Polycystin 1, the product of the PKD1 gene, is mutated in autosomal dominant polycystic kidney disease, a disease characterized by renal cyst formation and progressive renal failure. We show that expression of the C-terminal domain of human polycystin-1 (PKD1-CT) triggers spreading of isolated inner medullary collecting duct cells, a process mediated by Erk. As inner medullary collecting duct cells spread, PKD1-CT localizes to cell-extracellular matrix contacts, interacts with focal adhesion proteins Fak and paxillin, and stimulates Fak phosphorylation, paxillin phosphorylation, Fak-paxillin association, and formation of small focal complexes. PKD1-CT-mediated spreading requires membrane localization and the integrity of the C-terminal protein binding sites. We additionally show that Pkd1 null proximal tubule cells generated from Pkd1Pkd1/H11546/TSLargeT mice by in vitro Cre recombinase transfection demonstrate diminished spreading when compared with Pkd1Pkd1/H11546/+ heterozygous parental cells. These findings suggest that membrane-bound PC1 has a central role in regulating morphogenic protein signaling at cell-matrix interfaces in non-confluent cells.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited monogenic disorders, occurring in ~1 in 600–1000 live births. It is a systemic disorder characterized by the development of multiple renal cysts that arise along the proximal and distal tubules and collecting ducts. Large fluid-filled cysts develop and disrupt the normal architecture, leading to loss of kidney function. In Western countries, ADPKD accounts for 5–10% of end stage kidney failure (1).

ADPKD is caused by mutations in the PKD1 gene (85% of families) (2) or in the PKD2 gene (10–15% of families) (3), which encode the polycystin 1 (Pc-1) and 2 (Pc-2) proteins, respectively. Pc-1 is a membrane-spanning glycoprotein with a large N-terminal extracellular region, multiple transmembrane domains, and a short C-terminal cytoplasmic tail of 226 amino acids that has been found to be the site of major signaling interactions via its coiled-coil domain and putative phosphorylation sites. Pc-2 is an integral membrane protein with six transmembrane domains and cytoplasmic N and C termini. Pc-1 and -2 are able to interact and can function together as a Ca²⁺-ion channel with ion selectivity similar to other transient receptor potential family members (4, 5).

In epithelial cells, the Pc-1/Pc-2 complex has been located in the primary cilium at the apical pole of the cell (6), where it may act as a mechanoreceptor (7), and Pc-1 has been located at cell-cell junctions, where it could modulate adhesion of adjacent cells (8, 9). Typically, these subcellular localizations of polycystins require the cells to be grown to complete confluence and to become fully polarized (10, 11). In subconfluent cells, Pc-1 has been located at sites of cell-matrix interactions, where it associates with several focal adhesions proteins, such as integrins, vinculin, paxillin, and Fak (12). However, the functional role of Pc-1 at focal adhesions has yet to be fully elucidated.

Currently, more than 50 proteins have been reported to be associated with focal contacts and related extracellular matrix adhesions, forming a dense, heterogeneous, and dynamic protein network at the cytoplasmic face of the adhesion site that regulates integrin adhesion, signaling, and the actin cytoskeleton (13). Focal adhesion regulation and signaling ultimately control many cellular behaviors, including cell adhesion, spreading, migration, and proliferation (14).

To study the role of Pc-1 at focal adhesions, we used murine inner medullary collecting duct (IMCD) cells overexpressing a heterologous integral membrane protein fused to the C-terminal cytoplasmic domain of Pc-1 (PKD1-CT). This approach has been used to show heterodimeric interaction between Pc-1 and Pc-2 and to delineate the role of the Pc-1 cytoplasmic domain in signaling through the Wnt (15) and AP-1 pathways (16). We report that expression of PKD1-CT triggers spreading in isolated IMCD cells plated on fibronectin in a MEK-dependent manner. We also show that during the initial phase of cell spreading, PKD1-CT localized at the cell membrane is necessary for regulating interactions of paxillin and Fak as well as activating Erk, paxillin, and Fak. The role of the endogenous protein in mediating normal cell spreading is addressed by experiments demonstrating decreased spreading in PKD1 null cells.
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EXPERIMENTAL PROCEDURES

