Compromised Cytoarchitecture and Polarized Trafficking in Autosomal Dominant Polycystic Kidney Disease Cells

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Abstract. Cystogenesis associated with autosomal dominant polycystic kidney disease (ADPKD) is characterized by perturbations in the polarized phenotype and function of cyst-lining epithelial cells. The polycystins, the protein products of the genes mutated in the majority of ADPKD cases, have been described recently, but the pathological mechanism by which causal mutations result in the mislocalization of cell membrane proteins has remained unclear. This report documents the dissociation from the ADPKD cell basolateral membrane of three molecules essential for spatial organization and exocytosis. The adherens junction protein E-cadherin, the subcellular disposition of which governs intercellular and intracellular architecture, was discovered sequestered in an internal ADPKD cell compartment. At the same time, sec6 and sec8, components of a complex critical for basolateral cargo delivery normally arrayed at the apico-lateral apex, were depleted from the ADPKD cell plasma membrane. An analysis of membrane transport revealed that basolateral trafficking of proteins and lipids was impaired as a result of delayed cargo exit from the ADPKD cell Golgi apparatus. A pical transport proceeded normally. Taken together with recent documentation of an association between polycystin-1 and E-cadherin (Huan and van Adelsberg, 1999), the data suggest that causal mutations disrupt E-cadherin–dependent cytoarchitecture, adversely affecting protein assemblies crucial for basolateral trafficking.

Key words: basolateral • adherens junction • epithelia • autosomal dominant polycystic kidney disease (ADPKD) • polycystin

Introduction

The efficacy with which epithelial cells absorb, filter, and secrete metabolites is predicated upon a polarized cell architecture and vectorial molecular trafficking (Yeaman et al., 1999). Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited disease characterized by numerous morphological and functional alterations, many of which may be explained by deficits in the cyst epithelial cell differentiation program. The aberrant expression of select basolateral proteins on the apical ADPKD cell surface (Wilson, 1997) is associated with the progressive accumulation and enlargement of fluid-filled cysts, which ultimately abrogates renal function (Carone et al., 1994). Although it has long been supposed that modulations in cytoarchitecture and the fidelity of molecular targeting are central to ADPKD pathology (for review see Wilson, 1997), a mechanistic link between the occurrence of causal mutations and dysmorphogenesis has yet to be discovered.

Recent progress concerning the genes responsible for ADPKD has been instrumental in identifying the molecular genetic basis of this disorder. Genetic lesions associated with >85% of ADPKD cases have been mapped to the polycystic kidney disease (PKD) 1 gene (Reders et al., 1985), whereas the remaining cases are due to mutations in PKD2 (Mochizuki et al., 1996), and in rare instances a third undescribed locus (Doust et al., 1995). The PKD1 and PKD2 genes encode polycystin-1 and polycystin-2, re-
E-cadherin–mediated adhesion. As intercellular E-cadherin interactions stabilize cytoskeletal tethered membrane proteins, a subset of cytosolic proteins are recruited to the contacting membranes. The sec proteins comprising the multimeric exocyst (TerBush et al., 1996) are among those cytosolic proteins recruited to the basolateral membrane in response to E-cadherin ligation (Grindstaff et al., 1998). These proteins demarcate a basolateral targeting patch that cooperates with the soluble N-ethylmaleimide-sensitive attachment protein receptor (SNARE) family of vesicular and target membrane receptors to ensure the proficiency of basolateral trafficking (Grindstaff et al., 1998). The maintenance of this highly defined cellular organization is crucial in the continued performance of epithelial tissues.

Because E-cadherin is critical for epithelial cell organization, it is interesting to consider the possibility that mutations in PKD1 or PKD2 disrupt normal E-cadherin–polycystin assemblies, and consequently impact A D P K D cell morphology and performance. Therefore, an assessment of A D P K D cell architecture and molecular trafficking was undertaken in order to identify specific derangements that lead to the compromised phenotypic state typical of these cells.

Materials and Methods

Chemical Reagents and Antibodies

Super Signal chemiluminescent substrate was supplied by Pierce. 4-(2-aminoethyl)benzenesulfonylfluoride, HCl (A E B S F ) and Mowiol 4-88 were obtained from Calbiochem. Mouse mAbs against polycystin-1 and polycystin-2 were purchased from Stresgen Biotechnologies Corp. A rabbit polyclonal antibody (p a b ) against the human polycystin-1 was provided by the International Polycystic Kidney Disease Consortium. Individual cDNA was amplified as cDNA from cDNA libraries (International Polycystic Kidney Disease Consortium, 1999), which serves as the primary pathogenic stimulus that precipitates deterioration of previously normal epithelial tissue. Singular homozygous mutant cells are thought to undergo partial dedifferentiation and proliferation, leading to repopulation of the tubule wall and development of a clonal cyst (Carone et al., 1994; Qian et al., 1996).

