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Award Number: W81XWH-09-1-0269

TITLE: Role of Integrin-Beta 1 in Polycystic Kidney Disease

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REPORT DATE: April 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188		
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1. REPORT DATE (DD-MM-YYYY) 3 -0 -2 1		2. REPORT TYPE Annual		3. DATES COVERED (From - To) !µ0 a t0	
4. TITLE AND SUBTITLE Role of Integrin-Beta 1 in Polycystic Kidney Disease			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER W81XWH-09-1-0269		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Gabriele Luca Gusella luca.gusella@mssm.edu			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Mount Sinai School of Medicine New York, NY 10029			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Material Command Fort Detrick, MD 21702-5012			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Autosomal Dominant Polycystic Kidney Disease (ADPKD) is caused by the dysregulation of the PKD1 or PKD2 genes. Among the multiple molecular and biological changes associated with the cystogenic conversion are the amplification of the centrosome, genomic instability and aneuploidy, as well as an increase in the expression of the adhesion molecule integrinβ1. The scope of the study is to elucidate in the molecular mechanism underlying these events and the role of Intβ1 in ADPKD pathogenesis. Our recent work has confirmed that Intβ1 inhibition reverses the centrosome amplification and the increased fibronectin deposition that distinguish cystic as well PC1 knockdown cells. We have also characterized a novel cell line from human loop of Henle epithelium that can serve as a unique model to study medullary cystic kidney disease-2 (MCKD2) and familial juvenile hyperuricemic nephropathy (FJHN). MCKD2 and FJHN are autosomal dominant diseases with renal cystic involvement that are caused by aberrant function of the ciliary uromodulin gene. Using this model we have observed similar centrosomal amplification and genetic instability upon knockdown of uromodulin, suggesting that these biological changes may be a common denominator of the renal cystic phenotype. Breeding of the mice with floxed Pkd1 and Itgb1 (Intβ1) genes to be crossed with the specific transgenic mouse expressing the Cre recombinase under the control of the kidney-restricted Aqp2 promoter is progressing on schedule.					
15. SUBJECT TERMS Kidney, integrin, cyst, polycystic, siRNA, cell cycle, centrosome, uromodulin					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 25	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U		19b. TELEPHONE NUMBER (include area code)	

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INTRODUCTION

Our previous findings indicated that fluctuations of expression of PC1, the product of the *Pkd1* gene, from its physiological range causes centrosome amplification, genetic instability and the emergence of genotypic heterogeneity. Based on our observations, we proposed a genomic instability model of cystogenesis. This model is supported by the evidence that cysts form in a relatively low number of nephrons and that apoptotic cells are present in both tubular and cystic cells in ADPKD kidneys, and is consistent with the clonality of cystic epithelia. Our model predicts the great variability of ADPKD manifestations observed among individuals sharing identical germline mutations and provides a PC1-dependent mechanism for the generation of somatic mutation that may trigger cyst initiation¹. Since our first description of the role of PC1 expression on centrosome integrity, similar effects have been observed by us upon downregulation of *Pkd2* gene, and by others following the inhibition of other cystic genes, the autosomal recessive polycystic kidney disease gene, *Phkd1* (fibrocystin/polyductin), and the Meckel-Gruber syndrome genes, *Mks1* and *Mks3*^{2,3}. Genomically aberrant cells are usually eliminated through mitotic catastrophe or apoptosis. Nevertheless, similarly to carcinogenic transformation, some cells may escape negative selection at low frequency and in a stochastic fashion, thus allowing the emergence of the cystic phenotype.

The molecular mechanisms driving the cystic transformation remain unknown and likely involve molecules that support the survival of genetically abnormal cells. In agreement with in vivo observations, we showed that integrin- α 2 and integrin- β 1 (Int β 1) are upregulated after the knockdown of *Pkd1* in mouse cells and that integrin- α 2 β 1 mediated the resistance of *Pkd1* knockdown (*Pkd1*^{kd}) cells to anoikis⁴.

We hypothesized that Int β 1 is a necessary mediator of cystogenesis. The objective of the application is to dissect the role of Int β 1 in the control of centrosome integrity, the genomic instability and the cystogenetic progression pathways triggered by the loss of PC1. We proposed that Int β 1 plays a protective role in the genetic adaptation of the PC1 deficient cells allowing the survival of the cystogenic phenotype.

The identification of the factors promoting the adaptation of *Pkd1* defective cells and the elucidation of their effector mechanisms will provide a better understanding of the pathogenesis of renal cystic diseases and help identify novel potential therapeutic targets.

BODY

Establishment of a human renal epithelial cell line. In order to generate a human model to study the role of Int β 1 in cystic development, we engineered a lentiviral vector for the expression of the human telomerase reverse transcriptase (hTERT) (VVPW/Bleo/hTERT) and used it to immortalize primary human renal epithelial cells with the purpose to isolate a stable cell line with characteristics of the distal tubules and collecting duct cells.

We initially screened the clones according to their karyogram with the rationale that clones with a near-normal genetic organization would more faithfully behave as primary cells. Of the initial eight clones that we obtained, one clone, hereafter referred to as TIRE131, presented a seemingly normal 46-chromosome male karyotype and was therefore subjected to further characterization. Southern hybridization of genomic TIRE131 DNA indicated that it contained 2 copies of the VVPW/Bleo/hTERT lentivirus used for immortalizing the primary epithelial cells. Comparative genomic hybridization performed on TIRE131 cells identified a near normal karyotype with an amplified region on the long arm of chromosome 5 and a deletion of one long arm of chromosome 11. These findings were further confirmed by spectral karyotyping.

Morphologically, TIRE131 monolayers displayed a characteristic epithelial appearance. To better define their anatomical origin, we used markers specific for different segments of the nephron. TIRE131 stained positively for the epithelial antigens cytokeratin-8 and for cytokeratin-19. The latter is a member of the cytokeratin family that is prevalently present on cells of the renal distal tubules and collecting ducts, suggesting that TIRE131 originated from distal nephron. Indeed, no expression of markers of proximal tubule, such as alkaline phosphatase and AQP1 antigen, was detected in TIRE131 cells. TIRE131 cells

appeared to stain very weakly for AQP2 but were highly positive for uromodulin (Tamm-Horsfall antigen). These findings were confirmed by immunoblot and strongly suggested that TIRE131 cells originated from the thick ascending limb of the loop of Henle.

Though we were originally trying to derive a collecting duct cell line, these results presented the unique opportunity to study the biology of uromodulin, the product of the UMOD gene whose mutations account for other autosomal dominant disorders with renal cystic involvement: medullary cystic kidney disease-2 (MCKD2) and familial juvenile hyperuricemic nephropathy (FJHN).

Therefore, we further characterized the TIRE131 clone to confirm their loop of Henle origin. Similarly to the loop of Henle epithelium, the TIRE131 cells: 1) possessed a significant resistance to hyperosmotic growth conditions; 2) formed a functional epithelium with tight junction and apicobasal polarization as indicated by the apical staining with ZO1, a marker of tight junction; and 3) displayed a progressive and significant increase in the transepithelial electric resistance (TER) when cultured on transwell. Moreover, TIRE131 cells maintained the tubulocystogenic potential, when grown in collagen type I matrix with clearly detectable lumina in some of the tubular segments and in cystic formations, some of which displayed cilia on their apical side facing the lumen. Uromodulin was found to localize on the cilia of TIRE1 cells, in agreement with previous observations.

To our knowledge, this is the first human cell line derived from the thick ascending limb of the loop of Henle and our data indicated that TIRE131 may be a useful model to study the pathogenetic mechanisms of uromodulin-associated kidney diseases. Further studies indicated that inhibition of uromodulin expression in TIRE131 lead to centrosome amplification, genomic instability, and cystic growth in three-dimensional collagen culture, thus supporting the notion that these events may be common to all renal cystic diseases.

These data have been presented at the last American Society of Nephrology 43rd Annual Meeting, 2010, (Battini *et al*, American Society of Nephrology 43rd Annual Meeting, 2010, Poster # SA-PO2455, Appendix 1) and a manuscript detailing these results is under preparation.

We are planning to explore the effects of uromodulin suppression on the Int β 1 and matrix protein expression to determine whether this mechanism is conserved in the cystic phenotype independently of the causative gene.

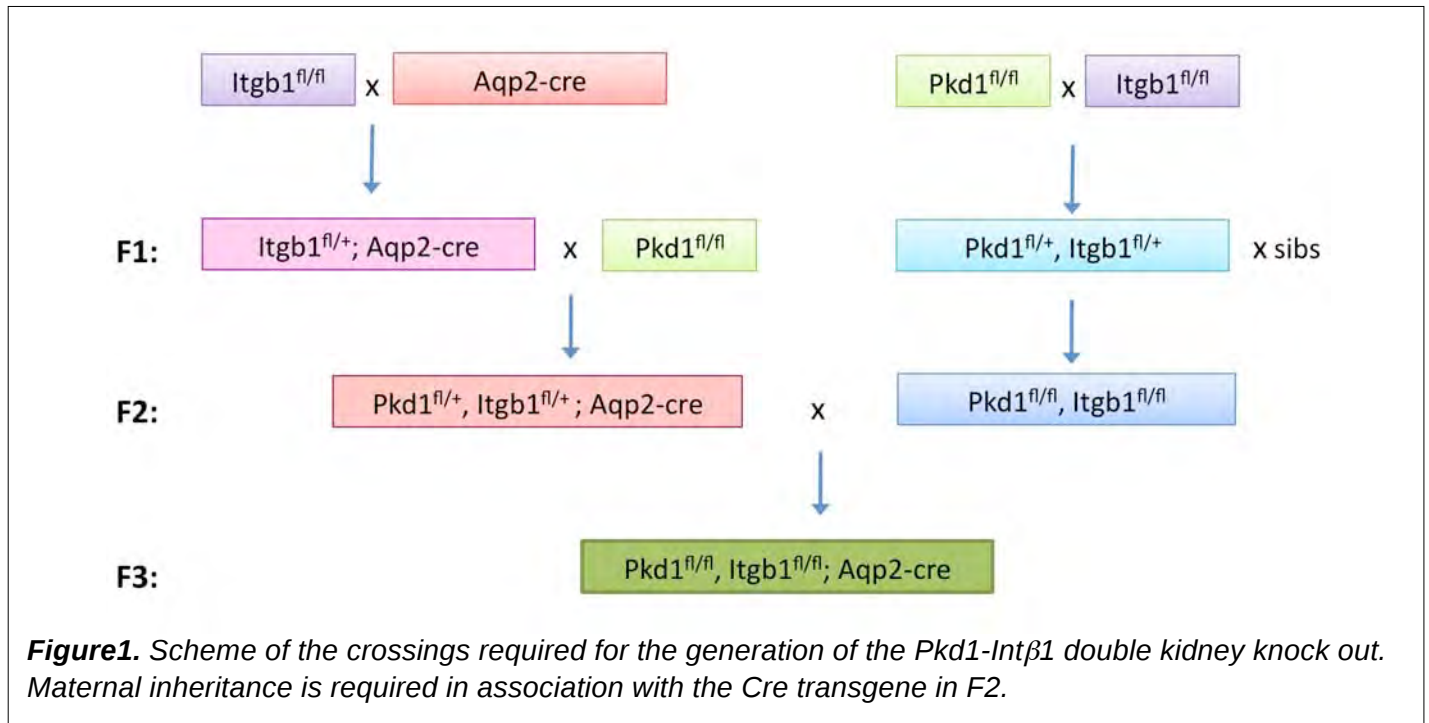
In the meantime, from a second round of cloning of the hTERT immortalized human renal epithelial cells, we have also succeeded in isolating a clone that is negative for alkaline phosphatase activity but highly resistant to growth in hyperosmolar conditions and displaying high AQP2 expression levels. We think that this clone likely originates from collecting ducts and we are currently characterizing its functional properties further.

