Original Article

PKC α regulates vasopressin-induced aquaporin-2 trafficking in mouse kidney collecting duct cells *in vitro* via altering microtubule assembly

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Aim: Aquaporin-2 (AQP2) is a vasopressin-regulated water channel located in the collecting tubule and collecting duct cells of mammalian kidney. The aim of this study is to investigate whether PKC α plays a role in vasopressin-induced AQP2 trafficking in mouse inner medullary collecting duct 3 (mIMCD3) cells.

Methods: AQP2-mIMCD3 stable cell line was constructed by transfection of mouse inner medullary collecting duct 3 (mIMCD3) cells with AQP2-GFP construct. Then the cells were transfected with PKC α shRNA, PKC α A/25E, or PKC α scrambled shRNA. The expression levels of PKC α , AQP2, and phospho-S256-AQP2 were analyzed using Western blot. The interaction between AQP2 and PKC α was examined using immunoprecipitation. The distribution of AQP2 and microtubules was studied using immunocytochemistry. The AQP2 trafficking was examined using the biotinylation of surface membranes.

Results: Treatment of AQP2-mIMCD3 cells with 100 μ mol/L of 1-desamino-8-D-arginine vasopressin (DdAVP) for 30 min stimulated the translocation of AQP2 from the cytoplasm to plasma membrane through influencing the microtubule assembly. Upregulation of active PKC α by transfection with PKC α A/25E plasmids resulted in de-polymerization of α -tubulin and redistributed AQP2 in the cytoplasm. Down-regulation of PKC α by PKC α shRNA partially inhibited DdAVP-stimulated AQP2 trafficking without altering α -tubulin distribution. Although 100 μ mol/L of DdAVP increased AQP2 phosphorylation at serine 256, down-regulation of PKC α by PKC α shRNA did not influence DdAVP-induced AQP2 phosphorylation, suggesting that AQP2 phosphorylation at serine 256 was independent of PKC α . Moreover, PKC α did not physically interact with AQP2 in the presence or absence of DdAVP.

Conclusion: Our results suggested that PKC α regulates AQP2 trafficking induced by DdAVP via microtubule assembly.

Keywords: PKC α ; 1-desamino-8-D-arginine vasopressin (DdAVP); aquaporin-2; microtubule; kidney; medullary collecting duct

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Introduction

Protein kinase C alpha (PKC α) is a member of the classic PKC family^[1], which is widely expressed in the mammalian kidney^[2–4] and contributes to various kidney functions, including substrate absorption and urine concentration^[5, 6]. PKC α -mediated cytoskeleton remodeling results in endocytosis in epithelial cells, which increases the uptake of various substrates, such as NHE3 and albumin^[7–9]. PKC α is localized in glomeruli, the intercalated cells of the cortical collecting duct, and the medullary-collecting duct in the mouse kidney^[4]. Genetic knockout mice that lack PKC α exhibit decreased

glomerular filtration rate, increased urinary output and lower urinary osmolarity, accompanied by normal water intake and normal levels of the plasma antidiuretic hormone arginine vasopressin (AVP)^[5]. These results strongly suggest that PKC α -mediated urine concentration primarily occurs within the medullary collecting duct.

Aquaporin-2 (AQP2) is a vasopressin-regulated water channel in the principal cells of the connecting tubule and the collecting duct in the kidney^[10, 11]. AQP2 is stored in an intracellular compartment and plays an important role in the regulation of urine concentration^[12–15]. AVP stimulates AQP2 translocation to the plasma membrane for the re-absorption of water. AQP2 is removed from the plasma membrane and returned to the intracellular compartment when the stimulation is terminated^[16–18]. In addition, AVP also regulates AQP2 mRNA and protein levels, which mediates long-term regula-

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tion^[19, 20]. Both short- and long-term regulation mechanisms are involved in the pathophysiology of AQP2-mediated urine concentration^[21]. Although PKC is involved in angiotensin II-mediated AQP2 expression and trafficking^[22], our previous study demonstrated that AQP2 expression is only slightly different between PKC α knockout mice and wild-type mice^[5], which strongly suggests that the long-term AQP2 regulation mechanism does not contribute to PKC α -mediated urine concentration. AQP2 transportation is partially regulated by the cytoskeleton^[23–25], and PKC α -mediated remodeling of the actin cytoskeleton is involved in constitutive albumin uptake in the renal proximal tubule^[9]. However, the contribution of PKC α to AQP2 trafficking, the role of the cytoskeleton in PKC α -mediated AQP2 transportation and the mechanism of PKC α regulation of AQP2 translocation are less well understood.