Cells, Antibodies, and Reagents—Experiments were conducted using immortalized mIMCD-3 cells of ureteric bud origin (referred to as IMCD cells) (17). High efficiency retroviral transfer of CD16-, CD7-, and PKD1-containing plasmids has been described (18). CD16.7 denotes the extracellular domain of CD16 and the transmembrane domain of CD7; PKD1-CT denotes the C-terminal cytoplasmic domain of human polycystin-1 (amino acids 4077–4302). The C-terminal domain of PKD1 was expressed as a CD16.7 fusion protein (referred to as CD16.7-PKD1). These cells represent a mixture of thousands of individual cells that originally were stably transduced with the retrovirus rather than an individual clone of cells. In a subset of experiments, we used a patient-derived truncation mutation of PKD1 (amino acids 4077–4302). The C-terminal domain of PKD1-CT was fused to the N terminus of amino acids 4077–4227 of PKD1 and the cytoplasmic domain of Pc-1, lacking amino acids 4077–4227 (CD16.7-R4227X). The same technique was used to generate IMCD cells expressing the FLAG epitope (F-) or FLAG fused to the N terminus of amino acids 4077–4227 of PKD1 (F-PKD1).

Anti-phospho-p42/44 and p42/44 MAPK antibodies were obtained from Cell Signaling; anti-phospho-Pkca and anti-phospho-397 Fak (α–P-FAK) were obtained from Upstate Biotechnology; and anti-Fak was obtained from Santa Cruz Biotechnology, anti-paxillin was from Transduction Laboratories, and anti-GAPDH was from Chemicon. Polyclonal anti-phospho-paxillin antibody (p-S83) was generated as described previously (19). Anti-BD3, directed against the C terminus of polycystin 1, was a kind gift of Dr Oxana Ibraghimov (Genzyme Corp.) (20). Cells were grown in Dulbecco’s modified Eagle’s medium-F12 medium supplemented with 10% fetal bovine serum. All other reagents were obtained from Sigma unless otherwise noted.

Immunocytochemistry—Quiescent cells were detached with cell dissociation buffer (Invitrogen), washed three times with PBS, resuspended in serum-free Dulbecco’s modified Eagle’s medium/F12 medium, and plated on fibronectin (20 μg/ml) -coated coverslips for the indicated times. Cells were then fixed in 4% paraformaldehyde in PBS, washed, and permeabilized with −20 °C methanol. After blocking with 1% bovine serum albumin and 5% goat serum in PBS, immunostaining was performed with anti-BD3 (4 °C overnight, 1:500), anti-paxillin (1 h at room temperature, 1:1000), or anti-Fak (30 min at room temperature, 1:100), and cells were probed at room temperature with the appropriate secondary antibodies coupled to Alexa Fluor 488 or 594 (Molecular Probes). 4,6-Diamidino-2-phenylindole was included in the mounting medium as a counterstain (Vector Laboratories). Fluorescent signals were visualized and acquired using a Zeiss epifluorescence confocal microscope.

Western Blot and Immunoprecipitation Analysis—Quiescent cells were detached with cell dissociation buffer, washed three times with PBS, and resuspended in serum-free Dulbecco’s modified Eagle’s medium/F12 medium; cells were then either kept in suspension or plated on fibronectin (20 μg/ml) -coated tissue culture dishes for the indicated times (15–60 min) and washed gently to remove non-adherent cells. For Erk and Pkcα inhibition, cells were preincubated for 20 min with the MEK inhibitor UO126 (10 μM, Promega) or the Pkcα inhibitor Gö6976 (100 μM). In a subset of experiments, 70–80% confluent cells were made quiescent by serum starvation overnight directly followed by stimulation with hepatocyte growth factor (HGF, 40 ng/ml) for the indicated times. For Western blot analysis, cells were lysed in modified radioimmune precipitation buffer (0.16 M NaCl, 20 mM Trizma (Tris base), 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1% Triton X-100, 1% sodium deoxycholate, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml pepstatin). The protein content was determined using the Bradford assay. For immunoprecipitation, cells were lysed with Triton X-100 buffer (0.16 M NaCl, 20 mM Trizma base, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1% Triton X-100, 0.5% Tween 20, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml pepstatin), and 1 mg of cell lysate was immunoprecipitated by the appropriate antibody overnight at 4 °C, collected by adding the appropriate Sepharose-coupled protein, and washed three times with lysis buffer. Proteins were separated using 10% SDS-PAGE, transferred to Immobilon-P membranes (Millipore), immunoblotted with the appropriate antibody, and visualized by enhanced chemiluminescence (Amersham Biosciences). To compare individual conditions, the level of phosphorylation of the protein in question was quantitated by density scanning using NIH Image software and normalized to an internal control (either the non-phosphorylated protein or GAPDH), and the maximal signal detected was arbitrarily defined as 100% phosphorylation. Statistical significance (mean ± S.D.) was determined using the Student’s t test.