Inhibition of Int β 1 reverts centrosome amplification and aberrant matrix deposition. We have shown that the suppression of Int β 1 expression during the inactivation of Pkd1 not only inhibits the hyperproliferative nature of the PC1 knockdown cells, but also significantly reduces the number of cells with increased centrosome amplification and the deposition of fibronectin (Lee *et al*, American Society of Nephrology 43rd Annual Meeting, 2010, Poster # F-PO1769, Appendix 2).

Experiments with time-lapse microscopy that are necessary to assess whether the centrosome defects are a result of the decreased rate of cell division or the direct effect of the reduced Int β 1 expression have been delayed by a mycoplasma contamination of our murine cell lines. We have performed extensive tests and we have now re-derived all the cell lines from clean batches and we are currently assessing them for characteristic biological responses (e.g. growth and Int β 1 overexpression upon Pkd1 knockdown) before proceeding with the live imaging.

In vivo analysis of $\text{Int}\beta 1$ cystogenic role: mouse breeding strategy. To overcome the early lethality associated with the loss of *Pkd1* or *Itgb1* ($\text{Int}\beta 1$) genes, we originally proposed to generate an inducible $\text{Int}\beta 1$ knockdown mouse model to determine the cystogenic role of integrin- $\beta 1$ *in vivo*. This strategy relies on the time-consuming characterization of an entirely new mouse model. Since the time of the proposal, mouse strains with floxed *Pkd1* or *Itgb1*, as well as a transgenic mouse in which the expression of the Cre recombinase is under the control of the kidney-specific *Aqp2* promoter have become commercially available, thus offering an the possibility to more quickly address the function of $\text{Int}\beta 1$ in cystic kidneys. Therefore, we have taken the approach of conditionally inactivating *Pkd1* and *Itgb1* genes specifically in the kidney by generating mice homozygous for the floxed *Pkd1*, *Itgb1* alleles and carrying the kidney-specific AQP2-cre transgene (*Pkd1*^{fl/fl}, *Itgb1*^{fl/fl}; AQP2-cre).

To generate *Pkd1*^{fl/fl}, *Itgb1*^{fl/fl}, AQP2-cre mice, from Jackson Laboratory three murine strains were obtained carrying either *Pkd1*^{fl/fl} (B6.129S4-*Pkd1*^{tm2Ggg}/J), the *Itgb1*^{fl/fl} allele (B6;129-*Itgb1*^{tm1Efu}/J), or the hemizygous AQP2-cre transgene (B6.Cg-Tg(Aqp2-cre)1Dek/J). The *Pkd1*^{fl/fl} mice possess *loxP* sites in introns 1 and 4 of the *Pkd1* gene⁵; the *Itgb1*^{fl/fl} mice possess *loxP* sites on either side of exon 3 of the *Itgb1* gene. Both strains of mice have been used to generate effective null alleles when bred with cre transgenic mouse^{6,7}. The *cre* activity in Aqp2-cre transgenic mouse, directed by the mouse aquaporin 2 promoter, has been observed in kidney collecting duct cells and in testes⁷. Therefore, our breeding scheme is devised to ensure maternal inheritance of the transgene, since paternal inheritance would result in gene recombination not only in the kidney, but also in sperm and the resulting fertilized oocytes. Our breeding scheme is briefly outlined in Figure 1. We currently have obtained the F2 generation of newborn mice and expect to have the F3 generation of mice ready for analysis in the next few months.



*Establishment of murine *Pkd1*^{fl/fl} cell lines.* We have constructed two lentiviral vectors expressing the murine mTert gene or the temperature sensitive SV40 large T antigen. These vectors have been used for the transduction of epithelial cells isolated from the renal corticomedullary region and papillae of *Pkd1*^{fl/+} and *Pkd1*^{fl/null} mice derived from the crossing of the *Pkd1*^{fl/fl} mice with the heterozygous *Pkd1*^{null/+} strain⁸. These cells are currently being expanded and will be then functionally characterized to produce clonal populations of distal tubule and collecting duct cells. To achieve the complete conditional inactivation/knock out of the *Pkd1* gene in vitro, the floxed region of the gene will be excised upon transduction with a lentivector co-expressing the Cre recombinase and a fluorescent histone 2B-Cherry red (H2B-Cherry) chimeric traceable gene that we have recently engineered. To prevent possible adverse effects of the constitutive expression of the Cre recombinase, this lentivector will be packaged

using an integrase-defective complementary plasmids that does not allow the integration of the provirus while permitting the transient expression of the Cre and the marker H2B-Cherry protein. Lack of stable Cre expression will be tested by observing the disappearance of the H2B-Cherry protein and confirmed by PCR. This system will allow us to study the early events of *Pkd1* loss in cells with normal or knockdown expression of Int β 1.

KEY RESEARCH ACCOMPLISHMENTS

Established the first human cell line from the loop of Henle epithelial cells.

Demonstrated the role of uromodulin in the control of centrosome integrity and genomic stability.

Proved that the suppression of Int β 1 reverses centrosome amplification and fibronectin expression in *Pkd1* knockdown cells.

REPORTABLE OUTCOMES

The current studies produced the following presented/published papers:

- Uromodulin Controls Centrosome Integrity in Cells of the Thick Ascending Limb of the Loop of Henle. Lorenzo Battini, Kim Lee, Carles Martinez-Romero, Lin Geng, and G. Luca Gusella. American Society of Nephrology 43rd Annual Meeting, 2010, Poster #SA-PO2455.
- Role of Integrin- β 1 in cystogenesis and hyperproliferation of ADPKD cells. Kim Lee, Lorenzo Battini, Carles Martinez-Romero, Lin Geng, and G. Luca Gusella. American Society of Nephrology 43rd Annual Meeting, 2010, Poster #F-PO1769.
- Blitzer A.L., Panagis L., Gusella G.L., Danias J., Mlodzik M., and Iomini, C.: Primary cilia dynamics instruct tissue patterning and repair of corneal endothelium. *Proc. Natl. Acad. Sci. U.S.A.* 108(7):2819-24, 2011.
- Lee K., Battini L., and Gusella G.L. Cilium, centrosome and cell cycle regulation in polycystic kidneys disease. *Biochim. Biophys. Acta*, 2011, *In press*.

CONCLUSIONS

Centrosome amplification and genetic instability seem to be common events in cystic models independently of the cystic gene involved.

Increased Int β 1 expression in *Pkd1* knockdown cells is required for centrosomal amplification and changes in matrix deposition.

The role of Int β 1 in these events suggests that targeting Int β 1 could be potentially beneficial to interfere with the progression of the renal cystogenesis.

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Role of Integrin-β1 in cystogenesis and hyperproliferation of ADPKD cells

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic kidney disease that is characterized by formation and progressive expansion of renal cysts, leading to end-stage kidney disease. Majority of ADPKD cases are caused by mutation of PKD1 gene that encodes polycystin-1 (PC1) or PKD2 gene that encodes polycystin-2 (PC2). PC1 and PC2 are transmembrane proteins whose function is involved in various biological processes, such as proliferation, differentiation, migration, and cell polarity. Consequently, ADPKD epithelia show alterations in all of these processes. In addition, our previous work showed that the cystogenic changes following the inhibition of PC1 expression are preceded by hyperproliferation of the cells, suggesting that the hyperproliferation phenotype is a primary event in the pathogenesis of the hyperproliferation phenotype. Furthermore, we showed that reduced PC1 function is associated with increased expression of integrin-β1 (Intβ1), and that Intβ1 signaling mediates the resistance of PC1 knock-down cells to anoikis, an apoptotic mechanism induced by the loss of attachment to the ECM.

ADPKD is associated with an increased deposition of extracellular matrix (ECM) proteins that contribute to renal cyst formation. It has been shown that PC1 is involved in the regulation of the integrin signaling pathway controlled by integrin overexpression in ADPKD cells, suggesting that integrin signaling may be involved in pathogenesis of ADPKD. The purpose of this study is to determine the role of Intβ1 in hyperproliferation and cystogenesis progression in ADPKD cells.

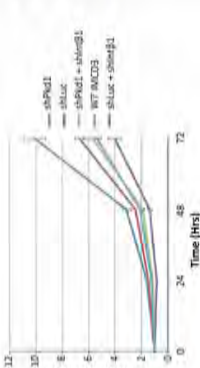
Methods

As an *in vitro* model system of ADPKD, we used two renal collecting duct cell lines (IMCD3 and MDCK cells), in which the expression of PC1 is constitutively knocked down by the use of lentivirus carrying a specific anti-PC1 shRNA. This model system recapitulates features that characterize ADPKD epithelia, such as hyperproliferation and resistance to anoikis. Cells stably expressing specific shRNAs against PC1 (shPC1), Intβ1 (shIntβ1), or against luciferase (shLuc) as a negative control were compared in different biological assays. Cells were transfected with one or two lentiviruses to express the following shRNAs:

- 1. shPC1 (constitutive)
- 2. shIntβ1 (constitutive)
- 3. shIntβ1 (inducible or not-inducible)
- 4. shLuc + shIntβ1 (constitutive or not-inducible)

Results

Fig.1



Intβ1 is required for the hyperproliferation of Pkd1 KD cells.

Cell proliferation assay was conducted in IMCD3 cells transfected with a lentiviral vector constitutively expressing a specific shRNA against PC1 (shPC1), luciferase negative control (shLuc), or Intβ1 (shIntβ1). Values collected at different time points are expressed relative to the initial number of cells at 0 hours post-transfection.

Fig.3



Inhibition of Intβ1 expression reduces the genomic aberration and centrosome amplification in Pkd1 KD cells.

A) Cell cycle analysis of IMCD3 cells co-expressing shIntβ1 or shLuc with a lentivirus expressing shIntβ1 under a tetracycline-inducible promoter. Peaks of abnormal ploidy in PC1-deficient cells (indicated by black arrowhead) are reduced with simultaneous inhibition of Intβ1 expression (red arrow). B) Number of cells with amplified centrosomes, stained by α -tubulin immunoreactivity, is also significantly reduced in PC1-deficient cells with Intβ1 KD.

Conclusion

Using an *in vitro* model of ADPKD we show that Intβ1 signaling is required in hyperproliferation and genomic aberration and centrosome amplification in PC1-deficient cells. In addition, the inhibition of Intβ1 signaling reduces the hyperproliferation and genomic aberration and centrosome amplification in PC1-deficient cells. These results suggest a positive feedback loop that may mediate the hyperproliferation phenotype in ADPKD cells that is mediated by Intβ1 signaling.

Both cell lines utilized in our study exhibit an enhanced Intβ1 signaling following the PC1 knockdown (KD), as well as concomitant increase in extracellular deposition of fibronectin (FN), a ligand of Intβ1 heterodimers. These results suggest a positive feedback loop that may mediate the hyperproliferation phenotype in ADPKD cells that is mediated by Intβ1 signaling. We are currently conducting an *in vivo* approach to study the role of Intβ1 signaling in the absence of PC1 function to corroborate these findings.

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Acknowledgement

This work is supported by the National Institutes of Health and the Department of Defense.

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Inhibition of Intβ1 expression reduces the increased deposition of fibronectin in Pkd1 KD cells.

IMCD3 cells constitutively expressing shRNAs against PC1, Luc, and/or Intβ1 were grown on glass coverslips and immunostained for expression of fibronectin using confocal microscopy (3x obj). DAPI was used for counterstaining of the nuclei.



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Uromodulin Controls Centrosome Integrity in Cells of the Thick Ascending Limb of the Loop of Henle

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ABSTRACT

Mutations of the uromodulin gene, UMOD, are responsible for familial juvenile hyperuricemic nephropathy (FJHN) and medullary cystic kidney disease type 2 (MCKD2). Both diseases are autosomal dominant tubulointerstitial nephropathies, characterized by hyperuricemia, gout, hypertension, cysts formation, and end-stage renal disease. In spite of the fact that uromodulin is the most abundant protein present in the urine, its biological function is not completely understood. We have created a novel cell line, TIRE (Tetronast-Immortalized Renal Epithelial cells), by infecting human primary epithelial cells with a lentiviral vector overexpressing human telomerase. Characterization of a specific clone, TIRE 13-1, showed characteristic morphological and functional properties of the thick ascending limb of the loop of Henle. To date, this is the only human cell line with this specific characteristic and therefore represents a valuable tool to study UMOD-associated diseases.

