Polycystin-1 and Gα12 regulate the cleavage of E-cadherin in kidney epithelial cells

Jen X. Xu,1,2* Tzong-Shi Lu,1,2* Suyan Li,1 Yong Wu,1 Lai Ding,3 Bradley M. Denker,4 Joseph V. Bonventre,1 and Tianqing Kong1

1Renal Division, Department of Medicine, Brigham and Women’s Hospital, Boston, Massachusetts; 2Division of Basic Neuroscience, McLean Hospital, Belmont, Massachusetts; 3Harvard NeuroDiscovery Center, Boston, Massachusetts; and 4Beth Israel Deaconess Medical Center, Boston, Massachusetts

Submitted 6 August 2014; accepted in final form 6 December 2014

Physiol Genomics 47: 24–32, 2015. First published December 9, 2014; doi:10.1152/physiolgenomics.00090.2014.—Interaction of polycystin-1 (PC1) and Gα12 is important for development of kidney cysts in autosomal dominant polycystic kidney disease (ADPKD). The integrity of cell polarity and cell-cell adhesion is altered in the renal epithelial cells of ADPKD. However, the key signaling pathway for this alteration is not fully understood. Madin-Darby canine kidney (MDCK) cells maintain the normal integrity of epithelial cell polarity and adherens junctions. Here, we found that deletion of Pkd1 increased activation of Gα12, which then promoted the cystogenesis of MDCK cells. The morphology of these cells was altered after the activation of Gα12. By using liquid chromatography-mass spectrometry, we found several proteins that could be related this change in the extracellular milieu. E-cadherin was one of the most abundant peptides after active Gα12 was induced. Gα12 activation or Pkd1 deletion increased the shedding of E-cadherin, which was mediated via increased ADAM10 activity. The increased shedding of E-cadherin was blocked by knockdown of ADAM10 or specific ADAM10 inhibitor GI254023X. Pkd1 deletion or Gα12 activation also changed the distribution of E-cadherin in kidney epithelial cells and caused β-catenin to shift from cell membrane to nucleus. Finally, ADAM10 inhibitor, GI254023X, blocked the cystogenesis induced by PC1 knockdown or Gα12 activation in renal epithelial cells. Our results demonstrate that the E-cadherin/β-catenin signaling pathway is regulated by PC1 and Gα12 via ADAM10. Specific inhibition of this pathway, especially ADAM10 activity, could be a novel therapeutic regimen for ADPKD.

G proteins; polycystin-1; E-cadherin; ADAM10; ADPKD

HETEROHTRIMERIC G PROTEINS contain a Gα and Gβγ subunit and are classically coupled to seven plasma membrane-bound transmembrane receptors. However, they are also associated with atypical receptors or modulators and transduce signaling via nontraditional mechanisms. G proteins are composed of four major families, Gs, Gi/o, Gq, and G12. The G12 family includes Gα12 and Gα13. They share some common downstream signaling molecules, but their functions are not completely redundant during embryonic development. Gα13-deficient mice are embryonic lethal at around embryonic day 10, whereas Gα12-deficient mice are apparently normal (42). Gα12 is ubiquitously expressed and signals through seven transmembrane receptors and atypical receptors on the cell membrane. Activation of G12 plays a role in cell growth and proliferation, cytoskeleton rearrangement, cell polarity, paracellular permeability, cell-cell adhesion, and migration and invasion, particularly in pathological situations (54, 61). In tumor cells, Gα12 is very important for regulating cell migration, invasion, and probably tumor metastasis. Activation of Gα12 triggers downstream signaling, such as Rho activation, cofilin and myosin light chain-2 phosphorylation, and stress fiber formation (13, 14). There are several signaling molecules related to Gα12 such as monomeric GTPases, mitogen-activated protein kinases, and nonreceptor tyrosine kinases (non-RTKs). Their effectors include cadherins, radixin of the ezrin/radixin/moesin protein family, non-RTKs, protein phosphatases, A-kinase anchoring proteins, the tight junction protein zonula occludens-1, Hsp90, and regulators of G protein signaling RGS1, RGS16, and axin, etc. (15, 19).