A n exciting insight was provided by the recent description of intercellular and intermolecular interactions have yielded important information regarding the localization and potential roles of the polycystins. Polycystin-1 is a 480,000-mol wt putative transmembrane protein (Hughes et al., 1995; International Polycystic Kidney Disease Consortium, 1995) localized solely along lateral contacting membranes of cultured cells (Ibrahim-Beskrovnaya et al., 1997). A predicted 2,500-amino acid extracellular domain is comprised of diverse motifs with putative functions in cell–cell and cell–extracellular matrix interactions (Hughes et al., 1995; International Polycystic Kidney Disease Consortium, 1995; Moy et al., 1996), suggesting that polycystin-1 plays a role in adhesion (Hughes et al., 1995). Polycystin-2 is an integral membrane protein consisting of domains with significant homology to polycystin-1 as well as to a family of voltage-sensitive attachment protein receptors (SNARE) family of vesicular and target membrane receptors to ensure the proficiency of basolateral trafficking (Grindstaff et al., 1998). The maintenance of this highly defined cellular organization is crucial in the continued performance of epithelial tissues.

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munofluorescence experiments, cells were also contained for the tight junction marker occludin to visually demonstrate cell confluence. Every experiment described in this study was performed in triplicate, using primary cells derived from normal or polycystic kidneys, in each case from three unrelated patients. The same patient samples (expanded no further than passage 4) were used throughout the study to minimize any effects of phenotypic variation.

**Fluorescent Lipid Transport Assay**

250 nmol C₆-NBD-ceramide (Molecular Probes) in chloroform and methanol was dried to a powder under dry nitrogen and prepared as a BSA complex as described (Pagano, 1989). The methods used to label live cells with C₆-NBD-ceramide and monitor lipid transport were based upon published procedures (Lipsky and Pagano, 1985; van Meer et al., 1987). Immediately before the experiment, an aliquot of prepared C₆-NBD-ceramide was thawed and divided 1:3 in plasmol red-free Heps-buffers M (HEM) containing 0.35 gliter NaHCO₃ and 60 mg/ml K₂HPO₄ (HEM_susan). Filter-grown cells were rinsed twice with PBS⁺ and 300 µl of the diluted fluorescent lipid was applied to the apical surface, whereas 2 ml of HMEM was added to the apical surface, whereas 3 ml of the same medium without virus was added basolaterally. After the 1-hour virus adsorption phase, cells were washed and incubated for 4 h at 37°C in DME/BSA before either metabolic labeling or steady-state biotinylation assays.

**Recombinant Adenovirus Infection**

Recombinant adenoviruses encoding either LDL-R or p75[TR] were kindly provided by Drs. J. Roger E. Her (University of Texas Southwestern Medical Center, Dallas, TX) (Her and Gerard, 1993) and Moses Chao (Cornell University Medical College, New York) (Yoon et al., 1996), respectively. Large-scale virus stocks were prepared as described previously (Spector et al., 1998). Stock virus preparations had titers of ~10¹⁰ pfu/ml and infected the cells with 90% efficiency at a dilution of 1:10. Confluent filter-grown cells (72 h after initial seeding) were infected with adenoviruses (~2 pfu/cell) for 1 h at 37°C. Viruses were diluted in serum-free DME and 300 µl was added to the apical surface, whereas 3 ml of the same medium without virus was added basolaterally. After the virus adsorption phase, media were replaced with serum-containing culture media and the cells were returned to a humidified 5% CO₂ incubator for 36 h before metabolic labeling.

**Cell Surface Biotinylation**

Filter-grown cells were starved for 30 min at 37°C in DME lacking cysteine and methionine and then radiolabeled for 30 min at 37°C with 0.2 mCi/ml of ³⁵S-Trans-label (ICN) diluted in the same medium and added to the basolateral surface (300 µl total volume on a piece of parafilm placed in a tissue culture dish). Medium without radiolabel (1 ml) was added to the apical surface. After this radiolabeling period, cells were washed and incubated at 37°C in DME supplemented with 2 mM cysteine and methionine from 0-150 min. The chase media was collected separately from the apical and basolateral sides at each timepoint to evaluate the secreted protein profiles.

**Immunoprecipitations**

Cells were scraped from the insert in 100 µl of 1% (vol/vol) TX-100, 150 mM NaCl, 35 mM Tris-Cl, pH 8.0, 4 mM EDTA, 1 µM CLAP, and 1 µM AEBFS. Detergent extracts were incubated with agitation for 1 h at 4°C, after which time insoluble material was removed by centrifugation at 15,000 g for 5 min at room temperature.