The siRNA lentiviral-mediated knock-down of UMOD showed centrosome amplification and genomic instability in TIRE 13-1 cells, but not in MCKD or IMCD cells. Interestingly, the knock-down of PND1 had the opposite effects, causing centrosomes amplification in MCKD and IMCD cells but not in TIRE 13-1. It has been well established that in ACPD patients cysts seem to be derived largely from collecting ducts and distal tubules, where polycystin-1 is mostly abundant in the adult kidney, whereas in UMOD-related diseases, cysts arise from the medulla or at the corticomedullary junction. These results raise the intriguing possibility that in different segment of the nephron in the adult kidney the cytoarchitecture is controlled by different genes.

MATERIALS AND METHODS

Cell culture and reagents

Cell lines were maintained at 37°C in 5% CO₂ in DMEM supplemented with 10% FBS (MCKD cells) or in a 1:1 (vol/vol) of DMEM/F12 medium supplemented with 10% FBS (Telomerase-Immortalized Renal Epithelial cells), TIRE13-1, and the transduced cell lines, TIREΔUMOD, TIREΔUMOD17 and TIREΔLuc.

Immunoblotting

Cells were harvested by scraping, washed twice in PBS and lysed in RIPA buffer containing Complete Protease and Phosphatase Inhibitors Cocktail. Protein extracts (100 µg) from each sample were resolved by SDS-polyacrylamide gel electrophoresis on a 4-20% gradient gel and electrophoretically blotted onto Immobilon-P membrane, and incubated with primary rabbit polyclonal anti-Uromodulin or rabbit polyclonal anti-Aquaporin-2 (pH2), or H40 (Santa Cruz Biotechnology, Santa Cruz, CA) in 5% milk in PBS containing 0.05% Tween-20. Immunocomplexes were detected with peroxidase-conjugated goat anti-rabbit IgG antibody (1:5000) and the enhanced chemiluminescence detection kit (ECL-Plus, Amersham Pharmacia Biotech).

Cell cycle analysis

At the indicated time points post-transduction, cells were washed twice with PBS, trypsinized and resuspended at 10⁶ cells/ml in DNEMF2 supplemented with 10% FCS. The samples were then processed using the CyclonEST PLUS following the manufacturer's protocol, and run through a FACScan flow cytometer. Data were analyzed using the FlowJo software.

Immunofluorescence

Cells grown on glass coverslips were fixed with -20°C methanol for 10 min, and then blocked in 10% BSA for 60 min at room temperature. Samples were then incubated with the indicated primary antibodies in PBS, 0.1% BSA for 60 min and then washed three times for 5 min with PBS and the incubated for 30 minutes with fluorescent antibodies in the dark. The slides were then mounted in Vectashield anti-fade reagent with DAPI and analyzed with a Leica confocal fluorescence microscope.

Three-Dimensional Collagen Gels

TIRE13-1 and stably transduced cells (TIREΔUMOD17 and TIREΔLuc) were trypsinized to a single-cell suspension and resuspended at a concentration of 1 × 10⁶ cells/ml in an ice-cold collagen solution. Briefly, collagen type I solution was mixed with 10% DMEM and 7.5% (vol/vol) bovine serum albumin in a ratio of 1:1. The cell suspension then was dispersed in droplets of 25 µl in six-well plates and allowed to gel for 20 min at 37°C before being overlaid with 1.5 ml DNEMF2 medium that was changed every 3 days, and pictures were taken at day 14.

TIRE13-1 cells are derived from the thick ascending limb of the loop of Henle

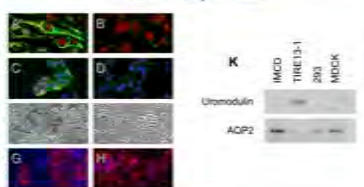


Fig. 1 Characterization of TIRE13-1 cells. Immunofluorescence staining with anti-Cytokeratin-8 Ab (green) A, anti-Cytokeratin-19 Ab (green) B, anti-Aquaporin-2 Ab (green) C, Alkaline Phosphatase F, anti-Uromodulin Ab (red) H, or control cell lines, MCKD with anti-Aquaporin-2 Ab (green) C, UMOD with Alkaline Phosphatase E, and Medullary section of a mouse kidney with anti-Uromodulin Ab (red) G. Immunoblotting of IMCD, TIRE13-1, 253, and MCKD cells K, with anti-Uromodulin (top panel) or anti-Aquaporin-2 Ab (lower panel) (AQP2).

Uromodulin knockdown causes centrosome amplification, genomic instability, and impairs the process of tubulogenesis in TIRE13-1 cells

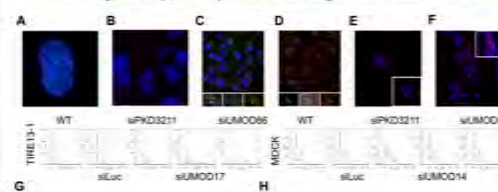


Fig. 3 Immunofluorescence staining five days post-transduction of Uromodulin or control (Luciferase) knockdown TIRE13-1 cells with centrosome protein γ-Tubulin (green), Uromodulin (red), and Acetylated Tubulin (green). A, B, and C, immunostaining of UMOD or control siLuc knockdown TIRE13-1 cells in three-dimensional collagen gels, after 14 days of culture, with Phalloidin anti-actin (red). D, E, F, Cell cycle analysis of TIRE13-1 or MCKD cells, infected with different siRNA five days post-transduction, G, H.

TIRE13-1 conserve their original phenotypal and physiological characteristics

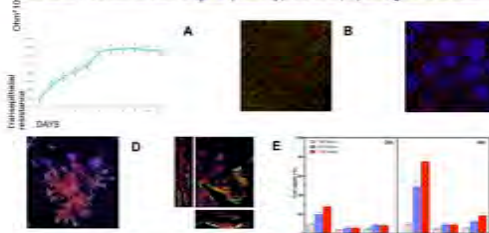


Fig. 2 Polarization curve of TIRE13-1 cells after 12 day of being cultured at confluency on a transwell. A, error bars represent an average of 3 independent experiments. Immunofluorescence staining of TIRE13-1 cells cultured on transwell with anti-CD11 Ab (green) B, or anti-AcetylatedTubulin Ab (red) C. Immunofluorescence staining of TIRE13-1 tubules formed in three-dimensional collagen gels with anti-AcetylatedTubulin (green) Ab, Phalloidin anti-Actin (red) and DAPI counterstaining (10X) Confocal microscopy D, or (50X) Confocal microscopy E. Hyperosmotic Resistance F, of 253, TIRE13-1, or IMCD cells, performed at 24 and 48h with 3 different osmolarity.

CONCLUSIONS

- TIRE13-1 is a unique human renal epithelial cell line with phenotypic and physiological characteristics of the thick ascending limb of the loop of Henle.
- TIRE13-1 undergo spontaneous tubulogenesis when grown in 3D collagen gels, and may be used as a novel *in vitro* model for the study for cystogenesis process of uromodulin-related diseases.
- Knockdown of UMOD resulted in centrosome amplification and genomic instability in TIRE13-1 cells, but not in MCKD. Conversely, PND1 knockdown causes centrosome amplification in MCKD cells but not in TIRE13-1.

Overall, these results suggest that centrosome instability and genomic instability may be a common event of the cystogenic transformation, regardless of the causative cystic gene. Furthermore, they raise the intriguing possibility that different genes control the cytoarchitecture of different segments of the nephron.

Primary cilia dynamics instruct tissue patterning and repair of corneal endothelium

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Edited* by Kathryn V. Anderson, Sloan-Kettering Institute, New York, NY, and approved January 11, 2011 (received for review November 10, 2010)

Primary cilia are required for several signaling pathways, but their function in cellular morphogenesis is poorly understood. Here we show that emergence of an hexagonal cellular pattern during development of the corneal endothelium (CE), a monolayer of neural crest-derived cells that maintains corneal transparency, depends on a precise temporal control of assembly of primary cilia that subsequently disassemble in adult corneal endothelial cells (CECs). However, cilia reassembly occurs rapidly in response to an in vivo mechanical injury and precedes basal body polarization and cellular elongation in mature CECs neighboring the wound. In contrast, CE from hypomorphic *IFT88* mutants (*Tg737^{orp4}*) or following in vivo lentiviral-mediated *IFT88* knockdown display dysfunctional cilia and show disorganized patterning, mislocalization of junctional markers, and accumulation of cytoplasmic acetylated tubulin. Our results indicate an active role of cilia in orchestrating coordinated morphogenesis of CECs during development and repair and define the murine CE as a powerful in vivo system to study ciliary-based cellular dynamics.

intraflagellar transport | eye development | ciliary length | microtubules

During development and tissue repair, groups of cells achieve or reconstitute highly organized patterns to form complex tissues by precisely integrating extracellular cues. How each cell converts such cues into events of cellular morphogenesis in a timely and coordinated manner is not understood (1). Primary cilia are microtubule-based antenna-like organelles protruding from the surface of vertebrate cells that mediate a number of signaling pathways during development and tissue homeostasis (2–4). The assembly, maintenance, and disassembly of cilia depend on intraflagellar transport (IFT) (5, 6). Null mutations affecting components of the IFT lead to patterning defects and midgestation lethality of the mouse embryo (7, 8). Cilia malfunction due to abnormal composition or function results in a spectrum of human syndromes referred to as ciliopathies (9–11).

As the cilium is reabsorbed before cell division, it is generally assumed that cilia appear in cells that have exited the cell cycle and entered quiescence or differentiation (12). Whereas this is true for many adult tissues, in some epithelial cells, such as the hair cells in the cochlea and the luminal cells of the mammary gland, the primary cilium regresses in the adult stage (13, 14). This led us to hypothesize that the resorption of the cilium may represent, in specific cell types, a signal of complete maturation and achievement of the normal adult cell shape and configuration in a tissue. Therefore, by controlling the assembly and disassembly of their primary cilium, cells could modulate the response to extracellular morphogenetic signals and determine their cellular architecture. Given the tridimensional nature of complex tissues, often comprising multiple cellular layers of different embryological origins, it has been difficult to follow the dynamics of ciliary assembly and disassembly in specific groups of cells and determine how these events correlate with cellular morphogenesis during development or tissue repair.

The corneal endothelium (CE), which plays an essential role in maintaining corneal transparency, offers the advantage of being organized in a 2D monolayer of hexagonal cells facing the anterior chamber of the vertebrate eye (15, 16). Because of its accessible location and simple well-defined organization, this tissue

constitutes a valuable model system to study cellular morphogenesis in the context of a complex tissue during development and repair.

Although the patterning and cell density of this tissue are critical in maintaining the normal physiology of CECs, the developmental steps that control these processes remain largely unknown. Here we provide evidence that primary cilia of the mouse corneal endothelium are required to allow proper morphogenesis toward the characteristic hexagonal cell patterning of the CE during postnatal development. Furthermore, we demonstrate that primary cilia assemble only when corneal endothelial cells undergo morphogenesis during development and tissue repair but disassemble during tissue homeostasis in adult animals.

