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Cilia and Polycystic Kidney Disease, Kith and Kin

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Abstract

In the past decade, cilia have been found to play important roles in renal cystogenesis. Many genes, such as PKD1 and PKD2 which, when mutated, cause autosomal dominant polycystic kidney disease (ADPKD), have been found to localize to primary cilia. The cilium functions as a sensor to transmit extracellular signals into the cell. Abnormal cilia structure and function are associated with the development of polycystic kidney disease (PKD). Cilia assembly includes centriole migration to the apical surface of the cell, ciliary vesicle docking and fusion with the cell membrane at the intended site of cilium outgrowth, and microtubule growth from the basal body. This review summarizes the most recent advances in cilia and PKD research, with special emphasis on the mechanisms of cytoplasmic and intraciliary protein transport during ciliogenesis.

Keywords

polycystic kidney disease; cilia; planar cell polarity; exocyst

Introduction

Cilia are thin rod-like organelles found on the surface of human eukaryotic cells. First described by Anthony van Leeuwenhoek in 1675 (Dobell, 1932), they were originally defined by their motility, being structurally and functionally similar to eukaryotic flagella. In 1876 and 1898 (Langerhans, 1876; Zimmermann, 1898), another class of cilia was described, the solitary (or nonmotile) cilia, which were renamed primary cilia in 1968 (Sorokin, 1968). Motile cilia/flagella have been studied for many years in single-celled organisms, such as protozoa, and important insights into the physiology and biochemistry of these organelles have resulted. However, despite the established anatomical presence of primary cilia in eukaryotic cells, until recently, little has been known about their specific function, and they had even been considered vestigial organelles (Webber and Lee, 1975). Over the past decade, the primary cilium began to receive increasing attention, after it was discovered that proteins mutated in different forms of polycystic kidney disease (PKD) were tightly associated with primary cilia.

PKD is a group of genetic disorders characterized by the growth of numerous cysts in the kidney. Cysts are normal “building blocks” for epithelial organs, but abnormal regulation of cystogenesis in the kidney results in cystic kidney disease. Autosomal dominant PKD (ADPKD), which affects approximately 500,000 Americans and 12,000,000 people world wide, is the most common potentially lethal genetic disorder in humans (Grantham, 2001). Mutations in PKD1, the gene encoding polycystin-1, and PKD2, the gene encoding polycystin-2, have been identified as the cause of ADPKD (The International Polycystic Kidney Disease Consortium, 1995; Mochizuki et al., 1996). Autosomal recessive PKD (ARPKD), a severe form of PKD that presents primarily in infancy and childhood, is caused by a mutation in the polycystic kidney and hepatic disease1 (*Pkhd1*) gene (Zerres et al., 1994). Nephronophthisis, a form of PKD that is the most common genetic cause of renal failure through age 30, is caused by mutations in multiple (16 and counting) different nephronophthisis (*NPHP*) genes (Hildebrandt et al., 2009).

More and more evidence has accumulated showing that gene mutations resulting in structural or functional defects of the primary cilium cause PKD. For example, ADP-ribosylation factor-like protein 13b (Arl13b), a small GTPase of the Arf family, is highly enriched in cilia and is required for cilia formation. Knockdown of the Arl13b gene results in disorganized cilia structure and kidney cyst formation in zebrafish (Duldulao et al., 2009). Mutations in Arl13b in patients lead to the nephronophthisis form of PKD (Cantagrel et al., 2008). Another example is kinesin-like protein 3A (KIF3A), one of the subunits of the cilia motor protein, kinesin-2, which is essential for cilia formation. Inactivation of KIF3A in renal epithelia induced kidney cyst formation and severe PKD (Lin et al., 2003). Virtually all forms of PKD in human patients and animal models are associated with perturbations in renal primary cilia structure and/or function. Although maintenance of primary cilia structure and function appears to be key to preventing/treating PKD, it is not entirely clear how aberrant ciliogenesis promotes the disease.