We have previously shown that Gα12 activation affects renal epithelial cell-matrix adhesions and cystogenesis (20, 21). The activation of Gα12 regulates apoptosis and tight junctions in renal epithelial cells (67), and knockout of Gα12 protects kidney epithelial cells from ischemic injury (65). Gα12 is directly associated with the cytoplasmic tail of polycystin-1 (PC1, encoded by PKD1) (68). Taken together, these findings indicate that Gα12 could be downstream signaling molecule for PC1 and very important for development of kidney cysts induced by PKD1 mutation in autosomal dominant polycystic kidney disease (ADPKD). ADPKD is one of the most common life-threatening genetic diseases and is characterized by early formation and gradual enlargement of multiple kidney cysts, which eventually results in end-stage renal disease. Mutation in PKD1 or PKD2 (polycystin-2, PC2) accounts for 85 and 15% of this disease, respectively (10, 50, 58, 70). PC1 acts as a G protein-coupled receptor and activates all four families of heterotrimeric G proteins (39, 68). PC1 also regulates mTOR (mammalian target of rapamycin) (16), planar cell polarity and Wnt signaling (15, 36), and focal adhesions (17, 60). Renal cysts initiate from a focal area within a tubule (usually within the distal tubule or collecting duct) and lead to tubular widening. In ADPKD, cyst formation begins in utero (59). As a cyst expands in size, it fills with fluid derived from unreabsorbed glomerular filtrate and fluid secreted from surrounding cells. Once cysts expand to ~2 mm in diameter, they detach from their parental tubules and become isolated fluid-filled sacs lined by an epithelial cell layer. These isolated cysts continue to expand in size at a relatively constant rate after birth (59). Over time, the kidneys become enlarged to four to eight times their normal size. By
the fifth to seventh decade of life, there is significant loss of renal function. Approximately one-half of ADPKD patients progress to chronic renal failure by age 60 yr and require dialysis or transplantation (43, 59). Regardless of the initial pathogenetic mechanism, kidney cysts are accompanied by partial differentiation of the epithelial cells, dysregulation of epithelial cell proliferation and apoptosis, and disruption of cell polarity, cell-matrix and cell-cell contacts. There is also chronic focal ischemia, inflammation, and fibrosis (6, 31).

E-cadherin is a cell membrane protein with a single transmembrane domain. It forms adherens junction between cell-cell contact via homophilic interaction of its ectodomain. This special structure between epithelial cells is important for maintaining the integrity of planar polarity and cell-cell adhesions (9, 23). Following the ectodomain interactions, the cytoplasmic domain of E-cadherin forms a complex with p120-α-, β-, and γ-catenin (plakoglobin), which connects to the actin cytoskeleton and promotes the maturation of adherens junctions in epithelia (37, 38). E-cadherin-mediated adhesion is also involved in Wnt signaling cascades that regulate gene expression and cytoskeleton remodeling (8). The link between the cytoplasmic tail of E-cadherin and the actin cytoskeleton is very important for regulating morphology, junction stabilization, cell-cell adhesion, cell migration, and tissue remodeling (8, 53).

On the cytoplasmic side, E-cadherin function is regulated by altering the composition of the cadherin-catenin complex, the presence of growth factors, tyrosine phosphorylation of the cadherin-catenin complex, p120 binding, and the activity of small GTPases (5, 55). In addition, E-cadherin can be removed from the cell surface by proteolytic cleavage, resulting in an 80 kDa fragment as a soluble E-cadherin (sE-cad). sE-cad was initially found in breast cancer cells (57), but it has also been reported in patients with viral and bacterial infections, organ failure, and other benign diseases. There are several proteases that cleave E-cadherin, such as the A disintegrin and metalloprotease (ADAM) family, bacterial proteases (gingipains and BFT/fragilysin), cathepsins (B, L, S), the family of matrix metalloproteases (MMPs) (MMP-2, 3, 7, 9, and 14), KLK7, and plasmin (2, 18, 27, 35, 62).

PC1 forms multiple protein complexes in cell membrane, which including E-cadherin and the catenins (15). In human renal cystic epithelial cells, PC1 deletion is associated with the concomitant loss of surface E-cadherin (44, 49). However, it is still unclear how E-cadherin is regulated in renal cystic epithelial cells after Pkd1 inactivation. We demonstrate here that Pkd1 deletion increases the activation of Go12, which then promotes the maturation of ADAM10 that is subsequently responsible for cleaving the ectodomain of E-cadherin in kidney epithelial cells.