Results and Discussion

Primary Cilia Transiently Assemble on CECs from the Periphery to the Center of the CE. Several studies describe a primary cilium of unknown function on CECs of different vertebrates; however, there are discrepancies about whether all or only a subpopulation of adult CECs are ciliated (17–19). Because CECs undergo profound age-dependent morphological changes, we asked whether the presence or absence of primary cilia is associated with a specific developmental stage (or age) (20). We analyzed the CE of mice from E17.5 to adulthood. Primary cilia and basal bodies were identified by immunofluorescence using a monoclonal antibody to acetylated- α -tubulin (21), a major component of the primary cilium, and a polyclonal antibody to γ -tubulin, respectively. Cell–cell contacts were visualized with a polyclonal antibody against the tight junction protein ZO-1. Only structures stemming from basal bodies were counted as primary cilia. Primary cilia were undetectable in CE of E18.5 embryos (Fig. S1). They started to assemble as early as postnatal day 1 (1 d) but were absent in adult animals (>45–75 d; Fig. 1). In 1- to 2-d-old mice, primary cilia of about 2–3.5 μ m were predominantly present on CECs located at the periphery of the cornea (Fig. 1B), whereas CECs present at the center of the CE only displayed a very short cilium (Fig. 1A). By 4 d, cilia of CECs at the center also reached a length of about 2–3 μ m that persisted through 12 d, when most of the peripheral cilia began to shorten (Fig. 1C and D). By 33–35 d, the majority of peripheral and central CECs had lost their cilia. The remaining ciliated cells presented a cilium no longer than 2 μ m and were more frequent at the center than at the periphery of CE (Fig. 1E and F). The CE of a 70-d mouse had virtually no cilia (Fig. 1G). Fig. 1H summarizes the dynamic changes of cilia length at the center and periphery of the CE at various postnatal stages.

Author contributions: C.I. designed research; A.L.B., L.P., G.L.G., J.D., and C.I. performed research; G.L.G. and J.D. contributed new reagents/analytic tools; A.L.B., G.L.G., J.D., M.M., and C.I. analyzed data; and C.I. wrote the paper.

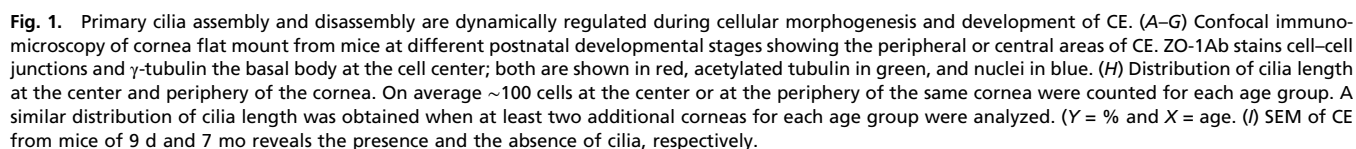
The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1016702108/-DCSupplemental.



Our data suggest that assembly of primary cilia in the CE is temporally regulated, starting perinatally first on peripheral CECs and then proceeding gradually to centrally located CECs in a wave-like manner. Likewise, cilia progressively disassemble in CECs of mice older than 15 d starting from the periphery toward the center of the CE until almost complete absence in 45-d-old mice. Cilia disassembly was confirmed by analyzing CE at different postnatal time points by scanning electron microscopy (SEM) (Fig. 1*A* and Fig. S2). Interestingly, re-sorption of cilia coincided precisely with the progressive formation of a hexagonal CEC pattern. Early after birth, CECs appear as irregular polygons with a variable number of sides and no detectable acetylated microtubules in the cytoplasm. By day 30–35, CECs achieved a hexagonal shape with characteristic bundles of acetylated microtubules around the nucleus (Fig. 1*A–F*).

CE Cellular Pattern Is Altered in *Tg737^{orp}* Mice, a Hypomorphic Allele of IFT88. The tight correlation between shape changes, cytoskeletal remodeling, and cilia assembly and disassembly in CECs during postnatal development led us to hypothesize that the cilium of CECs controls CE patterning. Null mutations that completely abolish cilia lead to midgestation lethality, before the CE layer is established (22). The IFT88 protein is a component of the IFT, the bidirectional movement of particles between the tip and the base of the cilium required for cilia assembly and maintenance first described in *Chlamydomonas* (23). The hypomorphic *Ift88* allele in the *Tg737^{orp}* mouse leads to cilia that are stunted and malformed, but not completely abolished, thus allowing these homozygous mutants to survive within the weaning period (24). In contrast, mice that are heterozygous for this mutation appear indistinguishable from wild-type littermates (25). To determine CE patterning in tissues with aberrant cilia, we isolated corneas from *Tg737^{orp}* mice ($n = 5$) and wild-type or heterozygous littermates at different postnatal developmental ages between 4 d and 19 d. To visualize the cell contacts, primary cilia, and nuclei of CECs, flat mounted corneas were stained with antibodies against ZO-1, acetylated tubulin, and DAPI. Cilia length and distribution differed significantly between *Tg737^{orp}* homozygotes compared with control littermates. Whereas some areas of CE in the corneas of *Tg737^{orp}* homozygotes exhibited cilia of fairly normal length, other areas presented short or absent cilia (Fig. 2*A*, *Middle* and *Bottom*, respectively). Within CE areas with defective cilia, CECs displayed a disorganized cellular pattern, ZO-1 mislocalization, and cytoplasmic accumulation of acetylated tubulin. SEM micrographs occasionally showed incomplete cellular junction formation between CECs with short cilia in *Tg737^{orp}* mice (Fig. 2*C*). In contrast, all CECs in wild-type or heterozygous littermates showed normal cilia and cellular distribution (Fig. 2*A*, *Top*). The patterning of the CECs in mutant mice was compared with that in wild-type using an image analysis approach and quantified (Fig. 2*B*). Notice that the nearest neighbor distance (NND) distribution of the CEC nuclei in *Tg737^{orp}* was much wider than that of wild-type controls. To determine whether the aberrant spacing of the nuclei in the CE of *Tg737^{orp}* was a consequence of cell death we performed TUNEL analysis on developing CE. No TUNEL-positive cells were detected on CE of 4-d *Tg737^{orp}* and wild-type littermate mice (Fig. S3). In addition, the CEC density is similar in both wild-type and mutant strains as assessed in 6-d old mice [wild type, 154 cells/0.02 mm² ($n = 3$) and *Tg737^{orp}*, 151.3 cells/0.02 mm² ($n = 3$)]. These results suggest that apoptosis does not account for CE patterning defects detected in the *Tg737^{orp}* mouse. Next, we tested whether the abnormal tissue patterning in *Tg737^{orp}* CE is due to defective morphology of individual CECs. We measured the area and the “shape factor” of the polygons identified by the cell–cell contacts in the CE labeled by the ZO-1 antibody in both strains. The distribution of the polygonal areas in the CE of 6-d *Tg737^{orp}* mice was wider than that of wild-type littermates and the means of the CEC shape factor

was 0.64 and 0.71 in the *Tg737^{orp}* and wild-type mice, respectively, implying an intrinsic defect of the overall architecture of the CECs in the *Tg737^{orp}* mice (Fig. S4). Taken together, these results suggest a critical role of IFT88 in the cytoskeletal remodeling and cellular morphogenesis of CECs required for tissue patterning in the developing CE.

Given the nonconditional nature of the *Tg737^{orp}* mutation, we cannot however exclude that the CE phenotype of newborn *Tg737^{orp}* mice resulted from defective interactions between neural crest progenitors of CECs and other ciliated cells early in development or from a role of IFT88 in neural crest cell migration similar to that described for other basal body and ciliary proteins (26). To exclude these possibilities, we knocked down IFT88 expression in CECs in vivo using a lentiviral-mediated RNAi approach.

Lentiviral-Mediated IFT88RNAi Phenocopies *Tg737^{orp}* Defects in CE.

As previously shown, primary cilia persist on mouse CECs for about 4 wk after birth. During this time, CECs undergo morphogenesis to acquire a hexagonal shape (Fig. 1). To specifically inactivate IFT88 in the CE, we injected the anterior chamber of 5- to 6-d old mice with 2 μ L of VIRHD/E/siLuc or VIRHD/E/siIFT88 lentivectors expressing an siRNA hairpin against luciferase (control) or the murine IFT88 gene, respectively (27). Transduction rates in vivo and cell-type specificity were monitored by injecting the VVPW/H2B-cherry lentivirus expressing cherry-tagged histone H2B under the constitutive CMV promoter. Virtually all CECs, but no other corneal cell types, expressed H2B-cherry in the nucleus (Fig. S5).

Corneas were isolated 5–7 d after injection and analyzed by immunofluorescence. Several areas of the CE from eyes injected with VIRHD/E/siIFT88 showed defects similar to those affecting the CE of *Tg737^{orp}* mice: absent or shortened cilia and corresponding abnormal patterning of CECs (Fig. 2*D*). In contrast, none of these defects were observed in CEs of mice injected with the control lentivector VIRHD/E/siLuc. These results indicate that primary cilia play an instructive role in determining the normal patterning of the CE during postnatal development.

Primary Cilia Assemble During in Vivo CE Repair in Adult Mice.

We next asked whether components of the IFT machinery that are required for ciliary assembly were still present in CECs of adult mice. Staining with antibodies specific for IFT72/74 (also known as CMG-1Ab) (28) and IFT88 was detected along the cilium and at the basal body of CECs in 4-d-old mice and at the basal body of CECs in 7-mo-old mice (Fig. 3 and Fig. S6). Therefore, IFT components persist at the basal body of CECs in adult mice,

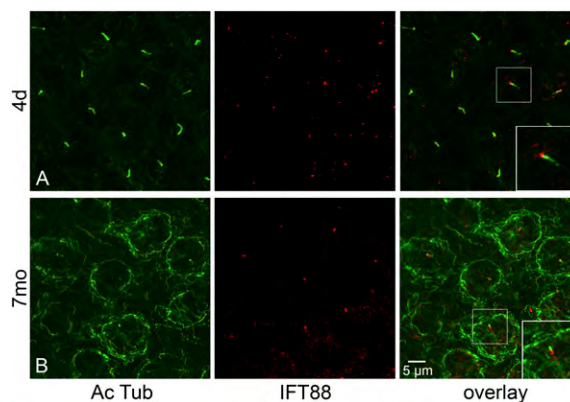


Fig. 3. IFT proteins in CE of young and adult mice. Immunofluorescence of flat mount of CE from 4-d- and 7-mo-old mouse stained with acetylated tubulin Ab (green) and IFT88Ab (red).

suggesting that CECs maintain the ability to reassemble a primary cilium.

To further address the notion that cilia orchestrate cellular morphogenesis of CECs, we tested the hypothesis that the cilium reassembles when cellular morphogenesis is reinduced in the CE of adult mice by wounding. As CECs show little or no proliferation during wound repair, they stretch and migrate in a direction perpendicular to the wound to cover the exposed Descemet's membrane, losing their normal hexagonal shape (29). Typically, after 48 h, a wound of about 3 mm is repaired by a complete layer of enlarged CECs, characterized by an irregular shape, which persists for several months (30). To investigate a possible role of primary cilia in endothelial repair *in vivo*, we produced a linear endothelial wound in the CE of adult mice. The wound was generated by gently scraping cells with a 10.0

suture surgical needle inserted into the anterior chamber of the eye of 5- to 6-mo-old mice and passed across the CE. After 18 h and 30 h of healing time, mice were killed and corneas were removed and analyzed. Cilia of about 2–3 μm were detected on CECs near the wound (4–5 cell rows) as early as 18 h after wounding (Fig. 4*A*). Although newly ciliated CECs within 4–5 cell rows next to the wound maintained a shape close to a hexagon, the perinuclear bundles of acetylated microtubules were disorganized or absent and the basal bodies localized at the median of the cell (Fig. 4*A* and *D*). In corneas isolated 30-h postwounding, CECs neighboring the wound still showed a primary cilium and lacked cytoplasmic acetylated microtubules. In addition, their shape was elongated in the axis perpendicular to the wound and the basal bodies polarized to the cell side closer to the wound (Fig. 4*B* and *D*). In contrast, CECs away from the

