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Polycystin-1, 2, and STIM1 Interact with IP_3R to Modulate ER Ca^{2+} Release through the PI3K/Akt Pathway

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Key Words

Polycystic Kidney Disease (ADPKD) • Cyst • Calcium • PI3K/Akt • Polycystin-1 • Polycystin-2 • IP_3R • STIM1

Abstract

Dysregulation of Ca^{2+} signaling and homeostasis has been linked to the development of ADPKD through aberrant functioning of the polycystins. In this study, we investigated the role of the polycystins in modulating Ca^{2+} signaling. Expression of full-length PC1 in MDCK cells inhibited intracellular Ca^{2+} release in response to ATP when compared to control cells. This phenotype correlated with reduced interaction of endogenous PC2 and IP_3R in PC1-containing cells. We also found that endogenous STIM1 also interacted with the IP_3R, and this interaction was enhanced by PC1 expression. Increased interaction between STIM1 and IP_3R inhibited Ca^{2+} release. PC1 regulates intracellular Ca^{2+} release and the interaction of PC2-IP_3R-STIM1 through the PI3K/Akt signaling pathway. Inhibition of the PI3K/Akt pathway in PC1 containing cells restored intracellular Ca^{2+} release, increased the interaction between PC2 and IP_3R and disrupted the STIM1-IP_3R complex. Conversely, activation of the PI3K/Akt signaling pathway by HGF in control MDCK cells gave the reverse effects. It reduced the release of Ca^{2+} to levels comparable to the PC1 cells, inhibited the association PC2 and IP_3R, and increased the interaction between STIM and IP_3R. Overall, our studies provide a potential mechanism for the modulation of intracellular Ca^{2+} signaling by the polycystins.

Introduction

Autosomal Dominant Polycystic Kidney disease (ADPKD), caused by mutations in either PKD1 and PKD2, is characterized by formation of fluid-filled cysts in kidney tubules [1]. Development of cysts, interferes with normal kidney function and eventually leads to kidney failure [2]. About 85% of ADPKD cases are caused by mutations in the *PKD1* gene, whereas the remaining 15% are caused by mutations in the *PKD2* gene [3]. The *PKD1* gene, encodes a transmembrane protein Polycystin-1 (PC1), and *PKD2* gene encodes another membrane protein called Polycystin-2 (PC2) [4, 5]. Currently, molecular mechanisms of how mutations in either of these genes can cause development of fluid-filled cysts in the

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kidney are still not well understood. However, the clinical course resulting from mutations in either PC1 or 2 is similar suggesting that both proteins function in a common pathway. Possible treatment for the disease is mainly to inhibit enlargement of the existing cysts, but does not prevent new cystogenesis. Therefore understanding the function of PC1 and PC2 in the normal kidney tubules is important for the development of a more effective treatment of ADPKD.

PC1 is a large transmembrane protein that has 4302 amino acids and 11 transmembrane domains. PC1 has a long extracellular N terminal domain compared to the intracellular C-terminus [6]. It is hypothesized that the N-terminal domain of PC1 is involved in fluid flow sensing, whereas the C-terminal domain containing the coiled-coil domain is important for signaling and interaction with other proteins, such as PC2 [7-9]. PC1 can be found at the cell-cell junction, plasma membrane, primary cilia, and also the endoplasmic reticulum (ER) [8, 10-12].

PC2 is a smaller transmembrane protein than PC1. It has 968 amino acids with 6 transmembrane domains. Its N-and C-terminal domains are intracellular. This protein is similar to other members of the transient receptor potential (TRP) channel family of proteins [13]. As with other TRP proteins, PC2 functions as a cation channel. PC2 is located in the ER, where it modulates the functioning of IP_3R [14, 15]. PC2 interacts with inositol 1,4,5 triphosphate (IP_3R) to prolong the IP_3-induced Ca^{2+} release [16]. PC2 is also located both at the plasma membrane and at the primary cilia, where it participates in flow-dependent Ca^{2+} signaling [8, 12].

While function of PC2 is more clearly defined, the precise function of PC1 in Ca^{2+} homeostasis is still elusive. Patch clamp and other electrophysiology studies suggest that PC1 does not by itself function as a cation channel [8, 17]. However, since mutations of either PC1 or PC2 cause the same phenotype, and alterations of Ca^{2+} homeostasis has been observed in ADPKD cells [18], one might hypothesize that PC1 can also regulate intracellular Ca^{2+} signaling. Indeed we showed that PC1 inhibits ER Ca^{2+} release by binding to IP_3R [11]. We also showed that PC1 can bind to stromal interaction molecule 1 (STIM1), sequestering it to the ER membrane and inhibiting store operated Ca^{2+} entry (SOCE) [19]. Thus, PC1 modulates intracellular Ca^{2+} signaling by binding to key elements well known to be involved both in receptor-mediated Ca^{2+} release and SOCE.

Given that both PC1 and 2 can modulate pathways involved in Ca^{2+} homeostasis, the question arises regarding of how malfunctioning proteins in ADPKD cause aberrations in intracellular Ca^{2+} and lead to cyst formation? Increased cell proliferation is associated with cyst growth [2], and frequently increases in intracellular Ca^{2+} result in enhanced cell proliferation [20]. For example expression of the C-terminus of PC1 enhanced cell proliferation of HEK293 cells through an increase in intracellular Ca^{2+} release [21]. Whereas, inhibition of receptor-mediated-increases in intracellular Ca^{2+} due to loss of PC2 in ADPKD lymphoblastoid cells actually dampens cell proliferation [22]. However, some studies have shown otherwise that the steady state levels of intracellular Ca^{2+} are lower in cyst cells a condition that is associated with an increase in cell proliferation when stimulated with cyclic adenosine monophsophate (cAMP) [23, 24]. Furthermore, another study shows when cyst cells were treated with a Ca^{2+} ionophore to increase intracellular Ca^{2+}, the ability of cAMP to inhibit cell proliferation was restored [25]. How both high and low intracellular Ca^{2+} can result in enhanced cell proliferation in ADPKD cells is still an open question. Clearly maintenance of intracellular Ca^{2+} homeostasis is important for normal tubule development. In this study, we investigated how the polycystins work together to modulate Ca^{2+} signaling.

Materials and Methods

Materials

Monoclonal antibodies recognizing STIM1 and IP_3R-3 were purchased from BD Biosciences (San Jose, CA), polyclonal antibody against STIM1 was from Sigma (St. Louis, MO). Monoclonal antibody for PC1 (7E12), monoclonal and polyclonal antibodies for PC2 were all from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibody against p-Akt and total Akt were purchased from Cell Signaling Technology. Protein A/G agarose beads were purchased from Santa Cruz Biotechnology. Hepatocyte growth factor was from Biomol. LY294002 was from Cell Signaling Technology, and wortmannin was purchased from Sigma. Both ionomycin and thapsigargin (TG) were purchased from Invitrogen, ATP (adenosine triphosphate) was from Sigma. The fluorescent Ca^{2+} indicator, Fura-2/AM was from Invitrogen. Plasmid STIM1-YFP was a gift from Dr. Paul Worley's lab, while PKD1-GFP, R4227X plasmids, and antibody to PC1 (LRR) were gifts from Dr. Gregory Germino's group.

Cell culture and transfections

Stable MDCK cell lines were a gift from Dr. Gregory Germino lab (Johns Hopkins, Baltimore). The cells were cultured as described previously [26]. M1 cells were cultured in Dulbecco's modified Eagle's medium/F-12 (DMEM/F-12) supplemented with 10% fetal bovine serum and 5% Penicilin/ Streptomycin. For transfection, cells were split and grown until