**Hemagglutinin**

Cells were lysed by addition of 100 µl of SDS lysis buffer [1% (wt/vol) SDS, 15 mM Tris-Cl, pH 8.0, 4 mM EDTA, 1 µM CLAP, and 1 µM AEBFS] containing the appropriate dilution of primary antibody. Samples were incubated with agitation for 1 h at 4°C, after which time insoluble material was removed by centrifugation at 15,000 g for 5 min at room temperature. Protein A-Sepharose-bound antibodies were recovered after the incubation by centrifugation at 15,000 g for 5 min at room temperature. Immunoprecipitates were incubated with 900 µl of incubation buffer (0.5% [vol/vol] TX-100, 150 mM NaCl, 35 mM Tris-Cl, pH 8.0, 4 mM EDTA, 1 µM CLAP, and 1 µM AEBFS) containing the appropriate dilution of primary antibody. Samples were incubated with agitation for 1 h at 4°C with agitation and for an additional 30 min with a rabbit pAb against mouse IgG as a linker antibody when monoclonal primary antibodies were used for immunoprecipitation. Immune complexes were recovered by incubation with 30 µl of protein A-Sepharose (100 µg total IgG binding capacity) at 1 h 4°C with agitation. Protein A-Sepharose-bound antibody complexes were recovered after the incubation by centrifugation at 15,000 g for 5 min at room temperature.
Results

Cells were seeded at 250,000 cells/cm² and passaged twice weekly with 0.25% trypsin-EDTA. They grew as colonies, as described previously (Wilson, 1997). The morphology of ADPKD cells was indistinguishable from normal kidney cells. However, the cells were slightly larger and exhibited more granularity than the normal kidney cells.

APKDK Cell E-Cadherin Is Not Expressed at the Cell Surface and Is Sequestered in an Intracellular Compartment

The adherens junctions are disposed along the lateral contacting membranes subjacent to the tight junctions, where they play a crucial role in maintaining a polarized epithelium (Drebin and Nelson, 1996). The immunolocalization of the integral membrane protein E-cadherin was examined to assess adherens junction architecture. Polarized monolayers were stained with antibodies against occludin and E-cadherin and imaged by epifluorescence microscopy. The contacting membranes of adjacent normal kidney and ADPKD cells were comparably demarcated by occludin (Fig. 2, upper panels). Normal kidney cell E-cadherin staining (Fig. 2, upper N panel, inset) coincided with that of occludin (Fig. 2, upper N panel) along the lateral cell membranes. Surprisingly, the lateral membranes of ADPKD cells, defined by the disposition of occludin (Fig. 2, upper P panels), were strikingly devoid of E-cadherin (Fig. 2, upper P panels, insets). Because the compartment with which E-cadherin was associated in ADPKD cells was ill-defined by epifluorescence microscopy, the same samples were examined by confocal microscopy to eliminate out-of-focus information (Fig. 2, lower panels). This investigation revealed that whereas E-cadherin was localized solely at the lateral membranes of normal kidney cells (Fig. 2, lower N panel), E-cadherin in ADPKD cells was exclusively sequestered in perinuclear vesicular structures, completely absent from the lateral membranes (Fig. 2, lower P panels). Thus, E-cadherin localization was markedly abnormal in ADPKD cells.

Immunoblot analysis of biotinylated cell surface proteins corroborated the depletion of E-cadherin from the ADPKD cell membrane. E-cadherin was abundant and properly polarized at the basolateral membrane of normal kidney cells (Fig. 3A, N samples). This contrasted sharply with the absence of any detectable E-cadherin on either the apical or basolateral membrane domains of ADPKD cells.

The integrity of tight junction fence function was ascertained by monitoring the domain-specific localization of the apical membrane protein influenza HA (M. Atlin et al., 1983). Cell surface HA was detected by membrane domain-specific biotinylation and subsequent immunoblot analysis of streptavidin-precipitated proteins. At steady state, influenza HA was correctly localized on the apical cell membrane of ADPKD cells (Fig. 1B), indicating that just as in normal kidney cells, diffusion of proteins between membrane domains was prevented. This finding is in agreement with the observation that only select proteins exhibited altered membrane polarity in ADPKD cells in situ (Wilson, 1997). Thus, using these morphological, electrical, and biochemical criteria, ADPKD cells in culture were judged to possess tight junctions indistinguishable from those of their normal kidney counterparts.

Polarized epithelial cells restrict the paracellular flow of solutes (gate function) and the intermixing of apical and basolateral membrane molecules (fence function) by virtue of their apico-lateral tight junctions (for review see Diamond, 1977). Tight junction integrity was assessed as one measure of the ability of ADPKD cells to establish a polarized cell architecture.