We hypothesized that PKC α mediates AQP2 trafficking via cytoskeleton distribution because of the important role of PKC α in urine concentration and cytoskeleton remodeling. Therefore, this study revealed the crosstalk among PKC α , AVP and AQP2 in trafficking and cytoskeletal remodeling in mIMCD3 cell.

Materials and methods

Constructs, antibodies, and reagents

The pEF-nero-PKC α A/25E vector was a gift from Dr Gotfried BAIER (Institute for Medical Biology and Human Genetics, University of Innsbruck, Innsbruck, Austria). The AQP2-GFP-pCMV6 construct was purchased from Origene (Rockville, MD, USA). Lipofectamine plus and geneticin (G418) were purchased from Invitrogen (Shanghai, China). Protein A/G-agarose beads and primary antibodies against phospho-S256-AQP2, AQP2, PKC α , and GAPDH were purchased from Santa Cruz (Heidelberg, Germany). The primary antibody against α -tubulin was purchased from Calbiochem (Darmstadt, Germany). The secondary goat anti-rabbit IgG conjugated with Cy3 antibody and the analogue 1-desamino-8-D-arginine vasopressin (DdAVP) were purchased from Sigma (Shanghai, China). DAPI was purchased from Vector Burlingame (San Diego, CA, USA). Sulfo-Link NHS-LC-biotin and streptavidin-agarose beads were purchased from Pierce (Beijing, China). Major apparatuses included a Mini-PROTEAN II Electrophoresis Cell (Bio-Rad, Shanghai, China) and a confocal laser-scanning microscope (Olympus FV500, Japan).

Cells and cell culture

Immortalized mouse inner medullary collecting duct 3 (mIMCD3) cells were kindly provided by Dr John M LUK (Department of Urology, University of Hong Kong, Hong Kong, China). mIMCD3 cells were maintained in Dulbecco's modified Eagle's medium/Ham's F12 (1:1) (GIBCO, Invitrogen, Shanghai, China) supplemented with 10% fetal bovine serum (GIBCO, Invitrogen, Shanghai, China) and 2% penicillin-streptomycin (Amresco, Shanghai, China) in a humidified atmosphere with 5% CO₂ at 37°C.

Generation of pGCsi-U6/Neo PKC α shRNA plasmid

Three siRNAs were designed and synthesized by Invitrogen (Shanghai, China) according to the cDNA sequence of PKC α (NM_011101): #1, GTCCTTCACGTTCAAATTA; #2, GTG-CAGTATGAAACTCAAA; and #3, CCATCCAACAACCTG-GACA. The siRNAs were cloned into the eukaryotic expression plasmid pGCsi-U6/Neo (Genechem, Shanghai, China). The constructed pGCsi-U6/Neo-PKC α -siRNA vector was transfected into mIMCD3 cells. Western blotting was used to evaluate the suppression of PKC α expression in different cell groups.

Transfection

Transfection of mIMCD3 cells with the AQP2-GFP construct was performed using Lipofectamine 2000 (Shanghai, China) according to the manufacturer's instructions. The cells were maintained in medium containing 400 μ g/mL geneticin for 24 h after transfection. Individual neomycin-resistant colonies were selected and expanded for 14 d after transfection.

AQP2-GFP was stably expressed in mIMCD3 cells (AQP2-mIMCD3 cells) that were transiently transfected with a eukaryotic expression vector encoding the constitutively activated form of PKC α A/25E (pEF-nero-PKC α A/25E)^[26] using Lipofectamine 2000 according to the manufacturer's instructions. AQP2-mIMCD3 cells were transfected with three pGCsi-U6/Neo-PKC α shRNAs and scrambled shRNA using Lipofectamine 2000 according to the manufacturer's instructions to evaluate the inhibition efficiency of the siRNAs. The cells were harvested 48 h after transfection, and Western blot and immunofluorescence were used to evaluate the transfection efficiency.

