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The role of the cilium in normal and abnormal cell cycles: emphasis on renal cystic pathologies

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Abstract

The primary cilium protrudes from the cell surface and acts as a sensor for chemical and mechanical growth cues, with receptors for a number of growth factors (PDGF α , Hedgehog, Wnt, Notch) concentrated within the ciliary membrane. In normal tissues, the cilium assembles after cells exit mitosis and is resorbed as part of cell cycle re-entry. Although regulation of the cilium by cell cycle transitions has been appreciated for over 100 years, only recently have data emerged to indicate the cilium also exerts influence on the cell cycle. The resorption/protrusion cycle, regulated by proteins including Aurora-A, VHL, and GSK-3 β , influences cell responsiveness to growth cues involving cilia-linked receptors; further, resorption liberates the ciliary basal body to differentiate into the centrosome, which performs discrete functions in S-, G2-, and M-phase. Besides these roles, the cilium provides a positional cue that regulates polarity of cell division, and thus directs cells towards fates of differentiation versus proliferation. In this review, we summarize the specific mechanisms mediating the cilia-cell cycle dialog. We then emphasize the examples of polycystic kidney disease (PKD), nephronophthisis (NPHP), and VHL-linked renal cysts as cases in which defects of ciliary function influence disease pathology, and may also condition response to treatment.

Keywords

cell cycle; cilia resorption; polycystic kidney disease; signaling; protein phosphorylation

1. Introduction

The primary cilium is a microtubule-based organelle that extends from a modified centriolar anchor, termed the basal body, which is located below the surface of the plasma membrane. Although related to the motile flagella found in lower eukaryotes and specialized cell types such as sperm, in mammals most cilia are non-motile. Non-motile or “primary cilia” are organized around a central cytoskeletal core termed the axoneme, composed of 9 microtubule doublets arranged in a ring (a 9+0 configuration) [1]. The axoneme is coated with a lipid bilayer and typically protrudes 3–10 μ m from the cell surface [2]. Ultrastructural features of the cilium have recently been reviewed in detail by Ishikawa and Marshall [3]. In humans, cells in the majority of tissues have a single non-motile cilium protruding; lymphocytes have none, while a small number of specialized cell types, such as the lung epithelial lining and the reproductive tract, have motile cilia [4, 5].

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In tissue culture, the primary cilium is readily observed in non-transformed cells that are serum-starved and confluent, in the stationary (G_0) phase of the cell cycle. A number of early studies noted the consistent absence of cilia in mitotic cells, with resorption occurring around the G2/M boundary [6–8]. More recent work, including investigation of the ciliary cycle in many cell types, indicates that ciliary resorption sometimes occurs at earlier stages in the cell cycle, prior to S phase [9–12]. Subsequent studies demonstrated that ciliary resorption occurs in two waves in quiescent cells stimulated to reenter cycle, with a first wave occurring within 1–3 hours during G1, and a second wave occurring after ~18 hours, as cells enter mitosis [10, 13]. This dynamic profile of ciliary resorption and protrusion led to early debate as to whether the ciliary cycle passively reflected or actively contributed to cell cycle [14]. This debate was given fuel by the observations that oncogenically transformed cancer cell lines and cells in primary tumors, which have deregulated cell growth, commonly lack cilia [15–20]; and that mutations eliminating proteins such as IFT88 both eliminate ciliary assembly and are associated with deregulated cell growth [21, 22]. Defects in cilia are now appreciated as contributing to numerous pathological conditions involving cell growth deficiencies, with these including not only cystic kidney diseases, but also retinal degeneration, obesity, mental retardation, and developmental malformation [23–28]. For these reasons, understanding the function of cilia is now appreciated to have high medical relevance.

The past decade has been marked by enormous growth in studies of cilia, with the net benefit of elucidating multiple discrete mechanisms for bidirectional signaling between cilia and cell cycle. The field has become too large to summarize in a single review: recent relevant reviews addressing different aspects of ciliary formation and function include [3, 23]. In this article, we first briefly summarize mechanisms by which cell cycle cues regulate the ciliary cycle, and reciprocally, mechanisms by which the presence or absence of cilia may influence the cell cycle and oriented cell division. As our main focus, we then describe the relevance of these mechanisms to pathological conditions, focusing in particular on the numerous diseases of the kidney in which a ciliary role is well documented [29–32]. We then summarize the current status of clinical management for these diseases, and explore the hypothesis that defects arising from compromised ciliary function may specifically influence response to treatment.

2. Regulation of ciliary protrusion and resorption during cell cycle

Cilia extend from the cell surface soon after completion of cytokinesis. As protrusion commences, the centrioles undergo specialized differentiation to become basal bodies. There is a fundamental asymmetry in the age of centrioles in individual post-mitotic cells, arising from the mother-daughter relationship in centriolar duplication [33]. Former mother centrioles are morphologically distinct, containing distal and subdistal appendages, while the daughter does not. After cytokinesis, cilia arise more rapidly in cells inheriting a former mother centriole, and these cells are initially more responsive to Sonic Hedgehog (Shh), which binds receptors displayed on the cilia [34], although this asynchrony is lost over time. Distal appendages contain CEP164, a protein that accumulates at the centrosome at G2/M and has been functionally defined as required for ciliary formation [35], suggesting a role for marks placed on the centriole prior to M phase as contributing to ciliary emergence in G0/G1. The process of protrusion involves the interaction of the interflagellar transport (IFT) machinery with polarized protein secretion, coupled with modification of the ciliary axoneme by acetylation [36], and has been thoroughly reviewed [37–39]. As a number of the proteins that have been shown to trigger ciliary resorption (e.g. AURKA, NDE1, discussed below) are abundant in mitosis but inactivated or degraded at cytokinesis, removal of these proteins is a likely prerequisite of effective ciliogenesis.

Quiescent (G0) cells maintained in culture or organized in tissues, and cells transitioning through G1, retain cilia. Depending on cell type, cilia are preferentially resorbed either at G1/S transition, or prior to mitotic entry, with the latter pattern more commonly reported [9–13] (Fig. 1). In studies with deciliation induced in G0-synchronized hTERT1-RPE1 cells, resorption occurred in two waves, with loss of ~ 20% of cell cilia in late G1, and the remainder during G2/M transition [13]. While serum is commonly used to induce cell cycle reentry, a number of studies have parsed out serum components that are sufficient to trigger ciliary resorption. Among those tested to date, the most critical growth factor for induction of resorption is platelet-derived growth factor (PDGF) [40], which can independently trigger ciliary resorption, and which is required for the process. Insulin growth factor (IGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) lack this capacity when applied independently but augment PDGF activity, as can treatment of cells with calcium ionophores to increase cytoplasmic Ca^{2+} levels [40].

The action of a number of proteins has been more proximally linked to the regulation of ciliary resorption (Figure 2). Tctex-1 is a light chain of cytoplasmic dynein complex, which participates in intraflagellar transport (IFT) [41], but also has dynein-independent functions [42]. Two hours following serum-treatment of quiescent, ciliated cells, Tctex-1 is phosphorylated and targeted to the ciliary base, at the transition zone [12]. Although Tctex-1-deficient cells are able to generate primary cilia, these cells are unable to resorb cilia. Tctex-1 is phosphorylated on T94 during the resorption process; a non-phosphorylatable mutant cannot resorb cilia, while overexpression of a T94E phosphomimic accelerates ciliary disassembly. Although Tctex-1 action is not yet well understood, at least part of its activity involves integrity of the actin cytoskeleton, as cytochalasin-D, which inhibits actin polymerization, blocked Tctex-1-dependent disassembly.

Aurora-A (AURKA) in mammals and CALK, an AURKA-like kinase in *Chlamydomonas*, are each activated at the ciliary basal body immediately prior to ciliary (flagellar) resorption [13, 43]. Experiments involving measurement of ciliary resorption following treatment of mammalian cells with small molecule inhibitors or siRNAs to deplete AURKA, or microinjection of inactive or constitutively active mutants of AURKA, demonstrated that AURKA activity was necessary and sufficient to induce ciliary resorption, paralleling genetic experiments performed in *Chlamydomonas*. AURKA activity in resorption was reported to involve the phosphorylation and activation of HDAC6, leading to deacetylation and destabilization of the ciliary axoneme. AURKA expression and activity in ciliary resorption are based on interactions with NEDD9, a multifunctional scaffolding protein [44, 45]. AURKA was recently defined to function in some contexts as a Ca^{2+} -regulated kinase [46], although the relationship of this activation mechanism to ciliary resorption has not yet been addressed. More recently, Pitchfork (PIFO) was identified as expressed in the embryonic node, and found to accumulate at the basal body during the disassembly process. Haploinsufficiency of PIFO causes left-right asymmetry and other developmental defects associated with ciliary anomalies. Providing one mechanistic explanation for these defects, a naturally occurring pathogenic R80K mutation of PIFO was found to be unable to activate AURKA, and cells expressing this mutation failed to resorb cilia during cell cycle [47].

