Int J Clin Exp Pathol 2018;11(1):208-215
www.ijcep.com /ISSN:1936-2625/IJCEP0065869

Original Article

PKCδ reveals a tumor promoter function by promoting cell proliferation and migration in somatotropinomas

Zhuowei Lei[1], Junwen Wang[2], Wei Sun[2], Xi Chen[2], Wei Jiao[2], Huaqiu Zhang[2], Ting Lei[2], Feng Li[1]

[1]*Department of Orthopedics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China;* [2]*Sino-German Neuro-Oncology Molecular Laboratory, Department of Neurosurgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

Received September 19, 2017; Accepted November 13, 2017; Epub January 1, 2018; Published January 15, 2018

Abstract: Protein kinase C δ (PKCδ), a subtype of PKC family, has been recognized as a tumor promoter or suppressor depending on different tissue specificities in various tumor types. However, the effects of PKCδ on somatotropinomas are poorly understood. This study aims to explore the precise role of PKCδ in promoting tumor progression in somatotropinomas. In the present study, we examined the expression levels of PKCδ in clinical specimens of human somatotropinomas to show that PKCδ overexpression correlated with invasive properties of somatotropinomas. Furthermore, we employed rat anterior pituitary GH3 cells as the experiment model to demonstrate that PKCδ activation by PKC agonist (Phorbol-12-myristate-13-acetate, PMA) significantly promoted the proliferation and migration potential of GH3 cells, and these effects could be abolished following PKCδ inhibition by specific inhibitor Rottlerin. Mechanistically, PKCδ activated ERK1/2 signaling, which was responsible for PKCδ-induced promotion of GH3 cell proliferation and migration. Taken together, our results indicated that PKCδ functions as a tumor promoter by promoting cell proliferation and migration in somatotropinomas.

Keywords: PKCδ, somatotropinomas, proliferation, migration

Introduction

Somatotropinomas cause acromegaly by producing excessive growth hormone (GH) and insulin growth factor 1 (IGF1) [1-3]. Surgery is the first-line therapy for somatotropinomas [4]. However, somatotropinomas often invade into the surrounding structures, such as cavernous sinus, sphenoid sinus, clivus, orbit, and brain tissue. It has been well recognized that cellular invasion of somatotropinomas is the main cause for incomplete tumor resection and postoperative recurrence [5]. Therefore, there is an urgent requirement to understand the invasive mechanism in somatotropinomas for targeted therapy and prognosis.

PKC is a Ser/Thr kinase family, including more than 12 subtypes [6]. PKC plays a multitude of physiological and pathological roles through its ability to phosphorylate target proteins involved in various cellular processes such as hormone secretion [7, 8], immune response [9], signal transduction [10, 11], cell proliferation [12], differentiation [13], migration [14], invasion [15] and apoptosis [16]. PKCδ, a subtype of PKC family, is involved in tumor progression of various tumor types. The expression levels and activation patterns of PKCδ vary depending on different cell specificities. Surprisingly, PKCδ could function as a tumor promoter or suppressor depending on different tissue specificities [17, 18]. Studies have implied that PKCδ is also expressed in somatotropinomas [19], however, little is known about its functions in somatotropinomas. The aim of this study is to investigate the precise role of PKCδ in somatotropinomas.

Materials and methods

Human somatotropinoma samples

Between June 2014 and October 2015, a series of 32 somatotropinoma samples were collected from patients who underwent transsphenoidal surgery at the Department of Neurosurgery, Tongji Hospital affiliated to Tongji Me-

dical College, Huazhong University of Science and Technology. Tumor invasiveness was classified on the basis of intraoperative findings and according to the Knosp grading scheme [20]. In case of a discrepancy between intraoperative findings and Knosp classification, the intraoperative findings were considered as gold standard. Generally, we included 18 females and 14 males, with a mean age of 41.0 years (range 22-61 years) at the time of surgery. Tumor sizes ≤10 mm were identified in 6 cases, 10-30 mm in 21 cases and ≥30 in 5 cases. 18 samples were classified to be invasive, while 14 were noninvasive. This work was approved by the ethical committees at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was obtained from all patients.