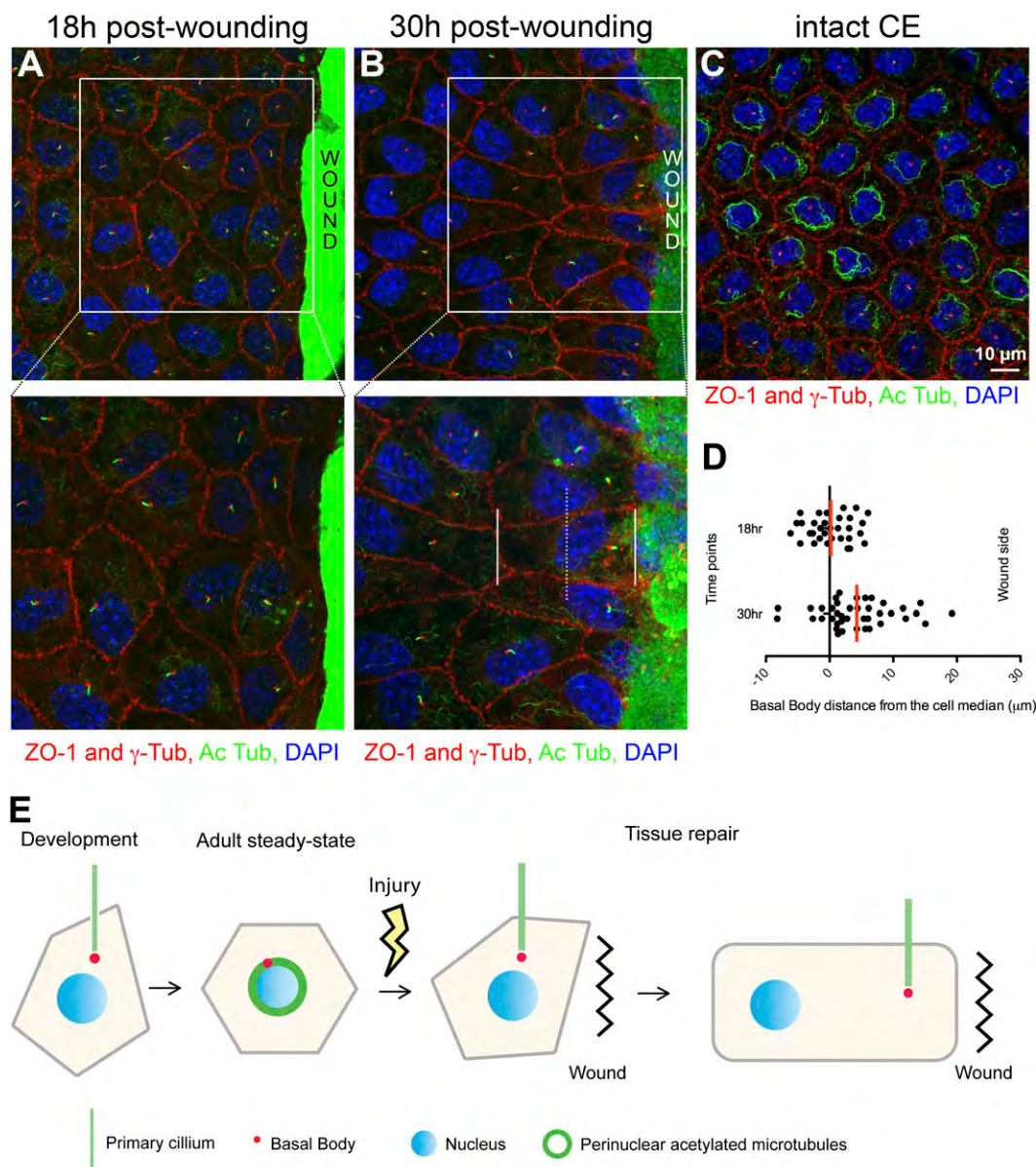


Fig. 4. Cilia assembly, cellular remodeling, and basal body polarization of CECs during CE repair. (A and B) Wounded area of CE 18 h and 30 h after in vivo recovery. Corneas were dissected from 3-mo-old mice. (C) Intact area of the same CE shown in B, located ~150–200 cell rows away from the wounded area. (D) Basal body distance from the cell median, measured as shown by the dotted line in B, *Bottom*. (E) Model of the cilia dynamics during cellular morphogenesis in development and repair (see text). Acetylated tubulin is shown in green, ZO-1 and γ -tubulin in red, DAPI in blue. Data corroborating the reorientation of the basal body were obtained in additional independent experiments and are shown in Fig. S7.

wounded area (~150–200 cell rows) remained aciliated and maintained their hexagonal shape, the perinuclear bundle of acetylated microtubules, and the basal bodies localized near the cell center (Fig. 4C).

Taken together, our data indicate that cilia are present on CECs during events of cellular remodeling, occurring in development or tissue repair of the CE, but disassemble during steady state in normal adult CE (Fig. 4E). In support of this idea, we have shown that ciliary dysfunction leads to defective patterning of CE during postnatal development. Intriguingly, findings in other tissues support similar conclusions. The kinocilium, the primary cilium found in inner ear hair cells, is required for proper development of the organ of Corti but it is reabsorbed about 10 d after birth. However, following trauma, hair cells from differentiated cochlea reassemble their kinocilia regardless of age, as shown in *in vivo* and *ex vivo* experiments in mice and rabbits (31, 32). During development and repair, inner ear hair cells undergo morphogenesis and show intense remodeling of the microtubular and actin cytoskeleton, and the kinocilium controls the localization of the basal bodies within hair cells (31, 33). In addition, primary cilia in adult kidney cells not only act as flow sensors, but may also be involved in epithelial differentiation during renal injury and repair. For example in mice, tubular damage causes an increase and a subsequent regression of cilium length during renal repair (34). Moreover *foxf1a*, the transcriptional regulator of cilia genes, is rapidly induced in response to epithelial stretch or injury (35). Thus, in general, the reassembly of primary cilia in adult tissues seems to represent a very early response to an injury. Interestingly, *Tg737^{oprk}* mice show defects in wound closure (36). Recent studies have shown that spatial constraint and actin cytoskeleton remodeling can control cilia elongation and resorption (37). It is tempting to speculate that mechanical forces

generated during tissue patterning and disrupted by an injury can modulate the cellular response to cilia-mediated signaling by controlling primary cilia dynamics. Identifying the cues and the mechanism promoting ciliary assembly upon tissue damage, and the signaling pathways mediated by this organelle during the healing process, could improve our understanding of how to treat injuries. Due to its simple organization as a monolayer facing the ocular anterior chamber and its accessibility to lentivector-mediated gene transfer that can be monitored *in vivo* by confocal microscopy, the CE provides a unique valuable model system to study the dynamic properties and function(s) of primary cilia in cellular morphogenesis during tissue development and repair.

Materials and Methods

The mouse strains and oligonucleotide sequences used in this study as well as a detailed description of lentiviral production, microscopy and morphometric analysis, and *in vivo* wound healing experiments are provided in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Brad Yoder (Alabama University), Beth Zaviowitz, and Lisa Satlin [Mount Sinai School of Medicine (MSSM)] for providing and breeding *Tg737^{oprk}* mice, and all members of the Mlodzik laboratory for helpful discussion. We are grateful to Dr. Ron Gordon and Heather Bell at the Mount Sinai Electron Microscopy Facility for helping with SEM analysis, Lizhen Ren for technical support, and Susanna Franks for assisting with the illustrations. Confocal laser scanning microscopy was performed at the MSSM-Microscopy Shared Resources Facility, supported with funding from National Institutes of Health (NIH)-National Cancer Institute Shared Resources Grant 5R24 CA095823-04, National Science Foundation Major Research Instrumentation Grant DBI-9724504, and NIH Shared Instrumentation Grant 1 S10 RR0 9145-01. This research was supported by NIH Grants HD058039 (to C.I.), DK78231 (to G.L.G.), EY014592 (to M.M.), and Department of Defense Grant W81XWH-09-1-0269 (to G.L.G.). C.I. is a Young Investigator of the National Kidney Foundation (Y1B1110).

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Biochimica et Biophysica Acta

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Review

Cilium, centrosome and cell cycle regulation in polycystic kidney disease[☆]Kyung Lee, Lorenzo Battini, G. Luca Gusella^{*}

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ARTICLE INFO

Article history:

Received 17 August 2010
 Received in revised form 10 January 2011
 Accepted 16 February 2011
 Available online xxxx

Keywords:

Cystogenesis
 Kidney
 Centrosome
 Cell cycle
 Cilium
 Planar cell polarity

ABSTRACT

Polycystic kidney disease is the defining condition of a group of common life-threatening genetic disorders characterized by the bilateral formation and progressive expansion of renal cysts that lead to end stage kidney disease. Although a large body of information has been acquired in the past years about the cellular functions that characterize the cystic cells, the mechanisms triggering the cystogenic conversion are just starting to emerge. Recent findings link defects in ciliary functions, planar cell polarity pathway, and centrosome integrity in early cystic development. Many of the signals dysregulated during cystogenesis may converge on the centrosome for its central function as a structural support for cilia formation and a coordinator of protein trafficking, polarity, and cell division. Here, we will discuss the contribution of proliferation, cilium and planar cell polarity to the cystic signal and will analyze in particular the possible role that the basal bodies/centrosome may play in the cystogenetic mechanisms. This article is part of a Special Issue entitled: Polycystic Kidney Disease.

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1. Introduction

Hereditary cystic kidney diseases comprise a heterogeneous group of monogenic disorders [1]. In some instances the bilateral development of multiple fluid-filled cysts in kidneys is part of a more complex syndromic clinical manifestation, whereas in others it is a distinctive feature of the disease and an important cause of end stage kidney disease. We will focus on the latter disorders, hereafter referred to as polycystic kidney disease.

Polycystic kidney disease is characterized by the hyperproliferation of tubular epithelial cells, the alterations of their fluid secretion functions, and changes in the extracellular matrix deposition and fibrosis, all of which profoundly alter the organ architecture and impair

renal function. Autosomal dominant and autosomal recessive forms of polycystic kidney disease have been recognized with an incidence of 1:800 and 1:20,000, respectively.

Autosomal dominant polycystic kidney disease (ADPKD) is caused by the dysregulation of the PKD1 or PKD2 genes, which code for polycystin-1 (PC1) and polycystin-2 (PC2), respectively. PC1 and PC2 may form a complex through the interaction of the respective carboxyl termini, thus establishing reciprocal regulatory functions. Consequently, regardless of the genotype, the clinical manifestations of ADPKD largely overlap, with few notable exceptions: on average, individuals with mutation in the PKD1 gene reach end stage kidney disease 20 years earlier than patients carrying mutations in the PKD2 gene, and PKD2 mutations result in more severe disease in males than in females.

Autosomal recessive polycystic kidney disease (ARPKD) results from mutations in the polycystic kidney and hepatic disease 1 gene (PKHD1), encoding fibrocystin/polyductin (FPC) [2,3]. ARPKD generally manifests earlier in life with the most severe cases resulting in

[☆] This article is part of a Special Issue entitled: Polycystic Kidney Disease.

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perinatal or neonatal death. In addition, collecting duct ectasia results in cysts that remain connected with the nephrons of origin. Unlike ADPKD, in which cysts are prevalent in the collecting ducts but may develop everywhere along the nephron, in ARPKD cystogenesis is restricted to the collecting ducts.

PC1 is a large integral membrane protein with receptor-like structural characteristics [4], which undergoes a complex Notch-like processing [5,6]. Abundant evidence supports the role of the PC1 carboxyl terminus in signaling mechanisms. The C terminal tail of PC1 contains phosphorylation sites for different tyrosine and serine/threonine kinases [7] and a domain for the interaction with G proteins and the activation of the JNK/AP1 pathway [8,9]. Importantly, in response to changes in mechanical stimulation, the carboxyl terminal tail undergoes regulated intramembrane proteolysis and translocates into the nucleus to activate the AP1 pathway through a process negatively regulated by PC2 [10].