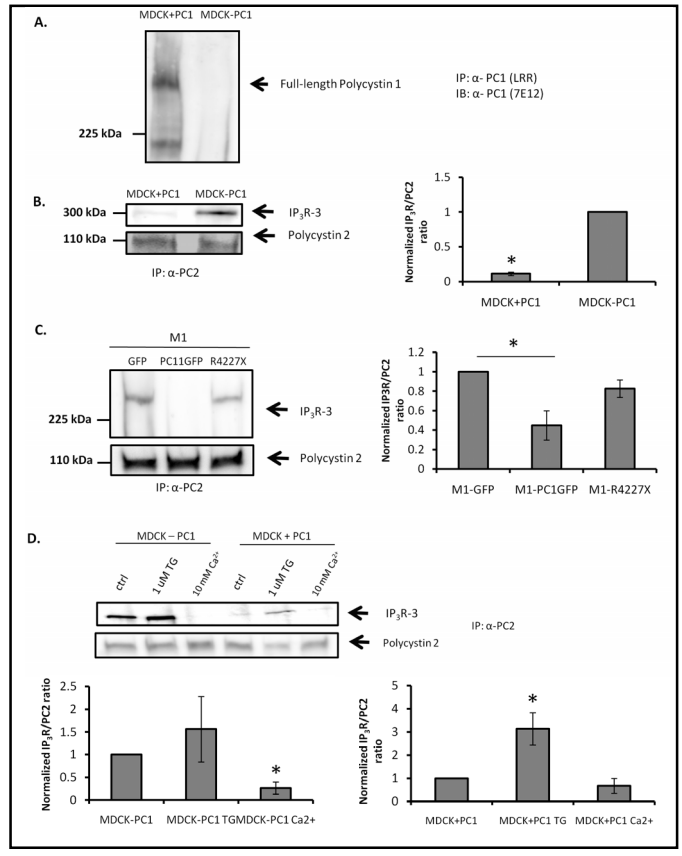

Fig. 1. Expression of PC1 decreases the PC2 and IP_3R interaction. A. Expression of full-length PC1 in MDCK stable cell line. PC1 antibody was able to pull down full-length PC1 only in MDCK+PC1 cells but not in control cells, MDCK – PC1. B. LEFT BLOTS: Co-immunoprecepitation of endogenous IP_3R and Polycystin 2 by PC2 antibody. Note that there was more IP_3R pulled down in control cells without PC1 expression (MDCK–PC1). RIGHT GRAPH: Densitometry analysis for the co-immunoprecipitation experiments (MDCK + PC1 n=5 and MDCK – PC1 n=5). Density of the co-immunoprecipitated IP_3R was normalized against density of the immunoprecipitated PC2. The resulting ratio for control cells without PC1 expression was considered 1, whereas the ratio for PC1 cells was normalized against the ratio from control cells to calculate the final value. Asterisk indicate significance between the two groups (student's t-test, $p<0.001$). C. LEFT: Transient transfection of PC1 (M1-PKD1GFP) inhibited pull-down of endogenous IP_3R in M-1 cells, while control (M1-GFP) and mutant PC1 (M1-R4227X) did not. RIGHT: Densitometry analysis of the co-immunoprecipitation (GFP n=4; PKD1GFP n=4, R4227X n=4) showed a reduction of the PC2-IP_3R association in PC1-transfected M-1 cells (M1-PKD1GFP). The ratio for GFP-transfected cell was considered 1, while the ratios for the other groups were normalized against the GFP cells. Asterisks indicates the significance between PKD1GFP group and control (student's t-test, $p<0.05$). D. Co-immunoprecipitation of endogenous IP_3R by PC2 antibody in MDCK stable cell lines after treatment with Thapsigargin (TG) and high extracellular Ca^{2+} (10 mM Ca^{2+}). LOWER PANEL: The graphs show the densitometry analysis of the co-immunoprecipitation. The data is the average of three independent experiments. Asterisk indicates statistical significance in the treatment groups (TG and 10 mM Ca^{2+}) against control (student's t-test, $p<0.05$) in each cell line. The ratio for MDCK cells without treatment was considered 1, while the ratios for the treatment groups were normalized against this group.

confluence. One or two days after confluence, cells were transfected with various amounts of DNA with Lipofectamine 2000 (Invitrogen). Transfection procedure was carried out according to the manufacturer's instructions. Cells were then harvested and assayed 24-48 hrs after transfection.

Adenoviral infection

Cells were grown until confluent and completely polarized. Adenovirus stock was diluted in the regular cell medium to achieve a final concentration of 50 m.o.i.(multiplicities of infection). Cells were then harvested or assayed two days after the infection.

PI3K-HGF assay

For PI3K inhibitors and HGF treatment, cells were cultured until confluent and polarized. Cells were then serum-starved two hours before treatment. PI3K inhibitors (15 μM LY294002 and 50 nM wortmannin) and 5 ng/ml of HGF were added into the media without serum for the treatment. Cells were incubated overnight before analyzing.

Western blot and co-IP

For western blot, cells were harvested and lysed with lysis buffer (composition: 50 mM NaCl, 150 mM Tris-HCl, 1% Nonidet P-40, 10% glycerol, pH 7.4, protease inhibitor was added before using). After incubating on ice for 30 mins, cells were then spun down for 15 mins at 14,000 *x g* at 4°C to remove the insoluble material. Supernatant of the samples were collected. Sample concentration was measured with the BCA reagent, and denatured in 2x Laemmli buffer at 42°C for 30 mins. Samples were then run in SDS-PAGE gel before transferring to polyvinylidene fluoride membrane. For co-IP experiments, samples were obtained as in the western blot assay (lysis buffer composition: 50 mM NaCl, 150 mM Tris-HCl, 0.1% Nonidet P-40, 10% glycerol, pH 7.4, protease inhibitor was added before using), monoclonal antibody to either PC2 or STIM1 was added to the samples afterwards and allowed to rotate overnight. Protein A/G agarose beads were then added the next day before rotating for 2 additional hours. The beads were collected by centrifugation and washed three times with phosphate buffer solution (PBS). After the final wash, the collected beads were resuspended in the 2x sample buffer (equal volume of sample buffer was added to the sample) and incubated at 42°C for 30 mins for the immunoblot assay. For TG and Ca^{2+} addition experiments, cells were treated with either 1 μM TG or 10 mM extracellular Ca^{2+} for 10 mins before lysing.

Fura-2 Ca^{2+} imaging assay

Fura-2 ratiometric imaging assay was performed as described previously [11]. Cells were bathed in zero Ca^{2+} ringer's

buffer, with composition as follow: 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 10 mM HEPES (pH adjusted to 7.4 with NaOH). 100 μM of ATP was given through perfusion system with the ATP stock was previously dissolved in the same Ringer's buffer. For ionomycin experiment, 2 μM ionomycin was added directly to the cells on the coverslip during the recording without disturbing the recording process. Image acquisition and analysis were done with the IPLab software.

Results

Both PC1 and ER Ca^{2+} modulate the interaction of PC2 and IP_3R

Previously, our lab showed that PC2 is present in the ER and interacts with IP_3R [16]. To extend this observation, here we addressed whether PC1 plays any role in modulating the interaction between PC2 and IP_3R. To address this question, we used a previously described MDCK cell line [27] that stably expresses full-length PC1 (Fig. 1A). This cell line contains PC2 and IP_3R type 3 endogenously. We first confirmed that PC2 does indeed interact with IP_3R in MDCK cells, as reported previously. However, the results also demonstrate a significant reduction in the interaction between endogenous PC2 and IP_3R in PC1-expressing cells (Fig. 1B; PC1 cell MDCK+PC1 in comparison to control cells MDCK-PC1). The same result was also seen in different clones of this stable cell line [26] (data not shown). We further confirmed the observation by transiently transfecting GFP-tagged PC1 into M1 cells, a cell line derived from mouse collecting duct [28] Similar to MDCK stable cell lines, expression of full-length PC1 in M1 cells also reduced the interaction of endogenous PC2 and IP_3R compared to the GFP-transfected cells (Fig. 1C). In contrast expression of the disease-causing mutation of PC1, R4227X, which destroys the coiled-coil domain and the C-terminus tail, did not interfere with interaction between endogenous PC2 and IP_3R in M1 cells (Fig. 1C).

Because we observed previously that PC2 prolongs the $t_{1/2}$ of the Ca^{2+} release from the ER, we asked whether the interaction of PC2 with IP_3R also depends on the level of intracellular Ca^{2+}. Treatment with thapsigargin (TG) that depletes ER Ca^{2+} stores by blocking the Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump [29] increased the association of endogenous PC2 to IP_3R specifically in PC1 cells, while addition of 10 mM Ca^{2+} in regular medium to prevent ER Ca^{2+} depletion [30] reduced the interaction in cells without PC1 (Fig . 1D). We also validated the results with different clones of the stable cell line (data not shown). Taken together, we conclude that expression of full-length PC1 and intracellular Ca^{2+} level modulate the interaction of PC2 and IP_3R. In addition, the C-terminus of PC1 is important in mediating inhibition of the interaction of PC2 and IP_3R.