Immunohistochemistry (IHC) staining

Sections were deparaffinized in xylene, rehydrated through an ethanol gradient, following a washed for 15 min with phosphate-buffered saline. Thereafter, sections were heated in 0.01 M citrate buffer (pH 6.0) at 95°C for 20 min for antigen retrieval. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase in methanol for 10 min. Nonspecific antibody binding was blocked by incubation with the goat serum for 20 min. Primary antibody (Rabbit monoclonal to PKCδ, Abcam, Cambridge, UK) was applied to sections overnight at 4°C in a humidified chamber. After that, sections were treated with biotinylated goat secondary antibodies against rabbit IgGs for 30 min (ABC Elite, Vector Laboratories, Burlingame, CA) and for 30 min with avidin-biotin complex (ABC Elite), followed by treatment with 0.06% diaminobenzidine (Sigma Chemical, St Louis, MO) and 0.01% hydrogen peroxidase for 5 min. Sections were counterstained with hematoxylin. Antibody specificity control stains were performed by omitting primary antibodies. The percentage of positively stained cells was determined using Image J software (NIH) by counting around 400 tumor cells at 400× magnification.

Cell line and cell culture

Rat somatotropinoma cells (GH3 cells) were cultured in DMEM medium containing 10% FCS and Penicillin/Streptomycin (10 mg/ml solution diluted 1:100). Cells were grown in a humidified atmosphere at 37°C under 5% CO_2.

Drug preparation

PKCδ activation in GH3 cells was performed using PKC agonist PMA (Selleckchem, Shanghai, China) at the concentration of 1×10^{-5} M for 48 h. PKCδ inhibition was achieved by specific inhibitor Rottlerin (Selleckchem) at the at the concentration of 1×10^{-5} M for 48 h. ERK1/2 blocking was performed by specific inhibitor U0126 at the concentration of 1.5×10^{-5} M for 48 h.

Protein extraction and western blot

Membrane and cytosolic protein extraction was performed using Mem-PER™ Plus Membrane Protein Extraction Kit according to the manufacturer's instructions (ThermoFisher, Shanghai, China). Protein concentrations were determined by BCA (bicinchoninic acid) assay (Sigma, Munich, Germany). Equal amounts of proteins were separated by SDS-PAGE (10%) and transferred onto nitrocellulose membranes. To block nonspecific binding, membranes were immersed in 5% non-fat, dried milk in Tris-buffered saline plus Tween (TBST, 50 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween-20) for 1 h at room temperature and incubated with primary antibodies at 48°C overnight. After three washes for 5 min, blots were incubated with the appropriate secondary HRP-antibodies at 37°C for 1 h. Signals were detected by Super-Signal chemiluminescent substrate (Pierce, Rockford, IL). Band positions and band intensities were calculated and standardized to GAPDH, using a computerized image analysis system (Image J, NIH, Bethesda, MA).

Cell proliferation assay

Cells were seeded into 96-well plates in triplicates. After 12 h incubation, cells were treated with PMA, Rottlerin and their combination for another 48 h. Subsequently, CCK-8 solution were added into each well and incubated for 2 h at 37°C under 5% CO_2. Absorbance at 490 nm was assessed in a plate reader (Tecan Group Ltd., Männedorf, Switzerland).

Cell migration assay

To determine the migration potential of GH3 cells, scratch wound healing assay was performed in culture-insert micro-dishes according to the manufacturer's manual (Ibidi GmbH,

Table 1. The relationship between PKCδ expression and clinical parameters

	Cases	PKCδ (positive cells %)	*P* value
Variable			
Age (years)			
≤41	13	38.96 ± 17.63	0.059
>41	19	24.99 ± 1.07	
Gender			
Male	14	28.94 ± 18.37	0.685
Female	18	32.00 ± 22.71	
Tumor size (mm)			
≤10	6	5.87 ± 3.25	<0.0001
10-30	21	30.41 ± 14.02	
≥30	5	61.46 ± 14.26	
Invasiveness			
Invasive	14	13.94 ± 10.23	<0.0001
Noninvasive	18	43.67 ± 17.00	

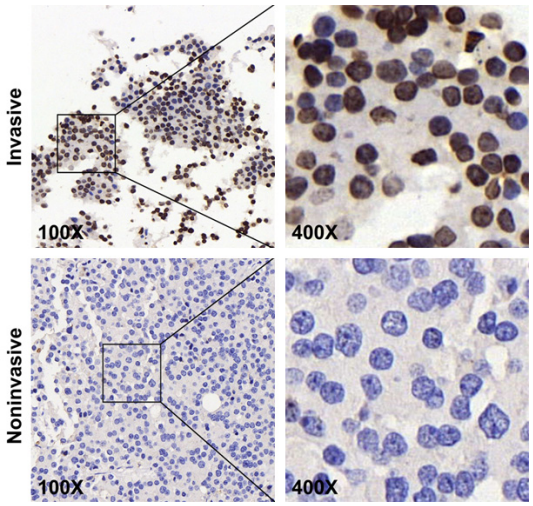

Figure 1. Representative IHC images for PKCδ staining in human invasive and noninvasive somatotropinomas.