Balancing the action of these proteins that promote resorption, other proteins act as brakes on the disassembly process. The tumor suppressor encoded by the von Hippel-Lindau (*VHL*) locus interacts with glycogen synthase kinase beta (GSK β) to support ciliary maintenance [48]. Interestingly, mutation-induced loss of VHL induces the expression of the AURKA-NEDD9 complex [49], and destabilizes cilia. The lipid phosphatase INPP5E, mutated in MORM syndrome, also stabilizes cilia during the disassembly process. Pathogenic mutants of INPP5E both inhibit the ciliary localization of INPP5E, and influence ciliary stability during disassembly [50, 51]. There is growing evidence that genes

controlling the length of cilia thereby affect the timing of ciliary resorption, given a cell cycle cue. Proteins in this category include members of the NIMA kinase family [52, 53], and the centrosomal protein NDE1 [11].

3. Influence of ciliary dynamics on cell cycle progression

Whether the presence or absence of a primary cilium controls cell cycle entry, or instead passively reflects cell cycle progression, has long been a topic of discussion [54–57]. This issue is difficult to resolve, because many proteins shown to influence ciliary dynamics have complex functions that might independently explain concurrent changes in cell cycle.

Several studies suggest that the forced retention of cilia imposes a brake on cell cycle progression [11, 12, 58]. For example, the NDE1 phosphoprotein localizes to the mother centriole [11, 59]. The expression of NDE1 is high in M phase but low after cytokinesis, timing which coincides with the burst of primary cilium formation in G0/G1 phase. Following the depletion of NDE1 in hTERT-RPE1 cells, cells have longer cilia, and cell cycle re-entry is delayed. Importantly, abrogation of ciliogenesis by knocking down expression of IFT88 or IFT20 genes was sufficient to reverse this delay in cell cycle re-entry, suggesting the ciliary function of NDE1 was an essential cell cycle determinant [11]. On the other hand, NDE1 also functions cytoplasmically, with roles in mitosis, organellar positioning, and other processes; potentially, knockdown of the IFT genes counteracts a non-ciliary NDE1 function.

In a separate study, knockdown of *Tctex-1* expression blocked ciliary resorption and cell cycle progression in G1 upstream of the phosphorylation and inactivation of pRb [12]. This block was seen in the ciliated NIH3T3 or RPE1 cell lines, but not in non-ciliated HeLa cells, and not in RPE1 cell lines with IFT20 or IFT88 knocked down. The authors of this study further showed that knockdown of AURKA or HDAC6 not only blocked *Tctex-1*-associated ciliary resorption, but also blocked new DNA synthesis; as AURKA is not known to have any other essential functions in G1 phase, this suggested the role of AURKA in ciliary resorption was the critical limit on DNA synthesis. As with NDE1, these results may suggest cilia disassembly is a prerequisite for G1-S transition, or alternatively indicate a cytoplasmic action of *Tctex-1*. Certainly, a mechanistic explanation for how the presence of cilium would constraints activation of G1-S transition is not currently available. Potentially, cilia or the ciliary basal body have the capacity to sequester proteins or other factors that activate G1-S transition, and the resorption of cilia and differentiation of basal body to centrosome releases and/or activates these factors.

4. Indirect regulation of cell cycle progression through ciliary signaling: growth factor receptors and mechanosensation

Under normal conditions of organismal growth, the primary cilium serves as a unique platform for sensory functions in many organs, including the kidney, eye, nose, and brain. The signaling pathways mediated by cilia are summarized in Figure 3. Stimulation of cilia-localized receptors by diffusible cues, and mechanical stimulation of the cilia by fluid flow, activate a number of effector pathways that independently or cooperatively contribute to cell cycle control. Some of the better studied of these pathways include receptor tyrosine kinases (RTKs) such as PDGFR, cAMP/mTOR, polycystin/Ca²⁺, Hedgehog, Wnt, and Notch [60–65].

a. PDGF signaling

PDGF (platelet-derived growth factor) regulates cell growth and proliferation for many cell types [66]. In NIH3T3 cells and *in vitro* culture of mouse embryo fibroblasts (MEFs), serum

starvation concurrently induces primary cilium formation and expression of PDGFR α , the receptor for the PDGF $\alpha\alpha$ ligand isoform in this signaling pathway, predominantly within the nascent primary cilium. Ligand binding to PDGFR α activates downstream ERK signaling within the cilium and at the basal body [67], and triggers cells to re-enter cell cycle, as demonstrated by protein phosphorylation of the G1-S checkpoint protein retinoblastoma (Rb). The evidence for the importance of ciliary location for this receptor-ligand interaction is robust. In serum-starved mutant MEF cells derived from the Tg737 mouse, which has no or stumpy cilia due to deficiency in the intraflagellar transport protein *ift88*, PDGFR α is instead localized in the basal body region. In these cells, addition of ligand does not induce protein phosphorylation of MEK, and cells remain in growth arrest. Ongoing work in normal MEFs and the *ift88* model has shown PDGFR α also acts at the cilium to activate the Na⁺/H⁺ exchange protein NHE1 to control growth factor-induced chemotaxis [68, 69], implying an organization function for the cytoskeleton that may also support cell cycle signaling.

b. Polycystins, mechanosensation, and calcium signaling

In the kidney, the cilium serves as a flow sensor in the kidney tubules, with flow-induced ciliary bending causing a transient increase in intracellular calcium [70]. Polycystins (PC) 1 and 2, gene products of *PKD1* and *PKD2*, are large multi-pass transmembrane proteins of the TRPC family of calcium transporters [71]. PC1 and PC2 heterodimerize in the primary cilium to mediate flow-induced calcium flux, with the large extracellular domain of PC1 receiving mechanical cues, then triggering the activity of the PC2 calcium ion channel [72, 73]. Inhibition of PC1 and PC2 activity either by gene mutation (as discussed below) or experimentally through use of antibodies interrupts the calcium flux generated by fluid flow [74]. Because mutations of *PKD1* and *PKD2* induce cyst formation in the kidney, and cyst formation in part arises because of de-restricted cell proliferation, PC1 and PC2 at least indirectly participate in cell cycle control. Signaling downstream of PC1 and PC2 is complicated. In normal cells, these proteins negatively regulate the cAMP and Raf-MEK-ERK signaling pathways [75], both of which are hyperactivated in renal epithelial cysts [76–78].

Interestingly, it was recently shown that loss of cilia is sufficient to increase intracellular cAMP levels [78]. This was observed when cilia were lost due to null mutation of the IFT motor protein KIF3a, due to maintenance of cells in non-confluent growth conditions, or following other stimuli, specifically linking the phenotype to ciliary loss. The mechanism involved requires further investigation. However, adenylyl cyclases (AC) 5 and 6, the A-kinase anchoring protein AKAP150, and phosphodiesterase (PDE) 4C, a negative regulator of cAMP, are all localized in the cilium and form a complex [78], implying cilia-dependent, spatially localized regulation of cAMP levels. Supporting the relevance of this pathway to disease, the transcription factor HNF-1b regulates PDE4C transcription, and mutation of HNF-1b results in kidney cysts [79]. It is known that calcium regulates AC5/6 activities [80]. Further, the PC2 calcium channel directly interacts with AC5/6, and regulates AC activities [78]. In PC2 mutant mice, the cAMP level in kidney cysts increases, and phosphorylation of the cAMP responsive transcription factor CREB is detected. AC5/6 is not detected in renal cilia expressing a mutant, inactive form of PC2, indicating that AC5/6 requires PC2 for ciliary localization [78]. Assembling this information, the primary cilium constrains cAMP signaling by tethering AC5/6 and regulating PDE4C in the cilium, with AC5/6 activation suppressed by calcium flux mediated by PC2 under fluid flow. The loss or dysfunction of cilia may abrogate calcium signaling to allow activation of cAMP pathway, which promotes cell cycle progression.