Immunocytochemical staining

AQP2-mIMCD3 cells were divided into 3 groups: (1) Scrambled shRNA transfected; (2) PKC α A/25E vector transfected; and (3) PKC α shRNA transfected. The cells in each group were treated with 100 μ mol/L DdAVP for 30 min. DdAVP was removed, and the cells were incubated with culture medium for 2 h (washout). The cells were then fixed in 4% paraformaldehyde for 10 min, rinsed twice in PBS, and blocked for 15 min in a blocking/permeabilization solution (PBS containing 0.1% BSA and 0.3% Triton X-100). The cells were washed with PBS and pre-incubated in PBS containing 1% BSA for 20 min. The cells were incubated with an α -tubulin antibody (at a final concentration of 2 μ g/mL) at 4°C overnight. The cells were washed with PBS and incubated in the secondary goat anti-rabbit IgG conjugated with Cy3 antibody (at a final concentration of 2 μ g/mL) for 1.5 h at RT. Finally, the cells were washed twice with PBS and mounted with DAPI for confocal laser-scanning microscopy equipped with a CoolSNAP HQ camera.

Biotinylation of surface membrane proteins

Identifiably transfected AQP2-mIMCD3 cells were cultured on polylysine-coated 60-mm dishes for 48 h and treated with

DdAVP for 0 min, 30 min, or 30 min followed by a 2-h wash-out. The cells were washed three times with PBS/glycine (137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4 , 1.8 mmol/L KH_2PO_4 , pH 8.0, and 5 mmol/L glycine) and twice with PBS^[27]. An ice-cold biotinylation reagent (0.5 mg/mL in PBS) was immediately added to cells. The biotinylation reagent was removed after a 10-min incubation on ice, and the cells were washed three times with PBS/glycine.

Immunoblotting and immunoprecipitation

Transfected and DdAVP-treated cells were lysed in RIPA buffer (10 mmol/L Tris-HCl, 0.15 mol/L NaCl, 1% NP-40, 1% Na-deoxycholate, 0.5% SDS, 0.02% sodium azide, and 1 mmol/L EDTA, pH 7.4). The cell lysates were clarified by centrifugation at $1000\times g$ for 10 min at 4°C. Protein concentrations were measured using the bicinchoninic acid protein assay reagent kit (Pierce, Thermo Fisher Scientific, Beijing, China) and adjusted to the same concentration using lysine buffer. A 0.5-mL aliquot of the cell lysates was incubated with 50 μL of streptavidin-agarose beads at 4°C overnight to capture biotinylated proteins, 50 μL of protein A/G-agarose beads plus the AQP2 antibody to capture total AQP2, or 50 μL of protein A/G-agarose beads plus the PKC α antibody to capture total PKC α . The beads were pelleted, washed, and boiled for 5 min in 50 μL of cracking buffer (50 mmol/L Tris-HCl [pH 7.0], 10% glycerol, 2% SDS, and 2% β -mercaptoethanol). The eluted immunoprecipitates were separated on 10% polyacrylamide gels and transferred electrophoretically to PVDF membrane. The membranes were blocked for 90 min with blocking buffer during gentle shaking and incubated overnight with antibodies against AQP2 (0.5 $\mu\text{g/mL}$), phospho-S256-AQP2 (0.5

$\mu\text{g/mL}$), PKC α (0.5 $\mu\text{g/mL}$) or GAPDH (0.5 $\mu\text{g/mL}$). The membranes were incubated with an HRP-conjugated IgG (0.1 $\mu\text{g/mL}$) for 2 h at room temperature. Finally, the immunoreactive signals were visualized using an ECL reagent (Beyotime, Shanghai, China) and quantified using the Image J program (NIH, Bethesda, MD, USA).

Statistical analyses

The values are presented as the means $\pm$ SEM. The data were analyzed using SPSS software. Statistical significance of differences was assessed using unpaired *t*-tests or two-way ANOVAs. A value of $P<0.05$ was viewed as statistically significant.

Results

Inhibition of PKC α and the overexpression of AQP2 in mIMCD3 cells

All three PKC α shRNA plasmids (#1, #2, and #3) and PKC α scrambled shRNA plasmids were transfected into mIMCD3 cells to select the shRNAs with the highest inhibition efficiency to PKC α . Crude proteins were extracted from the harvested cells 48 h after transfection and subjected to Western blotting using the PKC α antibody. PKC α shRNA #1 plasmids exhibited the greatest inhibitory effect on PKC α , but all three PKC α shRNAs demonstrated visible inhibition ($P<0.01$) (Figure 1A).