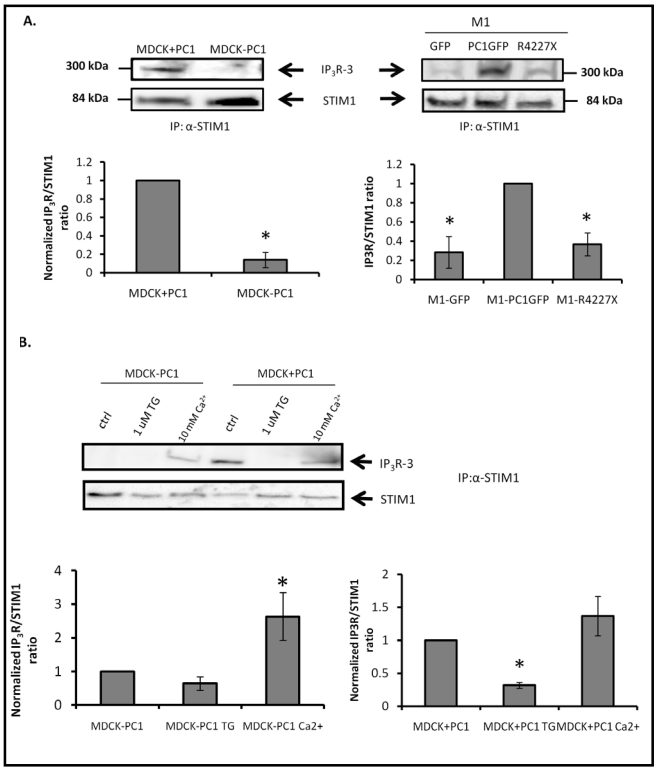

Fig. 2. Expression of PC1 enhances the association of STIM1 and IP_3R. A. LEFT BLOT: Western blot shows co-immunoprecipitation of endogenous IP_3R by STIM1 antibody in PC1 (MDCK+PC1) and control (MDCK-PC1) cells. There were more IP_3R being pulled down by STIM1 antibody in PC1 cells compared to control cells. RIGHT BLOT: Transient transfection of PC1 into M-1 cells (M1-PKD1GFP) increased STIM1-IP_3R co-immunoprecipitation, whereas transfection of GFP (M1-GFP) and PC1 mutant plasmids (M1-R4227X) did not increase the STIM1-IP_3R interaction. LOWER PANEL: Densitometry analysis of STIM1-IP_3R co-immunoprecipitation in MDCK stable cell line (left graph), and in M1 cell line (right graph). The data is the average of three sets of independent experiments. Asterisk indicates significance between PC1 cells and control (student's t-test, $p<0.001$). For M-1, asterisk shows statistical significance between GFP and R4227X-transfected cells when compared to PKD1GFP ($p<0.005$, student's t-test). B. Western blot shows co-immunoprecipitation of endogenous IP_3R by STIM1 antibody after treatment with TG and high extracellular Ca^{2+}. LOWER PANEL: The graphs show the densitometry analysis of the co-immunoprecipitation. The data is the average of three sets of experiments. Asterisk indicates significance between the treatment groups (TG and 10 mM Ca^{2+}) and control (student's t-test, $p<0.05$) in each cell line. The ratio for MDCK cells without treatment (ctrl) was considered 1, while the ratios for the treatment groups were normalized against the ctrl.

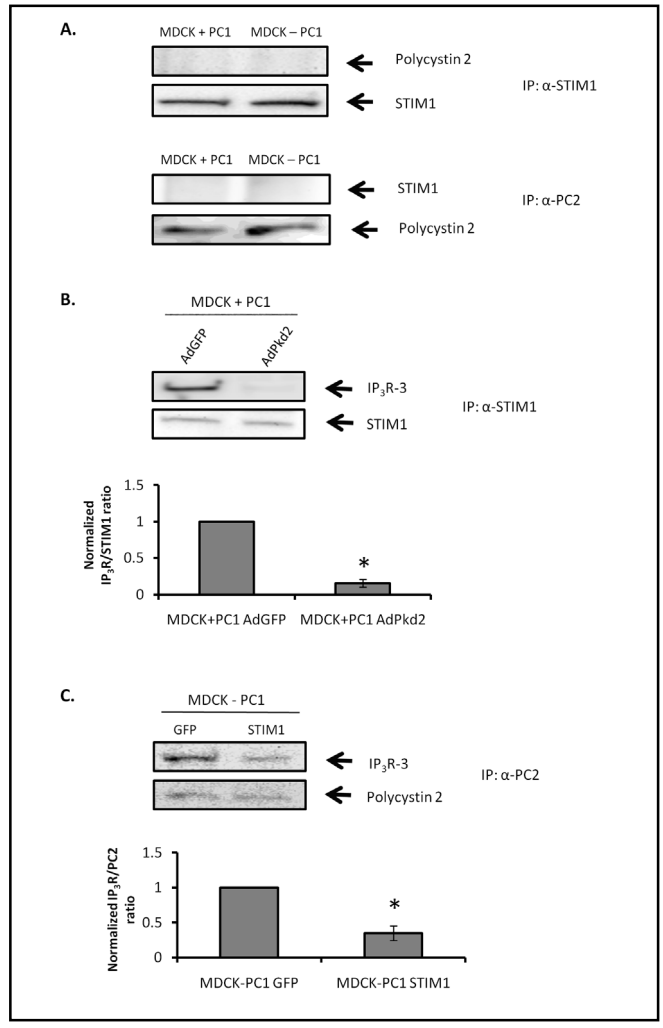

Fig. 3. PC2 competes with STIM1 for forming a complex with IP_3R. A. TOP BLOTS: Western blot shows co-immunoprecipitation of endogenous PC2 by STIM1 antibody in MDCK stable cell line. STIM1 antibody did not pull-down PC2 both in PC1 and control cells. BOTTOM BLOTS: Co-immunoprecipitation of endogenous STIM1 by PC2 antibody. PC2 antibody did not pull-down STIM1 both in PC1-containing and control cells. B. Western blot shows co-immunoprecipitation of endogenous IP_3R by STIM1 antibody in PC1 cells. Infection of PC2 adenovirus reduced IP_3R pulldown by STIM1 antibody, while GFP infection did not. LOWER PANEL: The graph shows the densitometry analysis of the co-immunoprecipitation experiments. Asterisk indicates statistical significance between the two groups (student's t-test, $p<0.005$). The data represents three set of independent experiments. The ratio for GFP adenovirus group was considered 1, and the ratio for PKD2 adenovirus group was normalized against this group. C. The blot shows co-immunoprecipitation of endogenous IP_3R by PC2 antibody in control cells. Transfection of STIM1 plasmid reduced co-immunoprecipitation of IP_3R by PC2 antibody, while PC2 still pulled-down IP_3R in the GFP-transfected cells. LOWER PANEL: Densitometry analysis of the co-immunoprecipitation. The data is the average of three sets of experiments. Asterisk indicates statistical significance between the two marked groups (student's t-test, $p<0.001$). The ratio for GFP-transfected group was considered 1, and the ratio for STIM1-transfected group was normalized against this group.

PC1 expression increases the interaction of STIM1 and IP_3R

We were intrigued by the observation that the interaction of PC2 and IP_3R is regulated by ER Ca^{2+} because both PC2 and IP_3R have their Ca^{2+} sensors, the EF-hand, on their cytoplasmic side [31, 32]. Another ER protein, STIM1, has been shown to act as an ER Ca^{2+} sensor. When ER Ca^{2+} is low, STIM1 translocates near to the plasma membrane and activates the store-operated Ca^{2+} channel. In addition, STIM1 has its EF-hand domain on the ER luminal side [33], therefore, it serves as a good candidate for the ER Ca^{2+} sensor that regulates the PC2-IP_3R interaction.

We then tested whether STIM1 is involved in regulating the PC2-IP_3R interaction. We first confirmed that the STIM1 antibody indeed could pull down endogenous IP_3R. Interestingly, the data showed an increased association of STIM1 and IP_3R in PC1-expressing cells (Fig. 2A). Thus, we hypothesized that PC1 expression increased the interaction of STIM1 and IP_3R. We tested this hypothesis by transiently transfecting full-length PC1 into M-1 cells. The data showed an increase of the STIM1 and IP_3R interaction, in comparison to control GFP transfection. Again, transfection of the PC1 mutant, R4227X, did not enhance the ability of STIM1 to pull down IP_3R (Fig. 2A, right blot). Thus, similar to PC2, the C-terminus of PC1 is also important in regulating the interaction of STIM1 and IP_3R, although in the reverse way. Finally, we observed that the interaction between endogenous STIM1 and IP_3R was also regulated by ER Ca^{2+}, however in a manner opposite to what occurs when PC2 and IP_3R interact. Depletion of the ER Ca^{2+} stores with TG decreased the interaction of STIM1 with IP_3R in PC1 cells, while addition of high extracellular Ca^{2+} increased the STIM-IP_3R complex in control cells without PC1 expression (Fig. 2B). Overall, we found that STIM1 interacts with IP_3R, and this interaction is enhanced by PC1 expression.