Actions of PC1 and PC2 at the cilium do not only involve controlling activity of cAMP. For example, under some conditions, PC1 interacts with PC2 and JAK2 to positively regulate

the JAK-STAT pathway; this induces p21, which inhibits Cdk2, leading to cell cycle arrest [81]. PC2 interacts directly with AURKA, which governs ciliary resorption; the increase in cytoplasmic Ca^{2+} mediated by PC2 very transiently activates AURKA, while AURKA phosphorylation of PC2 limits its Ca^{2+} channel activity [46, 82]. PC2 may also prevent cell proliferation by physical interaction with the helix-loop-helix protein ID2, an inhibitor of cell differentiation, and blocking its translocation into the nucleus [83]. Interestingly, NEDD9, which supports the action of AURKA in ciliary resorption, has separately been shown to interact with ID2 [84]. In other work, PC1 was found to be cleaved in response to changes in mechanical stimuli, generating a C-terminal fragment that is translocated into the nucleus where it activates AP-1 signaling [85], but also inhibit Wnt/TCF signaling [86]. Apart from their well-described functions at the primary cilium, PC1 and PC2 also localize to the plasma membrane, with a large population of PC2 also found in the endoplasmic reticulum (reviewed in detail in [63]). Although it is clear that localization of a functional PC1/PC2 heterodimer to the cilium is critical for preventing cystogenesis, it is possible that roles in other compartments are also important. Targeted experiments blocking traffic of PC1 or PC2 to the primary cilium may provide further insight [87, 88].

c. LKb1-AMPK-mTOR pathway

Although the mTOR (mammalian target of rapamycin) pathway is well recognized for its role in metabolism, cell cycle and size control, and cell polarity, it has only recently been recognized that mTOR signaling may have an important connection to cilia. A screen for yeast mutants that escaped the cell cycle arrest induced by rapamycin, an inhibitor of the yeast ortholog of mTOR [89, 90], led to the initial identification of the yeast TOR gene [91, 92] mTOR forms two distinct complexes: the rapamycin-sensitive mTORC1 complex and the rapamycin-insensitive mTORC2 complex. mTORC1 predominantly regulates metabolism and cell growth while mTORC2 regulates cell polarity [89, 90]. Increased intracellular AMP levels activates AMPK to phosphorylates Raptor, which binds and inactivates mTORC1 signaling [93]. AMPK can also indirectly suppress the mTORC1 pathway by phosphorylating the Tuberous Sclerosis Complex (TSC) proteins TSC1 and TSC2, which also negatively regulate mTORC1 [94, 95].

The link of the mTOR pathway to cilia, and the importance of this connection in ciliopathies, has been suggested by several studies. Mutation of the *TSC2* gene induces a cystic kidney disease, among other symptoms [96]. Hyperactivation of mTORC1 signaling is observed in PKD-associated cysts, and treatment with rapamycin reduces cyst formation [97]. In a recent study, it was shown that the tumor suppressor LKB1 (also called STK11) kinase phosphorylates AMPK and negatively regulates the mTOR pathway [89]. Intriguingly, a genome wide RNAi screen identified loss of LKB1 as stimulating ciliary disassembly [98]. Although a specific connection to mTOR or cilia has not yet been explored, it is suggestive that in lung cancers associated with loss of LKB1, the AURKA activator NEDD9 is highly upregulated [99]. LKB1-deficient MEFs double more rapidly than control MEFs [98, 100], while activation [101] or overexpression [102] of LKB1 induces cell cycle arrest at G1.

The above data demonstrates the role of LKB1 in cell cycle arrest and that LKB1 affects cilia maintenance. Reciprocally, several studies suggest that changes in cilia affect LKB1 functionality. Boehlke et al. have shown the LKB1-AMPK-mTOR pathway is regulated by cilia [64]. First, LKB1 is localized in the primary cilium. Second, under fluid flow conditions, LKB1 activates AMPK at the basal body to suppress the mTOR pathway and down-regulate cell size. Since fluid flow induces calcium signaling through PC1 and PC2, but a PC2 mutant does not abrogate activity of the mTORC1 pathway [64], this suggests that calcium signaling is not involved in LKB1 activation. How mechanical sensation by the primary cilium triggered by fluid flow activates LKB1 remains unclear. Collectively, the

above data shows that LKB1-AMPK-mTOR pathway is mediated by cilium and regulates both cell size and cell cycle arrest; more study of these signaling relations are merited.

d. Hedgehog and Notch

Cilia control additional pathways with important roles in regulation of cell cycle, cell differentiation, and organogenesis. The obligate existence of cilia for cellular response to Hedgehog (Hh) family proteins was first established by Huangfu and Anderson in 2005, following their observation that mice with mutations in the IFT proteins IFT172 or IFT88 lacked cilia and failed to respond to Hh [103]. Hh is a soluble ligand for the transmembrane receptor protein Patched (Ptc), which in its inactive state localizes to the cilium. Upon Hh binding to Ptc, Ptc moves out of the cilium and ceases to repress a third protein, Smoothened (Smo), which now enters the cilia. Ciliary Smo is then able to activate the Gli transcription factors, allowing their translocation to the nucleus [104]. The Gli transcription factors activate a suite of genes, including cyclins D and E, that contribute to cell cycle transition from G1 to S, and support cell survival [105]. Upregulation of Hh/Gli signaling is observed in a number of forms of cancer, and may be linked in part to the typical loss of cilia in transformed cells. This upregulation, together with the observation that Hh/Gli signaling collaborates with RTK signaling to promote the malignant state, have caused some to propose combination therapies targeting Hh and EGFR [106]. The majority of studies of Hh signaling at cilia have focused on clear developmental roles for these proteins at the neural tube and in the central nervous system (reviewed in [107, 108]), with the potential role of this pathway in cystic kidney disorders not well addressed. Interestingly, a recent RNA interference study identified LKB1 as essential both for Hedgehog and for Wnt signaling (discussed in section 5), emphasizing the coordinate regulation of these growth regulatory pathways at intact cilia [98].

A role for the developmental regulator Notch in supporting the differentiation of multiciliated cells was initially established in studies of the development of *Xenopus* skin and the zebrafish pronephros [109–111], and subsequently shown to be important for the development of the motile multiciliated cells in the mammalian lung [112, 113]. In one recent study, microRNA (miR)-dependent inhibition of Notch was necessary for the emergence of multi-ciliated cells [114]. Notch receptors and processing enzymes localize to the cilia during epidermal differentiation. Cells with mutated IFT genes that lacked cilia were deficient in Notch signaling, failed to differentiate, and were hyperproliferative, with these latter defects rescued by re-expression of pre-activated Notch [62]. Notch represses Multicilin, a recently identified nuclear coiled-coil protein that appears to be a key inducer of a group of genes essential for the formation of multiciliated airway epithelial cells [115]. While Notch is emerging as an important ciliary regulator of the cell proliferation versus differentiation in cell types relevant to ciliopathies, particularly kidney [116], studies of the Notch-cilia connection are at an early stage, with mechanistic details still to emerge. It is important also to note that the Notch studies cited above are predominantly performed in multiciliated cells of the lung epithelium, rather than in cells with non-motile primary cilia: the signaling in these two cell types is not necessarily equivalent.

5. Oriented cell division and planar cell polarity

During cytokinesis, the cleavage furrow forms in perpendicular to the orientation of the spindle, determining the outcome of the spatial arrangement of two daughter cells. In multicellular organisms, appropriate orientation of cell division is an essential determinant in the morphogenetic process leading to the final architecture for many organized tissues. The kidney, an organ often affected in ciliopathies, is composed of a repeated unit in which a glomerulus (the site of blood filtration) is connected by an extended tubular structure (the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule) to the collecting

duct. During tubular development, the proliferation of tubular cells must be precisely controlled to allow tubular lengthening while maintaining a constant tube diameter. A number of studies have suggested the idea that the enforced orientation of cell division parallel to the tubular axis supports normal morphogenesis, while misoriented cell division may cause enlargement rather than lengthening of the tubule [31]. Examination of kidney tubular development in PKD models in the mouse reveals that the orientation of mitotic spindles of tubular cells is parallel to the tubular axis in normal tissues, but significantly distorted in the context of mutations of *Tcf2*, associated with PKD. Such mis-oriented mitoses appear to occur at an early step in disease pathology, preceding tubular dilation and cyst formation [117].

Defects involving the cilium can influence the orientation of the cell division plane in several ways. The first is based on perturbation of the cilia-centrosome cycle. The resorption of the primary cilium prior to mitosis seems to be essential to release the centriole for maturation into a centrosome capable of nucleating the bipolar mitotic spindle [33]. Normally, the mother centriole that forms the basal body is constrained to the apical cell surface based on interactions of the ciliary distal appendages with the membrane, as well as polarization of secretory apparatus in support of the ciliary membranes [27]. These mechanical constraints are reduced during the ordered disassembly of the primary cilia as cells approach mitosis; nevertheless, some cues governing the localization of the centrosomes are maintained. The loss or structural defects in primary cilia arising from many cyst-associated mutations may result in uncontrolled centrosome positioning, thus increasing randomness in orientation of cell division.