METHODS

Cell culture and materials. Tet-off inducible Go12 and Go12QL (constitutively active) Madin-Darby canine kidney (MDCK) cell lines were cultured and maintained as previously described (20, 32). Plastic ware and tissue culture supplies were from BD Bioscience (San Jose, CA). Chemicals were from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Go12 antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). Collagen type I (rat tail) and Matrigel were from BD Bioscences. E-cadherin antibody was from Cell Signaling (cat. #3195, clone: 24E10), ADAM10 antibody was from Abcam (ab1997), and β-catenin antibody from Cell Signaling (cat. #9582, clone: 6B3). The secondary antibodies were from Life Technologies (Grand Island, NY).

Animals and kidney specimens. Inducible Pkd1 knockout mice (Mx1Cre+Pkd1fl/fl) were described elsewhere (51). In brief, exon 2 through exon 6 of Pkd1 were flanked by two LoxP sites. These mice were crossed with the mice expressing transgenic Cre recombinase under the control of the INF-inducible Mx1 promoter (Mx1Cre mice). All genotyping was done via PCR on tail DNA. The primers were as follows: Go12WT, 5′-GTT CTC ATC ATC GGT TTC C-3′; 5′-CGG GTC GCC CAT GAA ATC TGG-3′. Pkd1fl/fl, 5′-TGT CTG CCA GCT CTT TGT AT-3′; 5′-CAC AGC GTC AGG AAG AGG AGG AG-3′. Mx1Cre, 5′-TCC CAA CCT CAG TAC GAA GCC AAG-3′; 5′-ACG ACC GGC AAA CGG ACA GAA GCA-3′. In all experiments, littermates without Cre recombinase or Mx1Cre Pkd1fl/fl mice without inducible deletion of Pkd1 were used as controls. Cre recombinase was induced to conditionally knockout Pkd1 by intraperitoneal injection of 62.5 μg, 250 μg of IFN inducer plpC (Sigma) at consecutive 5 days at 1 or 5 wk of age. Most of these mice were kept to 9 wk of age. Animal protocols were approved by the Standing Committee on Animals of Harvard Medical School.

Kidney specimens (including cystic fluid and paraffin-embedded tissue) from control patients and ADPKD patients were obtained from Drs. J. Zhou and B. Denker (Harvard Polycystic Kidney Disease Center). The specimens had been taken from routine diagnostic or therapeutic samples and patient data had been made anonymous. The sample collection was approved by the ethical committee and the institutional review board of Harvard Medical School, and each patient gave written informed consent.

Liquid chromatography-tandem mass spectrometry. MDCK cells were grown to 90% confluence in 100 mm plates in the presence or absence of doxycycline (Dox) without serum for 48 h. Then the culture media were collected and centrifuged at 2,000 g at 4°C for 5 min. The supernatants were collected and filtered through Amicon (Millipore) from 5 ml to ~500 μl. Concentrated supernatants were resolved by SDS-PAGE using reducing condition. The gel was stained with Coomassie blue (Bio-Rad, Hercules, CA). All of the stained bands were excised and sent to the Taplin Biological Mass Spectrometry Facility (Harvard Medical School). Excised SDS-polyacrylamide gel bands were cut into small pieces and digested in gels with trypsin. Then the samples were run in a mass spectrometer. All of the criteria and data were acquired and analyzed as reported previously (63).

Immunoblotting. Monolayers were scratched in lysis buffer (150 mM NaCl, 2.5 mM EDTA, 25 mM HEPES, pH 7.5, 1 mM PMSF, 1% Triton X-100, and protease inhibitors; Roche, Indianapolis, IN) and phosphatase inhibitor cocktail (Sigma-Aldrich) plus 1 mM NaVO4 and 25 mM NaF. Cells were lysed at 4°C for 2 h and centrifuged. Lysates were used for GST-fused tetradecapetide repeat (TPR) pull-down or Western blots as detailed previously (20, 67). Mouse primary kidney epithelial cells and collecting duct epithelial cells were separated and purified as previously described (36). The density of Western blot bands was quantified by with NIH ImageJ (Version 1.48) after subtracting background.