Planegg, Germany). After PMA and Rottlerin treatments, cells were harvested and seeded into each chamber at densities of 1×10^5 cells/ml in 100 µl culture medium. Following incubation at 37°C for 12 h and serum starving for another 6 h, inserts were removed and 1 ml DMEM medium with 2% FBS was added into each well. Images were recorded immediately after insert removal and 9 h later. Rates of cell migration were assessed by counting cells in the gaps using Image J software (NIH, Bethesda, MA).

Transwell invasion assay

Transwell invasion assay was performed to examine the invasion ability of GH3 cells. Briefly, 8 mm pore transwell inserts (BD Biosciences, Heidelberg, Germany) were used as upper chambers in a 24-well plate. Inserts were coated with 75 µl Geltrex Matrix (Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix, Life Technologies, Frankfurt, Germany). After PMA and Rottlerin treatments, cells were harvested in serum free medium and added to the upper chambers. Simultaneously, 750 µl DMEM medium supplemented with 10% FBS was loaded into the 24 well plate and incubated for 48 h at 37°C. Thereafter, remaining cells in the upper chamber were removed gently with cotton swabs and cells that migrated through the Geltrex Matrix to the bottom membrane of the insert were fixed with 10% formalin and stained with hematoxylin (Carl Roth GmbH, Karlsruhe, Germany). Numbers of invaded cells were counted microscopically at 200× magnification.

Cell apoptosis assessment

Cell apoptosis was assessed by flow cytometry with Annexin V-FITC/PI double staining. Cells were harvested after PMA and Rottlerin treatments and incubated with FITC-conjugated Annexin V and PI according to the manufacturer's instructions (KeyGEN Biotech, Nanjing, China), thereafter, cells were subjected to Facscaliber II sorter machine (BD Biosciences, Shanghai, China) and data were analyzed with BD Cell Quest Pro software (BD Biosciences).

Statistical analysis

Data are expressed as mean ± standard derivation and analyzed with statistical software GraphPad Prism Ver. 6.05 (GraphPad Software, San Diego, CA). Unless stated otherwise, statistical significance was determined for intergroup comparison using Student's t-test and statistical significance was achieved when the *p* value is <0.05.

Results

PKCδ is overexpressed in invasive somatotropinomas

To determine the precise role of PKCδ in somatotropinomas, we examined the expression