One important recent study has shown that the intraflagellar transport protein IFT88 directly controls spindle orientation through regulation of astral microtubule formation [21]. Although IFT88 was long known to localize to cilia and basal bodies, it has now been recognized as a constituent of spindle poles [21, 22]. Cells with mutated or RNAi depleted IFT88 are characterized by spindle misorientation, and spindle poles lacking the astral microtubule arrays that interact with the cell cortex to orient the spindle [118, 119]. The formation of astral microtubule arrays requires the movement of peripheral microtubule clusters, dependent upon formation of a large protein complex containing cytoplasmic dynein1 and IFT52. This complex is lost in the absence of IFT88. Thus, IFT88 mediates orientation of cell division by controlling formation of astral microtubules to ensure proper spindle orientation (Fig. 4).

In contrast, another recent study has addressed function of the IFT protein IFT140 [120]. This work found that although mutation of IFT140 causes defects in cilia formation and formation of renal cysts, in this case pre-cystic dilated tubules have normal orientation of cell division. IFT140 has been much less studied than IFT88; how and if it interacts with the mitotic spindle is not well understood. However, in contrast to IFT88, which functions as a component of IFT complex B, which controls anterograde transport, IFT140 is a component of the IFT complex A, which controls retrograde signaling [121, 122]. Other studies comparing defects linked to mutation or depletion of IFT complex members have indicated weaker phenotypes associated with the targeting of complex A than with the loss of complex B. For example, Tsujikawa and Malicki found degeneration of sensory cells following targeting of the IFT complex B proteins IFT88, IFT52 or IFT57, but not after targeting IFT140 [123]. Earlier work studying *C. elegans* formation of amphid cilia following mutations of orthologs of IFT88 (*osm-5*), IFT52 (*osm-6*), IFT57 (*che-13*) versus IFT140 (*che-11*) or IFT122 (*daf-10*) also indicated greater ciliary defects among the complex B mutants [124]. In this context, the difference between the phenotypes associated with IFT88 and IFT140 mutation for cystogenesis either implies less severe consequences of IFT140, or

alternatively, suggests implies that defects in cell division orientation are sufficient, but not necessary, for cyst formation.

Tissue architecture is also influenced by planar cell polarity (PCP) controls, which provide an additional means of regulating cell distribution relative to an axis within a plane of growing cells. One mechanism by which this regulation is accomplished is by influencing the migration of cells during development, such that controlled, oriented interdigitation of cells leads to elongation of a tissue along a single axis. This process, termed convergent extension, is important in the formation of multiple tissue types, and was only recently documented as important in kidney morphogenesis, with defects in this process suggested as a cause of cystogenesis [125–129]. A growing body of evidence suggests that cilia and some cilia-associated proteins are intimately involved in control of PCP, while some PCP regulatory proteins reciprocally influence ciliogenesis and ciliary orientation. This topic has recently been comprehensively reviewed by Wallingford and Mitchell [130].

Canonical [131] and non-canonical [132] signaling downstream of the soluble factor Wnt has an essential role in conditioning many aspects of cell growth and differentiation. PCP signaling involves the activity of the non-canonical Wnt/Frizzled (Fz)/Disheveled (Dvl1) signaling pathway [133, 134]. Inversin, also known as NPHP2, localizes to the cilia and basal body, and is mutated in the cystic kidney disease nephronophthisis. Simons et al first showed a direct interaction between inversin and Dvl1 promotes non-canonical Wnt signaling, with defects in this pathway proposed to support the abnormal cell growth that characterizes cyst formation [125]. In contrast, cilia downregulate the amplitude of canonical Wnt signaling, in part through the action of the ciliopathy-associated protein Jouberin in blocking nuclear entry of the canonical Wnt effector β -catenin [135]. BBS-associated proteins are associated with control of cilia; some BBS proteins interact directly with and regulate the function of the PCP signaling effector Vangl2 [136]. Conversely, PCP signaling proteins can influence ciliary dynamics. For example, knockdown of the PCP effector Fritz in *Xenopus* embryos brings about defects in PCP mediated convergent extension as well as defects in ciliogenesis [137]. However, as discussed in depth in [130], the interactions between PCP signaling proteins and cilia are complicated, and in some cases do not support crosstalk as a direct cause of cystogenesis.

Alternatively, defects in cilia may affect PCP downstream of non-canonical Wnt signaling, at the level of control of the polarization of the cytoskeleton to support directional movement. The centrosome has many functions that impinge upon cell polarization in migration (reviewed in [138]). A number of proteins that affect ciliary formation and function have also been shown to affect placement of the centrosome. Besides regulating Dvl1, loss of Inversin also induces randomly oriented cell division prior to the detection of increased rates of proliferation in nascent kidney cysts [139]. Analysis of precystic tubules in *Kif3a* mutant mice reveals that cells lacking primary cilia have an abnormality in PCP that was suggested to initiate cyst formation [140]. IFT20 [141] and IFT88 [142] have both been shown to directly impact cell polarization in some tissues. MKS1 and meckelin are essential for ciliogenesis, but also influence centrosomal migration based on coordinated interactions with the actin cytoskeleton [143]. Given the various roles of basal body/centrosomal structures in polarizing the mitotic plane, organizing polarized cell migration, and providing a platform to integrate the function of proteins that signal to indirectly influence these processes, it is hard to prioritize which if any functions are dominant.

We note that in sum, the polarity controls discussed in this section would seem to address the qualitative features of cell organization rather than control of cell proliferation. However, these qualitative changes result in dilated tubules in which cells are exposed to different fluid pressures, and have altered ability to respond to chemotactic cues because of

defects in orientation of the internal cytoskeleton to the cilia, or in orientation of the cilia to the direction of fluid flow. Hence, they ultimately have the potential to support the abnormal proliferation that occurs in cystic growth.

6. The contribution of ciliary defects to human renal diseases

Given the dense web of connections between cilia and control of cell growth described above, it is not surprising that defects in cilia have been implicated in multiple clinically significant pathologies, involving developmental defects and later onset syndromes. Established ciliopathies include Bardet-Biedl Syndrome (BBS), Joubert syndrome (JBTS), Meckel-Gruber syndrome (MKS), polycystic kidney disease (PKD), and nephronophthisis (NPHP) [32]. Many of these rare autosomal recessive diseases present with phenotypes affecting multiple organs, with symptoms that include renal cysts, neural tube defects, retinal degeneration, obesity, and situs inversus. A recent proteomic analysis of proteins co-purifying with 9 proteins known to be mutated in NPHP, JBTS, or MKS syndrome identified 850 interactive partners, with dense physical connections to proteins functioning in cell polarity (apical positioning) and centrosome control, and Hedgehog signaling [144]. A targeted search for mutations affecting these genes in a first set of 250 patients with symptoms associated with NPHP, JBTS, or MKS identified several novel causative lesions [144]; it is likely that more will be identified in the future. We here take cystic kidney diseases as an example of how ciliary defects translate to pathological response, and discuss how current approaches to treatment align with what is known about ciliary biology.

a. PKD and NPHP

Cystic kidney diseases represent a significant group of genetically inherited renal disorders [145]. A kidney cyst is a sac-like structure comprised of a liquid-filled monolayer of cells. Formation of a cyst represents the integrated endpoint of multiple stimuli leading to abnormal cell division among the epithelial cells lining the collecting ducts and convoluted tubules of the kidney. The development of cysts eventually leads to end-stage renal disease (ESRD) [146], at which point patients afflicted with these conditions must either undergo dialysis or receive kidney transplants. The majority of cystic kidney diseases arise from inheritance of mutations associated with autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD), or NPHP, with more sporadic linkage to BBS [145, 147].