PC2 and STIM1 compete for IP_3R

Up to this point, our data showed that the amount of ER Ca^{2+} regulates interactions between PC2-IP_3R and STIM-IP_3R in the opposite way and that expression of PC1 increased the interaction of STIM1 and IP_3R but reduced the association of PC2 with IP_3R. We wondered whether STIM1 and PC2 actually compete to interact with IP_3R. We first addressed whether PC2 and STIM1

can also make a complex. Co-immunoprecipitation of PC2 by STIM1 antibodies failed to pull-down PC2, and PC2 antibody could not pull down STIM1 (Fig. 3A). These results showed that binding of PC2 and STIM1 as detected by this pull-down assay does not occur, although both can interact with IP_3R. To further confirm the competition of both proteins for the IP_3R, we over expressed either STIM1 or PC2 to see whether over expression of one of the proteins disrupts the other protein's interaction with the IP_3R. Indeed, we found that over expression of PC2 by adenovirus infection in PC1-expressing MDCK cells disrupted the endogenous association of STIM1 and IP_3R. There was less pull-down of endogenous IP_3R by STIM1 in PC2-infected cells (MDCK+PC1 AdPkd2) in comparison to GFP-infected cells (MDCK+PC1 AdGFP) (Fig. 3B). Similarly, transfection of STIM1-YFP plasmid into control MDCK cells (MDCK-PC1 STIM1) also reduced pull down of endogenous IP_3R by PC2 antibodies, which suggests interference in the ability of PC2 to interact with IP_3R (Fig. 3C). Thus, these results further establish that PC2 and STIM1 compete for interaction with IP_3R.

ER Ca^{2+} release is altered depending upon whether STIM1 or PC2 is associated with the IP_3R

Since PC1 affects interaction of PC2 and IP_3R, we then asked whether it also affects release of Ca^{2+} from the ER. To answer this question we treated the cells with adenosine triphosphate (ATP). ATP induces a release of Ca^{2+} in MDCK cells through activation of a purinergic receptor in the plasma membrane. Downstream signaling produces IP_3 which activates the IP_3R [34]. We conducted experiments in the absence of extracellular Ca^{2+} so that when the cells are stimulated with ATP, most of the increase in cytoplasmic Ca^{2+} comes from Ca^{2+} released through the IP_3R in the ER. The data showed that intracellular Ca^{2+} release following ATP-stimulation was significantly smaller in the cells containing PC1 (Fig. 4A). To rule out the possibility that the reduced release of Ca^{2+} was caused by alterations in the level of Ca^{2+} within the ER, we measured the response to the Ca^{2+} ionophore, ionomycin, in the absence of extracellular Ca^{2+}. We chose to use ionomycin because Weber et al. already showed by using the same cell line that the TG response was smaller in PC1 cells due to a smaller ER Ca^{2+} leak. Direct measurements of ER Ca^{2+} with Mag-Fura 2 by this group did not show any difference in ER Ca^{2+} level despite a change in the leak rate [35]. In line with their result, the response to ionomycin was similar in control and PC1-expressing cells (sup. Fig. 1A). This suggests

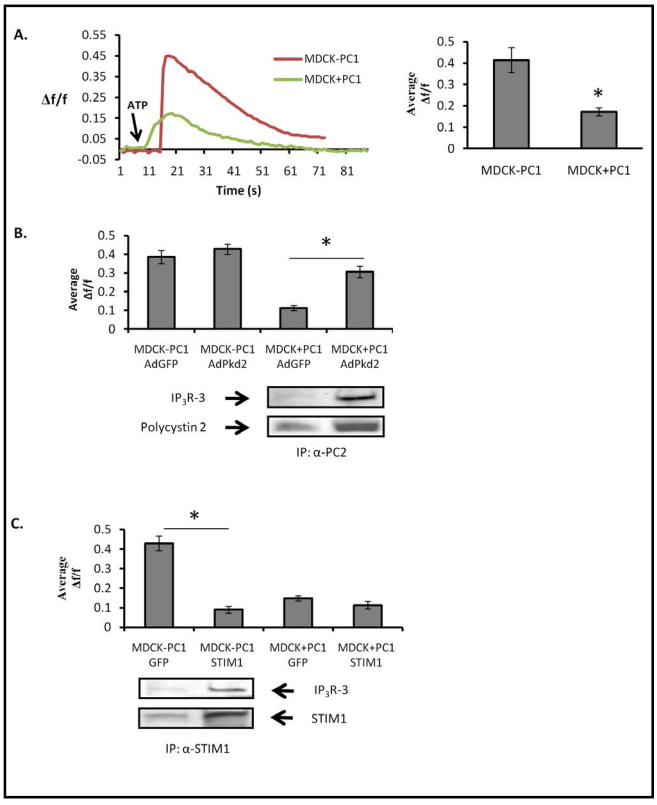

Fig. 4. Expression of PC1 inhibits ER Ca^{2+} release by increasing the STIM1-IP_3R interaction and disrupting the PC2-IP_3R interaction. A. Representative traces of intracellular Ca^{2+} release in response to 100 µM ATP in PC1-expressing and control MDCK cells. PC1 cells showed a reduction of Ca^{2+} release after stimulation with ATP. LEFT GRAPH: The graph summarizes the average amplitude of ER Ca^{2+} release in response to ATP. Asterisk indicates significance between the two groups (student's t-test, $p<0.001$, MDCK+PC1 n=7, MDCK – PC1 n=7). B. The graph summarizes the average amplitude of ER Ca^{2+} release after addition of ATP in PC2-infected cells compared to GFP-infected cells. Infection of PC2 adenovirus in PC1 cells restored the amplitude of ER Ca^{2+} release in response to ATP. Asterisk indicates significance between the two indicated groups (student's t-test, $p<0.001$, MDCK-PC1 AdGFP n=7, MDCK-PC1 AdPkd2 n=6, MDCK+PC1 AdGFP n=6, MDCK+ PC1 AdPkd2 n=5). BOTTOM PANEL: Western blot shows an increase of co-immunoprecipitation of endogenous IP_3R by PC2 antibody in PC1 cells after infection with PC2 adenovirus. C. The graph shows the average amplitude of ER Ca^{2+} release in response to ATP after transfection with STIM1-YFP plasmid.Transfection of STIM1 significantly reduced ATP-induced Ca^{2+} release in control MDCK cells. Asterisk indicates statistical significance between the two marked groups (student's t-test, $p<0.001$, MDCK-PC1GFP n=6, MDCK-PC1 STIM1 n=6, MDCK+PC1 GFP n=5, MDCK+PC1 STIM1 n=6). BOTTOM PANEL: The blot shows an increase of co-immunoprecipitation of endogenous IP_3R by STIM1 antibody in control cells after transfection with STIM1 plasmid.

that the reduced ability of ATP to increase intracellular Ca^{2+} in PC1 cells was not due to a change in the amount of Ca^{2+} in the ER, but rather due to changes in IP_3R-mediated Ca^{2+} release from the ER.

To address whether PC1's reduction of Ca^{2+} release through the IP_3R was caused by disruption of the association of PC2 and IP_3R, we over expressed PC2 by adenoviral infection, and measured intracellular Ca^{2+} release stimulated by ATP. Overexpression of PC2 rescued the association of PC2 with IP_3R especially in PC1-expressing cells (Fig. 4B). The transient peak of the ATP-mediated increase in Ca^{2+} was significantly bigger in PC2 –infected cells compared to control, which was infected with GFP adenovirus. Again, we measured the ionomycin response in the GFP- and PKD2- infected-PC1 cells, and we found that there was no significant difference between the two groups (sup. Fig. 1B, left graph). Therefore, our results are consistent with the previous observations that expression of PC2 enhances intracellular Ca^{2+} release from the ER [15, 16]. Furthermore we confirm the recent finding that the interaction of PC2 with IP_3R is important in facilitating the release of Ca^{2+} from the ER [36].