Immunohistochemistry. Histopathological analysis was performed after hematoxylin and eosin staining on formalin-fixed, paraffin-embedded tissues. Immunofluorescence of cells was performed as described previously (20). In brief, cells grown on slide chambers were washed with PBS and fixed (3.7% paraformaldehyde, 15 min). After blocking (1 h in PBS containing 1% BSA and 2% goat serum at 25°C), primary antibody (1:50–100 dilution with 1% BSA in PBS) was added for 1 h at room temperature. After three washes, secondary antibodies conjugated with Alexa Fluor-430 (green), and Alexa Fluor-532 (red) were added for 1 h at 25°C (Invitrogen). After three washes, the slides were air-dried and mounted in solution containing 4’,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA).
Selected frozen mouse kidney tissue was sectioned in 4 μm thickness and air dried at room temperature for 10 min. Sections were washed for 10 min in PBS and fixed in 95% alcohol for 10 min followed by two PBS washes of 5 min each. The sections were incubated in 1% BSA/3% normal donkey serum/PBS-Tween for 40 min to block nonspecific protein-protein interaction. Primary rabbit polyclonal Adam10 antibody (Abcam, ab1997) was applied on the sections at 1:400 dilution. E-cadherin (Cell Signaling, cat. #3195, clone: 24E10) and β-catenin (Cell Signaling, cat. #9582, clone: 6B3) were applied on the sections at 1:50 dilution. The sections were incubated overnight at 4°C for 20 h followed by two PBS washes of 5 min each. Then the sections were incubated for 30 min at room temperature with Alexa Fluor 568 donkey anti-rabbit IgG (Life Technologies A21206) at 1:250 dilution, with DAPI, 1 μg/ml, and Alexa Fluor 488 donkey anti-rabbit IgG (Life Technologies A21206) at 1:250 dilution, with DAPI, 1 μg/ml, and DBA-1:500 incubated 30 min. The sections were then washed in PBS twice for 5 min each and mounted with Fluorescent Mounting Medium (Vector Laboratories). Fluorescence images were acquired by using confocal microscopy (Zeiss LSM 510).

Three-dimensional cell culture. MDCK Tet-off, inducible Gα12WT [wild type (WT)] and Gα12QL cell lines were characterized and cultured as before (20). For three-dimensional (3D) culture, MDCK cells were grown to 60–80% confluence on 100 mm dishes, trypsinized, and resuspended at a concentration of 4 × 10⁴ cells/ml in the matrix with 40% collagen-I and 60% Matrigel mixture, 10× DMEM, and HEPES (at 8:1:1) on ice. The single-cell suspension was plated onto six-well plates for 30 min at 37°C until solid. We added 2 ml of media with 10% FBS into each chamber (three wells for each cell line) together with or without HGF (20 ng/ml, Sigma) and with or without Dox (40 ng/ml). ADAM10 inhibitor GI254023X (Sigma-Aldrich) was added into the culture medium to a final concentration of 5 μM. DMSO was used as vehicle control. This experiment was repeated at least three times per cell line.

Statistical analyses. Statistics were done in GraphPad Prism (San Diego, CA). Significance was determined by ANOVA. *P < 0.05 was considered statistically significant.

RESULTS

Activation of Gα12 blocks the tubulogenesis in PC1 overexpressing MDCK cells. In a 3D culture system, MDCK cells grew in tubular patterns (3). We have reported that activation of Gα12 promoted cystic growth of these cells (20). To explore the biological links between Gα12 and PC1, we used MDCK cells in this 3D culture system. Both vector control and PC1-overexpressing cells formed tubular growth patterns (Fig. 1A). PC1-silenced MDCK cells spontaneously formed cysts, similar to the effect caused by thrombin-stimulated Gα12 activation. As previously reported, thrombin stimulation also changed the tubular growth to cystic growth in PC1-overexpressing cells (Fig. 1A). Gα12 was associated with PC1 in kidney epithelial cells (67). Therefore, we hypothesize that PC1 regulates Gα12 activity and then results in subsequent biological changes in kidney epithelial cells.