Although the genetic deficiencies leading to formation of kidney cysts are diverse, most mutated genes have a close connection to control of ciliary structure and function [29, 145, 148, 149], and localization to the cilia or basal body [29, 31, 145, 150–153], although many also function at other cellular locations (e.g. PC1 at cell junctions, [154]). Mutations in the *PKD1* or *PKD2* genes cause ADPKD, which is the most common cystic kidney disorder, affecting approximately 1:500. The *PKD1* and *PKD2*-encoded protein products (polycystins 1 and 2) heterodimerize on cilia to integrate growth-limiting signals initiating from fluid pressure; defects in cilia impair this function [74]. Reciprocally, mutations in these genes have recently been found to cause defects in formation of cilia and/or centrosomes [155–157]. ARPKD and NPHP are much less common than ADPKD, with incidence estimated at around 1:5,000 for ARPKD and less than 1:50,000 for NPHP. ARPKD arises from mutations in the *PKHD1* gene, which encodes the ciliary protein fibrocystin [158]. Mutations in at least 11 independent genes of the *NPHP* group have been found to lead to NPHP. Many of these proteins have already been shown to localize to cilia or centrioles, and contribute to signaling at these structures [159].

b. von Hippel-Lindau (VHL) disease

VHL disease, caused by germline mutation in the *VHL* tumor suppressor gene, is an autosomal dominant genetic disease that has in the past been thought of predominantly in terms of hereditary cancer. The most clinically significant manifestation of the disease includes tumors in the central nervous system, retina and kidney, including hemangioblastomas, pheochromocytomas, and renal cell carcinomas [160]. Interestingly, around 70% of *VHL* patients also develop renal cysts [161, 162], a fact that has more recently led to targeted investigations of whether *VHL* might have a specific function at cilia, as with other cyst-associated proteins. Indeed, pVHL localizes to cilia [48, 163]. Further, both the kidney cysts found in *VHL* patients as well as renal clear cell carcinoma cell lines lacking pVHL have either no cilia or sparse, rudimentary cilia [164]. Importantly, ectopic expression of *VHL* gene in renal clear cell carcinoma cell lines restored cilia formation [163–165], implying pVHL might directly support ciliogenesis.

In one elegant study, combined inactivation of *VHL* and *GSK3b* was required to allow loss of cilia, based on cooperative function of these proteins in ciliary maintenance [48]. Although the mechanism by which these proteins interact is not yet clear, *VHL* normally promotes the proteasomal degradation of HIF-1 α , a protein that activates transcription in hypoxic cells. Maxwell and colleagues showed that suppression of HIF-1 α in *VHL*-deficient cell lines restored cilia [164]; separately, GSK3 β triggers HIF-1 α degradation [166]. The absence of cilia mediated by pVHL may be due to loss of cilia maintenance or defects in ciliogenesis. Benzing and colleagues found that pVHL regulates microtubule orientation during ciliogenesis and also interacts with the Par3-Par6-atypical PKC complex, which supports ciliogenesis [163, 167]. pVHL also associates with kinesin 2, allowing pVHL to influence microtubule dynamics in support of cilia [168, 169]. Some evidence suggests that pVHL also negatively regulates ciliary disassembly. AURKA and its homologue CALK in *Chlamydomonas* are necessary and sufficient for cilia disassembly [13, 43]. Loss of *VHL* activates HIF signaling to increase the expression of AURKA and its activating partner NEDD9 [170]; suppression of this pathway improves formation of cilia [49].

c. Cilia and cysts

Cementing the requirement for intact ciliary signaling in cystic disease, cyst formation can be experimentally induced by mutation of genes that are essential for cilia assembly and maintenance such as IFT88 [22] and the cilia-associated kinesin II, KIF3A [171]. Subsequent investigation of the consequences of the inactivation of KIF3A in young versus adult mice emphasized the requirement of kidney injury as a co-factor, and suggested an important consequence of ciliary inactivation was loss of PCP controls rather than a change in proliferation [140]. As noted above, one consequence of mutations relevant to clinically significant human cystic diseases is to impair ciliary function, and by extension to influence all of the cilia-associated, cell division regulating processes summarized throughout this review. In spite of the complexity this already invokes, it is important to note that proteins such as the polycystins, targeted in PKD, also have well-documented functions in cellular compartments other than cilia, acting at the endoplasmic reticulum, the plasma membrane, and at focal adhesions [63, 172]. The inevitably pleiotropic consequences of mutations eliminating polycystins makes it essential to move cautiously in assigning proliferation defects solely to ciliary deficiency, when considering the clinical management of "ciliary" diseases. Certainly, much more careful study is required to dissect relevant functions.

7. Targeted treatments

With the above cautions in mind, it is instructive to consider how the rapidly increasing knowledge of the root causes of diseases such as renal cysts impacts their management in

the clinic, taking PKD as primary example. For additional perspective, recent reviews addressing this topic at length include [173–175]. For many years, the standard of care has been primarily management of the symptoms associated with the disease, rather than addressing the root causes. As of 2012, the patient-directed description of treatment options assembled by the National Kidney and Urologic Disease Information Clearinghouse (NKUDIC), managed by the US National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) suggested to patients that they manage pain associated with growing cysts by using aspirin or acetaminophen; control associated urinary tract infections with antibiotics; control high blood pressure by lifestyle changes or standard medications; and prepare for ultimate ESRD, which will require dialysis or kidney transplant (<http://kidney.niddk.nih.gov/kudiseases/pubs/polycystic/#dominant>).

In contrast, Table 1 summarizes clinical and preclinical evaluation that have either recently been completed or are currently in progress; Table 2 summarizes rodent models used for preclinical work. Beginning with the more advanced clinical studies, a first theme has been to continue to address the symptoms, but with a more advanced underpinning of molecular biology. For example, the HALT-PKD study evaluates efficacy of the angiotensin converting enzyme (ACE) inhibitor lisinopril alone or in conjunction with the angiotensin II receptor antagonist Telmisartan, to more efficiently lower blood pressure (described in [176]).

Other studies have addressed signaling effects classically associated with PKD. Deficient activity of the PC2 Ca^{2+} channel at the cilia reduces intracellular Ca^{2+} levels in PKD. Reduced Ca^{2+} -dependent inhibition of adenylate cyclase (AC) helps elevate intracellular levels of cAMP; also see direct activation of AC through interaction with the AKAP150 complex and phosphodiesterase, discussed in [78] and above. In turn, elevation of cAMP contributes to activation of PKA signaling, which supports SRC and other RTK-dependent signaling effectors, and stimulates activity of the Cl^- efflux channel CFTR, among other consequences [177]. A number of studies experimentally limit AC activity by targeting proteins associated with the AC-activating complex, using therapies such as somatostatin [178, 179] and vasopressin V2R antagonists [180–183]. For the trials with somatostatin, a significant decrease in the rate of kidney growth was observed, but only limited efficacy in improving kidney function. PKA effector limitation is also the theme of more recent pre-clinical studies, in which the point of intervention is inhibition of CFTR [184]. Triptolide, the active ingredient of an ancient Chinese medicine known as Lei Gong Teng, enhances PC2 channel activity, potentially restoring normal homeostasis in cases of PKD not directly associated with inactivating mutations of PC2 [185]; a clinical trial of triptolide is in progress.

An alternative approach in designing trials has been to target signaling pathways canonically associated with the proliferative state of the cell, which are active in cystogenesis, and for which degree of connection to cilia has been discussed above. Bosutinib inhibits multiple tyrosine kinases, including SRC [186]; a trial is ongoing. Sirolimus and everolimus inhibit mTOR, which is essential for protein synthesis, and known to be regulated by PC1 and TSC, which have cilia-linked functions, making it natural to evaluate these compounds in PKD [187–189]. Disappointingly, although some activity was seen in reducing kidney growth, there was no significant change in kidney function; one study found an increased level of proteinuria and albuminuria in sirolimus-treated patients, and increased adverse events were reported with treatment. It has been suggested that a longer time of treatment would be beneficial; alternatively, this may increase the side-effects of mTOR inhibitors in non-renal cells. mTOR-directed therapies are discussed at length in [190] and [191]. For all of the advanced or completed trials, an emergent theme is the non-congruence between results in reducing the rate of kidney or cystic growth, which can often be partially achieved, and

results in improving renal function, which is clearly harder to achieve. Identifying the reasons for these differences requires more study.

As is clear from Table 1, a number of other pathway-directed therapies are now being pre-clinically evaluated for action in PKD, and may lead to trials in coming years. These generally follow the same approach as more advanced studies. Targets of therapies include metformin, targeting AMPK [192]; roscovitine, targeting CDK kinases [193]; STAT6 [194]; the PPAR- γ receptor [195–197]; HDACs [198, 199]; MEK [200]; Raf [201]; Cdc25A [202]; and other agents. A number of these agents were previously developed for other disease indications, such as treatment of cancer, and are being explored based on their ability to inhibit cell proliferation. Given the discussion above as to whether it is proliferation per se, or qualitatively altered cell growth associated with ciliary defects (e.g., PCP changes), that is the predominant cause of PKD, it is currently an open question as to how well these compounds will work.