PC2 is a Ca^{2+} regulated, non-selective cation channel that shares sequence and structure similarities with the superfamily of transient receptor potential channels [11–15]. PC2 is expressed predominantly in the ER, but it is also found in the Golgi, the plasma membrane, and on the cilium where with PC1, and likely FPC, it forms a mechanosensor complex that controls Ca^{2+} influx in response to flow [16,17]. On the plasma membrane, PC2 only partially co-localizes with PC1

and adhesion complexes, suggesting that it may function independently as homodimer or participate in different complexes with other members of the TRP family, thus expanding the functional characteristics of these channels [16]. The loose interaction of PC2 with PC1 and adhesion complexes may be important to confer PC2 more dynamic mechanosensory properties independent of or opposed to PC1. For example, *situs inversus*, the phenotype with reversed orientation of visceral organs, is associated with *Pkd2* but not *Pkd1* knockout mouse models, indicating the independent mechanosensing function of PC2 in the nodal cilia [18]. In the case of stretch-activated ion channels PC1 and PC2 exert opposing effects with PC2 inhibiting channel opening and PC1 reverting this suppression [19]. Though many aspects of the regulation of PC2 function remain unclear, the growing evidence of its multiple interactions with cytoskeleton organizing proteins supports its Ca^{2+} -dependent mechanosensory role at different cellular compartments (for a comprehensive review, see references [15,20,21]). Interestingly, the subcellular localization of PC1 at the cell adherens, desmosomes, focal adhesions, and cilia provides the proximity with cytoskeletal components, suggesting a possible role of PC1 in the control of cytoskeleton rearrangement (Fig. 1) [22–25].

Similar to PC1, FPC undergoes a complex proteolytic process at the ciliary membrane [26,27]. The large ectodomain is cleaved by a proprotein convertase and remains tethered to the carboxyl stalk via

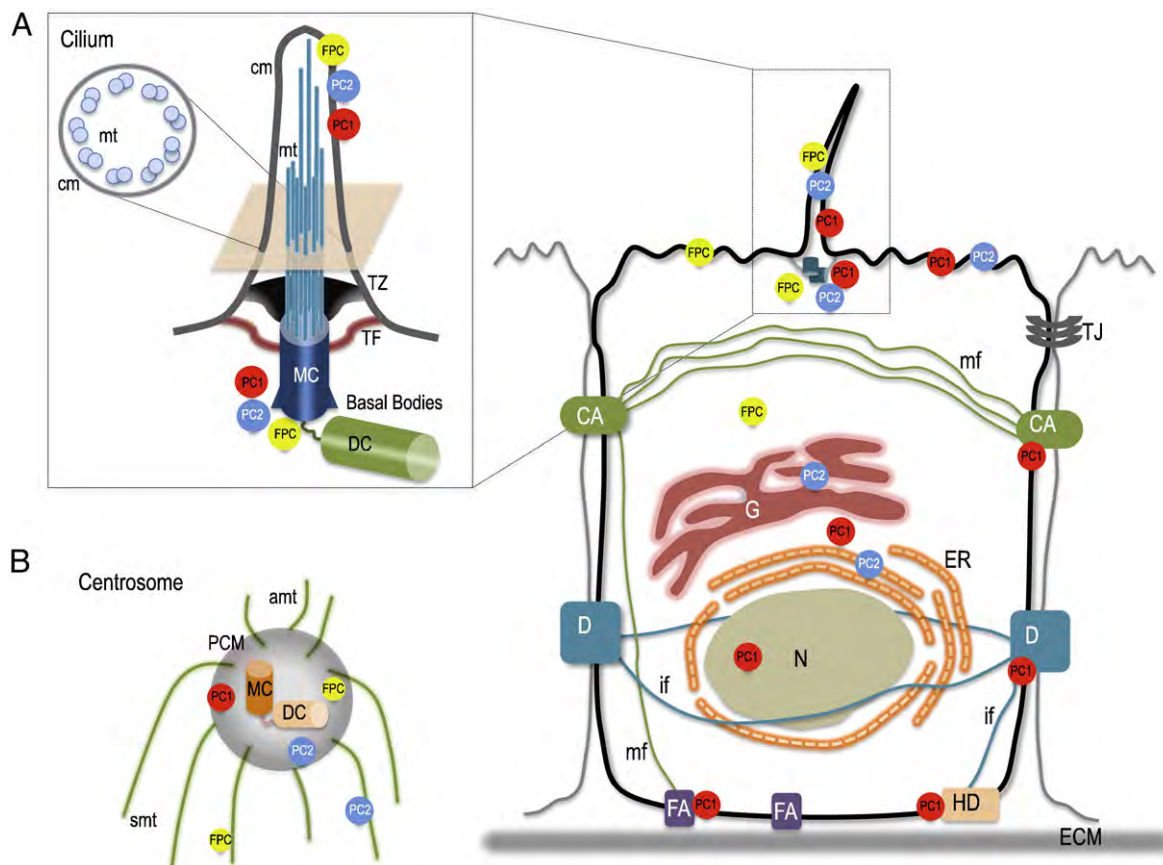


Fig. 1. Subcellular localization of PC1, PC2, and FPC. (A) Polycystic proteins localize to multiple compartments within the cell including the cilium in which they form a Ca^{2+} non-selective channel whose activity is essential during renal morphogenesis. In the kidney, the cilium protrudes from the apical side of renal epithelial cells into the luminal space. The cilium is supported by nine doublets of microtubules that nucleate from the basal body, a specialization of the mother centriole (MC), at the base of the cilium (1A, inset). (B) Following cilium resorption, pericentriolar material (PCM) organizes around the centrioles to form the centrosome. Microtubules emanating from the centrosome maintain cellular structure and are required for multiple cellular functions including spindle organization and cytokinesis. Polycystic proteins also localize to the centrosome and both PC2 and FPC are found to associate with the spindle microtubules during cell division. While the ciliary localization of polycystic proteins is important for fluid flow sensing, their function on the centrosome and mitotic spindle remains obscure. Similarly unclear is whether the localization at cell–cell and cell–matrix contacts plays a role in tension sensing and cytoskeletal rearrangement. N, nucleus; G, Golgi apparatus; ER, endoplasmic reticulum; CA, cell adherens; D, desmosomes; HD, hemidesmosomes; FA, focal adherens; ECM, extracellular matrix; TJ, tight junction; mt, microtubules; mf, actin microfilaments; if, intermediate filaments; MD, mother centriole; DC, daughter centriole; TZ, transition zone; TF, transition fiber; PCM, pericentriolar matrix; cm, ciliary membrane; amt, astral microtubules; smt, spindle microtubules.

disulfide bonds. Shedding of the ectodomain occurs concomitantly to the regulated intramembrane proteolysis that releases the intracellular cytoplasmic tail that then traffics to the nucleus or nucleolus. This process appears to be dependent on intracellular Ca^{2+} release, but it remains unknown whether a ligand or a mechanical change triggers the shedding of the ectodomain [26]. Similarly, the possible paracrine signaling function of the shed domain and the implication of the nuclear translocation on gene regulation remain unclear, though the Notch-like regulation and the ciliary localization of the process suggest that it may be involved in the maintenance of nephron architecture.

Excellent reviews have presented and discussed the characteristics and functions of polycystic genes and their encoded proteins in detail [28–31]. Here we will review the early events of renal cystogenesis and the relationship of polycystic proteins with the centrosome, its association with the cilium and its function in cell cycle control.

2. Proliferation of cystic cells

Cell hyperproliferation underlying continuous expansion of the cysts and renal enlargement is a hallmark of ADPKD and ARPKD and a determinant of renal failure [32,33]. Under normal conditions the mitotic index of the adult kidney is very low. However, in renal tissues from ADPKD or ARPKD patients, as well as from *Pkd1* or *Pkd2* mutant animal models, nuclei positive for proliferating cell nuclear antigen (PCNA) and Ki67 mitotic markers are readily detectable [34–36]. In fact, multiple mitogenic pathways may be constitutively activated in polycystic kidney disease as a consequence of altered Ca^{2+} homeostasis or abnormal protein trafficking.

Defects in PC2 Ca^{2+} channel activity that lead to low intracellular Ca^{2+} concentration, aberrant G-protein signaling by PC1 dysregulation [8,37], and decreased cyclic nucleotide catabolism [38] may contribute to the accumulation of cAMP and the abnormal activation of the Ca^{2+} inhibitable adenyl cyclase 5/6. Cystic cells proliferate in response to increased cAMP levels [39,40] and the activation of the PKA/B-Raf/MAPK pathway [41], in contrast to normal primary renal epithelial cells, whose growth is inhibited by cAMP [42]. Cyst expansion then accelerates partly through a mechanism promoting chloride-driven fluid secretion [43,44].

Altered protein trafficking may also contribute to cystogenic signals as in the case of the mislocalization of EGF receptors in renal epithelia. The EGF receptor (EGFR/HER1) is normally expressed apically during the embryonic mammalian kidney development, but its localization shifts to the basal side in the adult organ. In ADPKD and ARPKD, however, the EGFR/HER1 expression is increased and mislocalized to the apical membrane where it results in a paracrine loop of persistent stimulation by its ligand released in the filtrate or in the cystic fluid [45–47]. Other dedifferentiating processes characteristic of cystic cells may further reinforce this autostimulatory mechanism. For instance, the expression of ErbB2/Neu/HER2, a member of the EGFR superfamily, is developmentally regulated and restricted to the embryonic kidneys. However, the re-expression ErbB2/Neu/HER2 in the adult ADPKD renal epithelia allows it to heterodimerize with EGFR/HER1 on the apical membrane [48]. The interference with the autocrine/paracrine EGF/EGFR stimulatory loop reduced cystic lesions in organ culture [49], slowed down cyst expansion and ameliorated polycystic kidney disease in different, though not all, animal models [50–53].

Other proliferative pathways may also be activated. In particular, the evidence of the activation of the mammalian target of rapamycin (mTOR) signaling in the cyst lining cells of the kidneys from different mouse models of renal cystic disease (*MAL*, overexpressing myelin and lymphocyte protein; and the IFT88 hypomorph, *orpk*) and in human ADPKD specimens suggests that this may be a common pathway underlying cystic proliferation [54] (for an extensive review, see [55]).

The serine/threonine kinase mTOR is the key component of the multiprotein complexes mTORC1, which positively controls protein translation, cell metabolism and proliferation, and mTORC2, which is involved in actin cytoskeleton organization and cell survival [56,57]. The activation of mTORC1 is suppressed by the heterodimer of hamartin and tuberlin, encoded by the *TSC1* and *TSC2* genes, respectively. Stimulation of the PI3kinase/Akt or ERK pathways leads to the phosphorylation-mediated inhibition of TSC2/tuberlin and the activation of mTORC1 [58,59]. Tuberlin and PC1 functionally cooperate to regulate the mTOR pathway. PC1 interacts with tuberlin [54], sequesters it on the membrane and protects it from Akt phosphorylation, thus suppressing the activation of mTORC1 [60]. Conversely, tuberlin is necessary for the proper localization PC1, as seen in Eker rats that carry a homozygous mutation of the *Tsc2* gene. In the absence of functional tuberlin, PC1 accumulates in the Golgi and fails to properly traffic to the lateral cell membrane, demonstrating that tuberlin is necessary for correct PC1 localization [61].

In various animal models of polycystic kidney disease, a significant reduction of cystic growth has been obtained by pharmacologically preventing the cAMP increase, Ca^{2+} imbalance, EGF stimulation, mTOR activation [62–67] or by inhibiting cell cycle progression with the cyclin-dependent kinase inhibitor, roscovitine [68]. These *in vivo* results have provided the rationale for different experimental therapeutic approaches that are currently under investigation [69]. However, recently concluded clinical trials that tested the efficacy of mTOR inhibitors (rapamycin/sirolimus and the analog everolimus) on ADPKD patients at different stages of the disease yielded disappointing results. Treatment with these inhibitors showed no improvement in the renal function, despite a transient reduction in total kidney volume in patients with a more advanced stage of disease [70,71]. Unlike the findings from a shorter study with sirolimus on fewer patients [72], these trials also indicated that both mTOR inhibitors presented considerable side effects that severely limit their therapeutic value for ADPKD, even when administered at doses far lower than those used in the animal models.