The transfection of mIMCD3 with the AQP2-GFP-pCMV6 plasmid produced 12 geneticin-resistant clones, 10 of which were subjected to Western blot analysis (data not shown). The clone that amply expressed AQP2 was selected and named WT-7. A strong signal at 30 kDa in the selected AQP2-mIMCD3 stable cell line (WT-7) was observed, but no signal was detected in nontransfected mIMCD3 cells (Figure 1B).

Figure 1. Inhibition of PKC α and the overexpression of AQP2 in mIMCD3 cells. (A) PKC α shRNA plasmid #1, #2, #3, and PKC α scramble shRNA plasmid (control) were transfected into mIMCD3 cells respectively. Forty-eight hours after transfection, crude proteins extracted from harvested cells were subjected to Western blot using antibodies against PKC α . Representative blot was shown (left) and quantitative analysis of PKC α protein levels were normalized to control group ($n=3$ each group) (right). ^b $P<0.05$ vs control group. (B) AQP2-mIMCD3 stable cell line was generated by transfecting to express AQP2-GFP (See Materials and methods). The expression of AQP2-GFP was detected in selected WT7 stable cell lines. (C) AQP2-GFP stably expressed WT7 cells were transfected with scramble shRNA (Con), PKC α A/25E constructs or PKC α shRNA, respectively. Forty-eight hours after transfection, crude proteins extracted from harvested cells were subjected to Western blot using antibodies against PKC α (left). Quantitative analysis of PKC α protein levels were normalized to control group ($n=3$ each group) (right). ^b $P<0.05$ vs control group.

These results suggest the successful preparation of AQP2-mIMCD3 stable cell lines.

The WT-7 stable cell line was used to investigate PKC α expression following different transfections of PKC α scrambled shRNA, PKC α A/25E, and PKC α shRNA (#1) using Western blot. The transfection of PKC α A/25E significantly enhanced PKC α expression, but PKC α shRNA (#1) dramatically suppressed PKC α expression ($P < 0.05$) (Figure 1C). The WT-7 cell line and PKC α A/25E and PKC α shRNA (#1) plasmids were utilized in subsequent experiments.

DdAVP enhanced the phosphorylation of AQP2 and PKC α *in vitro*

The physical association of AQP2 with endogenous PKC α was examined *in vitro* in WT7 cell lines to reveal the direct role of PKC α on AQP2. Cells with or without DdAVP treatment for 30 min were lysed, and PKC α was immunoprecipitated using anti-PKC α antibodies. The immunoprecipitates were analyzed using Western blot and AQP2 antibodies. Strong signals for AQP2 and PKC α were detected in whole cell lysates and immunoprecipitates, but no AQP2 signal was detected in PKC α immunoprecipitates (Figure 2). These results suggest that PKC α did not interact with AQP2 *in vitro*. However, DdAVP treatment enhanced AQP2 phosphorylation at serine 256. Down-regulation of PKC α by shRNA did not alter DdAVP-mediated AQP2 phosphorylation at serine 256. These data indicated that PKC α did not influence DdAVP-induced AQP2 phosphorylation at serine 256 (Figure 2).

Figure 2. DdAVP enhanced the phosphorylation of AQP2 and AQP2 did not physically interact with PKC α *in vitro*. AQP2-GFP stably expressed WT7 cells were transfected with indicated constructs. Forty-eight hours after transfection, cells were treated with or without DdAVP for 30 min. Cells were then lysed and immunoprecipitation was performed with anti-PKC α and anti-AQP2 antibodies. Crude proteins from whole cell lysates and the immunoprecipitates were subjected to Western blot with indicated antibodies.

PKC α expression influenced the distribution of α -tubulin and DdAVP-mediated AQP2 trafficking in mIMCD cells

An increase in PKC α activity induces the re-organization of microtubules in proximal tubular cells^[9]. Therefore, PKC α -induced alterations in microtubule architecture were investigated. Most cells that were transfected with scrambled shRNA plasmid or PKC α shRNA plasmids exhibited a prominent dense network of microtubules around the nucleus with a considerably lower microtubule content in the cell periphery under basal conditions. The constitutive activation of PKC α increased microtubule formation in the cell periphery (Figure 3, upper panel and middle panel). AQP2-mIMCD3 cells (WT7 cells) were incubated with DdAVP for 30 min followed by a 2-h washout to evaluate the involvement of PKC α in AVP-induced microtubule formation in the cell periphery. Cells that were transfected with the control and PKC α A/25E plasmids displayed increased microtubule formation in the cell periphery following DdAVP stimulation. In contrast, the down-regulation of PKC α expression using shRNA inhibited DdAVP-induced microtubule depolymerization (Figure 3, lower panel). Taken together, these data strongly indicated that the expression of PKC α regulated microtubule distribution in mIMCD cells.