Immunofluorescence microscopy of the tight junction protein occludin (Furuse et al., 1993) was used to examine tight junction morphology. The tight junctions delimited by occludin appeared identical in all patient samples (Fig. 1A, upper panels). Tight junctions evident by EM consisted of closely apposed membrane contacts, which were morphologically similar in normal kidney and ADPKD cells (Fig. 1A, lower panels).

Although fluid accumulation within the developing cyst lumen would not be possible without an intact tight junction, paracellular gate function in explanted ADPKD cells has not been examined previously. ADPKD cells cultured on tissue culture inserts grew in tightly packed monolayers that reached a transepithelial resistance comparable to that of explanted normal kidney epithelial cells (~250 ohms/cm²). This measurement confirmed that the tight junction gate in ADPKD cells was intact.

Streptavidin Affinity Precipitation

Biotinylated samples used to analyze the steady-state distribution of cell surface proteins were solubilized in 100 µl of SDS lysis buffer. Detergent extracts were boiled for 5 min to denature nucleic acids. The lysate was subsequently diluted in 900 µl of incubation buffer containing 40 µl of streptavidin-agarose (sufficient to bind 125 µg of biotinylated protein) (Pierce), and rocked at 4°C for 1 h. Streptavidin-agarose beads were washed and recovered as described above, and boiled for 5 min in 40 µl of 2× sample buffer (100 mM Tris-Cl, pH 6.8, 4% [wt/vol] SDS, 0.2% [wt/vol] bromophenol blue, 20% [vol/vol] glycerol) containing 50 mM dithiothreitol.

Diluted immunoprecipitates from metabolically labeled samples were incubated with 40 µl of streptavidin-agarose while rocking at 4°C for 1 h. Streptavidin-agarose beads were washed and recovered as described above, and boiled for 5 min in 40 µl of 2× sample buffer containing 50 mM dithiothreitol.

SDS-PAGE and Immunoblot Analysis

Proteins were separated on 7 or 10% SDS polyacrylamide gels. A fter electrophoresis, metabolically labeled proteins were detected by drying the gels and subjecting them to phosphorimage analysis with a Fuji Phosphorimagager equipped with ImageQuant software. For immunoblot analyses, proteins resolved by SDS-PAGE were transferred to nitrocellulose membranes (A mercham Pharmacia Biotech). Nonspecific binding sites were blocked by a 1-h incubation at room temperature with 0.5% (wt/vol) nonfat dried milk dissolved in PBS containing 0.1% (vol/vol) Tween-20 (PBS-T). Blots were washed twice for 15 min in PBS-T, and incubated with the appropriate dilution of rabbit anti-HA (pAb) or mouse anti-E-cadherin, anti-rsec6, or anti-rsec8 mAbs for 1 h. Blots were washed twice for 15 min in PBS-T, and incubated with the appropriate dilution of rabbit anti-HA (pAb) or mouse anti-E-cadherin, anti-rsec6, or anti-rsec8 mAbs for 1 h. Blots were washed twice for 15 min in PBS-T. Bound antibodies were detected using chemiluminescent reagents.

Discussion

As a final step in evaluating the structural integrity of ADPKD tight junctions, we used an affinity precipitation assay to analyze the steady-state distribution of cell surface proteins. Biotinylated samples were incubated with streptavidin-agarose and the biotinylated proteins were studied by SDS-PAGE and immunoblot analysis. The results indicated that ADPKD cells contain an intact tight junction fence function.
cells (Fig. 3 A, P samples). Since ADPKD cells appeared to contain significant amounts of E-cadherin in an intracellular E-cadherin store as judged by confocal analysis (see Fig. 2), the E-cadherin content of cellular extracts was further examined by immunoblot analysis. Three different normal kidney samples expressed similar E-cadherin levels (Fig. 3 B, N samples). In contrast, ADPKD cell patient samples contained lower, variable levels of E-cadherin, although none of the three samples examined lacked the protein entirely (Fig. 3 B, P samples). These results demonstrated that ADPKD cells have reduced levels of E-cadherin, which is improperly sequestered in an intracellular pool. This could be attributed to either decreased synthesis or defective post-Golgi handling, prompting a more detailed analysis of E-cadherin biosynthesis, trafficking, and stability.