Another pragmatic issue is how patients will respond to long-term treatment with powerful agents that interfere with cell growth controls. Much care will be necessary to prevent compensatory changes that lead to severe adverse events, such as induction of cancer. Use of therapies targeted at proteins that specifically act in the kidney would reduce this last concern; as proteomic studies of cilia-associated proteins [144, 203–205], and tissue specific protein expression and activation [206] advance, these data may help guide target selection. On the other hand, because PKD arises from an autosomally inherited dominant mutation, the mutant allele for PKD is found in every cell of the body, and some recent studies have documented striking cell cycle defects in non-renal cells associated with PKD lesions. For instance, endothelial cells from humans and mice with PKD were characterized by abnormal cilia and deficient cytokinesis [174, 207]. Based on these recent findings, it is plausible that the hypertension associated with PKD may not just be a secondary consequence of aberrant cell growth and signaling in the kidney, but may arise directly from cilia-associated defects in the vasculature. If so, a kidney-focused therapeutic strategy would be inadequate. A potentially promising approach would be to try to correct the deficient function of PC2 specifically at the cilium. For instance, the observed AURKA activation in cysts is predicted to help suppress PC2 calcium channel activity [82]; inhibition of AURKA might be expected to enhance PC2 activity. Alternatively, as discussed at length above, cilia coordinate signaling of PDGF α , Wnt, Hedgehog, Notch, and other pathways; to date, there has been little effort to explore the consequences of manipulating these pathways for therapeutic gain in PKD. There is clearly much room for investigation of potential strategies.

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Fig. 1.

Primary cilia disassembly prior to mitosis. The primary cilium present in G0/G1 phase starts to disassemble at G1-S transition, and is completely resorbed at early prophase. In some descriptions of quiescent cultured cells stimulated to reenter cell cycle by administration of serum, the primary cilium reassembles after disassembly at G1-S and is present during DNA and centriole duplication at S phase, and centriole maturation at G2 phase. Other reports describe two waves of deciliation, with some cells losing cilia prior to G1/S transition, and the remainder losing cilia at the end of G2 phase.

Fig. 2. Cilia disassembly at the G1-S transition. Activation of aurora A, NIMA kinases and phosphorylation of Tectex-1 induces cilia resorption, whereas activity of Nde1 and INPPE5 blocks this process. The disassembly of cilia triggers phosphorylation and inactivation of pRb leading to cell cycle progression. Aurora A can be activated by PIFO and by inactivation of VHL to regulate cilia disassembly.

Fig. 3.

Ciliary signaling pathways implicated in control of cell proliferation. Mechanical sensation of cilia induced by fluid flow activates the LKb1-AMPK pathway in a calcium independent manner and inhibits mTOR1 pathway. Mechanical flow induces activation of PC1 and PC2 and results in elevation of internal calcium level, which suppresses cAMP signaling. In addition, calcium is required for PC1-CT targeting into the nucleus. PC1 also activates JAK2, which induces gene expression that promotes cell cycle progression. Ligand binding of PDGFR α in the cilia activates RAF-ERK pathway. Activation of cAMP signaling promotes PKA and AKAP activity, triggering cell cycle progression whereas calcium inhibits this pathway. In addition, aberrant activation of wnt or hedgehog pathway stimulates cell cycle progression.

Fig. 4. Oriented cell division regulated by cilia. Ciliary protein IFT88 (red ball, semi-transparent) is localized at the basal body of the cilium and at the centriole during spindle formation. Formation of astral microtubules (green lines) requires IFT88, which is essential for proper spindle orientation. Dysfunction of IFT88 results in malformation of astral microtubules leading to misoriented cell division.

Treatment strategies in ADPKD: Clinical trials and *in vivo* studies.

Table 1

Clinical trials: mTOR inhibitor	References; ClinicalTrials.gov numbers	Eligibility	Outcome	Duration (month)	Number of patients (drop out rate)
Everolimus (mTOR inhibitor)	[188] NCT00414440	eGFR 30–89 ml/min; age 18–65 years	Kidney volume increased less in everolimus group (significant after first year but not after second year); no significant difference in kidney function	24	433 randomized, 329 completed (vehicle: 14.7%; Everolimus: 32.7%)
Sirolimus (mTOR inhibitor)	[187] NCT00346918	eGFR at least 70ml/min; age 18–40 years	No significance in TKV and eGFR; urinary albumin-to-creatinine ratio higher in sirolimus group	18	100 randomized 96 completed (vehicle: 2 Sirolimus: 2)
Sirolimus (mTOR inhibitor) SIRENA Study	[189] NCT00491517	eGFR at least 40 ml/min; age 18–80 years	TKV and cyst volume increased less; parenchymal volume increased more; no significant difference in kidney function; albuminuria and proteinuria significantly increased with sirolimus	6	21 randomized 15 completed
Preclinical studies: mTOR inhibitor	References	<i>In vivo</i> model	<i>In vivo</i> results	Treatment schedule; administration of the drug	
Rapamycin (mTOR-Inhibitor)	[97]	Orpk-rescue mouse (Tg737orp/orp;TgRsq)	Decrease in cystic index and kidney volume (MRI: day P150, P164, P178); apoptosis increased in cyst lining epithelium	P150 to P178; daily intraperitoneal injection	
	[208]	Bpk mouse	Decrease in cystic index, kidney weight as % of BW, and blood urea nitrogen	P7 to P21; daily intraperitoneal injection	
		Pkd1 ^{cond/cond} ;NestinCre	Decrease in cystic index, kidney weight as % of BW, blood urea nitrogen, renal fibrosis, proliferation and apoptosis	P28 to P49; daily intraperitoneal injection	
Sirolimus (mTOR-Inhibitor)	[209]	Pkd2 ^{NS25/-} mouse	Decrease in kidney weight as % of BW and cystic volume density, fibrosis (no significant difference in blood urea nitrogen)	4 to 16 weeks of age; daily intraperitoneal injection	
Clinical trials: Somatostatin analogue	References; ClinicalTrials.gov numbers	Eligibility	Outcome	Duration (month)	Number of patients (drop out rate)
Octreotide (long-acting somatostatin analogue)	[178] NCT00426153	No criteria for eGFR; Polycystic Liver Disease associated with ADPKD or isolated Autosomal Dominant Polycystic Liver Disease; age 18–80 years	Significantly less increase of total kidney and liver volume; no significant difference in GFR	12	42 randomized 42 completed
Lanreotide (long-acting somatostatin analogue)	[179] NCT00565097	No patients requiring hemodialysis because of renal failure; patients with ADPKD and patients with Polycystic Liver Disease	Reduction of total kidney and liver volume; no significant difference in serum creatinine	6	54 randomized 53 completed (vehicle: 1)
Octreotide (long-acting somatostatin analogue)	NCT00309283 Estimated completion: Dec 2011	eGFR over 40 ml/min; age 18 to 75 years	Parameters measured: TKV; kidney parenchymal volume; kidney cyst/intermediate volume	36	78 (estimated)
Octreotide (long-acting somatostatin analogue)	NCT01377246 Estimated completion: Dec 2015	eGFR 15 to 40 ml/min; age 18 to 75 years	Parameters measured: TKV; GFR; kidney parenchymal, cyst and intermediate volume; liver and liver cyst volume	36	80 (estimated)