Cell Spreading Assay—Cells were serum-starved overnight prior to harvesting with cell dissociation buffer, washed three times with PBS, plated on fibronectin (20 μg/ml) -coated coverslips ± HGF (40 ng/ml), and left to adhere and spread for 1 or 3 h. Spreading of individual cells was quantitated after imaging by using the Photoshop 7 software to determine the individual surface area (in square pixels) of 200 different single cells per condition. Each experiment was repeated at least three times. Statistical significance (mean ± S.D.) was determined using the Student’s t test.

PKD1 Null Cells—For some experiments, PKD1 null cells isolated from the proximal tubule were used to assess cell spreading. Microscopically dissected and then dissociated proximal tubules from Pkd1<sup>flox</sup>−/−:TSLargeT (ImmortoMouse) mice were cultured under permissive conditions (33 °C with γ-interferon) and cloned by limiting dilution. Clones were evaluated at full confluency under non-permissive conditions (37 °C, no γ-interferon for >7 days) for epithelial properties including tight junction formation (ZO1), E-cadherin and β-catenin expression, primary cilia formation, and expression of the proximal tubule marker NHE3, and only clones meeting these criteria were utilized. Parental Pkd1<sup>flox</sup>−/− cell clones showing epithelial properties were then transiently transfected with a plasmid encoding Cre recombinase and cloned again by limiting dilution, giving rise to daughter cells that either expressed Cre and therefore had undergone transformation to Pkd1<sup>−/−</sup> or that had not expressed Cre and retained the parental Pkd1<sup>flox</sup>−/− genotype. To minimize the chance that differences in cell responses could be due to derivation from different original
cells, the present experiments were performed using \( Pkd^{−/−} \) clones PN18 and PN24 and \( Pkd^{flox/−} \) clones PH2 and PH3, all of which were derived from the same original parental \( Pkd^{flox/−} \) clone. The epithelial properties of the newly derived cell clones were evaluated, and they were found to have robust lateral localization of E-cadherin and \( \beta \)-catenin and formation of tight junctions and cilia (data not shown).

RESULTS AND DISCUSSION

Expression of the Membrane-anchored C-terminal Cytoplasmic Domain of Polycystin-1 Stimulates Spreading of IMCD Cells—To examine the role of polycystin signaling in regulating cell-matrix interactions, IMCD cells overexpressing the membrane-anchored PKD1-CT were utilized. This construct has been shown to mediate interactions with Pc-2 (21) and has been used previously in our laboratory to show that Pc-1 stimulates in vitro branching tubulogenesis in three-dimensional gels (18), consistent with findings seen following expression of full-length Pc-1 (22). To specifically focus on Pc-1 function at the cell-matrix interface, IMCD cells expressing either the cytoplasmic tail of polycystin-1 (CD16.7-PKD1) or the control construct (CD16.7) were plated on fibronectin-coated dishes at single cell density. Under these conditions, neither intercellular junctions nor primary cilia would be expected to form.

To determine whether PKD1-CT has a potential role in regulating signaling at cell-matrix interaction sites, we assessed individual cell spreading (see “Experimental Procedures”). Expression of the cytoplasmic tail of Pc-1 stimulates cell spreading of quiescent IMCD cells at both 60 and 180 min (Fig. 1). Stimulation of the cells with HGF, a well known stimulant of epithelial renal cell spreading (23), demonstrated that both cell types were able to reach equivalent levels of maximal spreading at 180 min. These results demonstrate that expression of PKD1-CT is able to accelerate IMCD cell spreading on fibronectin.