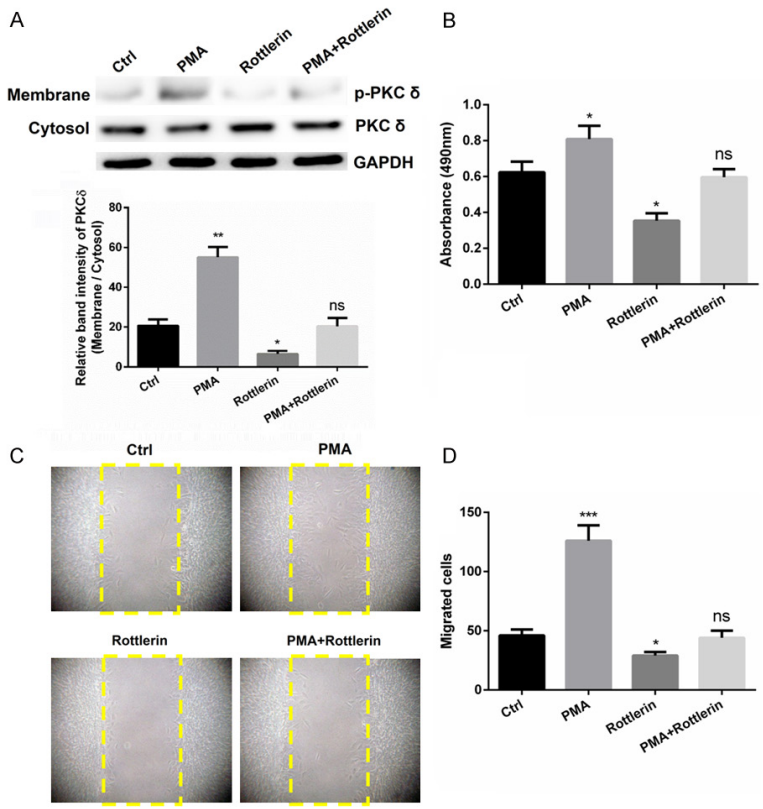

Figure 2. PKCδ promoted GH3 cell proliferation and migration potential. A. Western blot was performed to detect the PKCδ activities. Note that PKCδ was activated by PMA (10^{-5} for 48 h) and inhibited by Rottlerin (10^{-5} for 48 h) treatment in GH3 cells. Rottlerin could abolish PMA-induced activation of PKCδ. B. CCK-8 assays were performed to assess GH3 cell proliferation. Note that PMA treatment significantly increased cell proliferation, while Rottlerin significantly reduced cell proliferation; Rottlerin could abolish PMA-induced promotion of cell proliferation. C, D. Wound healing assays were employed to determine GH3 cell migration ability. Note that PMA treatment significantly increased the number of migrated cells, while Rottlerin significantly reduced migrated cells in somatotropinomas; Rottlerin could abolish PMA-induced promotion of cell migration. *P<0.05, ***P<0.001 vs. Ctrl group.

levels of PKCδ by IHC staining in human somatotropinoma tissues and analyzed the relationship between PKCδ expression and clinical characteristics. The results showed that PKCδ expression was not correlated with patient age or gender. However, a positive correlation was observed between PKCδ expression and tumor size as well as tumor invasiveness (**Table 1**; **Figure 1**). These findings indicate that PKCδ acts as a tumor promoter in somatotropinomas.

PKCδ promotes the proliferation and migration without influencing the invasion and apoptosis of GH3 cells

To further determine the functional role of PKCδ in somatotropinomas, we examined the impact of PKCδ on GH3 cell proliferation, migration, invasion and apoptosis. As a result, we found that PKCδ activation by PMA significantly promoted GH3 cell proliferation and migration, while PKCδ inhibition by Rottlerin exerted a significant suppression on GH3 cell proliferation and migration. Furthermore, the promotion of GH3 cell proliferation and migration induced by PKC agonist PMA could be abolished by specific PKCδ inhibitor Rottlerin (**Figure 2**). We also assessed whether PKCδ affected GH3 cell invasion and apoptosis potential. The results showed that activation or inhibition of PKCδ did not influence GH3 cell invasion and apoptosis (**Figure 3**). Taken together, our data suggest that PKCδ promotes cell proliferation and migration without influencing cell invasion and apoptosis in somatotropinomas.

PKCδ activates ERK1/2 signaling in GH3 cells

It is demonstrated that PKC is associated with ERK1/2 and NF-κB signaling. To determine the mechanistic role of PKCδ in somatotropinomas, we assessed the impact of PKCδ on ERK1/2 and NF-κB signaling in GH3 cells. We found that PKCδ activation by PMA significantly increased the phosphorylation of ERK1/2, while PKCδ inhibition by Rottlerin reduced the phosphorylation of ERK1/2. Moreover, Rottlerin could abolish PMA-induced promotion of ERK1/2 phosphorylation (**Figure 4**). However, activation or inhibition of PKCδ did not influence NFκB phosphorylation in GH3 cells (**Figure 4**). These findings indicate that of PKCδ activates ERK1/2 signaling in somatotropinomas.

PKCδ promotes GH3 cell proliferation and migration via ERK1/2 signaling pathway

Specific inhibitor for ERK1/2 (U0126) was employed to test whether PKCδ promoted GH3

