, PKD1 at Ser916, Akt at Ser473 and ERK at Thr202 and Tyr204. The expression level of total PKD1 was used to evaluate siRNA-mediated knockdown of PKD1 expression. (E) Negative control or GFP-tagged PKD1 ca (constitutively active, S744/748E) plasmid were transiently transfected in A549 cells. Forty-eight hours after transfection, cells were harvested and examined for the phosphorylation on S6K1, S6, Akt and ERK. Similar results were obtained in at least three independent experiments in each case.

whether phorbol ester stimulates mTORC1 via MEK/ERK/RSK, cultures of A549 cells which were exposed to Kb were treated with the MEK inhibitor U0126 (10 μM) or PD0325901 (10 μM) then stimulated with PMA. Our results showed that exposure of cells to U0126 and PD0325901 prevented S6K1 Thr389 and S6 Ser235/236 phosphorylation in response to PMA treatment (Fig. 5B). Similar results were obtained when we detected the S6K1 and S6 activation in H520 cells which were pretreated with PI3K or MEK inhibitors (Fig. 5C).

Then we determined the influence of KU63794 (KU), PP242 and metformin on Kb induced phosphorylation of S6K in A549 cells. KU and PP242 are highly specific ATP competitive inhibitors of mTOR. Recently, metformin which is most widely used drug in the treatment of type 2 diabetes mellitus, has been identified as an effective mTOR inhibitor. As shown in Fig. 5D, KU and PP242 significantly inhibited the phosphorylation of S6K1 and S6 in cells incubated in the absence (−) or presence (+) 5 μM Kb, while the phosphorylation of ERK was not influenced.

3.4. Knockdown of PKD1 by siRNAs increases the proliferation of NSCLC cells in vitro in response to PMA

Considering the mTORC1-S6K1 signaling plays an important role in the regulation of protein and lipid synthesis, processes which are critical for the cell proliferation. We therefore test related biological effects of PKD1 depletion on NSCLC cells. The effect of PKD1 knockdown on NSCLC cell proliferation was examined in vitro. As shown in Fig. 6A and B, the expression level of PKD1 protein in cells transfected with PKD1 specific siRNAs was significantly downregulated compared with cells transfected with negative control siRNAs. Next, we assessed the effect of PKD1 siRNA transfection on the increase in the number of A549 and H520 cells induced by stimulation with PMA in the presence of 0.25% serum for 72 h. The WST-8 assay showed a significant increase of cell number after transfected with PKD1 siRNA in comparison with siRNA control group and PKD1 siRNA + Rapamycin group at 48 h and 72 h after the stimulation of PMA (Fig. 6C and D, \( P < 0.05 \)). These results suggest that PKD1 inhibits the proliferation of NSCLC cells.

4. Discussion

Protein kinase D family contains 3 members with distinct structure and function, namely, PKD1, PKD2 and PKD3. As the founding member of this family, PKD1 has been widely studied in multiple types of cancers. It was shown that PKD1 was downregulated in prostate cancer (Jaggi et al., 2003; Du et al., 2009), breast cancer (Eiseler et al., 2009) and gastric cancer (Kim et al., 2008). On the other hand, overexpression of PKD1 has been shown to play an important role in the progression of pancreatic cancer (Kisfalvi et al., 2010) and skin cancer (Bollag et al., 2004; Ristich et al., 2006). However, the expression level and function of PKD1
in lung cancer still remains unknown. In the present study, we compared the expression level of PKD1 in the NSCLC tissues versus corresponding normal bronchiolar epitheliums and report for the first time that PKD1 expression is significantly decreased in NSCLC tissue. These results suggest that the expression patterns of PKD1 may be dependent on the tissue type. Furthermore, the T/N ratio of PKD1 expression in patients with lymph node metastasis or venous invasion was significantly lower than in those without them. Although the observation period was too short to evaluate the survivals of the patients enrolled in the present study, these results show that lower expression of PKD1 may therefore be associated with a poor outcome in NSCLC patients.

