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PRINCIPAL INVESTIGATOR: Gabriele Luca Gusella, Ph.D.

CONTRACTING ORGANIZATION:
Mount Sinai School of Medicine
New York, NY 10029

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# Role of Integrin-Beta 1 in Polycystic Kidney Disease

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 integrin β1. The scope of the study is to elucidate the molecular mechanism underlying these events and the role of Integrin β1 in ADPKD pathogenesis. Our recent work has confirmed that Integrin β1 inhibition reverses the centrosome amplification and the increased fibronectin deposition that distinguish cystic PKD1 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 (Integrin β1) 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.

## Subject Terms
Kidney, integrin, cyst, polycystic, siRNA, cell cycle, centrosome, uromodulin
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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 initiation. 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. 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-α2 and integrin-β1 (Intβ1) are upregulated after the knockdown of Pkd1 in mouse cells and that integrin-α2β1 mediated the resistance of Pkd1 knockdown (Pkd1kd) cells to anoikis.

We hypothesized that Intβ1 is a necessary mediator of cystogenesis. The objective of the application is to dissect the role of Intβ1 in the control of centrosome integrity, the genomic instability and the cystogenetic progression pathways triggered by the loss of PC1. We proposed that Intβ1 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 Intβ1 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 with the purpose to isolate a stable cell line with characteristics of the distal tubules and collecting duct cells.

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 Intβ1 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 Intβ1 reverts centrosome amplification and aberrant matrix deposition.** We have shown that the suppression of Intβ1 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 Intβ1 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 Intβ1 overexpression upon Pkd1 knockdown) before proceeding with the live imaging.
In vivo analysis of Intβ1 cystogenic role: mouse breeding strategy. To overcome the early lethality associated with the loss of Pkd1 or Itgb1 (Intβ1) genes, we originally proposed to generate an inducible Intβ1 knockdown mouse model to determine the cystogenic role of integrin-β1 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 Intβ1 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 (Pkd1fl/fl, Itgb1fl/fl, AQP2-cre).

To generate Pkd1fl/fl, Itgb1fl/fl, AQP2-cre mice, from Jackson Laboratory three murine strains were obtained carrying either Pkd1fl/fl (B6.129S4-Pkd1tm2Ggg/J), the Itgb1fl/fl allele (B6;129-Itgb1tm1Efu/J), or the hemizygous AQP2-cre transgene (B6.Cg-Tg(Aqp2-cre)1Dek/J). The Pkd1fl/fl mice possess loxP sites in introns 1 and 4 of the Pkd1 gene; the Itgb1fl/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. 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 testes. 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 Pkd1fl/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 Pkd1fl/+ and Pkd1fl/null mice derived from the crossing of the Pkd1fl/fl mice with the heterozygous Pkd1null/+ strain. 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 \textit{Pkd1} loss in cells with normal or knockdown expression of Int\textbeta 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\textbeta 1 reverses centrosome amplification and fibronectin expression in Pkd1 knockdown cells.

**REPORTABLE OUTCOMES**
The current studies produced the following presented/published papers:


**CONCLUSIONS**
Centrosome amplification and genetic instability seem to be common events in cystic models independently of the cystic gene involved.
Increased Int\textbeta 1 expression in \textit{Pkd1} knockdown cells is required for centrosomal amplification and changes in matrix deposition.
The role of Int\textbeta 1 in these events suggests that targeting Int\textbeta 1 could be potentially beneficial to interfere with the progression of the renal cystogenesis.

**REFERENCES**

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

Lorenzo Battini,*1 Kim Lee1, Carlos Martinez-Romero1, Lin Geng1 and G. Luca Gusella1

1Medicine, Division of Nephrology, Mount Sinai School of Medicine, New York, NY.

ABSTRACT

Members of the uromodulin gene (UMOD) are responsible for tubular protein dysfunction, nephropathy, and medullary cystic kidney disease type 2 (MCYD2). Both deficiencies are thought to cause tubulointerstitial nephropathy, although the precise molecular mechanisms remain unclear. Genetic studies have shown that uromodulin knockdown in various cell lines results in a loss of pericentriolar material and a reduction in pericentriolar matrix formation. In contrast, overexpression of uromodulin results in an increase in the size of the centrosome. Consistent with the notion that the pericentriolar matrix is dependent on the regulation of microtubule dynamics, treatment with an inhibitor of microtubule dynamics results in a loss of pericentriolar matrix and centrosome size. In this study, we have shown that uromodulin knockdown in HK-2 human proximal tubule epithelial cells results in a reduction in the size of the centrosome. We have also observed that uromodulin shows good localization to the centrosome and that the size of the centrosome is dependent on the integrity of the pericentriolar matrix. Our findings suggest that uromodulin has a role in the regulation of microtubule dynamics and pericentriolar matrix formation. These observations may have implications for the development of new therapeutic strategies for the treatment of tubular protein dysfunction and nephropathy.