The levels of Gα12 mRNA and protein were significantly increased in the kidney tissue after conditional Pkd1 deletion in mice (Fig. 1B). In normal kidney tissue, the level of Gα12 mRNA was very low. Its level was elevated in the kidney tissue of ADPKD mice. The level of Gα12 protein increased with the level of Gα12 mRNA in the kidney tissue of ADPKD mice. The levels of Gα12 mRNA and protein were significantly increased in the kidney tissue after conditional Pkd1 deletion in mice (Fig. 1B). In normal kidney tissue, the level of Gα12 mRNA was very low. Its level was elevated in the kidney tissue of ADPKD mice. The level of Gα12 protein increased with the level of Gα12 mRNA in the kidney tissue of ADPKD mice.
was barely detected. In kidney tissue from ADPKD patients, mRNA and protein of Gα12 were dramatically increased (Fig. 1C). The active form of Gα12 was barely detected in normal kidney epithelial cells. To confirm that active Gα12 was also elevated after Pkd1 deletion, we used GST-TPR (GST-fused TPR domain of protein phosphatase 5: only binding to active form of Gα12) to precipitate active Gα12 in mouse kidney tissue. Equal amount of lysates were used to pull down active Gα12. In kidney epithelial cells from Pkd1−/− mice, active Gα12 was significantly higher than in control (WT). However, after stimulation with thrombin, there was no significant difference of active Gα12 between Pkd1−/− and WT mice (Fig. 1, D and E).

Activation of Gα12 results in morphological changes in MDCK cells. The integrity of cell-cell contacts and polarity is disrupted in the kidney epithelial cells of ADPKD patients (44, 49). We noticed a dramatic change in the morphology of MDCK cells after we induced Gα12QL by removing Dox from the culture medium (Fig. 2A). Cell morphology is mostly determined by cell-cell contacts, polarity, and cellular stress fibers. To identify the proteins in the extracellular environment that could be related to these changes, we collected the conditioned medium from MDCK cells after Gα12QL was induced. We used mass spectrometry to identify these proteins (Table 1). Compared with the control (without inducible expression of Gα12QL), one of the most specific peptides was from E-cadherin (Table 1). In addition, two cell membrane metalloproteinases, ADAM10 and ADAM17 were found in the media, which could be also important for the shedding of these cell membrane proteins in kidney epithelial cells.

To confirm the result from mass spectrometry, we used kidney cell lines and mouse kidney tissue. In MDCK cells, the cleaved ectodomain of E-cadherin was barely detected in culture medium. However, inducible expression of Gα12 increased the shedding of E-cadherin (Fig. 2B). After endogenous Gα12 was activated by the addition of thrombin, the shed fragment of E-cadherin was also increased. In PC1 knockdown cells, the shed amount of E-cadherin was elevated, which was independent of thrombin stimulation (Fig. 2C). In the renal cysts induced by inducible inactivation of Pkd1 in mice, the cleaved fragments of E-cadherin were also present (Fig. 3D).