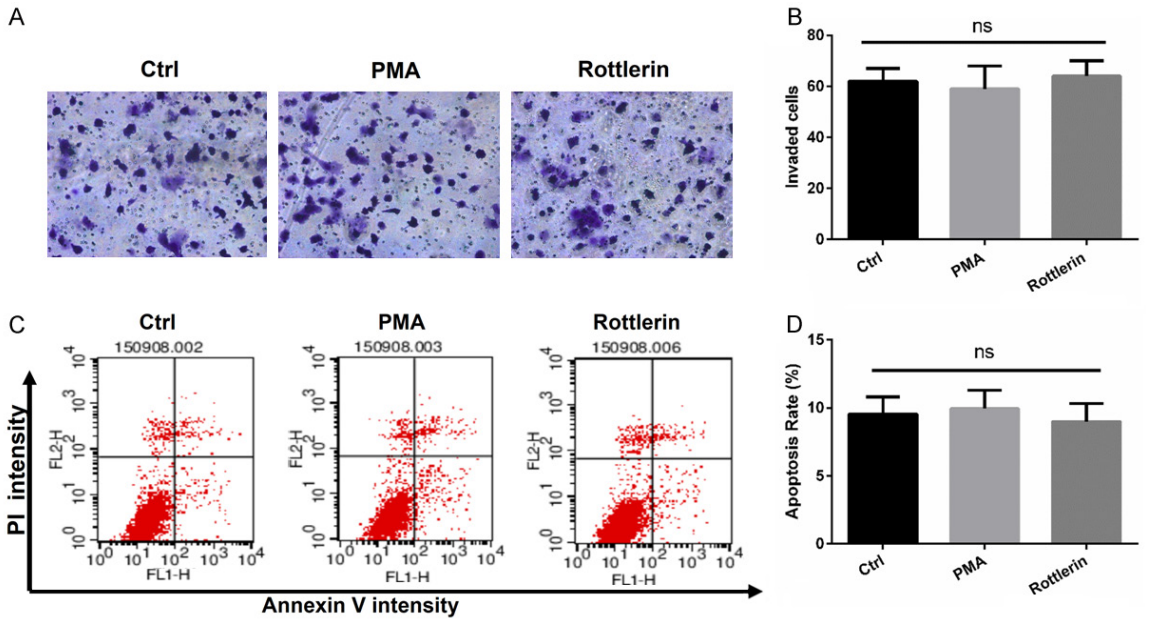

Figure 3. PKCδ did not influence GH3 cell invasion and apoptosis. A, B. Transwell invasion assays were used to detect cell invasion ability of GH3 cells. PKCδ activation or inhibition did not affect GH3 cell invasion capability. C, D. Flow cytometry with Annexin V-FITC/PI double staining was performed to examine GH3 cell apoptosis. PKCδ activation or inhibition did not influence GH3 cell apoptosis.

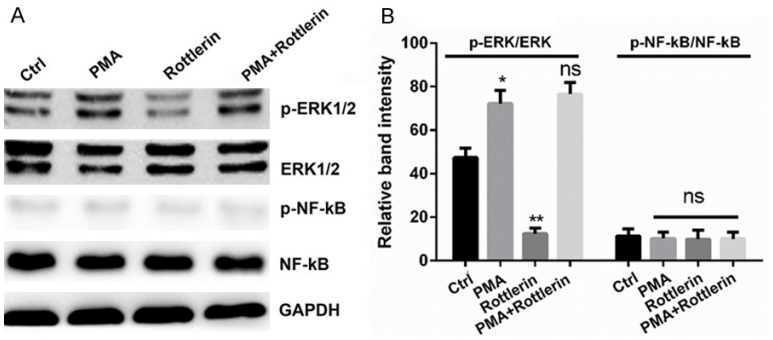

Figure 4. PKCδ activated ERK1/2 but not NF-κB signaling in GH3 cells. A, B. PKCδ was activated by PMA (10^{-5} for 48 h) and inhibited by Rottlerin (10^{-5} for 48 h) treatment in GH3 cells. Note that PMA significantly enhanced ERK1/2 signaling in GH3 cells, while Rottlerin remarkably attenuated ERK1/2 signaling; Rottlerin could cancel PMA-induced enhancement on ERK1/2 signaling. However, PKCδ activation and inhibition did not influence NF-κB signaling pathway. *P<0.05, **P<0.01 vs. Ctrl group.

cell proliferation and migration via ERK1/2 signaling pathway. The re-sults showed that ERK1/2 blocking by U0126 significantly reduced GH3 cell proliferation and migration. Furthermore, ERK1/2 blocking significantly abolished PMA-induced promotion of GH3 cell proliferation and migration (**Figure 5**). Taken together, these findings indicate that PKCδ promotes cell proliferation and migration via ERK1/2 signaling patway in somatotropinomas.

Discussion

PKC is expressed in numerous human tissues, and the expression and function of individual PKC subtypes vary according to different tissue specificities [6, 21, 22]. PKCδ, a special member of PKC family, plays a vital role in various cellular process, such as proliferation, differentiation, migration, invasion and apoptosis [18, 23-25]. Interest in PKCδ has increased because it could function as a tumor promoter or suppressor alternatively according to different tissue specificities. For example, Hai Li et al. found that PKCδ was overexpressed in gastric cancer and promoted tumor invasion and metastasis by enhancing tumor cell migration, invasion and proliferation [26], but Hsien-Ming Wu et al. demonstrated that PKCδ functioned as a tumor suppressor by inducing tumor cell apoptosis in human endometrial cancer [27]. Little is known about the precise role of PKCδ in somatotropinomas. In our study, we examined the expression of PKCδ