Another thing we noticed was when we overexpressed PC2 in PC1 cells the interaction of STIM1 and IP_3R was disrupted (Fig. 3B). To take this one step further we investigated whether the association of STIM1 and IP_3R also has functional consequences as is the case for the PC2-IP_3R interaction. STIM1 is widely known as a sensor of luminal ER Ca^{2+}. STIM1 translocates from the ER-PM junction to activate SOCE at the plasma membrane when the level of ER Ca^{2+} drops [33]. However, whether STIM1 functions in the basal condition, when ER Ca^{2+} is not depleted, is still not known yet. We over expressed STIM1 in control and PC1-expressing MDCK cells and measured the release of Ca^{2+} stimulated by ATP in the absence of extracellular Ca^{2+}. Transient transfection of STIM1-YFP cDNA into control cells with the purpose of increasing the STIM1-IP_3R interaction significantly reduced ATP-mediated-intracellular Ca^{2+} release to a level similar to PC1-expressing cells (Fig. 4C). This reduction was not due to pre-emptied ER Ca^{2+}, since the Ca^{2+} responses from ionomycin in both GFP and STIM1-transfected cells were similar (sup. Fig. 1B, right graph). Meanwhile, PC1-expressing cells did not show any significant reduction of the Ca^{2+} response to ATP when transfected with STIM1. Taken together, our results suggest that STIM1, by competing with PC2 for binding to IP_3R, also has a functional effect opposite to that of PC2. PC2's interaction with the IP_3R increases

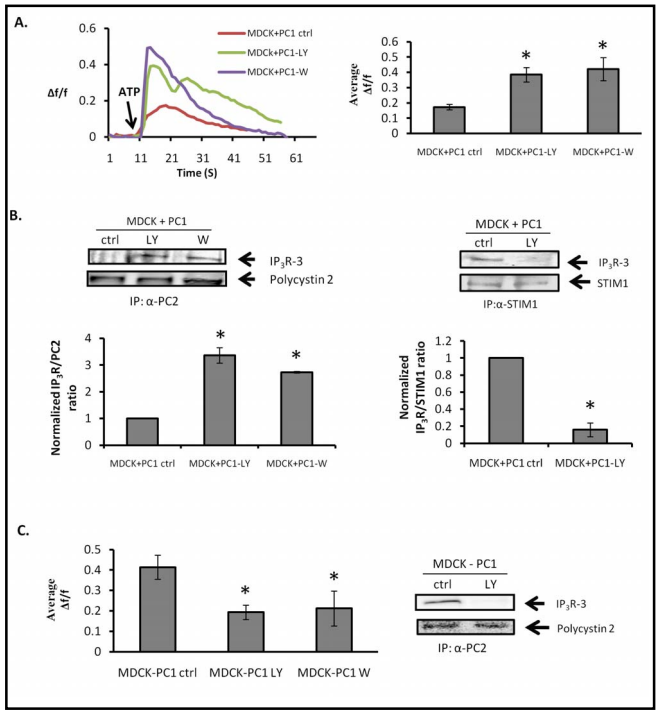

Fig. 5. Inhibition of the PI3K/Akt pathway in the PC1 cells increases ER Ca^{2+} release. A. Representative traces of ER Ca^{2+} release in response to ATP in PC1 cells that have been treated with PI3K inhibitors. Inhibition of the PI3K pathway restored ER Ca^{2+} release in response to ATP. RIGHT PANEL: Histogram shows the average amplitude of ER Ca^{2+} release in response to ATP in PC1 cells after inhibition of the PI3K pathway. Asterisk indicates statistical significance between treatment groups (LY or W) against the control (student's t-test, $p<0.001$, MDCK+ PC1 ctrl n=7, MDCK+PC1 LY n=6, MDCK+PC1 W n=6). B. The blots display co-immunoprecipitation of endogenous IP_3R by PC2 antibody (left blot) and by STIM1 antibody (right blot) in PC1 cells that were treated with PI3K inhibitors. Inhibition of the PI3K pathway in the PC1 cells restored PC2-IP_3R pulldown and inhibited STIM1-IP_3R co-immunoprecipitation. LOWER PANEL: The graphs show the densitometry analysis of co-immunoprecipitation of PC2 and IP_3R (left graph) and STIM1 and IP_3R (right graph). The ratio for the ctrl group was considered 1, and the ratios for the treatment group were normalized against control. The data is the average of three sets of experiments. Asterisk indicates significance between treatment group and control (student's t-test, $p<0.05$). C. Histogram shows the average amplitude of intracellular Ca^{2+} release in response to ATP in control cells that were treated with PI3K inhibitors (MDCK-PC1ctrl n=7, MDCK-PC1 LY n=7, MDCK-PC1 W n=6). Inhibition of the PI3K pathway in control cells also inhibited the increase in intracellular Ca^{2+} induced by ATP. Asterisk indicates statistical significance between the treatment group against control (student's t-test, $p<0.005$). RIGHT PANEL: Immunoblot shows reduction in co-immunoprecipitation of endogenous IP_3R by PC2 antibody in control MDCK cells after treatment with LY294002.

ER Ca^{2+} release, whereas STIM1-IP_3R inhibits it perhaps by directly inhibiting Ca^{2+} release or strengthening the interaction of PC1 with IP_3R.

The PI3K/Akt signaling regulates ER Ca^{2+} release and interactions between PC2, IP_3R and STIM

In PC1-containing cells, Akt phosphorylation is greater compared to control, suggesting that the PI3K/Akt pathway is already activated (Fig. 6C, Ser 473-Akt). In order to investigate the effect of activation of the PI3K/Akt pathway in PC1 cells, we inhibited this signaling pathway by exposing the cells to PI3K inhibitors, LY294002 and wortmannin. The inhibition restored intracellular Ca^{2+} release induced by ATP (Fig. 5A), whereas the ionomycin response were similar in all groups (sup. Fig. 1C, top panel). Furthermore, we also saw that treatment with LY294002 enhanced the interaction of PC2 and IP_3R, and inhibited the STIM1-IP_3R complex (Fig. 5B). On the other hand, treatment of cells without PC1 with PI3K inhibitor showed a reduction of ER Ca^{2+} release in response to ATP and a decrease in the PC2 and IP_3R interaction (Fig. 5C). This result was actually consistent with a previous study showing that inhibition of the PI3K pathway caused a reduction of Ca^{2+} released from the ER [37]. Our data also show that increased release of ER Ca^{2+}, caused by inhibition of the PI3K pathway, was specific only to PC1 cells.

Similarly, activation of the PI3K/Akt pathway by hepatocyte growth factor (HGF) in control cells reduced the ATP-induced Ca^{2+} release (Fig. 6A). Again, this reduction was not caused by ER Ca^{2+} depletion, as the ionomycin response in cells treated with HGF was similar to control (Sup Fig. 1C, bottom panel). Addition of HGF into the cell culture medium in control cells reduced the PC2-IP_3R complex and increased the interaction of STIM1 and IP_3R (Fig. 6B). Interestingly, a recent study by Mangos et al. demonstrates similar results, where they show that either depletion of ER Ca^{2+} or inhibition of the PI3K pathway causes the same phenotype as knocking down the polycystins in the zebrafish model [38]. In our study, we saw that either high ER Ca^{2+} or activation of the PI3K pathway mimicked the PC1 phenotype.

Finally, we also confirmed that there was no change of endogenous expression of PC2, STIM1 and IP_3R upon treatment with PI3K inhibitors or HGF (Fig. 6C). Therefore, any alterations of PC2-IP_3R and STIM1 interactions were not caused by changes in protein expression. From these observations, we conclude that activation of the PI3K/Akt pathway increases the