PKD1-CT Transiently Colocalizes with Focal Adhesion Proteins Fak and Paxillin—Cell spreading requires the dissolution of existing focal adhesions and the formation of new focal adhesions at the leading edge of the lamellipodia, a process known as focal adhesion turnover. Focal adhesion turnover requires the recruitment of activated Fak to focal adhesions, a process that is at least partially mediated by the interaction of Fak with the scaffolding protein paxillin (24, 25). To determine whether PKD1-CT-induced spreading could be mediated by interactions with focal adhesion proteins, communoprecipitation experiments were performed using cells plated on fibronectin for 60 min. These experiments revealed association of PKD1-CT with both Fak and paxillin in Triton X-100 cell lysates. This interaction was not supported under the harsher conditions of a modified radioimmune precipitation buffer (Fig. 2A), suggesting that it might be an indirect interaction.

To determine whether the PKD1-CT localized at sites of newly forming focal contacts, immunocytochemistry was per-
FIGURE 3. A, serum-starved untransfected (m-IMCD), control (CD16.7), and PKD1-CT (CD16.7-PKD1)-expressing cells were kept in suspension (S) or allowed to adhere and spread on fibronectin-coated dishes for the indicated times (15–60 min). Whole cell lysates (40 μg) from these cells were separated by SDS-PAGE and immuno-blotted with antibodies against activated Erk (α-pp44/42), activated Pkα (α-p-Pkα), and GAPDH (as loading control). B, densitometric quantification of activated Erk/GAPDH ratio from four independent experiments performed as in A. *, p < 0.05 versus pp42/GAPDH ratio of CD16.7-PKD1 cells plated for 15 min. C, CD16.7- and CD16.7-PKD1-expressing cells were kept in suspension pretreatment for 20 min with either U0126 (10 μM) or Gö6976 (10 μM) and then were plated and allowed to adhere and spread for 15 min. Cell lysates were processed as in A. D, CD16.7 and CD16.7-PKD1 cells were pretreated with U0126 or Gö6976 as in C and plated on fibronectin, and spreading was quantitated 60 min after plating. **, p < 0.01; data are representative of three independent experiments. E, serum-starved control (CD16.7) and PKD1 (CD16.7-PKD1) cells were treated with HGF (40 ng/ml) for 15 min to 2 h. Whole cell lysates (40 μg) were immunoblotted with activated Erk, activated Pkα, and α-GAPDH antibodies. F, densitometric quantification of pp42/GAPDH ratio (activated Erk/GAPDH ratio) from four independent experiments performed as in E.
FIGURE 4. A, CD16.7 control cells and CD16.7-PKD1 cells were kept in suspension (susp.) or plated for 30 min on fibronectin (fn), cell lysates were immunoprecipitated (IP) with α-Fak, and three-quarters of the immunoprecipitate were blotted with α-p-Fak (antibody directed against p-Tyr-397). One-quarter of the immunoprecipitate was immunoblotted (IB) with α-Fak to assess the equality of the starting material. The bar graph shows the densitometric quantification of p-Fak/Fak ratio from four independent experiments. *, p < 0.05 versus pFak/Fak ratio of CD16.7-PKD1 cells plated for 30 min, set as 100%. B, whole cell lysates prepared from cells treated as described in A were immunoblotted with anti-phospho-paxillin (α-pS83) antibody. The same membrane was stripped and reprobed with anti-paxillin (α-PAX) antibody. The bar graph shows the densitometric quantification of pS83/PAX ratio from four independent experiments. **, p < 0.01 versus pS83/PAX ratio of CD16.7-PKD1 cells plated for 30 min, set as 100%. C, CD16.7 and CD16.7-PKD1 cells were plated for 30 min on fibronectin. Lysates were immunoprecipitated with α-Fak and immunoblotted with α-PAX and α-Fak. The bar graph shows the densitometric quantification of PAX/Fak ratio from four independent experiments. *, p < 0.05 versus PAX/Fak ratio of CD16.7-PKD1 cells plated for 30 min, set as 100%. D, control (CD16.7) and PKD1 (CD16.7-PKD1) IMCD cells were allowed to spread for 1 h on fibronectin-coated coverslips, fixed, and immunostained with α-PAX and anti-Fak (α-Fak) antibodies and the corresponding fluorescent conjugated secondary antibodies. Bar = 2 μm. Note that paxillin and Fak colocalize to focal adhesions in both cell types, but in PKD1 cells, focal adhesions are consistently shorter. The length of each individual focal adhesion identified at the periphery of 20 cells for each cell type was determined (bar graph, ***, p < 0.001).
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Focal contact formation and dissolution allows cells to alter their attachment to the basement membrane and thus promotes cell morphogenesis and migration, events that occur in vivo during renal tubule development and in the early phase of repair after tubular injury. During tubulogenesis and tubule repair, cell confluency can range from individual cells to cell sheets, with the cells exhibiting a flattened phenotype due to the large proportion of the surface area that is in contact with the underlying matrix. As tubules mature, cell density increases, and the cells become more vertically oriented with a lesser amount of cell-matrix interactions and the formation of extensive cell-cell adherens junctions at the lateral borders. These fully confluent, differentiated cells develop an apical brush border and primary cilia. The present results suggest that under the less confluent, less differentiated conditions of the immature or injured tubule, PKD1 can localize to focal contacts rather than primary cilia and thus may play a role in the regulation of cell morphogenesis.