The role of PKC α in DdAVP-induced AQP2 trafficking was investigated because PKC α influences microtubule reorganization, and the DdAVP-induced depolymerization of microtubules affects the redistribution of AQP2 to the plasma membrane^[25, 28, 29]. Immunofluorescence analyses indicated that AQP2 was primarily localized around the nucleus under basal condition. Treatment with DdAVP for 30 min produced a pronounced increase in AQP2 localization in the plasma membrane. DdAVP washout restored the perinuclear localization of AQP2. The expression of constitutively activated PKC α produced a wide distribution of AQP2 throughout the cytoplasm in contrast to AQP2 localization around the nucleus under basal conditions. DdAVP also induced the translocation of AQP2 to the plasma membrane in the PKC α A/25E transfected group. The down-regulation of PKC α expression by shRNA interference inhibited DdAVP-induced AQP2 translocation to the plasma membrane (Figure 3).

Regulation of PKC α expression influenced DdAVP-stimulated plasma location of AQP2

mIMCD-3 surface membrane proteins were biotinylated following DdAVP treatment for 0 min, 30 min, and 30 min followed by a 2-h washout to confirm the immunofluorescence results. Biotinylated proteins were captured from whole-cell lysates using streptavidin beads, separated by SDS-PAGE and probed using an AQP2 antibody. Immunoprecipitation was performed in whole cell lysates in parallel using AQP2-specific antibodies. The DdAVP-induced increase in biotin-AQP2 was partially inhibited by PKC α down-regulation with shRNA transfection, but biotin-AQP2 was enhanced by the overexpression of PKC α A/25E (Figure 4). Moreover, different treatments only marginally affected the total amount of

Figure 3. PKC α expression influenced the distribution of α -tubulin and DdAVP-mediated AQP2 trafficking in mIMCD3 cells. AQP2-GFP stably expressed WT7 cells were transfected with PKC α cramble shRNA (Con), PKC α A/25E constructs or PKC α shRNA constructs. Forty-eight hours after transfection, cells were treated with or without DdAVP for 0 min, 30 min or 30 min followed by 2 h washout. Cells were then fixed, permeabilized and stained with antibodies against PKC α (green) and α -tubulin (red) for immunofluorescence. Nuclear was stained by DAPI (blue).

Figure 4. Regulation of PKC α expression influenced DdAVP stimulated plasma location of AQP2. (A) AQP2-GFP stably expressed WT7 cells were transfected with indicated constructs. Forty-eight hours after transfection, surface membrane proteins were biotinylated at 0 min, DdAVP treated 30 min, and DdAVP treated 30 min followed by 2 h washout. Whole cell lysates were subjected to avidin pull-down assay using streptavidin-agarose beads or immunoprecipitation (IP) using anti-AQP2 antibodies. The recovered proteins were separated by SDS-PAGE and analyzed by Western blot for AQP2 as described in Materials and Methods. For each condition, the detected signals from avidin-pull-down assay and AQP2 immunoprecipitation were from same SDS-PAGE gel. (B) Signals from 3 independent experiments were quantified by densitometry. The intensity of the AQP2 signals from the avidin pull-downs were normalized to the corresponding AQP2 IP signals under each condition. Data were represented as mean $\pm$ SEM. ^b $P<0.05$ vs DdAVP 0 min group. ^e $P<0.05$ vs DdAVP 30 min+2 h washout group. ^h $P<0.05$ vs DdAVP 30 min con group. ^k $P<0.05$ vs DdAVP 30 min PKC α A/25E group.

AQP2 as determined by AQP2 immunoprecipitation (Figure 4). These results were consistent with the AQP2 translocation observed by immunofluorescence detection and demonstrated that PKC α participated in DdAVP-mediated AQP2 translocation in mIMCD cells.