A further detailed analysis of these studies may help explain some of the differences within the human studies and the discrepancies with the experimental data on animal models as commented in references [73,74]. In light of the remarkably promising preclinical results, it would be premature to interpret the discouraging results of the human trials to confute the validity of targeting the mTOR pathway in cystic diseases. Rather, it may be necessary to explore alternative strategies in which mTOR inhibition is part of a combination therapy or in which mTOR inhibitors could be specifically targeted to the kidney.

A smaller clinical trial on ADPKD patients was also conducted to assess the efficacy of octreotide, a long-lasting somatostatin analog, that inhibits the intracellular accumulation of cAMP in renal epithelia [75]. In this 12-month study, results similar to those with mTOR inhibitors were obtained: octreotide arrested the increase of kidney volume but failed at improving kidney function. Differently from mTOR inhibitors, however, octreotide appeared to be well tolerated with no serious adverse effects.

Overall, these clinical trials underscore the complexity and variability of the disease progression, and question the use of kidney volume change as a surrogate marker of organ function [32,33,73]. They also suggest that at advanced stages of the disease, cell proliferation is dissociated from cellular and organ function. As such, proliferation may have different roles at different stages of cystogenesis. Findings of proliferating cells in normal tubular epithelia surrounding cysts suggest that cell growth is an early event in the cystogenic transformation. Nevertheless, no cysts derive from the active proliferation during normal organ morphogenesis, and active growth of renal carcinoma cells does not necessarily result in cyst formation. Therefore, it remains difficult to establish whether the activation of these pathways represents the cystogenic trigger or if it supports cyst expansion.

3. Cilium and cystogenesis

The observation that cystic proteins localize on the primary cilium and basal body [76–78] provided new insights into the mechanisms of renal cystic diseases. The intense focus on the cilium that followed unveiled the genetic determinants of numerous complex diseases that define a new class of disorders collectively referred to as ciliopathies (for comprehensive reviews, see references [79–82]).

The primary cilium is a highly compartmentalized organelle present in most cell types that functions as a sensor of extracellular environmental cues. It is formed as a single protrusion of the plasma membrane supported by the axoneme, a cytoskeletal component that is assembled as a ring of 9 microtubule doublets arranged tangentially to the center in a configuration known as 9 + 0 (Fig. 1A, inset). Defects in cilia formation result in complex phenotypes, which invariably include cystic kidneys [83]. In renal epithelia, cilia convert mechanical force of fluid flow into cellular functions [84]. PC1, PC2 and FPC are expressed in renal primary cilia where they are a part of a mechanosensor complex that translates the ciliary bending induced by flow into Ca^{2+} influx [85–87]. Their functional role was supported by observation that STAT6, whose ciliary localization depends on flow stimulation, is part of a complex that includes the cleaved carboxyl terminus of PC1 and the transcriptional coactivator P100. As the carboxyl tail of PC1 is proteolytically cleaved, the complex translocates into the nucleus and activates gene expression, thereby linking mechanical stimulation of the cilium by urine flow and cellular responses [88]. However, impaired mechanosensation of cilium as a primary defect in cystogenesis was challenged by the work on conditional knockout models of the intraflagellar transport *Ift88* (polaris) and the *Kif3a* subunit of kinesin-2 genes, which are essential for ciliogenesis [89]. While the deletion of *Ift88* or *Kif3a* during gestation prevented cilia formation and resulted in severe cystic disease within 2 weeks after birth, deletion of either gene in the adult animals did not immediately result in detectable cystic phenotype, despite the cilia ablation. Eventually, mild renal cyst formation was observed 6 months after the knockout, revealing different requirements of ciliary function during renal development and in the maintenance of adult kidney [89]. These results indicated that cilia are dispensable in adult mice and that other components may participate in the cystic process.

Interestingly, the conditional models of *Pkd1* inactivation similarly displayed greater susceptibility of young mice to develop severe cystic kidney disease as compared to the adult mice. These mouse models offered the opportunity to investigate the early cystogenetic events following the depletion of PC1 [90–92]. A detailed analysis of perinatal *Pkd1* inactivation demonstrated that the deletion of the gene within day P13 led to extensive cystogenesis and kidney enlargement, whereas inactivation of *Pkd1* from day P14 onward resulted in late onset cystic kidney disease [91]. These observations uncovered a window of susceptibility, which corresponds with the completion of mouse nephrogenesis when proliferation is actively ongoing and a specifically timed brake point. However, the study also showed that although proliferation abruptly decreased after day P14, it remained significantly higher at P16 as compared to the adult kidney. Nevertheless the course of cystic disease was comparable in the P16 and older mice, suggesting that proliferation *per se* may not be sufficient to trigger the cystogenic change [91]. As the brake point was characterized molecularly by a change in gene expression pattern consistent with a developmental switch, it was proposed that components of an early developmental program could in fact be the cystogenic triggers. Such a program may be recalled during the re-epithelialization process that follows renal injury. In support of this notion, in adult kidneys in which *Pkd1* is conditionally inactivated or in which no cilia can be formed because of the conditional *Kif3a* excision, the cystic phenotype can be accelerated by the induction of ischemia/reperfusion injury or pharmacological nephrotoxicity [36,93,94]. In addition, although cell

growth occurs rapidly following injury, it reverts to control levels before cystic expansion, again suggesting that proliferation cannot be the only cystogenic switch [94]. Nevertheless, even though proliferation may not be sufficient as the sole cystogenic trigger, it may yet provide the necessary context for such a trigger to arise as indicated in recent experiments on the conditional inactivation of the *Hnf1b* gene, which encodes a transcription factor involved in the expression of genes that include *Pkd2*, *Pkhd1* and *UMOD* (encoding uromodulin). Similar to the *IFT88*, *Kif3a*, and *Pkd1* models, the pre- or perinatal conditional inactivation of *Hnf1b* leads to rapid polycystic kidney disease, while the ablation of *Hnf1b* in the adult leads to slow onset cystic disease that can turn into rapidly progressing disease following renal injury. Careful analysis of proliferating cells using BrdU showed that tubular dilatation coincided with the regenerative proliferation burst and the loss of mitotic orientation only in the mutant dividing cells and not in those of wild type kidneys [95]. Therefore, cell proliferation may create the conditions for the cystogenic switch, which may include defects in oriented cell division, planar cell polarity (PCP), and changes in the centrosome positioning [93–95].

4. Role of planar cell polarity in cystogenesis

The organization and asymmetric distribution of protein content that cells maintain in parallel to the epithelial plane is called planar cell polarity (PCP). The mechanisms of PCP are fundamental for the developmental patterning of both invertebrates and vertebrates [96] and are regulated by the non-canonical Wnt pathway (for a comprehensive review of the Wnt signaling in cystic diseases, see reference [97]).

During kidney development, the spindle of the dividing cell organizes with an orientation parallel to the axis of the elongating tubule, revealing an intrinsic cell polarity. The evidence of a link between cilia and PCP came from the observation that the ciliary protein inversin, the product of the *NPHP2* gene whose mutations cause nephronophthisis, functioned as a switch from the canonical to the non-canonical Wnt pathway [98]. Whether PCP in turn played a role in cystic disease was first observed in kidneys in two rodent renal cystic models: the mouse with inactivation of the *Tcf2/HNF1 β* transcription factor [99], and the *pck* rat, which carries a mutated *PKHD1* gene ortholog [100]. In both cystic models a significant number of spindles in the dividing cells of the kidneys were misaligned, suggesting that the loss of proper spindle orientation and planar cell polarity are linked to cystogenesis [101].

Recently, a direct proof of the role of PCP in renal cystic development was provided by the knockout mouse model of *Fat4* gene, which encodes a PCP protein of the proto-cadherins family [102]. Homozygous *Fat4*^{−/−} mutants died at birth but displayed multiple characteristics of PCP protein defects including anomalies in the elongation of the cochlea and disruption of hair cell organization in the organ of Corti. *Fat4*^{−/−} mutants also displayed smaller kidneys with dilated and shorter tubules and significant defects in oriented cell division. Crossing *Fat4*^{−/−} mice with mutants for other PCP components, *Vangl2* and *Frx1*, exacerbated the cystic phenotype [102]. Together with the ciliary localization of FAT4, these findings further strengthened the link between PCP and cilium during cystogenesis. The interdependence of PCP and ciliary function is also supported by observations with other PCP core proteins, Dishevelled and Vangl2. Dishevelled is involved in the docking of the centrioles/basal bodies to the apical membrane that precedes ciliogenesis [103], and Vangl2 is required for the asymmetric positioning of motile cilia in cells of zebrafish neural tube [104]. Furthermore, the fluid flow influences centrioles' movement and contributes to the orientation of motile cilia in conjunction with PCP in ependymal cells [105,106].

It should be noted, however, that more recent reports question the role of oriented cell division as a primary cause of cystogenesis. In the hypomorphic mutant for *Wnt9b*, whose expression is required for

renal morphogenesis, cystogenesis starts *in utero*, leading to the development of grossly cystic kidneys within a month of age [107]. The analysis of the embryonic renal development revealed that during the period from E13.5 to P1, tubules lengthen through the movement of the cells that assume an elongated shape parallel to the tubule axis in a process of convergent extension, which is dependent on PCP and the activation of the Rho/Jnk signaling pathway. The impairment of this process in the *Wnt9b* mutants alters tubule diameter and triggers cyst formation. Interestingly, until P1, cell division appeared similarly misoriented in both *Wnt9b* mutants and wild type mice, suggesting that defects in oriented cell division alone cannot account for *in utero* cystogenesis [107]. Moreover, a study in *Pkd1*, *Pkd2* and *Phkd1* mouse mutants showed that changes in oriented cell division did not precede cystogenesis, but rather followed the cystic transformation [108]. While challenging the defects of oriented cell division as a driver of cystogenesis, these results nevertheless emphasize the role of PCP in cystogenesis.

5. Centrosome and cell cycle

The basal bodies located at the base of the cilium are a morphological specialization of the centrioles/centrosome, specifically the mother centriole from which the axoneme emanates to support the formation of the primary cilium (Fig. 1). Functionally, the basal bodies participate in the intraflagellar transport (IFT) through the organization of the transition zone and the control of vesicles trafficking to and from the cilium [109], thereby coupling the cilium and centrosome functions. The essential role played by the centrosome in coordinating the ciliary and PCP crosstalk is further emphasized by the alteration of the Wnt signaling following the disruption of basal bodies in zebrafish *bbs4* morphants [110].

The centrioles/centrosome serve as the microtubule-organizing center (MTOC), and thus play a major role in the spatial organization of the microtubular network required for not only the formation of primary cilia, but also cell polarity, migration, trafficking of cytoplasmic organelles, and organization of the mitotic spindle [111]. Because of these essential functions that it underlies, the centrosome integrity and duplication are tightly controlled. In most cells, under normal conditions the centrosome divides only once per cell cycle through a mechanism coupled to the cell cycle progression, so that each daughter cell receives only one centrosome [112,113]. Reciprocal interactions exist between IFT and centrosomal proteins to regulate their trafficking and localization. For example, IFT20 shuttles between the Golgi and the cilium, and is required for the localization of pericentrin to the centrosome [114,115]. Conversely, reduced expression of pericentrin also lowers the levels of IFT20, IFT88, IFT57 and PC2 in centrioles and inhibits cilia formation [115].

In cells preparing to cycle, the cilium is reabsorbed, leaving the basal bodies/centrioles free to anchor to the cell cortex and to be ready for centrosome duplication and the subsequent organization of the microtubule rearrangement that is required for the assembly of the spindle, mitosis, and cytokinesis. Cilium resorption may allow redistribution of ciliary components to the centrosome that can affect the cell cycle progression. For instance, IFT88/polaris remains tightly associated with the centrosome and modulates the G1–S transition by titrating out Che-1, an inhibitor of the growth suppressor function of Rb [116]. Consequently, interfering with various centrosome proteins leads to the p53-dependent block of cell cycle progression from G1 to S and failure to assemble cilia [117,118]. p53 is also a centrosomal protein, and its depletion increases centrosome amplification [119]. The control of cell cycle progression and restriction of centrosome overduplication by p53 is exerted partly via the transactivation of p21 and the direct association of p53 with the centrosome [120–122].