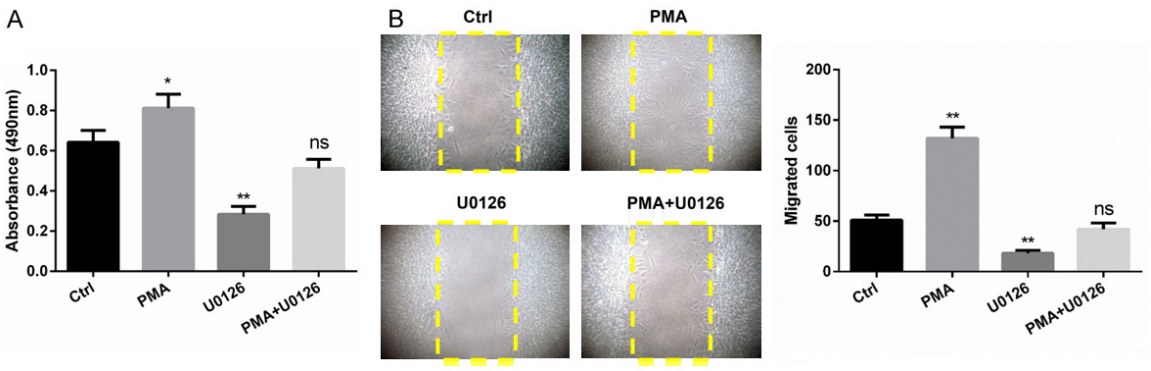

Figure 5. PKCδ promoted GH3 cell proliferation and migration via ERK1/2 signaling pathway. A. CCK-8 assays were performed to assess GH3 cell proliferation. Note that ERK1/2 blocking using specific inhibitor (U0126) abolished PMA-induced promotion of GH3 cell proliferation. B. Wound healing assays were employed to determine GH3 cell migration ability. Note that ERK1/2 blocking using specific inhibitor (U0126) abolished PMA-induced promotion of GH3 cell migration. *P<0.05, **P<0.01 vs. Ctrl group.

in human somatotropinoma tissues and demonstrated that PKCδ expression positively correlated with tumor size and invasiveness, indicating that PKCδ might function as a tumor promoter in somatotropinomas.

PKC is initially produced and released into cytoplasm, where it could be phosphorylated to a stable structure. The translocation to cell membrane with DAG (diacylglycerol) binding is considered to be the sign of PKC activation [22]. Active PKC can regulate various cellular functions by phosphorylating different target proteins. In this study, we evaluated the effects of PKCδ activities on cellular functions of somatotropinomas. As a result, PKCδ activation significantly enhanced GH3 cell proliferation and migration potential, while PKCδ inhibition exerted a significant suppression on GH3 cell proliferation and migration. However, activation or inhibition of PKCδ in somatotropinomas were not correlated with GH3 cell invasion and apoptosis potential. These results indicated that the cellular functions of PKCδ also varied according to different tissue specificities. An interesting finding in our study is that specific inhibitor of PKCδ (Rottlerin) could abolish the promotion of cell proliferation and migration induced by broad-spectrum PKC agonist (PMA), suggesting that PKCδ might be a major cause of tumor progression in somatotropinomas.

NFκB and ERK1/2 signaling pathways are associated with PKC activities [11, 26, 28-30]. In our study, we assessed the impact of PKCδ activation and inhibition on these signaling pathways. The results showed that PKCδ activation only enhanced ERK1/2 signaling, leaving NF-κB signaling intact. ERK1/2 blocking using specific inhibitor could abolish the promotion of cell proliferation and migration induced by PKCδ activation, indicating that promotes cell proliferation and migration via ERK1/2 signaling pathway in somatotropinomas.

Although inhibition of PKC has been achieved in vitro and in vivo [31-34], a broad spectrum inhibition of PKC subtypes is usually inappropriate because of their essential physiological functions. A precise inhibition of specific PKC subtype in targeted tissues might be a promising strategy and is a matter of future research activities.

Acknowledgements

This work was supported by National Natural Science Foundation of China (grant No. 81702478 and 81270865) and China Postdoctoral Science Foundation (grant No. 2016M600596).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ting Lei, Sino-German Neuro-Oncology Molecular Laboratory, Department of Neurosurgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Qiaokou District, Wuhan 430030, China. Tel: 0086-13720166117; Fax: 0086-8366-5200; E-mail: tlei@tjh.tjmu.edu.cn

References

[1] Kontogeorgos G. Classification and pathology of pituitary tumors. Endocrine 2005; 28: 27-35.