**Table 1. Peptides in conditioned media after Gα12 activation**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Gα12QL-Dox</th>
<th>Gα12QL−Dox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calsyntenin 1 isof orm 2 (4)</td>
<td>Calsyntenin 1 isof orm 2 (4)</td>
<td></td>
</tr>
<tr>
<td>Syndecan-4 (3)</td>
<td>Syndecan-4 (6)</td>
<td></td>
</tr>
<tr>
<td>JAM-A (2)</td>
<td>JAM-A (1)</td>
<td></td>
</tr>
<tr>
<td>Lactadherin (1)</td>
<td>Lactadherin (2)</td>
<td></td>
</tr>
<tr>
<td>APP (1)</td>
<td>APP (8)</td>
<td></td>
</tr>
<tr>
<td>E-cadherin (1)</td>
<td>E-cadherin (5)</td>
<td></td>
</tr>
<tr>
<td>L1CAM (1)</td>
<td>Cadherin-6 (2)</td>
<td></td>
</tr>
<tr>
<td>ADAM10 (1)</td>
<td>ADAM17 isof orm 2 (1)</td>
<td></td>
</tr>
<tr>
<td>ADAM10 (1)</td>
<td>Integrin beta-4 (2)</td>
<td></td>
</tr>
<tr>
<td>Clusterin (23)</td>
<td>CD44 (1)</td>
<td></td>
</tr>
<tr>
<td>Osteopontin (10)</td>
<td>Clusterin (21)</td>
<td></td>
</tr>
<tr>
<td>IGF-binding protein7 (6)</td>
<td>Osteopontin (10)</td>
<td></td>
</tr>
<tr>
<td>Stanniocalcin-1 (5)</td>
<td>IGF-binding protein7 (4)</td>
<td></td>
</tr>
<tr>
<td>Semaphorin 3C (5)</td>
<td>Stanniocalcin-1 (5)</td>
<td></td>
</tr>
<tr>
<td>Collagen alpha 2(V) (13)</td>
<td>Semaphorin 3C (3)</td>
<td></td>
</tr>
<tr>
<td>Galectin-3 binding protein (10)</td>
<td>Collagen alpha 2(V) (12)</td>
<td></td>
</tr>
<tr>
<td>Thombospondin 1 (3)</td>
<td>Galectin-3 binding protein (10)</td>
<td></td>
</tr>
<tr>
<td>Fibulin-1 (1)</td>
<td>Thombospondin 1 (4)</td>
<td></td>
</tr>
<tr>
<td>Fibronectin 1 isof orm 1 (5)</td>
<td>Fibulin-1 (4)</td>
<td></td>
</tr>
<tr>
<td>Urokinase plasminogen activator (1)</td>
<td>Fibronectin 1 isof orm 1 (5)</td>
<td></td>
</tr>
<tr>
<td>Galectin-1 (5)</td>
<td>Agrin (1)</td>
<td></td>
</tr>
<tr>
<td>Podocan isof orm 1 (1)</td>
<td>Podocan isof orm 1 (1)</td>
<td></td>
</tr>
<tr>
<td>Fibronectin (2)</td>
<td>Fibronectin (2)</td>
<td></td>
</tr>
</tbody>
</table>

Peptides were identified by liquid chromatography-tandem mass spectrometry in conditioned media. Only membrane and extracellular proteins are listed. Peptide numbers are shown in parentheses.
induced (data not shown). To confirm that ADAM10 is the major sheddase for E-cadherin, we first used siRNA to knock down the expression of ADAM10 in these cells. The siRNA oligos 2 and 3 were more efficient in inhibiting ADAM10 expression (Fig. 3B). Therefore, we transfected these two ADAM10 siRNA oligos into MDCK Gα12QL cells. After the expression of Gα12QL was induced, the shed fragment of E-cadherin was dramatically reduced with siRNA oligo 3, and no E-cadherin fragment in the conditioned medium was detected with the siRNA oligo 2 (Fig. 3C). After the ADAM10-specific inhibitor GI254023 was added into MDCK cells with the expression of Gα12QL, or knockdown of PC1, there was no cleaved fragment of E-cadherin detected in the conditioned media as well (Fig. 3D).

**DISCUSSION**

MDCK cells (12) are widely used for studying the biological function of epithelia since they maintain the integrity of apico-basolateral polarity and cell junctions (tight and adherens). They polarize in 2D and 3D cell culture and are suitable for confocal imaging (3). In our laboratory, we have used MDCK Tet-off cells to investigate the functional roles of Gα12 in kidney diseases for over 10 yr. Activation of Gα12 leads to cystic growth of these kidney epithelial cells in a 3D culture (20). The cytoplasmic tail of PC1 is directly associated with Gα12 (66, 67). We used this system with the initial goal to understand the relationship between Gα12 and PC1 and especially to determine if Gα12 is a downstream signaling molecule of PC1. Our results demonstrate that activation of Gα12 blocked tubulogenesis in the MDCK cells expressing ectopic PC1, which indicates that Gα12 could be a signaling mediator of PC1. We recently reported that the loss of PC1 led to hyperactive Gα12 signaling (66, 67), which was further supported by the GST-TPR immunoprecipitation of activated Gα12 (67) (Fig. 1D). The expression level of Gα12 was elevated in kidney tissue from Pkd1+/− null mice and ADPKD patients (Fig. 1, B and C). Although PC1 is physically associated with Gα12 (66, 67), a few possibilities may be responsible for loss of PC1 resulted in activation of Gα12. Deletion of PC1 increased the expression of Gα12 (Fig. 1, B and C), which may increase the basal level of the activation of Gα12. PC1 may affects the stability of Gα12 protein. Direct binding of PC1 to Gα12 may prevent the exchange of GTP. Further study is needed to address the underlying mechanism.