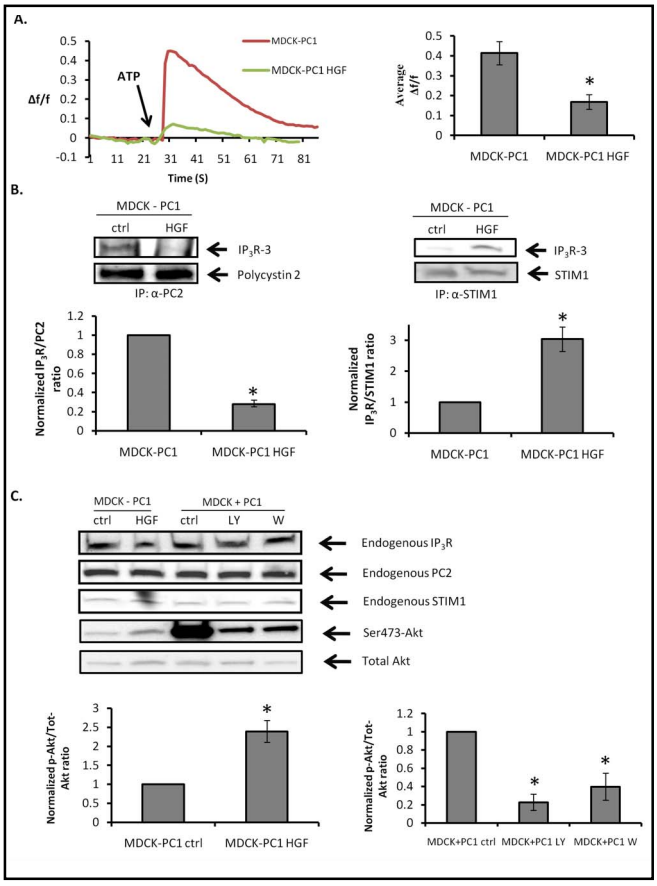

Fig. 6. Activation of the PI3K/Akt pathway in the control MDCK cells reduces ER Ca^{2+} release. A. Representative traces of ATP-stimulated Ca^{2+} release in control cells treated with HGF. RIGHT PANEL: The plot shows the average amplitude of Ca^{2+} release in response to ATP in control cells after treatment with HGF. HGF treatment inhibited increase of intracellular Ca^{2+} stimulated by ATP in cells without PC1. Asterisk indicates statistical significance between the two groups (student's t-test, $p< 0.005$, MDCK-PC1 n=7, MDCK-PC1HGF n=7). B. The immunoblots show HGF treatment in control cells reduced co-immunoprecipitation of PC2 and IP_3R (left blot) and increased co-immunoprecipitation of STIM1 and IP_3R (right blot). LOWER PANEL: The histograms show the densitometry analysis of co-immunoprecipitation of PC2 and IP_3R (left) and STIM1 and IP_3R (right). The ratio for the non-treated group was considered 1, and ratio for the HGF group was normalized against the non-treated group. The data is the average of three sets of experiments, and asterisk indicates significance between the two groups (student's t-test, $p<0.05$). C. Immunoblots show endogenous expression of IP_3R, PC2, STIM1, Ser473-Akt, and total Akt in MDCK stable cell line after treatment with HGF, and PI3K inhibitors. LOWER PANEL: The graphs summarize the densitometry analysis of Akt activation after treatment of control cells with HGF (left graph) and PC1 cells with PI3K inhibitors (right graph). Asterisk indicates significance between treatment groups compared to control (student's t-test, $p<0.005$, n=3 for all groups).

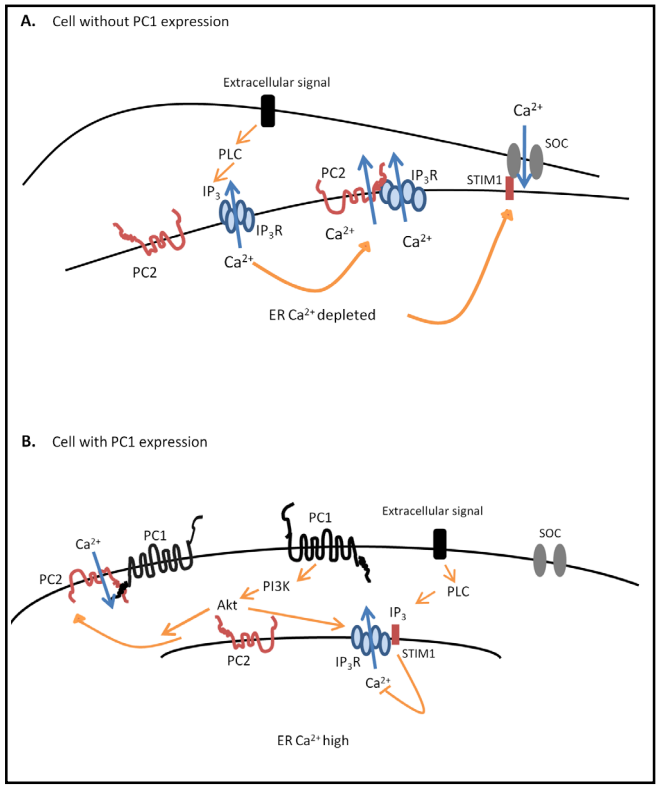

Fig. 7. Proposed model. A. In the cell without PC1 expression, the PI3K/Akt pathway is not active, and PC2 is retained in the ER. Extracellular signals that activate the phospholipase C (PLC) pathway induce production of IP_3 to activate the IP_3R. Activation of the IP_3R releases Ca^{2+} from within the ER to cytosol. PC2, which is retained in the ER interacts with IP_3R and facilitates the release of Ca^{2+}. Enhancement of Ca^{2+} release by PC2-IP_3R interaction eventually leads to Ca^{2+} depletion in the ER and activation of the store-operated Ca^{2+} influx (SOC). Blue arrow represents Ca^{2+} movement, and orange arrow represents activation of inhibition (arrow with flat head) of cellular activities. B. In the cell with PC1 expression, the PI3K/Akt pathway is activated. This signaling pathway leads to an increase of STIM1-IP_3R complex, reduction of the PC2-IP_3R interaction in the ER, and probably translocation of PC2 from the ER to the plasma membrane. PC2 interacts with PC1 at the plasma membrane to serve as a Ca^{2+} influx channel. Extracellular signals that activate the PLC pathway induce production of IP_3 to activate the IP_3R. Activation of the IP_3R will stimulate Ca^{2+} release, however, reduction of the PC2-IP_3R interaction and increased the association of STIM1-IP_3R inhibit release of intracellular Ca^{2+} that eventually keeps the Ca^{2+} level within the ER high, and therefore inactivate the SOC.

interaction of PC1 and STIM1 with IP_3R, reduces the ability of PC2 to bind to IP_3R, and therefore inhibits Ca^{2+} release from the ER.

Discussion

Previously, our group demonstrated that PC2 interacts with IP_3R to enhance the release of Ca^{2+} from the ER [16]. We then showed that PC1 also complexes with the IP_3R to suppress ER Ca^{2+} release [11]. In addition, our group also subsequently described how PC1 inhibits SOCE through interaction with STIM1 to prevent its translocation to the ER-PM junction [19]. These preceding studies show that two proteins, whose malfunctions cause the same cyst phenotype, when functioning individually have opposite effects on intracellular Ca^{2+} homeostasis. It also gives a clue that regulation of intracellular Ca^{2+} signaling is more sophisticated than what was previously believed.

In our current work, we describe how expression of PC1 and PC2 together regulates intracellular Ca^{2+} homeostasis in mammalian cells. We show that expression of full-length PC1 reduces the interaction of PC2 and IP_3R consistent with PC1's ability to inhibit the release of ER Ca^{2+}. Furthermore, we found that STIM1's association with IP_3R is increased by PC1. Our results show that unlike PC1, direct binding of PC2 to STIM1 cannot be detected using our pull down assay. However, both PC2 and STIM1 do interact with IP_3R. Importantly by competing for interaction with IP_3R, PC2 disrupts the complex of STIM1 and IP_3R. We also showed that increasing the interaction between STIM1 and IP_3R in cells without PC1 significantly reduces ATP-mediated increases in intracellular Ca^{2+}. Our results suggest that STIM1 by competing with PC2 for interaction with the IP_3R also has a functional effect opposite that of PC2. Thus, in the absence of PC1, PC2 interacts with IP_3R to enhance Ca^{2+} release. The presence of PC1 disrupts the complex by increasing STIM1's interaction with IP_3R to inhibit Ca^{2+} release from the ER. It probably also serves as a mechanism to sequester STIM1 to the ER membrane and consequently inhibits SOCE [19] (see Fig 7).

The question is whether these processes are regulated. Involvement of the PI3K/Akt pathway in tubulogenesis in MDCK cells has been discussed in several studies [26, 39]. In addition, activation of the PI3K/Akt pathway in MDCK cells is important for cell resistance to apoptosis [26]. We were able to show that inhibition of the PI3K/Akt pathway in PC1-expressing cells relieves inhibition of ER Ca^{2+} release, restores the interaction between PC2 and IP_3R, and reduces the ability of STIM1 and IP_3R to associate. Meanwhile,

activation of the PI3K/Akt pathway in cells without PC1 inhibits the PC2-IP_3R, and increases the STIM1-IP_3R interaction leading to a reduction of intracellular Ca^{2+} release induced by ATP. Our results are consistent with a previous study by Marchi et al., where they showed that activation of the Akt kinase inhibits ER Ca^{2+} release in Hela cells [40]. They further link this phenotype with cell protection from apoptosis [40]. Several other reports also support a tight relationship between activation of the PI3K/Akt pathway and inhibition of apoptosis [41]. These data lead us to propose that activation of the PI3K/Akt pathway modulates the PC2, IP_3R, and STIM1 interaction to regulate intracellular Ca^{2+} release as a protection mechanism against cell apoptosis.