**Cell Surface Delivery of E-cadherin Is Impaired, while Maturation and Sorting Proceed Efficiently in ADPKD Cells**

The absence of E-cadherin at the ADPKD cell membrane raised the possibility that the protein was prevented from effectively traversing the exocytic pathway. E-cadherin is initially synthesized in association with the ER as a high molecular weight precursor (Peyrieras et al., 1983; Vestweber and Kemler, 1984). A complex assembly of β-catenin enables transit of E-cadherin to the Golgi apparatus (Chen et al., 1999a), where it is subsequently processed to its mature form (Shore and Nelson, 1991). Information about the molecular maturation, polarized sorting, and cell surface delivery was obtained from metabolic labeling and domain-selective biotinylation experiments. The precursor form of ADPKD cell E-cadherin was synthesized in near-normal amounts and efficiently proteolytically cleaved, demonstrating that ER and Golgi apparatus functions were not generally disrupted in these cells (Fig. 4 A, Total N and P samples). A complex assembly of newly synthesized E-cadherin with the catenins was detected by coimmunoprecipitation under the nondenaturing conditions. Multimeric cadherin–catenin complex assembly appeared equally efficient in normal and ADPKD cells (Fig. 4 A, Total basolateral N and P samples). Diminished plasma membrane association of E-cadherin was therefore not due to defective catenin affiliation. However, it was noted that there was a
significant decrease in E-cadherin delivery to the basolateral surface of ADPKD cells despite near-normal synthesis levels (Fig. 4 A, compare N and P samples in basolateral Cell Surface panel). Importantly, no missorting to the apical membrane was evident at any timepoint (Fig. 4 A, apical Cell Surface panel). Quantification of basolateral delivery showed that at the latest timepoint, only 20% of the protein had arrived at the ADPKD cell surface, compared with 60% in normal kidney cells (Fig. 4 B). Thus, inefficient transport of E-cadherin from the late Golgi cisternae to the plasma membrane is partially responsible for the diminished cell surface–associated E-cadherin observed in ADPKD cells.

Cell Surface Transport of Basolateral Cargo Is Impaired in ADPKD Cells

The polarized sorting and transport of well-characterized apical and basolateral membrane proteins was analyzed to distinguish whether decreased basolateral delivery was unique to E-cadherin or whether a more general defect in vectorial transport existed in ADPKD cells. The cell surface arrival of newly synthesized molecules was scored by metabolic labeling and cell surface biotinylation as described above. Influenza HA was used as an apical marker that is sorted in association with glycosphingolipid rafts (Scheiffele et al., 1997). Within 30 min after pulse labeling, the mature form of influenza HA appeared at the apical cell surface, where it reached maximal levels by 120 min (Fig. 5 A, upper panel). Both the kinetics and amounts of HA delivered to the apical membrane as a percentage of total newly synthesized HA were similar in normal kidney and ADPKD cells (Fig. 5 B). Delivery of HA to the basolateral membrane was undetectable in both normal and ADPKD cells (Fig. 5 A, lower panel).

Neurotrophin receptor (p75NTR) served as a second apical marker that is sorted by virtue of its luminal O-linked glycosylation (Yeaman et al., 1997). A similar observation was made for influenza HA, p75NTR delivery to the apical membrane of ADPKD cells was identical to that documented in normal kidney cells (Fig. 5 C). Approximately 30% of the total 35S-labeled p75NTR was scored as having reached the cell surface during the chase period by biotinylation. Similar amounts of p75NTR were seen delivered to the surface of MDCK cells in an analogous experiment, where it was determined that this was an under-representation of cell surface delivery most likely due to inefficient biotinylation and/or streptavidin recovery (Grindstaff et al., 1998).

Complementary experiments were conducted to examine the cell surface delivery of the basolaterally targeted LDL-R, the sorting signal for which resides in the cytoplasmic domain (Matter et al., 1992). In normal kidney cells, LDL-R was first detected at the cell surface within 30 min after pulse labeling and increased steadily over the course of the 120-min chase period (Fig. 6 A, Cell Surface samples, N lanes). The synthesis of LDL-R and processing of the precursor to the mature form proceeded with comparable kinetics in ADPKD cells and normal kidney cells (Fig. 6 A, Total samples, compare N and P lanes). However, the rate of LDL-R cell surface delivery was diminished twofold in ADPKD as compared with normal kidney cells (Fig. 6 B). Missorting of LDL-R to the apical membrane domain was not evident (Fig. 6 B). In two of the three ADPKD patient samples analyzed, the mobility of the mature form of LDL-R during SDS-PAGE was slightly faster than LDL-R from normal kidney cell samples (Fig. 6 A and data not shown), likely indicative of minor differences in posttranslational processing. Since basolateral transport efficiency was similarly diminished in all three samples and in no case was there any impact on polarized LDL-R sorting, it is felt that processing differences are inconsequential with respect to the trafficking deficit. Instead, the reduced efficiency of basolateral LDL-R delivery was reminiscent of the impaired transport of nor-
mally assembled and processed E-cadherin to the ADPKD basolateral surface. Polarized secretion of newly synthesized proteins was examined as a further measure of overall protein trafficking to the apical and basolateral plasma membrane domains. This was accomplished by collecting the media separately from the apical and basolateral chambers of metabolically labeled, filter-grown cells. Comparisons of the secreted protein profiles after SDS-PAGE and autoradiography demonstrated that most apical proteins were secreted with identical kinetics in comparable amounts in both cell types up to the 60 min timepoint (Fig. 7 A). It was noted that apical proteins secreted by ADPKD cells failed to increase in amounts beyond those present at the 60 min timepoint, which may represent either enhanced protein degradation on account of enhanced internalization or elevated protease activity by polycystic kidney disease cells (Rankin et al., 1996). In marked contrast to apical protein secretion, comparisons of basolateral protein secretion revealed a disparity in the efficiency of basolateral exocytosis at all timepoints (Fig. 7 B). Normal kidney cells steadily secreted increasing amounts of several proteins into the basolateral medium (Fig. 7 B, arrowheads). Basolateral secretion from ADPKD cells was significantly reduced, with very little secreted protein detectable even at the latest timepoint (Fig. 7 B, P lanes), in keeping with the observations made in the analyses of individual basolateral membrane proteins. Based on this series of experiments, it was apparent that protein transport to the basolateral cell surface was impaired, whereas apical delivery proceeded relatively unimpeded.