The fate of the PKD1-CT construct in maximally spread, non-confluent cells remains to be determined. By careful confocal microscopy examination, we failed to detect PKD1-CT-paxillin interaction or colocalization with the more mature focal adhesion complexes seen in stable contacting fibroblasts to other subcellular structures such as the intermediate filament network. The functional significance of PKD1-CT translocating from focal adhesions to other subcellular structures such as the intermediate filament network remains to be elucidated.

**Spreading Induced by PKD1-CT Is Mediated by Erk**—To further delineate the mechanisms involved in PKD1-CT-induced spreading, we focused on two signaling pathways previously studied in our laboratory: Erk1/2 MAP kinase, which has been determined as an important pathway for regulating focal adhesion turnover (23), and Pkc, which was shown to be activated by PKD1-CT (16) and to regulate PKD1-CT-induced branching morphogenesis (18). Untransfected quiescent IMCD cells or control cells overexpressing CD16.7 were plated on fibronectin for 15, 30, and 60 min, resulting in a weak and transient Erk1 and Erk2 phosphorylation (Fig. 3A). In contrast, cells overexpressing CD16.7-PKD1 displayed an enhanced anchorage-dependent Erk activation at 15, 30, and 60 min (Fig. 3A, and quantitated in Fig. 3B). In these three cell lines, significant and comparable Pkalpha phosphorylation could be detected in suspension, with no modification during the first hour after plating. To study the functional role of Erk and Pkalpha pathways during PKD1-CT-induced cell spreading, we used the MEK-specific inhibitor U0126 to prevent Erk activation and G66976 for Pkc inhibition. U0126 and G66976 efficiently abrogated Erk1/2 and Pkalpha phosphorylation, respectively, in control and PKD1-CT-overexpressing cells plated for 15 min (Fig. 3C). Neither inhibitor had a significant effect on control cell spreading at 1 h, whereas PKD1-CT-induced spreading was inhibited by U0126 but not by G66976 (Fig. 3D). These results suggest that Pc-1 expression up-regulates anchorage-dependent Erk activation and that signaling by this pathway is required for Pc-1-mediated cell spreading.

formed on cells plated for 30 min, and the localization of the C terminus of Pc-1 was visualized using confocal microscopy. PKD1-CT-overexpressing IMCD cells stained with anti-BD3 antibody (specific for the C terminus of PKD1) indicate that PKD1-CT localized to discrete peripheral structures of isolated spreading cells (Fig. 2B). A similar staining pattern was observed in untransfected cells when the confocal signal intensity was increased sufficiently, suggesting that at least a fraction of endogenous polycystin 1 distributes in a similar subcellular localization. Confocal examination of cells immunostained with BD3 as well as anti-paxillin demonstrates that PKD1-CT colocalizes with paxillin at sites of newly forming focal contacts during the early stages of cell spreading (Fig. 2C, 30'). These results are in agreement with those published by Wilson et al. (12) demonstrating that endogenous Pc-1 can colocalize with alpha beta 1 integrin and the focal adhesion proteins vinculin and paxillin at sites of cell-matrix focal contacts. In the present work, we failed to detect PKD1-paxillin interaction or colocalization at focal adhesions after cells were maximally spread (Fig. 2C, 180'), suggesting that PKD1-CT may interact with focal adhesion proteins selectively during the initial stages of focal contact formation when focal adhesion turnover is required but not with the more mature focal adhesion complex seen in stable focal adhesions.
Anchorage-dependent Erk activation has been found to be mediated, at least in part, by integrin-dependent Fak and p21-associated kinase (Pak) activation followed by Pak-mediated Mek-Erk association (27, 28). Alternatively, the Erk-MAPK pathway can be activated by growth factor-dependent recruitment of Grb2-Sos to the membrane followed by Ras activation.