The purpose of this review is to summarize the most recent advances in cilia and PKD research, with an emphasis on ciliary protein trafficking. Proteins located on the primary cilium play important roles in cilia structure and assembly, and are also critical for the pathogenesis of PKD. Primary cilia function as sensors that transmit extracellular signals into the cell and regulate downstream signal pathways, such as the planar cell polarity (PCP) pathway, cell proliferation, and differentiation. The mechanisms of protein transport to, and inside, the primary cilia, and their role in ciliary function and PKD, are critical for proper ciliogenesis and function.

Ciliary Structure and Assembly

Cilia are organelles protruding from the surface of human eukaryotic cells, which extend outward from the basal body, a cellular organelle related to the centriole. Figure 1 shows the structure of the primary cilium. The membrane of the cilium is an extension of the cell membrane, although it is separated from the apical membrane by a septin band (Hu et al., 2010). Cilia contain a central axoneme composed of microtubules. The primary ciliary axoneme, a cytoskeletal component that extends from the basal body, consists of nine microtubule doublets arranged tangentially around the center in a configuration known as

9+0, whereas the motile ciliary axoneme is characterized by a 9+2 architecture with nine outer microtubule doublets plus a central pair of microtubules.

Cilia are rapidly assembled and disassembled at different stages of the cell cycle, indicating that ciliary dynamics are precisely coordinated with the cell cycle (Sorokin, 1962; Gilula and Satir, 1972). Most cells in the human body will enter the G0 phase, or quiescent stage, after cell division. Primary cilia present in G0 cells are regarded as postmitotic structures of quiescent cells. Cilia assembly includes several different stages, as observed by electron microscopy (Sorokin, 1962; Pedersen, 2008). To assemble primary cilia, the centrosomes, which are comprised of two centrioles (mother and daughter centrioles), migrate to the apical membrane of the cell where the mother centriole forms the basal body. This migration involves vesicles, which bind at the distal end of the mother centriole, the actin cytoskeleton, and the exocyst complex, which assists with basal body migration, membrane docking, and fusion of the ciliary vesicles at the intended site of cilium outgrowth (Dawe et al., 2007; Zuo et al., 2009; Garcia-Gonzalo and Reiter, 2012). The detailed mechanisms of how centrioles migrate during primary ciliogenesis, however, remain unclear. The Meckel–Gruber Syndrome proteins, MKS1 and MKS3, were found to be required for migration of the centrosomes to the plasma membrane (Dawe et al., 2007). After migration, basal bodies dock with the cell surface through vesicular fusion. Before docking, basal bodies acquire accessory structures, including basal feet and striated rootlets. The basal body also has other accessories, including centriolar satellites and transitional fibers, but the timing of acquisition of these structures is also unclear (Dawe et al., 2007). The PCP protein, Dishevelled (Dvl), is required to establish the apical actin network necessary for vesicle targeting, transport, and docking of basal bodies (Vladar and Axelrod, 2008). After basal body docking, post-translationally modified tubulin begins to polymerize from the basal body and grows away from the surface of the membrane. Tubulin then is transported from the cytoplasm to the ciliary tip by intraflagellar transport (IFT) to construct the cilium. At the same time, a disassembly process occurs, and the balance between these two processes determines the length of the cilia (Avasthi and Marshall, 2012). In cystic kidney disease, excessively long cilia and the absence of cilia have both been associated with cyst development (Mokrzan et al., 2007; Besschetnova et al., 2010; Hildebrandt et al., 2011).

Primary cilia do not contain the machinery for protein synthesis, so the cell must transport proteins that are required for cilia assembly, sensory perception, and signaling from the site of synthesis into the cilium. Trafficking of proteins from the cytosol and Golgi to the primary cilium, and the movement of proteins along the ciliary axoneme are critical for normal cilia structure and function, and are regulated by polarized vesicle trafficking and IFT, respectively.

