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Cilium, Centrosome and Cell Cycle Regulation in Polycystic Kidneys Disease

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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 mechanism(s) 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.

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

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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 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 (Figure1) [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 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 diseaseas 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 epitelia. 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 [50-52] [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 *Tsc2* 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 proper 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 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 [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 (Figure 1, inset A). 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 two 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 six 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 *Pkd*1 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-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, 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 *Hfn1b* 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 nephronophtisis, 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/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 (Figure 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 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 effector or a consequence of the cystogenic transformation.

Polycystic proteins localize on the centrosome and are important to maintain centrosome integrity (Figure 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 PC2ocalization 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-actin in 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 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 tuberosclerosis, 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 likely 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.

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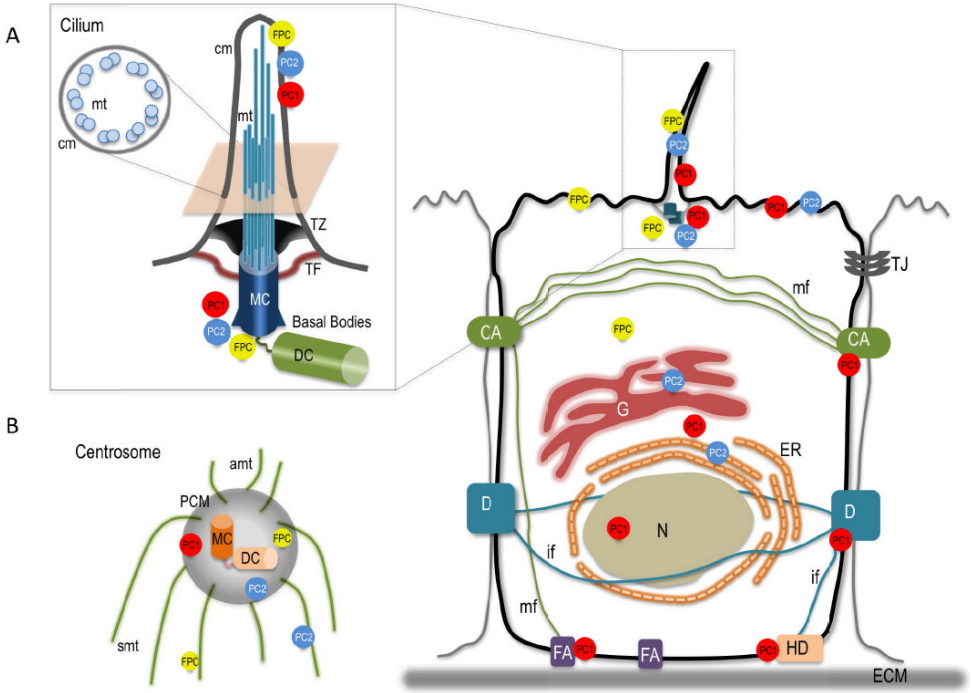

Figure 1.

Subcellular localization of PC1, PC2, and FPC. A) Polycystic proteins localize to multiple compartment 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.

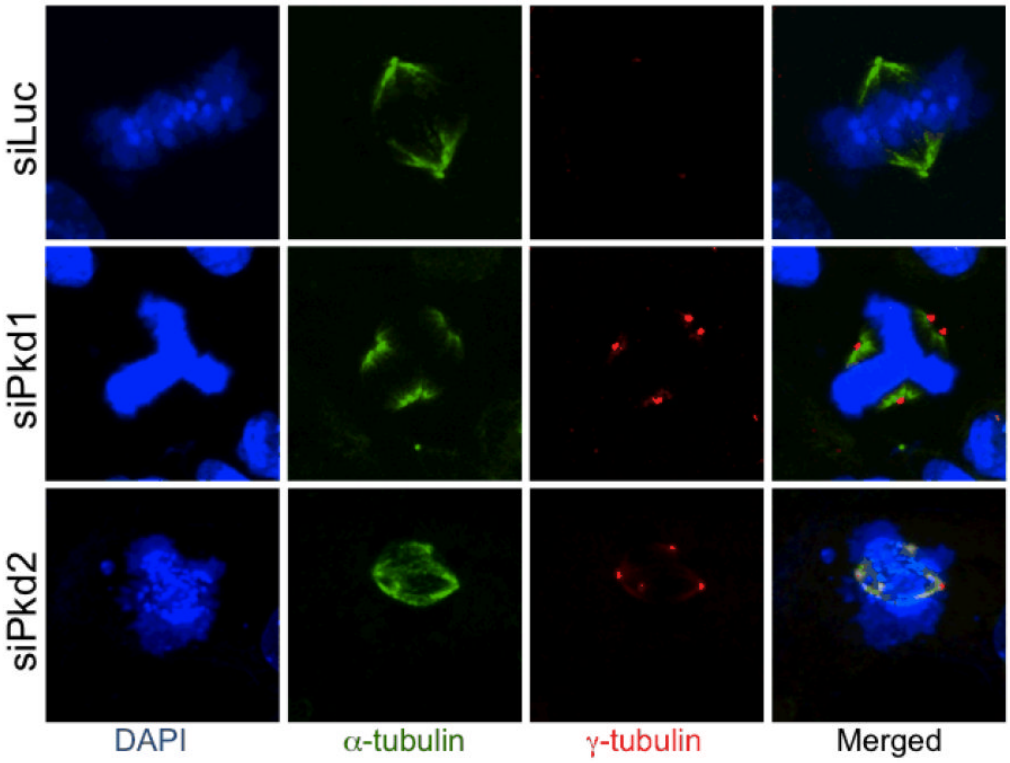

Figure 2.

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 three days following the transduction with a lentivirus constitutively expressing the shRNAs specific for Pkd1 and Pkd2, respectively. IMCD3 cells (or MDKC 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).