Exocytic Cargo Is Retained in the ADPKD Cell Golgi Apparatus

Diminished cell surface delivery of newly synthesized basolateral cargo in the absence of any posttranslational processing deficits or apical mistargeting prompted an investigation of whether molecules might be accumulating in the ADPKD cell Golgi apparatus. To explore this possibility, the fluorescent lipid analogue C₆-NBD-ceramide was used to assay Golgi-to-plasma membrane transport both mor-
phologically and biochemically as described (Lipsky and Pagano, 1985; van Meer et al., 1987). Cellular membranes were labeled with C6-NBD-ceramide at reduced temperature (20°C) for 90 min, after which time cell surface C6-NBD-ceramide was removed by back-exchange at low temperature. Under these conditions, C6-NBD-ceramide was delivered to the Golgi apparatus, where it was similarly metabolized to C6-NBD-glucosylceramide and C6-NBD-sphingomyelin in both normal kidney and ADPKD cells (data not shown). The inhibition of vesicular transport to the cell surface caused by the 20°C incubation resulted in the accumulation of these fluorescent lipid metabolites in the TGN of both normal and ADPKD cells (Fig. 8, 0 min). Golgi-to-cell surface transport of the C6-NBD-lipids was initiated by transferring the cells to 37°C and cell surface delivery was monitored by confocal microscopy (Fig. 8, 30–90 min). These experiments revealed a dramatic defect in the ability of ADPKD cells to transport the C6-NBD-lipids to the cell surface (Fig. 8, compare P samples with N samples). In normal kidney cells, the C6-NBD-lipids were first evident at the basolateral plasma membrane within 30 min after warming to 37°C, and cell surface delivery was complete by 90 min, with none remaining Golgi-associated at the latter timepoint. In contrast, basolateral delivery in ADPKD cells was not detectable after 90 min, and the C6-NBD-lipids remained associated with the ADPKD cell Golgi apparatus for as long as 150 min (Fig. 8, P 90 min sample; data not shown). Quantitative analyses of cell surface fluorescent lipids extracted from the apical or basolateral membrane domains indicated that lipid transport to the ADPKD cell basolateral membrane was reduced by 2.5–3-fold, whereas apical delivery was unimpeded (data not shown).

**Components of the Basolateral Targeting Patch Are Depleted from the ADPKD Cell Membrane**

The demonstration that C6-NBD-lipids failed to exit the Golgi apparatus, coupled with the observed basolateral trafficking defect, were indicative of defective vectorial trafficking from the ADPKD cell Golgi apparatus to the basolateral plasma membrane. Ineffective delivery of exocytic cargo to the ADPKD cell basolateral membrane raised the possibility that constituents of the basolateral targeting patch were improperly expressed or localized. The localization of sec6 and sec8 was therefore examined by confocal microscopy. Cells were stained to visualize sec6 or sec8 (red channel) in parallel with the tight junction protein occludin (green channel). In normal kidney cells, both sec6 and sec8 were localized in close apposition to the tight junction protein occludin (Fig. 9 A, left panels). In contrast, both proteins were depleted from the ADPKD cell lateral membranes and appeared diffusely dispersed throughout the cytoplasm (Fig. 9 A, right panels). Immunoblot analyses revealed that sec6 and sec8 protein levels were similar in all normal kidney and ADPKD samples analyzed (Fig. 9 B, compare N and P samples).
Therefore, sec protein redistribution was not accompanied by a decline in sec protein expression, as was observed for E-cadherin. These results substantiate a marked disruption in the integrity of the basolateral cargo delivery site in ADPKD cells, likely brought about by altered E-cadherin–based adherens junction assembly.