Cysts cells are known to have aberrant signal transduction and fluid secretion compared to the normal renal tubules [2, 42, 43]. Fluid secretion driven by chloride plays an important role in the maintenance and enlargement of the cysts. Two pathways lead to chloride secretion, one being ATP-stimulated chloride secretion and the other CFTR [42, 44]. Activation of purinergic receptors by ATP induces the release of Ca^{2+} from the ER. The key mechanism for ATP-mediated chloride secretion is intracellular Ca^{2+} release, which in turn will active calcium-activated chloride channels (CaCC) [34]. In fact, a truncated form of PC1 has been shown to enhance ATP-stimulated chloride secretion in the mouse collecting duct cells [44]. This corresponds with our results where we saw that expression of wild-type PC1 reduces intracellular Ca^{2+} release in response to ATP.

Besides CaCC, CFTR is also involved in fluid secretion in the cyst. CFTR is activated by cAMP, which is also a cyst promoter [42]. Inhibition of CFTR reduces cyst growth in both cell and animal models providing convincing evidence that CFTR does indeed play a role [45, 46]. MDCK cells which lack PC1 form cysts which are dependent upon chloride secretion but when PC1 is expressed in these cells, chloride secretion in response to cAMP is inhibited. The exact mechanism behind these changes is still not clear. Given that the reduced cAMP-dependent chloride secretion in PC1 cells was not caused by down regulation of CFTR protein expression suggests that PC1 either caused a change in the trafficking of CFTR to the plasma membrane or altered the signal transduction pathway leading to activation of CFTR [47]. Interestingly, a recent report by Lefkimmiatis et al. describes how depletion of ER Ca^{2+} leads to increased cAMP production. They show that blockage of STIM1 translocation from the ER to the plasma membrane reduced cAMP production [48]. In relation to our results, we observed an increase in the interaction between STIM1 and IP_3R in PC1-expressing cells, and a reduction of ER Ca^{2+} release upon this binding. This would lead to maintenance of high ER Ca^{2+} levels and consequently inhibition of STIM1 translocation to the ER-PM junction. In addition, our group also showed that PC1 expression impairs STIM1 translocation to the ER-PM junction [19]. From all these studies, we can speculate that the expression of PC1 inhibits cAMP-dependent chloride secretion in MDCK cells through a decrease in cAMP production and eventually inhibits cyst growth itself.

Although our results suggest that inhibition of ER Ca^{2+} release may be protective against cyst formation, several other groups have reported that lower levels of cytoplasmic Ca^{2+} increased cell proliferation [23-25]. One possible explanation for the difference is that PC1 might inhibit Ca^{2+} release from the ER, but it might also facilitate Ca^{2+} influx across the plasma membrane, probably via PC2. Our lab previously showed that PC1 promotes the trafficking of PC2 to the plasma membrane where PC2 can function as a Ca^{2+} influx channel [8]. Another study with primary cilia describes how PC1 complexes with PC2 in the cilia membrane to allow extracellular Ca^{2+} to flow into the cells [12]. Interestingly, it was reported that activation of the PI3K pathway enhances trafficking of TRP-like channels to the cell surface [49]. We found in the present study that PC1 expression reduced the interaction of PC2 and IP_3R and this reduction was mediated by the PI3K pathway. From our results and all these preceding studies, we can hypothesize that expression of PC1 activates the PI3K pathway to help trafficking of PC2 to the cell surface, therefore reduces the ability of PC2 to complex with IP_3R in the ER. In addition, PC1 by forming a complex with PC2 and functioning at the plasma membrane and/or cilia membrane may be the primary pathway for Ca^{2+} influx across the plasma membrane in renal cells. In ADPKD with mutations in PC1, interaction of PC2 with IP_3R would be increased to eventually cause ER Ca^{2+} depletion. In ADPKD with mutations in PC2, the lack of function of PC2 would reduce Ca^{2+} entry across the plasma membrane and strengthen the interaction of PC1 and STIM1 with IP_3R, which eventually leads to low intracellular Ca^{2+} in cyst cells. Thus, it is apparent that expression and proper functioning of both PC1 and PC2 are important for Ca^{2+} homeostasis both in the ER

and in the cytoplasm. Maintenance of high ER Ca^{2+} is important for normal cellular functions, such as protein translation [50], processing [51], and proper hormonal signaling [52]. It seems that the optimal concentration of Ca^{2+} in the cytoplasm as well as within the ER must be sustained to avoid cell over proliferation, apoptosis, and increased of fluid secretion, all of which contribute to cyst formation.

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References

1 Gabow PA: Autosomal-Dominant Polycystic Kidney-Disease. N Engl J Med 1993;329:332-342.

2 Wilson PD: Polycystic kidney disease. N Engl J Med 2004;350:151-164.

3 Ward CJ: The Polycystic Kidney-Disease-1 Gene Encodes A 14 Kb Transcript and Lies Within A Duplicated Region on Chromosome-16. Cell 1994;77:881-894.

4 Glucksmann-Kuis MA, Tayber O, Woolf EA, Bougueleret L, Deng NH, Alperin GD, Iris F, Hawkins F, Munro C, Lakey N, Duyk G, Schneider MC, Geng L, Zhang F, Zhao ZH, Torosian S, Zhou J, Reeders ST, Bork P, Pohlschmidt M, Lohning C, Kraus B, Nowicka U, Leung ALS, Frischauf AM: Polycystic Kidney-Disease - the Complete Structure of the Pkd1 Gene and Its Protein. Cell 1995;81:289-298.

5 Wu G, D'Agati V, Cai Y, Markowitz G, Park JH, Reynolds DM, Maeda Y, Le TC, Hou H, Kucherlapati R, Edelmann W, Somlo S: Somatic inactivation of Pkd2 results in polycystic kidney disease. Cell 1998;93:177-188.

6 Nims N, Vassmer D, Maser RL: Transmembrane domain analysis of polycystin-1, the product of the polycystic kidney disease-1 (PKD1) gene: evidence for 11 membrane-spanning domains. Biochemistry 2003;42:13035-13048.

7 Arnould T, Kim E, Tsiokas L, Jochimsen F, Gruning W, Chang JD, Walz G: The polycystic kidney disease 1 gene product mediates protein kinase C alpha-dependent and c-Jun N-terminal kinase-dependent activation of the transcription factor AP-1. J Biol Chem 1998;273:6013-6018.

8 Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, Germino GG: Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. Nature 2000;408:990-994.

9 Puri S, Magenheimer BS, Maser RL, Ryan EM, Zien CA, Walker DD, Wallace DP, Hempson SJ, Calvet JP: Polycystin-1 activates the calcineurin/NFAT (nuclear factor of activated T-cells) signaling pathway. J Biol Chem 2004;279:55455-55464.

10 Huan Y, van AJ: Polycystin-1, the PKD1 gene product, is in a complex containing E-cadherin and the catenins. J Clin Invest 1999;104:1459-1468.

11 Li Y, Santoso NG, Yu S, Woodward OM, Qian F, Guggino WB: Polycystin-1 interacts with inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca^{2+} signaling with implications for polycystic kidney disease. J Biol Chem 2009;284:36431-36441.

12 Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 2003;33:129-137.

13 Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, Kimberling WJ, Breuning MH, Deltas CC, Peters DJ, Somlo S: PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 1996;272:1339-1342.

14 Gonzalez-Perrett S, Kim K, Ibarra C, Damiano AE, Zotta E, Batelli M, Harris PC, Reisin IL, Arnaout MA, Cantiello HF: Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+}-permeable nonselective cation channel. Proc Natl Acad Sci USA 2001;98:1182-1187.

15 Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, Witzgall R, Ehrlich BE, Somlo S: Polycystin-2 is an intracellular calcium release channel. Nat Cell Biol 2002;4:191-197.

16 Li Y, Wright JM, Qian F, Germino GG, Guggino WB: Polycystin 2 interacts with type I inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca^{2+} signaling. J Biol Chem 2005;280:41298-41306.

17 Delmas P, Nomura H, Li X, Lakkis M, Luo Y, Segal Y, Fernandez-Fernandez JM, Harris P, Frischauf AM, Brown DA, Zhou J: Constitutive activation of G-proteins by polycystin-1 is antagonized by polycystin-2. J Biol Chem 2002;277:11276-11283.