[2] Larysz D, Blamek S and Rudnik A. Clinical aspects of molecular biology of pituitary adenomas. Folia Neuropathol 2012; 50: 110-117.

[3] Gadelha MR, Kasuki L and Korbonits M. The genetic background of acromegaly. Pituitary 2017; 20: 10-21.

[4] Gadelha MR, Wildemberg LE, Bronstein MD, Gatto F and Ferone D. Somatostatin receptor ligands in the treatment of acromegaly. Pituitary 2017; 20: 100-108.

[5] Bi WL, Greenwald NF, Ramkissoon SH, Abedalthagafi M, Coy SM, Ligon KL, Mei Y, MacConaill L, Ducar M, Min L, Santagata S, Kaiser UB, Beroukhim R, Laws ER Jr and Dunn IF. Clinical identification of oncogenic drivers and copy number alterations in pituitary tumors. Endocri-nology 2017; 158: 2284-2291.

[6] Singh RK, Kumar S, Gautam PK, Tomar MS, Verma PK, Singh SP, Kumar S and Acharya A. Protein kinase C-alpha and the regulation of diverse cell responses. Biomol Concepts 2017; 26: 143-153.

[7] Bergan-Roller HE and Sheridan MA. The growth hormone signaling system: Insights into coordinating the anabolic and catabolic actions of growth hormone. Gen Comp Endocrinol 2017; 17: 30282-30284.

[8] Lei T, Liu Q, Li L, Zhang L, Shu K and Xue D. Preliminary study on the relationship between cAMP level and gsp expression in cultured human pituitary somatotrophinomas. J Tongji Med Univ 2000; 20: 214-216.

[9] Zeng Q, Luo P, Gu J, Liang B, Liu Q and Zhang A. PKC theta-mediated Ca2+/NF-AT signalling pathway may be involved in T-cell immunosuppression in coal-burning arsenic-poisoned population. Environ Toxicol Pharmacol 2017; 55: 44-50.

[10] Zheng J, Kong C, Yang X, Cui X, Lin X and Zhang Z. Protein kinase C-alpha (PKCalpha) modulates cell apoptosis by stimulating nuclear translocation of NF-kappa-B p65 in urothelial cell carcinoma of the bladder. BMC Cancer 2017; 17: 432.

[11] He P, Shen N, Gao G, Jiang X, Sun H, Zhou D, Xu N, Nong L and Ren K. Periodic mechanical stress activates PKCdelta-Dependent EGFR mitogenic signals in rat chondrocytes via PI3K-Akt and ERK1/2. Cell Physiol Biochem 2016; 39: 1281-1294.

[12] He M, Wang G, Jiang L, Qiu C, Li B, Wang J and Fu Y. miR-486 suppresses the development of osteosarcoma by regulating PKC-delta pathway. Int J Oncol 2017; 50: 1590-1600.

[13] Kostyak JC, Liverani E and Kunapuli SP. PKC-epsilon deficiency alters progenitor cell populations in favor of megakaryopoiesis. PLoS One 2017; 12: e0182867.

[14] Peng J, Zheng H, Wang X and Cheng Z. Upregulation of TLR4 via PKC activation contributes to impaired wound healing in high-glucose-treated kidney proximal tubular cells. PLoS One 2017; 12: e0178147.

[15] Marquina-Sanchez B, Gonzalez-Jorge J, Hansberg-Pastor V, Wegman-Ostrosky T, Baranda-Avila N, Mejia-Perez S, Camacho-Arroyo I and Gonza-lez-Arenas A. The interplay between intracellular progesterone receptor and PKC plays a key role in migration and invasion of human glioblas-toma cells. J Steroid Biochem Mol Biol 2017; 172: 198-206.

[16] Wong VKW, Zeng W, Chen J, Yao XJ, Leung ELH, Wang QQ, Chiu P, Ko BCB and Law BYK. Tetrandrine, an activator of autophagy, induces autophagic cell death via PKC-alpha inhibition and mTOR-dependent mechanisms. Front Pharmacol 2017; 8: 351.

[17] Xu X, Sun S, Xie F, Ma J, Tang J, He S and Bai L. Advanced oxidation protein products induce epithelial-mesenchymal transition of intestinal epithelial cells via a PKC delta-Mediated, redox-dependent signaling pathway. Antioxid Redox Signal 2017; 27: 37-56.

[18] Zhang D, Fu M, Li L, Ye H, Song Z and Piao Y. PKC-delta attenuates the cancer stem cell among squamous cell carcinoma cells through down-regulating p63. Pathol Res Pract 2017; 9: 1119-1124.