To begin to define the mechanism of Erk activation in PKD1-CT-expressing cells, we examined the activation of both the Erk-MAPK and the Pkc pathways in stably adherent cells following growth factor stimulation with HGF. In contrast to the increase in anchorage-dependent Erk activation demonstrated in Fig. 3A, stably adherent CD16.7-PKD1-expressing cells displayed a basal and HGF-stimulated level of Erk activation that was indistinguishable from the control cells (Fig. 3E, and quantitated in Fig. 3F). As reported previously, Pkc phosphorylation was more pronounced in CD16.7-PKD1 cells (18). Thus, the enhanced Erk activation seen in newly plated PKD1-CT-overexpressing cells appears to be specifically dependent on signaling pathways that are transiently activated during the initial stages of cell spreading, supporting a role for integrin-dependent MAPK activation in this process.

As opposed to the Erk-dependent cell spreading shown here, PKD1-CT overexpression has been reported to stimulate other morphogenic events, such as haptotactic migration and branching morphogenesis, in a largely Erk-independent and Pkc-dependent manner (18). Based on our current studies, it is possible that transient localization of PKD1-CT to newly forming focal contacts stimulates Erk-dependent cell spreading, whereas different subcellular localization in stably adherent cells may result in distinct interacting partners and thus promote directed migration and branching tubulogenesis that are dependent on alternate signaling pathways.

**PKD1-CT Stimulates Fak and Paxillin Phosphorylation, Fak-Paxillin Association, and Small Focal Complex Formation**—Previous studies have shown that recruitment of paxillin and Fak to focal adhesions is critical for cell spreading, independent of their role in cell adhesion (29). This process requires Fak and paxillin tyrosine phosphorylation and Fak activation (30, 31). In addition, we have found that both growth factor-dependent and cell adhesion-dependent cell spreading are accelerated in the setting of Erk-MAPK pathway activation (24) and that this effect of MAPK activation involves Erk-dependent paxillin phosphorylation on serine 83 and subsequent recrui-
ment and activation of Fak signaling (19, 23, 30, 31). Since PKD1-CT localizes to focal adhesions and stimulates cell spreading in a MAP kinase-dependent manner, the role of Fak activation and MAPK-dependent paxillin-Fak association was assessed in PKD1-CT-dependent cell spreading.

Following plating on fibronectin, the PKD1-CT-expressing cells demonstrated a greater increase in Fak phosphorylation at Tyr-397 when compared with control cells expressing CD16.7 (Fig. 4A). Tyrosine 397 is the site known to be phosphorylated during Fak activation and to mediate downstream signaling via the phosphatidylinositol 3-kinase. Examination of paxillin in these cells revealed that phosphorylation at serine 83, the Erk phosphorylation site that is required for MAPK-dependent paxillin-Fak association, was also increased in PKD1-CT-ex-
pressed cells when compared with control cells (Fig. 4B). Consistent with this increase in serine 83 phosphorylation, paxillin-Fak association was also up-regulated in PKD1-CT-expressing cells (Fig. 4C). These results support the hypothesis that PKD1-CT regulates activation of the focal adhesion protein Fak and stimulates the Erk-dependent association of Fak with the focal adhesion scaffolding protein paxillin.

Dynamic changes of matrix adhesion structures occur during cell spreading, polarization, migration, and division. Stimuli that induce the turnover of focal adhesions, such as growth factor treatment or Src activation, lead to the turnover of stable focal adhesions and formation of new, smaller focal adhesions at the sites of membrane protrusion, thus stabilizing lamellipodia extension and supporting cell spreading and migration (32, 33). Small focal adhesions, often referred to as focal complexes, are seen at the periphery of spreading cells (34) and precede the formation of larger focal adhesions (35). Signaling events that induce large and dense focal adhesions typically inhibit membrane protrusion (36, 37). For example, Fak<sup>−/−</sup> fibroblasts display very large focal adhesions and fail to spread efficiently (38).