Both PC1 and PC2 exert a direct effect on cell cycle and centrosome duplication. The heterologous expression of PC1 or PC2 arrests the cell cycle in G1 through different mechanisms that converge on the

induction of the cyclin-dependent kinase (Cdk) inhibitor p21 and the inhibition of Cdk2 activity [123,124]. In the case of PC1, the expression of p21 results from the activation of the JAK2-dependent phosphorylation of STAT1, but not of p53 [123]. In contrast, PC2 functions by binding to Id2, a member of the helix–loop–helix (HLH) family of transcriptional regulators that antagonize basic HLH transcription factors that are involved in the control of cell cycle progression. The interaction with PC2 sequesters Id2 in the cytosol, thus preventing its translocation into the nucleus where it suppresses p21 transcription [124]. PC1 and PC2 exert a reciprocal control on the activation of these pathways. The physical interaction of PC1 with JAK2 is dependent on the presence of PC2 as a cofactor, whereas PC1 phosphorylation of PC2 is required for its interaction with Id2. Conversely, depletion of PC1 or PC2 results in faster G1 to S progression [124–126] and reduced expression of p53 in HEK293 cells [125]. Lowered p53 expression is also observed in embryonic kidneys of *Pkd1* $-/-$ mice [127], albeit in this case it is difficult to determine whether such downregulation is an effector or a consequence of the cystogenic transformation.

Polycystic proteins localize on the centrosome and are important to maintain centrosome integrity (Fig. 2). The inhibition of PC1 expression induces centrosome amplification *in vitro*, and supernumerary centrosomes were observed both in the kidneys of *Pkd1* conditional knockout animal model and in human renal tissue from ADPKD patients *in vivo* [128]. These centrosomes appeared fully functional, as they were able to organize multipolar spindles. However, the cells dividing with aberrant mitotic spindles entered mitotic catastrophe or produced genetically unstable progeny, characterized by significant apoptosis and aneuploidy [129,130]. Amplified centrosomes were noted on seemingly normal tubular cells, suggesting that centrosome aberrations may be an early event in the cystic conversion [128]. Similarly, centrosome amplification was also reported in fibroblast cell lines derived from *Pkd2* transgenic mice and in mesenchymal cells of *Pkd2* knockout embryos [131], indicating that PC2 dysregulation also affects centrosome integrity. Polycystins' broad tissue distribution and the effects of interference of PC1 or PC2 in centrosome integrity in non-renal cells suggest that polycystins play a fundamental role in the mechanisms controlling centrosome duplication and that centrosomal aberrations may be important in cystic development.

More recently, at least some FPC isoforms have also been shown to be required for the maintenance of centrosome integrity and proper spindle assembly [132]. Similarly to PC2, FPC is found on the spindle during cell division, but the mechanisms controlling its localization remain unknown. The spindle localization of PC2, however, was shown to require the interaction with Diaphanous (mDia)-related formin 1, mDia1 [133], a protein involved in actin polymerization and microtubule stabilization [134]. Depletion of mDia1 coincides with the loss of PC2 localization from the spindle and a decreased Ca^{2+} release in mitotic cells. The function of PC2 on the spindle is unclear, but the interaction of PC2 with the actin bundling protein α -actinin and with the microtubule-dependent motor kinesin-2 subunit KIF3A, both of which activate PC2 channel activity *in vitro*, lends support to the intriguing possibility that PC2-mediated Ca^{2+} transport may function in the cytoskeletal remodeling required for cell division [21,135,136]. Although the spindle localization of PC1 is unclear, its presence on the centrosome along with FPC may be important in the reconstitution and regulation of the PC2 Ca^{2+} channel activity [87,132,137]. Overall, these observations underline the interdependence of cilium, centrosome, and cytoskeletal rearrangement.

The mechanisms contributing to centrosome amplification remain speculative, but it might involve the altered expression of p53 and/or cyclin-A, as observed in PC1-deficient cells [125,138], as well as imbalanced Ca^{2+} homeostasis. Centrosome amplification can occur following cytokinesis failure or by reiterated centriole duplication within the same cycle. Evidence of multinucleation and enlarged nuclei in PC1- or PC2-deficient cells suggests that supernumerary

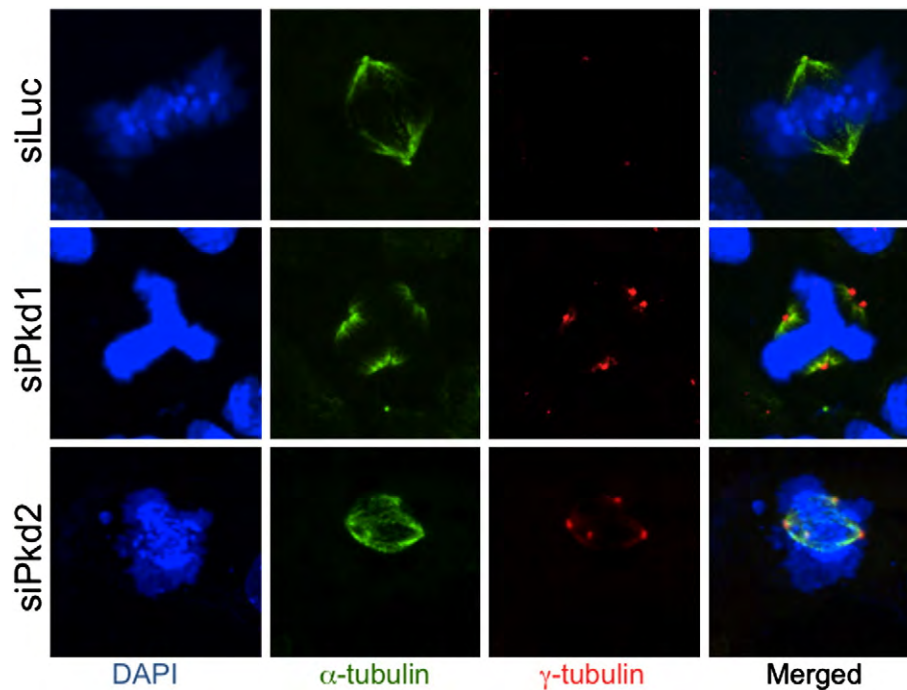


Fig. 2. Centrosome amplification following the suppression PC1 or PC2 expression. Centrosome amplification and mitotic spindle abnormalities occur rapidly after the knockdown of *Pkd1* or *Pkd2*. Shown are MDCK cells and IMCD3 cells 3 days following the transduction with a lentivirus constitutively expressing the shRNAs specific for *Pkd1* and *Pkd2*, respectively. IMCD3 cells (or MDCK cells, not shown) transduced with control lentivector expressing shLuc against luciferase (siLuc) maintain normal mitosis. Cells were immunostained with anti- α -tubulin and anti- γ -tubulin to specifically detect microtubules (green) and centrosomes (red), respectively, and then counterstained with DAPI to visualize DNA (blue).

centrosomes may result from endoreduplication. Cytokinesis depends on the accumulation of Ca^{2+} stores to the furrow and on the proper Ca^{2+} release before abscission [139]. It remains to be determined whether the reciprocal interaction of PC2 (or a polycystins complex) with cytoskeletal components has any function on this Ca^{2+} regulation. Furthermore, both centrosome duplication and cell growth processes depend on increased Ca^{2+} transients from internal Ca^{2+} stores [140], a requirement that seems to be at odds with the decreased intracellular Ca^{2+} content in polycystic cells [141,142]. However, experiments on HEK293 cells indicate that PC1 negatively regulates non-capacitative Ca^{2+} entry (NCCE) channels and that cell proliferation upon PC1 knockdown is sustained by an NCCE-dependent increase in Ca^{2+} oscillations [143]. Hence, it is possible that changes in the frequency and amplitude of Ca^{2+} oscillations may support also centrosomal amplification or centriole reduplication [144].

The centrosomal defects extend to other diseases with renal cystic manifestations. Loss of hamartin, the product of the *TSC1* gene whose mutations cause tuberous sclerosis, also leads to centrosome amplification [145]. The depletion of the centrosomal Mks1 or Mks3/meckelin proteins, which are mutated in the autosomal recessive Meckel-Gruber syndrome, results in centrosome amplification and, in the case of Mks3, in multiciliation [146]. Renal cysts develop following the loss of IFT20, which results in cilia ablation, centrosome amplification with loss of centrosome positioning, and mitotic spindle misorientation [147]. It will be of interest to determine whether centrosome defects are common to other renal cystic diseases.

Centrosome aberrations occur early after the inhibition of polycystic proteins and, similar to ciliary defects, they may be a common denominator in renal cystic disease. A causative role of centrosome defects in cystogenesis is difficult to establish, but its expected consequences are consistent with all the findings characteristic of ADPKD cells. Errors in centrosome duplication may result in the formation of monopolar or multipolar spindles, aberrations associated with chromosome missegregation, genomic instability, and apoptosis. Cells

that accumulate excessive genomic damage/imbalance become apoptotic, whereas others may survive carrying abnormal karyotypes [148,149], and altered physiological functions. A kidney-specific interference with effectors of the centrosome duplication process will be required to establish a causal link between centrosome anomalies and renal cystic development.

6. Conclusions

As intense research has focused on cystic cells, we have a better understanding of the mechanisms that support cystic expansion, including alterations of calcium homeostasis and changes in protein trafficking and interactions, which sustain the constitutive activation of mitogenic pathways. On the other hand, the signals (or lack thereof) that trigger the cystic conversion are unknown and the mechanisms underlying the early cystogenic events are just emerging in a picture of increasing complexity.

The view of the cilium as sensor of fluid flow has expanded to the regulation of planar cell polarity and defects in PCP-controlled mechanisms, convergent extension, and oriented cell division have been indicated as possible cystogenic triggers. However, it seems that ciliary functions and PCP in the cystogenic conversion cannot be clearly separated, as they exert a reciprocal regulation. Docking of centrioles/centrosome to the cortex is essential for the formation of the basal bodies and ciliogenesis as well as for the establishment of the spindle pole position [150]. Therefore, a cystogenic trigger driven by centrosome amplification is also conceivable as the presence of supernumerary centrioles, caused by the depletion or malfunction of different cystic proteins, can produce conflicting cues leading to improper attachment, misalignment of the spindle axis, or altered cilium positioning. These effects may be exposed by the dysregulation of cell cycle progression in cells with amplified centrosomes.

Very important has been the finding that a developmental switch limits the cystogenic susceptibility to ciliary defects to a period of time

largely overlapping with the completion of murine renal morphogenesis [91]. Whether PCP mutants are similarly constrained remains to be demonstrated, and experiments with conditional inactivation of PCP genes may provide a clue on whether and how ciliary and PCP functions follow an order of succession in cystogenesis. In the adult, tubular epithelia injury reestablishes the susceptibility to cystogenesis, although it has not been determined whether this depends on the reactivation of the same renal morphogenetic developmental program. Both early development and repair processes are characterized by the need for cell proliferation. Since proliferation shows a biphasic curve, that is, it subsides before starting again in the cystic cells, it cannot be the main cystogenic trigger [94]. Nevertheless, proliferation appears necessary for the trigger to be set off [95].

While we are gaining a better understanding of multiple cellular processes and cell components that play a role in cystogenesis, a unifying pathogenetic mechanism is still missing, largely due to our incomplete knowledge of the workings of polycystic proteins. Further efforts will be necessary to integrate the functions of cilium/centrosome, PCP, and cell proliferation and to determine the sequence of early events that initiate the cystogenic signal.

Acknowledgments

We thank Carlo Iomini and Debbie Hyink for the helpful comments. Work in the authors' laboratory is supported by the National Institutes of Health (5R01DK78231-2) and the Department of Defense (W81XWH-09-1-0269).

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