[19] Jin L, Maeda T, Chandler WF and Lloyd RV. Protein kinase C (PKC) activity and PKC messenger RNAs in human pituitary adenomas. Am J Pathol 1993; 142: 569-578.

[20] Knosp E, Steiner E, Kitz K and Matula C. Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery 1993; 33: 610-617; discussion 617-618.

[21] Storz P. Targeting protein kinase C subtypes in pancreatic cancer. Expert Rev Anticancer Ther 2015; 15: 433-438.

[22] Redig AJ and Platanias LC. The protein kinase C (PKC) family of proteins in cytokine signaling in hematopoiesis. J Interferon Cytokine Res 2007; 27: 623-636.

[23] Parihar SP, Ozturk M, Marakalala MJ, Loots DT, Hurdayal R, Beukes D, Van Reenen M, Zak DE, Mbandi SK, Darboe F, Penn-Nicholson A, Hanekom WA, Leitges M, Scriba TJ, Guler R and Brombacher F. Protein kinase C-delta (PKCdelta), a marker of inflammation and tuberculosis disease progression in humans, is important for optimal macrophage killing ef-

fector functions and survival in mice. Mucosal Immunol 2017; [Epub ahead of print].
[24] Wei SY, Lin TE, Wang WL, Lee PL, Tsai MC and Chiu JJ. Protein kinase C-delta and -beta coordinate flow-induced directionality and deformation of migratory human blood T-lymphocytes. J Mol Cell Biol 2014; 6: 458-472.
[25] Gan X, Wang J, Wang C, Sommer E, Kozasa T, Srinivasula S, Alessi D, Offermanns S, Simon MI and Wu D. PRR5L degradation promotes mTORC2-mediated PKC-delta phosphorylation and cell migration downstream of Galpha12. Nat Cell Biol 2012; 14: 686-696.
[26] Li H and Chen C. Quercetin has antimetastatic effects on gastric cancer cells via the interruption of uPA/uPAR function by modulating NF-kappab, PKC-delta, ERK1/2, and AMPKalpha. Integr Cancer Ther 2017; 1534735417696702.
[27] Wu HM, Schally AV, Cheng JC, Zarandi M, Varga J and Leung PC. Growth hormone-releasing hormone antagonist induces apoptosis of human endometrial cancer cells through PKCdelta-mediated activation of p53/p21. Cancer Lett 2010; 298: 16-25.
[28] Toriumi K, Horikoshi Y, Yoshiyuki Osamura R, Yamamoto Y, Nakamura N and Takekoshi S. Carbon tetrachloride-induced hepatic injury through formation of oxidized diacylglycerol and activation of the PKC/NF-kappaB pathway. Lab Invest 2013; 93: 218-229.
[29] Tian C, Ye F, Xu T, Wang S, Wang X, Wang H, Wan F and Lei T. GHRP-6 induces CREB phosphorylation and growth hormone secretion via a protein kinase Csigma-dependent pathway in GH3 cells. J Huazhong Univ Sci Technolog Med Sci 2010; 30: 183-187.
[30] Sun W, Jiao W, Huang Y, Li R, Zhang Z, Wang J and Lei T. Exchange proteins directly activated by cAMP induce the proliferation of rat anterior pituitary GH3 cells via the activation of extracellular signal-regulated kinase. Biochem Biophys Res Commun 2017; 485: 355-359.
[31] Muscella A, Giulio Cossa L, Vetrugno C, Antonaci G and Marsigliante S. Inhibition of ZL55 cell proliferation by ADP via PKC-dependent signalling pathway. J Cell Physiol 2018; 233: 2526-2536.
[32] Liu Z, Huang Y, Liu L and Zhang L. Inhibitions of PKC and CaMK-II synergistically rescue ischemia-induced astrocytic dysfunction. Neurosci Lett 2017; 657: 199-203.
[33] Lien LM, Lin KH, Huang LT, Tseng MF, Chiu HC, Chen RJ and Lu WJ. Licochalcone A prevents platelet activation and thrombus formation through the inhibition of PLCgamma2-PKC, Akt, and MAPK pathways. Int J Mol Sci 2017; 18: E1500.
[34] Xu T, Ye F, Wang B, Tian C, Wang S, Shu K, Guo D and Lei T. Elevation of growth hormone secretagogue receptor type 1a mRNA expression in human growth hormone-secreting pituitary adenoma harboring G protein alpha subunit mutation. Neuro Endocrinol Lett 2010; 31: 147-154.