PKD1 has been shown to play important roles in a variety of cellular functions that regulate intracellular signal transduction pathways, cell survival, motility and apoptosis. Since PKD1 is downregulated in NSCLC, we hypothesized PKD1 regulates cancer cells activities by modulating related signaling pathways. To test this hypothesis, we inhibited the activity of PKD1 by inhibitor Kb then stimulated with a strong PKD activator-phorbol ester PMA. Inhibition of PKD1 results in the hyperactivation of S6K1 in a dose-dependent manner. Furthermore, the enhancement of S6K1 activation was elicited in these cells challenged with PMA for various times, suggesting that inhibition of PKD1 activity converted mTORC1-S6K1 signaling from transient to persistent. Knockdown of PKD1 protein expression using siRNAs directed against PKD1 also enhanced PMA-induced S6K1 phosphorylation at Thr389. Moreover, constitutively active PKD1 resulted in the inhibition of S6K1 and its substrate S6 in line with Akt and ERK. Collectively, these results demonstrate for the first time that PKD1 mediates potent feedback inhibition of mTORC1-S6K1 activation in NSCLC cells. In most human tumors, the mTORC1 signaling pathway is aberrantly activated and correlated with the malignant progression. Particularly, mTOR has been implicated in the development of metastasis and blocking mTOR with the rapamycin derivative CCI-779 was sufficient to block lung tumor progression (Wislez et al., 2005). Therefore, the feedback inhibition of mTORC1-S6K1 activation by PKD1 points out a key regulatory role of PKD1 in lung cancer cells. Together with the results that PKD1 is downregulated in lung cancer tissues, it is plausible that PKD1 may function as a tumor suppressor in NSCLC.

Next, we examined the mechanism by which PKD1 attenuates mTORC1-S6K1 activation. In intact cells, two major intracellular
effector kinases – protein kinase B (Akt/PKB), extracellular-signal-regulated kinase1/2 (ERK1/2) directly phosphorylate the TSC1/TSC2 complex to inactivate it and thus activate mTORC1 (Wullschleger et al., 2006). Interestingly, pretreating cells with either PKD1 inhibitor or transfection with specific siRNA elicited significant elevated phosphorylation of Akt at Ser473 and ERK at Thr202 and Tyr204 in PMA stimulated cells. In order to explore the possible mechanism by which PKD1 attenuates mTORC1-S6K1 axis, we evaluated the S6K1 and S6 phosphorylation in cells exposed to PI3K and MEK inhibitors. Our results showed that the enhancement of S6K1 phosphorylation at Thr389 and S6 phosphorylation at Ser235/236 in cells exposed to Kb then challenged with PMA was completely blocked by PI3K and MEK inhibitors, which indicates PKD1 inhibits the activation of mTORC1-S6K1, at least in part, by regulating the kinase activity of Akt and ERK. As mentioned before, phorbol ester PMA has been shown to inhibit PI3K activation via phosphorylation of the p85α regulatory subunit by PKD1, predominantly at Ser652. The phosphorylation of this site impairs binding of p85α to Tyr-phosphorylated peptides then impairs the downstream activation of Akt (Lee et al., 2011). Also, our previous results showed that the inhibition of PKD1 enhances accumulation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) in the plasma membrane and strikingly increase the activation of Akt. Transgenic mice overexpressing PKD1 showed a reduced phosphorylation of Akt at Ser473 in intestinal epithelial cells compared to wild type littermates (Ni et al., 2013). However, whether PKD1 regulates the mTORC1-S6K1 axis via PI3K/Akt is not clear. Consistent with this prediction, our results show that inhibition or depletion of PKD1 induces aberrant phosphorylation of Akt at Ser473 in NSCLC and PKD1 mediates the feedback inhibition of mTORC1-S6K1 axis by inhibiting PI3K/Akt signaling pathway.

One the other hand, it was reported that ERK-dependent phosphorylation leads to TSC1-TSC2 dissociation and markedly impairs TSC2 ability to inhibit mTOR signaling, cell proliferation, and
In the study, the authors aimed to investigate the role of protein kinase D1 (PKD1) in the regulation of the MEK/ERK signaling pathway and its impact on tumor growth. The research focused on examining the phosphorylation of mTOR complex 1 (mTORC1) and its downstream targets, such as S6K1 and S6, in response to PKD1 depletion. These studies revealed that PKD1 mediates the phosphorylation of mTORC1-S6K1, which is crucial for the activation of the MEK/ERK pathway. Furthermore, the results showed that PKD1 plays a role in the negative feedback regulation of the MEK/ERK pathway, suggesting a potential role in cancer progression. The authors concluded that PKD1 could be a therapeutic target for cancer treatment.