**Discussion**

This study identified the loss of cell surface E-cadherin and the exocyst components sec6 and sec8 as critical molecular deficits affecting ADPKD epithelial cells. Although ADPKD cells possessed characteristics of polarized cells, the disease cells suffered from a lack of detectable cell surface E-cadherin. Total cellular E-cadherin levels were lower than those in normal kidney cells, and existing E-cadherin was sequestered in an intracellular pool. The cell surface depletion of sec6 and sec8 in ADPKD cells devoid of plasma membrane–associated E-cadherin was correlated with significantly impaired delivery of proteins and lipids to the basolateral cell surface. Vectorial transport to the apical ADPKD cell surface, on the other hand, was functionally intact. Together, the investigations presented here serve to clarify the molecular mechanisms whereby mutations in PKD1 or PKD2 may lead to downstream alterations in cytoarchitecture and molecular trafficking in ADPKD cells.

E-cadherin, the catenins, and the polycystins are all disposed within the basolateral membrane beneath the apicolateral tight junction, where recent data suggest they are engaged in a large multimeric complex that may coordinate regulation of adherens junctions. The calcium-dependent molecular link between adjacent cells membranes that implements cytoskeletal organization via cytosolic catenins (Drubin and Nelson, 1996). A association of polycystin-1 with the E-cadherin–catenin assembly (Huan and van Adelsberg, 1999) is expected to tether a subset of polycystin-1 molecules in close proximity to adherens junctions. Polycystin-1, with its numerous cell–cell and cell–extracellular matrix adhesion domains, is thus poised to facilitate interactions with neighboring cells or the matrix. Demonstrated interactions between polycystin-2 and polycystin-1 (Tsokas et al., 1997) merit the inclusion of polycystin-2 within this complex. Given the likelihood of an ordered assembly between adherens junction components and the polycystins, it is interesting to consider why E-cadherin and sec6/8 might be depleted from the cell surface of ADPKD cells, and what ramifications this may have on cellular organization.

**Mechanisms Underlying Loss of Cell Surface E-Cadherin**

It is plausible that the physical association of E-cadherin with mutant polycystin-1 might lead to the disruption of epithelial cell organization, particularly given the demonstrated interaction between polycystin-1 and E-cadherin (Huan and van A. delsberg, 1999). The developmental downregulation of polycystin-1 expression is altered in ADPKD so that mutant PKD1 products are often overexpressed (Geng et al., 1996, 1997). Conceivably, overexpressed mutant polycystin-1 may adversely impact E-cadherin stability. Indeed, the patient-to-patient variability in E-cadherin expression levels in ADPKD cells demonstrated here indicates that individual polycystin mutations...
differentially influence E-cadherin stability. Disturbances in either the stoichiometry of normal E-cadherin-polycystin-1 interactions or the structural characteristics of such complexes could account for the compromised efficiency with which E-cadherin is transported through the late exocytic pathway and stably retained at the plasma membrane in multiple ways. This report demonstrated that de novo membrane insertion, plasma membrane stabilization, and recycling or degradation of E-cadherin are all affected in ADPKD cells. Diminished E-cadherin cell surface delivery undoubtedly contributes to the reduced cell surface expression, although given the threefold decrease in delivery, this deficit cannot entirely account for the lack of ADPKD cell surface E-cadherin observed at steady state. E-cadherin was recently demonstrated to undergo dynamic recycling between the cell surface and early endosomes, traversing a circuit through which the plasma membrane disposition of E-cadherin may be regulated (Le et al., 1999). In light of this finding, it is interesting to consider that modulation of a regulatory circuit may contribute to reduced cell surface expression of E-cadherin in ADPKD cells. A aberrant assembly of multimeric E-cadherin-containing complexes may cause the newly synthesized E-cadherin arriving at the basolateral cell surface to be increasingly internalized and decreasingly recycled to the plasma membrane, resulting in the observed intracellular accumulation. The existence of a pathogenic variation in this circuit is further suggested by the diminished levels of E-cadherin in ADPKD cells: once internalized, unrecycled abnormal E-cadherin complexes may be transported to late endocytic organelles and degraded. Depletion of the exocyst complex from the basolateral membrane upon the acquisition of a second, somatic genetic lesion is likely a
downstream event that follows a reduction in cell surface E-cadherin. Dissociation of the basolateral cargo targeting patch from the apico-lateral apex may exacerbate the defect by precluding further delivery of newly synthesized E-cadherin, and by impacting recycling of E-cadherin to the basolateral membrane. Although future studies are needed to clarify the precise mechanisms by which mutations in PKD1 or PKD2 affect E-cadherin trafficking and stability, it is probable that deranged intermolecular interactions involving E-cadherin constitute potent stimuli culminating in cellular dysmorphogenesis in ADPKD.