Clinical trial: HMG-CoA reductase inhibitor	References; ClinicalTrials.gov numbers	Eligibility	Outcome	Duration (month)	Number of patients (drop out rate)
Parvastatin (HMG-CoA reductase inhibitor) in Lisinopril (ACE inhibitor) treated patients	[210] NCT00456365 Estimated completion: Apr 2011	Normal kidney function; age 8–21 years	Parameters measured: TKV, left ventricular mass index, urinary albumin excretion	36	100
Clinical trial: ACE-inhibitor/angiotensin II receptor antagonist	References; ClinicalTrials.gov numbers	Eligibility	Outcome	Duration (month)	Number of patients (drop out rate)
Lisinopril (ACE inhibitor) monotherapy vs Telmisartan (angiotensin II receptor antagonist)/Lisinopril combination therapy with low or standard blood pressure control HALT-PKD studies	NCT00283686 Estimated completion: April 2013	Study A: eGFR over 60 ml/min; Study B: eGFR 25–60 ml/min; Blood pressure over 130/80 or receiving treatment for hypertension; age 15–64 years	Study A: Change in TKV; Study B: Time to the 50% reduction of baseline eGFR, end-stage renal disease, or death	48	1,018
Clinical trials: Vasopressin V2R antagonists	References; ClinicalTrials.gov numbers	Eligibility	Outcome	Duration (month)	Number of patients (drop out rate)
Tolvaptan (vasopressin V2R antagonist) TEMPO 2/4 Trial	[211, 212] NCT00413777	eGFR at least 30 mL/min; no renal-replacement therapy; prior participation in designated tolvaptan ADPKD studies; age over 18 years	Assessment of long-term safety (48 months): all experienced adverse events; six of 12 withdrawals account for adverse events; Assessment of disease progression (36 month); less increase in TKV; no significantly reduced increase in eGFR; increasing TKV correlated with decreasing eGFR	36 to 48	63 (12)
Tolvaptan (vasopressin V2R antagonist) TEMPO 3/4 Trial	[211, 212] NCT00428948 Estimated completion: May 2012	eGFR at least 60 ml/min; TKV at least 750 cm ³ ; prior exposure to tolvaptan or other experimental PKD therapies; age 18 to 50 years	Parameters measured: TKV change; time to onset of multiple ADPKD outcomes (e.g. worsening of renal function, hypertension, albuminuria); evaluation of long-term safety	36	1,445
Tolvaptan (vasopressin V2R antagonist) TEMPO 4/4 Trial	[211, 212] NCT01214421 Estimated completion: Aug 2014	Successful completion of a phase 1, 2, or 3 tolvaptan ADPKD or renal impairment trial; age 18 years or older	Parameters measured: TKV; eGFR; evaluation of safety endpoints and multiple ADPKD outcomes	24	1,500
Preclinical studies: Vasopressin V2R antagonists	References	<i>In vivo</i> model	<i>In vivo</i> results	Treatment schedule; administration of the drug	
OPC31260 (vasopressin V2 receptor antagonist)	[180]	PCK rat	Decrease in kidney weight as % of BW, cyst volume as % of BW, blood urea nitrogen, fibrosis volume as % of BW, apoptosis index (%)	3 to 10 weeks of age; 10 to 18 weeks of age; added to chow	4 to 30 weeks of age; 15 to 30 weeks of age; added to chow
		Pcy mouse	mitotic index (%), and systemic blood pressure (PCK rat only)		
		Pkd2 ^{-tm1Som} mouse	Decrease in kidney weight as % of BW, cyst volume as % of BW, and blood urea nitrogen; no significant decrease in systemic blood pressure	3–16 weeks of age; added to chow	
Genetic arginine vasopressin (AVP) depletion (Brattleboro rats)	[182]	PCK; AVP+/+ PCK; AVP+/- PCK; AVP-/-	Decrease in kidney weight as % of BW, cyst volume (%), fibrosis volume (%), urine volume, and blood urea nitrogen	12–20 weeks of age; +/- V2 receptor agonist (1-deamino-8-D -arginine vasopressin); continuous subcutaneous administration	
Clinical trials: Triptolide	References; ClinicalTrials.gov numbers	Eligibility	Outcome	Duration (month)	Number of patients (drop out rate)
Triptolide (active diterpene in the traditional Chinese medicine <i>Tripterygium wilfordii</i>)	NCT00801268 Estimated completion: Sep 2012	eGFR over 30 ml/min; age 15 to 70 years	Parameters measured: TKV; eGFR; end-stage renal disease	36	150
Preclinical studies: Triptolide	References	<i>In vivo</i> model	<i>In vivo</i> results	Treatment schedule; administration of the drug	

Triptolide (<i>Tripterygium wilfordii</i>)	[185]		Pkd1 ^{-/-} mouse		Decrease in % cystic burden	E10.5 until birth; daily intraperitoneal injection of pregnant mice	
	[213]		Pkd1 ^{fllox/-} ;Ksp-Cre mouse		Decrease in kidney weight as % of BW, cystic burden, cyst number and size, proliferation	P1 – P4 : daily intraperitoneal injection of lactating mother	
					Decrease in kidney weight as % of BW and blood urea nitrogen	P1 – P8; daily intraperitoneal injection of lactating mother (P1–P5) and P6 to P8 intraperitoneal injections in pups	
	[214]		Pkd1 ^{fllox/fllox} ;Mx1Cre mouse; (cyst induction P10/P12)		Decrease in number of renal cysts, cystic burden, proliferation, and blood urea nitrogen (P22 and P35); reduction in microcysts	P16– P35; daily intraperitoneal injection	
Clinical trials: Bosutinib; multi kinase inhibitor	References; ClinicalTrials.gov numbers		Eligibility		Outcome	Duration (month)	Number of patients (drop out rate)
Bosutinib (primary targets: Abl, Src, HDAC)	NC/T01233869 Estimated completion: Feb 2019		eGFR at least 60 ml/min; TKV at least 750 cm ³ ; 18 to 50 years of age		Safety endpoints; rate of kidney enlargement; renal function	24	275
Preclinical studies: Src-inhibitor	References		<i>In vivo</i> model		<i>In vivo</i> results	Treatment schedule; administration of the drug	
SKI-606 (Src-inhibitor)	[186] }		Bpk mouse		Decrease in kidney weight as % of BW, cystic index, blood urea nitrogen and creatinine; increase in maximum urinary concentrating ability; less biliary ductal ectasia	P7–P21; daily intraperitoneal injection	
			PCK rat (male)		Decrease in kidney weight as % of BW, renal cystic volume, liver weight as % of BW, blood urea nitrogen and creatinine; increase in maximum urinary concentrating ability; reduction in biliary ductal cysts and hepatic fibrosis	P7– P89; daily intraperitoneal injection	
			C57/Pkd1 ^{+/-} mouse		Decrease in cyst number and kidney weight	5 to 8 month of age; (8 month to 11 month of age); daily added in drinking water	
Preclinical studies: HDAC inhibitor	References		<i>In vivo</i> model		<i>In vivo</i> results	Treatment schedule; administration of the drug	
Trichostatin A (pan HDAC inhibitor)	[199]		Pkd2 ^{-/-} mouse		Decrease in cystic area (%); HDAC5 heterozygosity reduces cyst formation	10.5 to 18.5 day post conception; daily subcutaneous injection in pregnant mice	
valproic acid (class I HDAC inhibitor)	[198]		Pkd1 ^{fllox/fllox} ;Pkd1 ⁻ Cre mouse		Decrease in kidney weight as % of BW, cystic index and blood urea nitrogen;	P10–P25; daily intraperitoneal injection	
Various preclinical studies:	References		<i>In vivo</i> model		<i>In vivo</i> results	Treatment schedule; administration of the drug	
Roscovitine (cyclin-dependant kinase-inhibitor)	[216]		Jck mouse		Decrease in kidney weight as % of BW, cyst volume as % of BW, blood urea nitrogen (significant only with (1) and (2)); decreased proliferation; decreased apoptosis	Starting at P26: 1 Treatment for 5 weeks; 2 treatment for 3 weeks, followed by 2 weeks without treatment; 3 1 week on treatment followed by 1 week off treatment for a total of 5 weeks; daily intraperitoneal injection	