Discussion

PKC α exhibits different expression patterns in mouse and rat kidneys. For example, PKC α localizes in the proximal tubule of mice but not rats^[3,4]. Our previous study demonstrated that PKC α contributes to urine concentration in the mouse kidney^[5]. The use of cell lines that originate from mouse IMCD cells is reasonable because AQP2 is primarily expressed in collecting duct principal cells, and the effect of PKC α on urine concentrating occurs in the inner medulla. In the present study, AQP2 trafficking was examined in an mIMCD3 cell line that was derived from mouse IMCD and exhibited the properties of IMCD cells. However, immortalized mIMCD3 cells

lack AQP2 expression^[30], and AQP2 is only briefly expressed in primary cultured IMCD cells^[31]. Therefore, we generated AQP2-mIMCD3 stable cell lines for the following experiments. Our results demonstrated that AQP2 was located in intracellular vesicles in non-stimulated mIMCD3 cells. DdAVP treatment induced the translocation of AQP2 from the cytoplasm to the plasma membrane, and DdAVP washout reversed AQP2 localization from the plasma membrane to the cytoplasm (Figure 4). Therefore, AQP2-mIMCD3 cells provide an optimal cell model to investigate AQP2 trafficking.

AQP2 phosphorylation at serine 256 by PKA plays an important role in AQP2 trafficking^[32, 33]. However, the phosphorylation of AQP2 at serine 256 is not sufficient to maintain the presence of the water channel at the plasma membrane^[34], and the PKC-induced internalization of AQP2 in collecting duct cells does not depend on the phosphorylation state of AQP2^[35, 36]. Our study demonstrated that DdAVP increased AQP2 phosphorylation, and the inhibition of PKC α expression did not influence DdAVP-mediated AQP2 phosphorylation at serine 256, which is consistent with previous reports. These results strongly suggest that AQP2 phosphorylation at serine 256 is PKC α independent. However, the ability of PKC α to phosphorylate AQP2 at other phosphorylation sites, such as serine 231, remains unclear. Our results indicated that PKC α did not interact with AQP2 *in vitro*. Therefore, the ability of PKC α to mediate the trafficking of phosphorylated AQP2 requires further investigation.

The disruption of microtubules induces AQP2 translocation to the plasma membrane^[13, 23, 29], and PKC α is involved in cytoskeletal remodeling during cell motility, phagocytosis, neurite outgrowth and the regulation of cytoskeleton-associated proteins^[37-40]. Therefore, the impact of PKC α on the cytoskeleton in AQP2-mIMCD3 cells was investigated. Microtubules are part of the cytoskeleton and consist of α , β , and γ tubulins^[41]. α -Tubulin is the major cytoskeletal protein related to cell trafficking. The role of PKC α in the assembly of α -tubulin was determined. PKC α overexpression or inhibition produced a constitutively active form of PKC α or down-regulated expression of PKC α , respectively, which resulted in the de- and re-polymerization of α -tubulin, respectively. Moreover, PKC α down-regulation prevented DdAVP-mediated α -tubulin depolymerization. These data indicated that PKC α is involved in the assembly of α -tubulin in AQP2-mIMCD3 cells, which is consistent with previous reports^[9].

The microtubule-dependent transport of AQP2 is predominantly responsible for AQP2 trafficking and localization inside of the cell after its internalization, but it is not responsible for the exocytic transport of the water channel^[25]. In the present study, the overexpression of constitutively active PKC α produced a wide distribution of AQP2 throughout the cytoplasm, which is in contrast to its translocation to the plasma membrane. This result indicates that other factors/proteins rather than α -tubulin are required for AQP2 translocation to the plasma membrane^[40]. Furthermore, the consistent inhibition of α -tubulin depolymerization by the down-regulation of PKC α expression produced a loss in DdAVP-mediated AQP2

translocation. This result confirms that α -tubulin is a key element for the proper localization of AQP2 in the cytoplasmic compartment.

In summary, the use of stably expressing AQP2-mIMCD3 cells demonstrated that PKC α did not functionally associate with AQP2. The down-regulation of PKC α expression altered the distribution of α -tubulin and inhibited DdAVP-mediated AQP2 trafficking. However, constitutively activated PKC α rescued or aggravated these changes. These data directly demonstrated that PKC α mediates AQP2 trafficking by influencing the assembly of α -tubulin and underscore the complexity of the molecular events of PKC α -mediated urine concentration.

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Author contribution

Dr Li-jun YAO designed the research and wrote the paper; Dr Hong ZHAO performed biotinylation of cell surface protein experiment; Xi YAO performed Western blot and immunocytochemistry experiment; Tao-xia WANG prepared plasmids and analysed data; Wen-min JIN performed cell culture experiment; Qian-qian JI contributed reagents preparation; Dr Xiao YANG wrote the paper; Dr Qiu-hong DUAN performed the research.

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