To determine whether the expression of the C terminus of PKD1 altered the morphology of cell-extracellular matrix contacts, confocal microscopy was utilized to quantitate focal adhesion length in cells expressing CD16.7-PKD1. Cells were plated for 60 min on fibronectin-coated dishes and stained with α-paxillin and α-Fak antibodies. Fak and paxillin were found to colocalize at sites of cell-extracellular matrix focal adhesions in both cell types, but in CD16.7-PKD1 cells, focal contacts appear smaller (Fig. 4D). The length of each individual focal adhesion seen at the periphery of 20 cells was determined (Fig. 4D, bar graph), demonstrating that focal adhesions were 36% shorter in PKD1-CT-expressing cells. The presence of smaller focal adhesions in these rapidly spreading cells is consistent with an accelerated rate of focal adhesion turnover, likely stimulated by MAPK-dependent Fak-paxillin association and Fak activation (32–37).

**PKD1-CT-induced Focal Adhesion Signaling and Cell Spreading Require Membrane Localization**—To determine whether membrane localization of PKD1-CT is important for cell spreading, a FLAG-tagged PKD1-CT construct was generated (F-PKD1) containing the cytoplasmic tail of polycystin 1 (amino acids 4077–4302) tagged at its N terminus with the FLAG epitope. As this construct lacks a signal sequence or transmembrane domain, its expression was found to be cytoplasmic without membrane localization (data not shown). As demonstrated by anti-BD3 immunoblotting, CD16.7-PKD1 and F-PKD1 cell lines expressed comparable amounts of PKD1-CT, and the proteins migrated at the expected sizes (Fig. 5A).

Examination of focal adhesion signaling in cells expressing either F-PKD1 or CD16.7-PKD1 and plated on fibronectin for 30 min revealed that expression of F-PKD1 failed to activate Erk or Fak, as judged by quantitation of phospho-specific antibody staining, when compared with CD16.7-PKD1 (Fig. 5B, and Fak phosphorylation quantitated in Fig. 5C). Consistent with the failure of F-PKD1 to mediate increased Erk activation, phosphorylation of the serine 83 site on paxillin was also not increased in F-PKD1-expressing cells, and paxillin-Fak association was not up-regulated (Fig. 5B, and paxillin phosphorylation quantitated in Fig. 5C). Moreover, when compared with CD16.7-PKD1-expressing cells, the F-PKD1 cell line did not demonstrate increased adhesion-dependent cell spreading, although maximal spreading in response to HGF was indistinguishable in all cell lines (Fig. 5D). These results indicate that in order for PKD1-CT to regulate focal adhesion signaling and subsequent spreading, membrane localization is required. This suggests that Pc-1 may interact with other membrane-associated proteins at focal adhesions, such as the α/β integrin heterodimers, proteoglycans, or glycosaminoglycan receptors (39, 40).

**Truncation of the C-terminal Domain of Polycystin-1 Prevents Spreading**—The C terminus of Pc-1 contains a coiled-coil domain that has been shown to mediate interactions with protein partners, including Pc-2 (41). To determine the importance of this domain in supporting increased cell spreading, IMCD cells expressing a truncation mutation of Pc-1 that disrupts the coiled-coil structure (CD16.7-R4227X) were utilized. This mutation is known to have functional consequences for Pc-1 signaling as it has been described in several families with ADPKD (42). As demonstrated by immunocytochemistry, the overall intensity of CD16 staining was comparable in CD16.7, CD16.7-PKD1, and CD16.7-R4227X cell lines.
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(Fig. 6A), suggesting equivalent amounts of expressed proteins. However, expression of CD16.7-R4227X failed to activate Erk or Fak and did not mediate increased paxillin phosphorylation at serine 83 (Fig. 6B). Consistent with these observations, cells expressing CD16.7-R4227X did not display increased adhesion-dependent cell spreading, although maximal spreading in response to HGF was still observed (Fig. 6C). Interestingly, we observed the same phenotype with a triple mutant of Pc-1 (L14196D/V4235D/L4238D) that selectively disrupts coiled-coil domain folding and thus disrupts PKD1-CT interaction with Pc-2 (18) (data not shown). Based on these results, it appears that specific Pc-1 protein interactions mediated by the C-terminal coiled-coil domain, possibly including the interaction with Pc-2, are required for PKD1-mediated regulation of focal adhesions and cell spreading.

Endogenous Polycystin-1 Stimulates Renal Epithelial Cell Spreading—The results obtained in PKD1-CT cells demonstrate that overexpressing the C-terminal domain of polycystin-1 at the membrane can induce cell spreading in isolated epithelial cells. To determine whether the endogenous cystin-1 at the membrane can induce cell spreading in iso-

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