MATERIALS AND METHODS

Cell lines and reagents

Cell lines were maintained in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% sodium pyruvate. Primary cultures of human proximal tubule cells were obtained from the American Type Culture Collection (ATCC). Uromodulin knockdown cells were generated using siRNA oligonucleotides (Dharmacon, Thermo Fisher Scientific). The siRNA pools were designed to target the human uromodulin gene (UMOD). The pools were transfected into cell lines using lipofectamine reagent (Thermo Fisher Scientific). The siRNA pools were transfected into cell lines using lipofectamine reagent (Thermo Fisher Scientific). The reagents were obtained from the ATCC. The siRNA pools were transfected into cell lines using lipofectamine reagent (Thermo Fisher Scientific). The reagents were obtained from the ATCC.

Immunofluorescence

Immunofluorescence was performed using standard protocols. Briefly, formalin-fixed tissues were incubated with primary antibodies overnight at 4°C. The following primary antibodies were used: anti-α-tubulin (clone DM1A, Sigma-Aldrich), anti-γ-tubulin (clone 3F3, Sigma-Aldrich), and anti-UMOD (clone U8G8, Santa Cruz Biotechnology). The secondary antibodies used were Alexa Fluor 488- and Alexa Fluor 594-conjugated antibodies (Invitrogen). Images were acquired using a confocal microscope (Zeiss LSM 880). The results were analyzed using ImageJ software (NIH).

CONCLUSIONS

Our findings suggest that uromodulin knockdown results in a loss of pericentriolar matrix and a reduction in the size of the centrosome. These observations may have implications for the development of new therapeutic strategies for the treatment of tubular protein dysfunction and nephropathy. Future studies are needed to further investigate the role of uromodulin in the regulation of microtubule dynamics and pericentriolar matrix formation.
Primary cilia dynamics instruct tissue patterning and repair of corneal endothelium

Andrea L. Blitzer, Lampros Panagis, G. Luca Gusella, John Danias, Marek Mlodzik, and Carlo Iomini

Departments of *Developmental and Regenerative Biology, †Ophthalmology, and ‡Medicine, Mount Sinai School of Medicine, New York, NY 10029

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Primary cilia are transiently assembled on cells of the corneal endothelium in a stage-specific manner. This finding highlights the importance of cilia in cellular morphogenesis and tissue repair.

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 CECs possess primary cilia. CECs are known to be highly dynamic and participate in morphogenetic processes during development and tissue repair.


The authors declare no conflict of interest.

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†Present address: Department of Cell Biology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203.

‡To whom correspondence may be addressed. E-mail: Marek.Mlodzik@mssm.edu or carlo.iomini@mssm.edu.

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Primary cilia assembly and disassembly are dynamically regulated during cellular morphogenesis and development of CE. (A–G) Confocal immunomicroscopy of cornea flat mount from mice at different postnatal developmental stages showing the peripheral or central areas of CE. ZO-1Ab stains cell–cell junctions and γ-tubulin the basal body at the cell center; both are shown in red, acetylated tubulin in green, and nuclei in blue. (H) Distribution of cilia length at the center and periphery of the cornea. On average ∼100 cells at the center or at the periphery of the same cornea were counted for each age group. A similar distribution of cilia length was obtained when at least two additional corneas for each age group were analyzed. (Y % and X = age. (I) SEM of CE from mice of 9 d and 7 mo reveals the presence and the absence of cilia, respectively.
Fig. 2. Ciliary dysfunction leads to defects in CE patterning. (A) Confocal immunomicroscopy of 6-d-old Tg737<sup>−/−</sup> and wild-type CE flat mount stained with indicated antibodies. (Middle) Only areas lacking cilia (Right of the dotted line) are defective in nuclei and ZO-1 distribution. (Bottom) Defect seems more severe and in addition to the "packing" phenotype there is also a remarkable accumulation of cytoplasmic acetylated tubulin. (B) NND analysis for each nucleus was obtained by comparing the thresholded image of the nuclei in A, Upper and Lower. Note the defect in cell–cell contact formation indicated by the arrow and blunted cilia. (C) SEM of 5-d-old wild-type and Tg737<sup>−/−</sup> CE, Upper and Lower, respectively. Mice were injected 6 d after birth and killed at age 15 d; the experiment was repeated twice with similar outcome. IFT88 knockdown phenocopies Tg737<sup>−/−</sup>.
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. 1I and Fig. S2). Interestingly, resorption 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. 1A–F).