We noticed that activation of Gα12 resulted in the morphology change of MDCK cells, which could result from the disruption of PC1, Gα12, ADAM10, and E-cadherin/β-catenin signaling. The signaling complex could be critical for renal cystogenesis in ADPKD.

In ADPKD, cell-cell adhesion, cell-matrix adhesion, and polarity are altered (43). We have reported that cell-matrix adhesion was disrupted after Gα12 activation, which was through changing the affinity of integrins and phosphorylation of focal adhesion molecules (20). The normal integrity of adherens junctions is dependent on intact E-cadherin. E-cad-
PC1 and Go_{12} Regulate the Shedding of E-Cadherin

E-cadherin forms complexes with a few intracellular proteins and is involved in Wnt/β-catenin signaling to regulate cell polarity and adherens junctions. PC1 is colocalized with E-cadherin and can be communoprecipitated with E-cadherin and the catenins (15). Go_{12} is directly associated with the cytoplasmic tail of PC1 (67). Go_{12} is associated with E-cadherin/catenins (30). Therefore, PC1/Go_{12}/E-cadherin/catenin is a unique complex in kidney epithelial cells and is important for regulating the polarity and adherens junction.

The ectodomain shedding of E-cadherin is a unique way to control the biological function of E-cadherin (44, 49). Metalloproteinases are the major sheddase of E-cadherin. In MDCK cells, ADAM10 and ADAM17 are the two major proteinases. Both ADAM10 and Go_{12} are involved in migration and invasion of tumor cells (19, 40). ADAM10 is responsible for shedding dozens of substrates that are related to cancer progression, inflammatory disease, and Alzheimer’s disease, such as Notch, E-cadherin, EGF, ErbB2, amyloid precursor protein (APP), and inflammatory cytokines (29, 40). APP is related to cell proliferation and Alzheimer’s disease. We also observed that syndecan-4 and cadherin-6 (K-cadherin) were present among the shed proteins (Table 1). However, we did not find any differences in the expression level of K-cadherin after active Go_{12} was induced (data not shown). Syndecan-4 is a cell surface protein that affects cellular proliferation, migration, mechanotransduction, and endocytosis via various signaling pathways such as growth factor receptors, mTOR, AKT1, and the Rho family of GTPases. Associated with extracellular matrix and cytoskeletal signaling proteins, syndecan-4 is also involved in regulating integrin turnover and cell-matrix adhesions (7). We have shown that Go_{12} regulates the cytoskeleton and integrin and focal adhesion (20, 21). Further studies are needed to elucidate any functional linkage between Go_{12} and syndecan-4 in kidney epithelial cells. In addition, there were dozens of extracellular proteins, which seems insignificant after active Go_{12} is induced (Table 1).

Fig. 4. Distribution of E-cadherin and β-catenin in kidney epithelial cells. A: active Go_{12} was induced for 48 h in MDCK cells. Green, E-cadherin; blue (DAPI), nucleus. Immunostaining of E-cadherin (B, white arrowheads) and β-catenin (C, white arrowheads) in the kidney tissue from WT and Pkd1^{-/-} (null) mice. White bar: 10 μm.
In MDCK cells, the majority of ADAM10 is the premature form. After removal of the prodomain from its premature form, ADAM10 becomes the mature form: enzymatically active. Our result showed that expression of Ga12WT or Ga12QL increased the mature form of ADAM10 to a similar level (Fig. 3A). Theoretically, expression of Ga12WT only elevates the amount of Ga12 but not the active form of Ga12. However, in the kidney tissue from WT mice, there was a decent amount of active Ga12 (Fig. 1D). In MDCK cells, we did not see dramatic difference of the ectodomain shedding of E-cadherin between Ga12WT and Ga12QL (Fig. 2B). In addition, there is no difference in the adhesion of MDCK cells on collagen-1 between the expression of Ga12WT and Ga12QL (20). So, we assume that certain amount of active Ga12 would be enough to activate ADAM10 or that another mechanism is involved, increasing stress fibers and disrupting integrins (20, 21). To confirm that the increased shedding of E-cadherin by active Ga12 or knockdown of PC is dependent on ADAM10, we used ADAM10-specific inhibitor GI254023X, which completely blocked its effects on the cleavage of E-cadherin (Fig. 3D). This result demonstrates that ADAM10 is the key proteolytic enzyme, which is regulated by PC1 and Ga12 and is responsible for cleaving the ectodomain of E-cadherin in kidney epithelial cells.