18 Peters DJ, Sandkuijl LA: Genetic heterogeneity of polycystic kidney disease in Europe. Contrib Nephrol 1992;97:128-139.

19 Woodward OM, Li Y, Yu S, Greenwell P, Wodarczyk C, Boletta A, Guggino WB, Qian F: Identification of a Polycystin-1 Cleavage Product, P100, That Regulates Store Operated Ca^{2+} Entry through Interactions with STIM1. PLoS ONE 2010;5:e12305.

20 Berridge MJ: Calcium Signaling and Cell-Proliferation. Bioessays 1995;17:491-500.

21 Manzati E, Aguiari G, Banzi M, Manzati M, Selvatici R, Falzarano S, Maestri I, Pinton P, Rizzuto R, del Senno L: The cytoplasmic C-terminus of polycystin-1 increases cell proliferation in kidney epithelial cells through serum-activated and Ca^{2+}-dependent pathway(s). Exp Cell Res 2005;304:391-406.

22 Aguiari G, Banzi M, Gessi S, Cai YQ, Zeggio E, Manzati E, Piva R, Lambertini E, Ferrari L, Peters DJ, Lanza F, Harris PC, Borea PA, Somlo S, del Senno L: Deficiency of polycystin-2 reduces Ca^{2+} channel activity and cell proliferation in ADPKD lymphoblastoid cells. FASEB J 2004;18:884-886.

23 Cowley BD: Calcium, cyclic AMP, and MAP kinases: dysregulation in polycystic kidney disease. Kidney Int 2008;73:251-253.

24 Kip SN, Hunter LW, Ren Q, Harris PC, Somlo S, Torres VE, Sieck GC, Qian Q: $[Ca^{2+}]_i$ reduction increases cellular proliferation and apoptosis in vascular smooth muscle cells: relevance to the ADPKD phenotype. Circ Res 2005;96:873-880.

25 Yamaguchi T, Hempson SJ, Reif GA, Hedge AM, Wallace DP: Calcium restores a normal proliferation phenotype in human polycystic kidney disease epithelial cells. J Am Soc Nephrol 2006;17:178-187.

26 Boca M, Distefano G, Qian F, Bhunia AK, Germino GG, Boletta A: Polycystin-1 induces resistance to apoptosis through the phosphatidylinositol 3-kinase/Akt signaling pathway. J Am Soc Nephrol 2006;17:637-647.

27 Boletta A, Qian F, Onuchic LF, Bhunia AK, Phakdeekitcharoen B, Hanaoka K, Guggino W, Monaco L, Germino GG: Polycystin-1, the gene product of PKD1, induces resistance to apoptosis and spontaneous tubulogenesis in MDCK cells. Mol Cell 2000;6:1267-1273.

28 Geng L, Boehmerle W, Maeda Y, Okuhara DY, Tian X, Yu ZH, Choe CU, Anyatonwu GI, Ehrlich BE, Somlo S: Syntaxin 5 regulates the endoplasmic reticulum channel-release properties of polycystin-2. Proc Natl Acad Sci USA 2008;105:15920-15925.

29 Thastrup O, Cullen PJ, Drobak BK, Hanley MR, Dawson AP: Thapsigargin, A Tumor Promoter, Discharges Intracellular Ca^{-2+} Stores by Specific-Inhibition of the Endoplasmic-Reticulum Ca^{-2+}-Atpase. Proc Natl Acad Sci USA 1990;87:2466-2470.

30 Yuan JP, Kiselyov K, Shin DM, Chen J, Shcheynikov N, Kang SH, Dehoff MH, Schwarz MK, Seeburg PH, Muallem S, Worley PF: Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors. Cell 2003;114:777-789.

31 Foskett JK, White C, Cheung KH, Mak DOD: Inositol trisphosphate receptor Ca^{2+} release channels. Physiol Rev 2007;87:593-658.

32 Schumann F, Hoffmeister H, Bader R, Schmidt M, Witzgall R, Kalbitzer HR: Ca^{2+}-dependent Conformational Changes in a C-terminal Cytosolic Domain of Polycystin-2. J Biol Chem 2009;284:24372-24383.

33 Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE, Meyer T: STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. Curr Biol 2005;15:1235-1241.

34 Nilius B, Sehrer J, Heinke S, Droogmans G: Ca^{2+} Release and Activation of K^+ and Cl^- Currents by Extracellular Atp in Distal Nephron Epithelial-Cells. Am J Physiol Cell Physiol 1995;38:C376-C384.

35 Weber KH, Lee EK, Basavanna U, Lindley S, Ziegelstein RC, Germino GG, Sutters M: Heterologous expression of polycystin-1 inhibits endoplasmic reticulum calcium leak in stably transfected MDCK cells. Am J Physiol Renal Physiol 2008;294:F1279-F1286.

36 Sammels E, Devogelaere B, Mekahli D, Bultynck G, Missiaen L, Parys JB, Cai Y, Somlo S, De SH: Polycystin-2 activation by inositol 1,4,5-trisphosphate-induced Ca^{2+} release requires its direct association with the inositol 1,4,5-trisphosphate receptor in a signaling microdomain. J Biol Chem 2010;285:18794-18805

37 Rameh LE, Rhee SG, Spokes K, Kazlauskas A, Cantley LC, Cantley LG: Phosphoinositide 3-kinase regulates phospholipase C gamma-mediated calcium signaling. J Biol Chem 1998;273:23750-23757.

38 Mangos S, Lam PY, Zhao A, Liu Y, Mudumana S, Vasilyev A, Liu A, Drummond IA: The ADPKD genes pkd1a/b and pkd2 regulate extracellular matrix formation. Dis Model Mech 2010;3:354-365.

39 Khwaja A, Lehmann K, Marte BM, Downward J: Phosphoinositide 3-kinase induces scattering and tubulogenesis in epithelial cells through a novel pathway. J Biol Chem 1998;273:18793-18801.

40 Marchi S, Rimessi A, Giorgi C, Baldini C, Ferroni L, Rizzuto R, Pinton P: Akt kinase reducing endoplasmic reticulum Ca^{2+} release protects cells from Ca^{2+}-dependent apoptotic stimuli. Biochem Biophys Res Commun 2008;375:501-505.

41 Franke TF, Kaplan DR, Cantley LC: PI3K: downstream AKTion blocks apoptosis. Cell 1997;88:435-437.

42 Hanaoka K, Devuyst O, Schwiebert EM, Wilson PD, Guggino WB: A role for CFTR in human autosomal dominant polycystic kidney disease. Am J Physiol 1996;270:C389-C399.

43 Mangookarim R, Uchic ME, Grant M, Shumate WA, Calvet JP, Park CH, Grantham JJ: Renal Epithelial Fluid Secretion and Cyst Growth - the Role of Cyclic-Amp. FASEB J 1989;3:2629-2632.

44 Chernova MN, Vandorpe DH, Clark JS, Alper SL: Expression of the polycystin-1 C-terminal cytoplasmic tail increases Cl channel activity in Xenopus oocytes. Kidney Int 2005;68:632-641.

45 Li H, Findlay IA, Sheppard DN: The relationship between cell proliferation, Cl^- secretion, and renal cyst growth: a study using CFTR inhibitors. Kidney Int 2004;66:1926-1938.

46 Yang BX, Sonawane ND, Zhao D, Somlo S, Verkman AS: Small-molecule CFTR inhibitors slow cyst growth in polycystic kidney disease. J Am Soc Nephrol 2008;19:1300-1310.

47 Ikeda M, Fong P, Cheng J, Boletta A, Qian F, Zhang XM, Cai H, Germino GG, Guggino WB: A regulatory role of polycystin-1 on cystic fibrosis transmembrane conductance regulator plasma membrane expression. Cell Physiol Biochem 2006;18:9-20.

48 Lefkimmiatis K, Srikanthan M, Maiellaro I, Moyer MP, Curci S, Hofer AM: Store-operated cyclic AMP signalling mediated by STIM1. Nat Cell Biol 2009;11:433-442.

49 Kanzaki M, Zhang YQ, Mashima H, Li L, Shibata H, Kojima I: Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. Nat Cell Biol 1999;1:165-170.

50 Brostrom CO, Brostrom MA: Calcium-dependent regulation of protein synthesis in intact mammalian cells. Annu Rev Physiol 1990;52:577-590.

51 Gething MJ, Sambrook J: Protein folding in the cell. Nature 1992;355:33-45.

52 Clapham DE: Calcium Signaling. Cell 1995;80:259-268.