Cell–Cell Adhesion in the Absence of Cell Surface E-Cadherin

E-cadherin has been suggested to occupy a central role in the nucleation and maintenance of epithelial cell polarity (Drubin and Nelson, 1996). When E-cadherin ligation was prevented, both adherens junction as well as tight junction and desmosomal junction assembly were inhibited (Gumbiner et al., 1998). Nevertheless, in the absence of detectable cell surface E-cadherin, explanted ADPKD cells are somehow capable of maintaining partially polarized, physically intact monolayers. The possibility that alternate cadherins are present on the ADPKD cell surface, sufficing to generate a partially polarized phenotype, was explored by immunocytochemical analyses of K-cadherin and the cadherins in general. K-cadherin/cadherin-6 is a cadherin family member expressed during renal development as well as in mature proximal tubule cells (Xiang et al., 1994; Shimoyama et al., 1995; Paul et al., 1997). K-cadherin protein levels in both normal and ADPKD cells were low, and no compensatory changes were evident (data not shown). However, immunostaining using a pan-cadherin antibody directed against a highly conserved region shared by cadherin proteins demonstrated that the lateral membranes of ADPKD cells contained an alternate cadherin (data not shown). Thus, adhesion through an alternate cadherin family member, in concert with integrin- and desmosome-mediated adhesion, may compensate for the loss of cell surface E-cadherin and serve to support ADPKD cell architecture.

Mechanisms Underlying Altered ADPKD Cell Polarity

The nonpolarized distribution of certain basolateral membrane proteins in ADPKD cyst-lining cells led to the tenable hypothesis that polarized trafficking is defective (Wilson, 1997), which has until now remained untested. Quite unexpectedly, even in the absence of efficient basolateral delivery, E-cadherin and LDL-R were not mistargeted to the apical ADPKD cell membrane. These findings exclude promiscuous packaging of exocytic cargo within the TGN and aberrant targeting of basolateral vesicles as contributing factors in abnormal ADPKD cell polarity. Therefore, it is necessary to consider alternative explanations for
The diagram depicts current knowledge regarding the localizations of the polycystins, adherens junction proteins, and exocyst components. A legend identifies each component and detailed functional descriptions are given in the text. Polycystin-1 is depicted in contact with E-cadherin and catenins, though it is not known whether the association is direct or involves intermediary proteins.

Figure 10. A multimeric complex involved in epithelial cell organization. The diagram depicts current knowledge regarding the localizations of the polycystins, adherens junction proteins, and exocyst components. A legend identifies each component and detailed functional descriptions are given in the text. Polycystin-1 is depicted in contact with E-cadherin and catenins, though it is not known whether the association is direct or involves intermediary proteins.
eraly destined molecules within the Golgi apparatus. Impediment of cargo export from the Golgi apparatus may be indicative of regulatory mechanisms coordinating vesicle budding and vesicular fusion with the target (basolateral) ADPKD cell membrane.

Docking and fusion of vesicles with the plasma membrane embodies the ultimate step in basolateral trafficking. This step is mediated by the concerted actions of the SNARE proteins and exocyt components (Chen et al., 1999b; Grindstaff et al., 1998). Examination of SNARE protein distribution using antisera against the basolateral SNARE protein syntaxin 4 did not reveal noticeable differences in its distribution between the two cell types (Charron, A. J., R. L. Bacalla, and A. W. Angenier-Ness, manuscript in preparation). This component of the basal docking and fusion machinery is therefore likely intact, and may partially compensate for the depletion of the basolateral targeting patch from the ADPKD cell membrane, mediating residual basolateral transport. However, the loss of sec6 and sec8 from the ADPKD cell membrane presumably impacts not only the final event in basolateral exocytosis, but also cellular organization per se. Given the central role of E-cadherin in maintaining epithelial cell organization, impaired docking and fusion of plasma membrane-bound E-cadherin-containing vesicles due to dissection of sec6/8 from the basolateral targeting patch likely directly impacts cytoarchitecture, as discussed above. In addition, it is also conceivable that the dispersal of exocyst components affects the exocytic organelles. Basolateral cargo-bearing vesicles that leave the ADPKD cell Golgi apparatus and are transported to the membrane region normally associated with the exocytic complex may become stalled, as efficient docking and fusion is prevented. By engaging basolateral trafficking effectors in futile post-Golgi transport, this effect is expected to eventually lead to diminished basolateral vesicle budding from the ADPKD cell Golgi apparatus. If such a retrograde pathogenic mechanism issuing from the cell surface back to the Golgi exists, it is reasonable to postulate that the acquisition of mutations in the PKD1 or PKD2 loci would set into motion a cascade of events, commencing with the destabilization of the E-cadherin/beta catenin complex to efficient endoplasmic reticulum exit and basolateral membrane targeting of E-cadherin in polarized M DCK cells. J. Cell Biol. 144:687–699.

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