The disruption of adherens junctions results in the dissociation of β-catenin from the cytoplasmic tail of E-cadherin and its translocation into the nucleus to regulate the expression of certain genes. Subsequently, this disruption causes the changes in morphology, polarity, and cytoskeleton (8, 38). We observed that activation of Ga12 altered the distribution of E-cadherin and β-catenin in MDCK cells (Fig. 4). In transgenic mice, the Wnt/β-catenin signaling pathway is activated in kidney epithelial cells of ADPKD. Targeted expression of a mutant β-catenin results in development of renal cysts similar to that seen in ADPKD (45). PC1 multiprotein complexes also comprise E-cadherin and β-catenin that are located at the plasma membrane and cell-cell contacts (15, 43). In addition, recent evidence also indicates that the proteolytic cleaved COOH-terminal tail (CTT) of PC1 binds to active β-catenin. Then this complex moves to the nucleus together (22). It is unclear if the cleavage is mediated via ADAM10 in a similar way to the shedding of E-cadherin.

The disrupted signaling pathways in ADPKD are mostly involved in Ca2+, cAMP, and mammalian target of rapamycin complex (mTORC), mTORC1 controls cell growth, proliferation, and autophagy (47, 49, 62). Ga12 is reportedly involved in mTORC2 signaling that regulates the cytoskeleton and resistance to apoptosis (11, 25, 26). Several medications that reduce cAMP levels inhibit mTOR, inhibit cell proliferation, and reduce fluid secretion have been through clinical trials for ADPKD (4, 52). Based on changes in total kidney volume (TKV), total cyst volume, renal function, and adverse effects, a recent meta-analysis of all major clinical trials for ADPKD shows that TORC1 inhibitors and eicosapentaenoic acid have no therapeutic effects; somatostatin analogs decrease TKV by 9% only; the vasopressin receptor antagonist slows TKV increase to 3%/yr and attenuates kidney function decline. All of their adverse events are significant in all trials compared with placebo (16, 34). At present, there are no safe and effective clinical therapeutic regimens for ADPKD. Further understanding and identification of the signaling pathways and cellular mechanisms associated with ADPKD are essential to develop new therapeutic targets that block or slow down renal cystogenesis. Inhibition of ADAM10 activity could be useful for blocking the proliferation, differentiation, adhesion, and migration of cancer cells (33). ADPKD epithelial cells also demonstrate abnormalities in proliferation, differentiation, adhesion, and migration (10, 50). Triptolide, a bioactive small molecule isolated from a Chinese herb, shows both antitumor activity (1) and reduction of renal cystogenesis in ADPKD mice (24). Triptolide has been reported to inhibit ADAM10 activity and affect Wnt/β-catenin signaling pathway (46, 48).

In summary, Pkd1 mutation or deletion leads to the activation of Ga12, which promotes the maturation of ADAM10 that increases the shedding of E-cadherin. Subsequently, the cleavage of the ectodomain fragment of E-cadherin causes the transition of β-catenin from cell membrane to the nucleus. Eventually cell polarity and cell-cell contacts could be disrupted, favoring the cystogenesis of renal epithelial cells. Blocking this signaling pathway, especially inhibiting ADAM10 activity, could be a novel target for ADPKD.

ACKNOWLEDGMENTS

We thank Dr. G. Germino for providing PC1-overexpressing MDCK cells, Dr. G. Gusella for providing PC1-silenced MDCK cells, and Dr. J. Zhou for Mx1Cre\(^+/\)Pkd1flox/flox mice.

GRANTS

These studies were supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants KO1 DK-080179, 3K01DK-080179, and DK-096160 (T. Kong). These studies were also supported by the Baltimore Polycystic Kidney Disease Research and Clinical Core Center, P30DK-090868.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

T.-S.L., B.M.D., J.V.B., and T.K. interpreted results of experiments; J.X.X.

REFERENCES