		Cpk mouse	Decrease in kidney weight as % of BW, cyst volume as % of BW, blood urea nitrogen, and creatinine	P7 to P21; daily intraperitoneal injection
Vitamin K3 and PM-20 (Cdc25A inhibition) or genetic inactivation of Cdc25A	[202]	PCK rat; Pkd2 ^{ws25/-} mouse; Cdc25A ^{+/-} Pkhd1 ^{del2/del2} mouse	Decrease in liver and kidney weights, hepato-renal cystic and fibrotic areas, and mitotic and apoptotic indices; reduced liver weights and liver fibrosis in Cdc25A ^{+/-} Pkhd1 ^{del2/del2} mice compared with Pkhd1 ^{del2/del2}	PCK rats starting age 3 weeks and Pkd2 ^{ws25/-} mice starting age 5 month; PM-20 : 4 weeks of treatment; daily intraperitoneal injection (Pkd2 ^{ws25/-} mice only) Vitamin K3 : 4 and 8 weeks of treatment; in drinking water every other day (PCK rats and Pkd2 ^{ws25/-} mice); Cdc25A ^{+/-} Pkhd1 ^{del2/del2} mouse: 7 to 9 month of age
PD184352 (MAP/ERK kinase inhibitor)	[217]	Pcy mouse	Decrease in kidney weight as % of BW, cystic index, serum creatinine, systemic blood pressure, and water intake; urine osmolality increased	10 to 17 weeks of age; added to chow; daily for the first week and then every third day for 6 additional weeks
Leflunomide/teriflunomide (STAT6-inhibitor) or genetic inactivation of STAT6	[194]	bpk/bpk mouse	Decrease in kidney weight as % of BW, cystic index, and blood urea nitrogen	P7 to P21; daily intraperitoneal injection, every 2 days
		bpk/bpk:STAT6 ^{+/+} bpk/bpk:STAT6 ^{+/-} bpk/bpk:STAT6 ^{-/-}	Decrease in kidney weight as % of BW, blood urea nitrogen, mean cyst diameter and number of renal proliferating cells; increase in number of normal tubules in bpk/bpk:STAT6 ^{-/-} compared to bpk/bpk:STAT6 ^{+/+} animals; no change in apoptosis rate in the kidney	No treatment
Curcumin (inhibits mTOR, Wnt/beta-actin and STAT3 signaling)	[218]	iKsp-Pkd1 ^{del} mice; (cyst induction P40–P42)	Short treatment: Decrease in proliferation index, cystic index and kidney weight as % of BW Long treatment: renal failure significantly postponed in mice with severe PKD	Starting 1 week after cyst induction; Short treatment: 11 weeks Long treatment: Until renal failure occurs; added to chow
Genz-123346 (glucosylceramide synthase inhibitor, Akt-mTOR inhibitor)	[219]	Jck mouse	Decrease in kidney weight as % of BW, cyst volume as % of BW, blood urea nitrogen (jck and PKD1 ^{cond} mouse), fibrosis (pcy mouse), apoptosis and proliferation (jck mouse)	4 to 9 weeks of age; added to chow
		Pcy mouse		4 to 15 weeks of age; added to chow
		Pkd1 ^{fllox/-} ; Tamoxifen inducible Cre (Pkd1 ^{tm1(Gzhd)} allele) (induction at day P5)		P7–P33; added to chow
Metformin (AMPK activator, mTOR and CFTR channel inhibitor)	[192]	Pkd1 ^{fllox/-} ;Ksp-Cre mouse	Decrease in cystic index	P4 to P6; daily intraperitoneal injection
		Pkd1 ^{fllox/-} ;pCX-CreER mouse;(cyst induction at P9 or P10)	Decrease in cystic index	P7 to P17; daily intraperitoneal injection
Pioglitazone (PPAR-γ receptor agonist; inhibits mTOR and ERK signaling; inhibits CFTR expression)	[220]	PCK rat	Decrease in kidney and liver weight as % of BW, renal cyst volume, renal and liver fibrosis, and serum albumin	3–10 weeks of age or 4–18 weeks of age; added to chow
	[196]	PCK rat	Decrease in kidney and liver weight as % of BW, renal and liver cystic area, serum urea nitrogen, renal and hepatic proliferation, and fibrotic index in liver	4 to 20 weeks of age; daily administration by gavage
Tetrazolo-CFTR _{inh} -172 and Ph-GlyH-101 (CFTR inhibitor)	[184]	Pkd1 ^{fllox/+} ;Ksp-Cre mouse	Decrease in renal cyst number, kidney weight as % of BW, serum urea and creatinine	P2 through P5 or P9; subcutaneous injection every 6h

R-568 (type II calcimimetic)	[221]	Pkd2 ^{-WS25} mouse; PCK rat	No detectable effect on cystogenesis; decrease in renal interstitial fibrosis (PCK rats only); increase in urine output and osmolar clearance	Starting at 3 weeks of age; treatment for 10 (PCK rats) or 16 (mice) weeks of age; added to chow
	[222]	Cy/+ male rat (Han:SPRD-Cy _{lu})	After 18 weeks: Decrease in kidney weight as % of BW, cyst volume, fibrosis (except calcium alone), blood urea nitrogen	Starting at 20 weeks of age; treatment for 14 to 18 weeks: <div>1no treatment,</div> <div>2R-568 only,</div> <div>3R-568 plus calcium</div> <div>calcium only; R-568 added in chow</div>

Abbreviations: eGFR = estimated glomerular filtration rate; TKV = total kidney volume; BW = body weight; MRI = magnetic resonance imaging; mTOR = mammalian target of rapamycin; HMG-CoA reductase = 3-hydroxy-3-methylglutaryl co-enzyme A reductase; ACE inhibitor = angiotensin-converting enzyme inhibitor; AVP = arginine vasopressin; HDAC = histone deacetylase inhibitor; MAP kinase = mitogen-activated protein kinase, ERK = extracellular signal-regulated kinase; STAT6 = signal transducer and activator of transcription 6; AMPK = 5' adenosine monophosphate-activated protein kinase; CFTR = cystic fibrosis transmembrane regulator; PPAR γ = peroxisome proliferator activator receptor γ

Table 2

PKD mouse models

Dominant cystic kidney disease model	Genotype description	Phenotype	Reference
Pkd1 ^{fllox/-} ;Ksp-Cre mouse	Kidney-specific inactivation of Pkd1 (in distal nephron segments including medullary thick ascending loop of Henle and collecting duct)	Rapidly progressive cysts in the neonatal period, resulting in renal failure and death by age 20 days.	[140, 223]
Pkd1 ^{fllox/flox} ;Pkhdl1-Cre mouse	Kidney-specific inactivation of Pkd1 (primarily in the collecting ducts)	Similar to Pkd1 ^{fllox/-} ;Ksp-Cre mouse but with longer survival of cystic mice	[140, 223]
Pkd1 ^{fllox/-} ;pCX-CreER mouse	Tamoxifen-inducible inactivation of Pkd1	Inactivation before day P13 leads to rapidly progressive cyst formation with severe disease at 3 weeks of age. In contrast inactivation after day P13 shows slow cyst progression with cysts starting to develop at about 4 months of age.	[224]
Pkd1 ^{fllox/flox} ;Mx1Cre mouse	IFN- inducible inactivation of Pkd1	Progression of cyst formation dependent on time of inactivation (see Pkd1 ^{fllox/-} ;pCX-CreER mouse).	[225]
C57/Pkd1 ^{+/-} mouse	Heterozygous for null mutation in Pkd1	Adult onset of cystic kidney disease with cysts developing as early as 2.5 months of age.	[226]
Pkd1 ^{-/-} mouse	Homozygous for null mutation in Pkd1	Aggressive embryonal cyst progression starting at E15.5	[226]
Pkd1 ^{cond/cond} ;NestinCre mouse	Mosaic inactivation of the Pkd1 gene (predominantly in collecting duct/ distal tubules)	Development of highly cystic kidneys within less than 2 months with elevated apoptosis, proliferation, and fibrosis.	[208]
iKsp-Pkd1 ^{del} mouse	Tamoxifen inducible kidney specific inactivation of Pkd1	Progression of cyst formation dependant on time point of inactivation (see Pkd1 ^{fllox/-} ;pCX-CreER mouse).	[227, 228]
Pkd2 ^{-tm1Som} mouse = Pkd2 ^{WS25/-} mouse	Targeted mutation of Pkd2 resulting in a hypomorphic allele	Development of renal cysts within 3 months	[229]
Han:SPRD-Cy _{III} mouse	Missense mutation in Anks6 (also called Pkdr1)	Slowly progressive PKD	[230]
Recessive cystic kidney disease model	Genotype description	Phenotype	Reference
Pcy mouse	Spontaneous missense mutation in NPHP3	Late onset of PKD, equivalent to adolescent nephronophthisis.	[231, 232]
Jck mouse	Spontaneous mutation in Nek8 (NPHP9)	Slowly progressive renal cystic disease resembles human ADPKD phenotypically, despite its autosomal recessive mode of inheritance; gender dimorphism (males more severe) and cysts in multiple parts of the nephron.	[233, 234]
PCK rat	Splicing mutation, which leads to a framshift in pkhd1	Renal cysts derived from collecting ducts and congenital hepatic fibrosis associated with biliary cysts. Life span is 1 year in males and 1.5 year in females.	[235]
Bpk mouse	Spontaneous mutation in bicaudal C (Bicc1)	Model for ARPKD: Homozygous animals typically die 1 month postnatal due to renal failure with progressively enlarged kidneys.	[236]
Orkp-rescue mouse (Tg737orp/orkp;TgRsq)	Homozygous mutation in the poaris/IFT88 gene; hemizygous for the transgene expressing wild type polaris	Develops an adult-onset form of ARPKD	[237]

Cpk mouse	Spontaneous recessive mutation (cpk locus on chromosome 12) in the C57BL/6J strain, disrupting the cystin gene	Aggressive PKD with cysts becoming apparent by 8–9 days of age, resulting in renal failure and death by age 4 to 5 weeks.	[238, 239]
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Abbreviations: ADKPD = autosomal dominant polycystic kidney disease; ARPKD = autosomal recessive polycystic kidney disease