**CE Cellular Pattern Is Altered in Tg737orpk 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 Tg737orpk 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 Tg737orpk 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 Tg737orpk homozygotes compared with control littermates. Whereas some areas of CE in the corneas of Tg737orpk/uniFB01 homozygotes exhibited cilia of fairly normal length, other areas presented short or absent cilia (Fig. 2A, 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 Tg737orpk mice (Fig. 2C). In contrast, all CECs in wild-type or heterozygous littermates showed normal cilia and cellular distribution (Fig. 2A, Top). The patterning of the CECs in mutant mice was compared with that in wild-type using an image analysis approach and quantified (Fig. 2B). Notice that the nearest neighbor distance (NND) distribution of the CEC nuclei in Tg737orpk was much wider than that of wild-type controls. To determine whether the aberrant spacing of the nuclei in the CE of Tg737orpk was a consequence of cell death we performed TUNEL analysis on developing CE. No TUNEL-positive cells were detected on CE of 4-d Tg737orpk 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 Tg737orpk, 151.3 cells/0.02 mm² (n = 3)]. These results suggest that apoptosis does not account for CE patterning defects detected in the Tg737orpk mice. Next, we tested whether the abnormal tissue patterning in Tg737orpk 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 Tg737orpk 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 Tg737orpk and wild-type mice, respectively, implying an intrinsic defect of the overall architecture of the CECs in the Tg737orpk 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 Tg737orpk mutation, we cannot however exclude that the CE phenotype of newborn Tg737orpk 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 Tg737orpk 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 μL 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 Tg737orpk mice: absent or shortened cilia and corresponding abnormal patterning of CECs (Fig. 2D). 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.
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. 4A). 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. 4A 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.](image-url)
Thus, in general, the reassembly of primary cilia in adult tissues foxj1a ample in mice, tubular damage causes an increase and a sub-epithelial differentiation during renal injury and repair. For ex- cepts not only act as hair cells (31, 33). In addition, primary cilia in adult kidney 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 sub-sequent regression of cilium length during renal repair (34). Moreover foxj1a, 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. In- terestingly, foxj1a mRNA in mice show defects in wound closure (36). Recent studies have shown that spatial constraint and actin cytoskeleton remodeling can control cilia elongation and re-sorption (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 lenti-vector-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.

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Cilium, centrosome and cell cycle regulation in polycystic kidney disease

Kyung Lee, Lorenzo Battini, G. Luca Gusella*

Division of Renal Medicine, Department of Medicine, The Mount Sinai School of Medicine, New York, NY 10029, USA

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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
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 mechanosensorial 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 mechanosensorial 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 lumenal 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 Ca2+ 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 proteins 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 Ca2+ homeostasis or abnormal protein trafficking.

Defects in PC2 Ca2+ channel activity that lead to low intracellular Ca2+ concentration, aberrant G-protein signaling by PC1 dysregulation [8,37], and decreased cyclic nucleotide catalysis [38] contribute to the accumulation of cAMP and the abnormal activation of the Ca2+ 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 of 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 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 tuberin, encoded by the TSC1 and TSC2 genes, respectively. Stimulation of the PI3kinase/Akt or ERK pathways leads to the phosphorylation-mediated inhibition of TSC2/tuberin and the activation of mTORC1 [58,59]. Tuberin and PC1 functionally cooperate to regulate the mTOR pathway. PC1 interacts with tuberin [54], sequesters it on the membrane and protects it from Akt phosphorylation, thus suppressing the activation of mTORC1 [60]. Conversely, tuberin is necessary for the proper localization PC1, as seen in Eker rats that carry a homozygous mutation of the fsc2 gene. In the absence of functional tuberin, PC1 accumulates in the Golgi and fails to properly traffic to the lateral cell membrane, demonstrating that tuberin 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, Ca2+ 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 but failed at improving kidney function. Differently from mTOR inhibitors, however, octreotide appeared to be well tolerated with no serious adverse effects.

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 ciliary 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 cilium 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]. PCK1, 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 Ca2+ influx [85–87]. Their functional role was supported by observation that STAT6, whose cilary localization depends on flow stimulation, is part of a complex that includes the cleaved carboxyl terminus of PC1 and the transcriptional activator 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 JKB89 (polars) and the Kif3a subunit of kinesin-2 genes, which are essential for ciliogenesis [89]. While the deletion of JKB88 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 cilary 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 cystogenic events following the depletion of PCK1 [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 be not 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-epithelization 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, Phkd1 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 dilation 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 nephronphthisis, 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 Tc2/HNF1β 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 Fjx1, 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 cellular 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 titering 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 effect 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 Diaphanos (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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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
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 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. 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. 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.

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