IFT refers to the process of transporting vital proteins within the cilium. Because of the similarity to the processes required to build flagella, it is named “intraflagellar” transport. IFT describes the bidirectional movement of nonmembrane-bound particles along the doublet microtubules of the flagellar or ciliary axoneme, and between the axoneme and plasma membrane. Movement of IFT particles along the microtubule is carried out by two different microtubule-based motors: movement towards the tip of the cilium is termed anterograde IFT and depends upon kinesin-2 microtubule motors, while the reverse

retrograde transport requires dynein (Goetz and Anderson, 2010). The IFT particle consists of two subcomplexes, each made up of several individual IFT proteins. Complex A has five IFT subunits and complex B is composed of 12 IFT subunits. Collingridge et al. found that compartmentalized intraciliary Ca^{+1} signaling can regulate the movement of IFT particles and is therefore likely to play a central role in directing the movement and distribution of many ciliary proteins (Collingridge et al., 2013). Bhogaraju et al., recently identified the transport mechanism of the key protein tubulin, the most abundant protein and the main backbone of the cilium, and found that two proteins, IFT74 and IFT81, work together to form a tubulin-binding molecule (Bhogaraju et al., 2013).

Normal Ciliary Function in Embryonic Kidney Development and in the Adult Kidney

The mammalian kidney originates from intermediate mesoderm, and three kidneys form in succession during embryogenesis: the pronephros, mesonephros, and metanephros (Lipschutz, 1998; Vize et al., 2003). The metanephros becomes the permanent kidney in mammals. It forms when the epithelial ureteric bud grows out of the mesonephric (aka Wolffian) duct and contacts the loose metanephric mesenchyme in the caudal region of the embryo. The ureteric bud then elongates and branches to form a tree-like structure, which becomes the highly branched collecting duct system of the mature kidney (Saxen and Sariola, 1987). The metanephric mesenchyme cells condense around the tip of the ureteric bud, aggregate, epithelialize, and undergo morphogenetic movement and differentiation to form the podocytes, and proximal and distal renal tubules. Stromal mesenchymal cells surround the condensed mesenchyme and differentiate into the pericytes of the mature kidney (Cullen-McEwen et al., 2005).

It has been known for over a hundred years that epithelial cells along the length of the nephron possess primary cilia (Zimmermann, 1898). In the mammalian kidney, cilia have been observed on cells in the parietal layer of the Bowman's capsule, the proximal tubule, the distal tubule, and in the principal, but not intercalated, cells of the collecting duct (Webber and Lee, 1975). Their specific function, however, has only recently begun to be understood.

Primary cilia play key roles in the development of many organs by coordinating extracellular signaling with cellular physiology (Corbit et al., 2005; Haycraft et al., 2005; Simons et al., 2005). The ciliary membrane is rich in receptors, ion channels, and signaling proteins, which can be activated by mechanical and/or chemical stimuli (Rosenbaum, 2002). In adult kidney, cilia function as mechanosensors for luminal flow [as covered in a recent review by Kotsis et al. (2013)]. Aside from their important sensory functions, primary cilia also play important roles in the control of cell proliferation, and are involved in several developmental signaling pathways, including Hedgehog, Wnt, and mammalian target of rapamycin (mTOR) (Morgan et al., 2002; Cano et al., 2004; Haycraft et al., 2005).

Ciliary Dysfunction in ADPKD

Work with *C. elegans* provided early clues that the gene products for PKD1 and PKD2 might also be involved with cilia structure and/or function. During the examination of mutations that affect mating behavior in *C. elegans*, Barr et al. (1999, 2001) identified worm homologs of PKD1 as *lov-1* (for location of vulva), and PKD2 as *pkd2*. The protein products for *lov-1* and *pkd2* localized to the cilia of sensory neurons in *C. elegans* (Barr and Sternberg, 1999; Barr et al., 2001).

The relationship between primary renal cilia and the pathogenesis of PKD was further suggested by the Oak Ridge polycystic kidney (*orpk*) mouse. In these animals, insertional mutation of the *Tg737* gene product resulted in a hypomorphic allele, and mice carrying this mutation developed complex phenotypes, including polycystic kidney and pancreas, and portal fibrosis. Kidney cysts and hepatic fibrosis are also seen in humans with ARPKD, and this mouse became one of the models for this disease (Moyer et al., 1994). The *Tg737* gene is a homolog of *Chlamydomonas* IFT88, a subunit of the intraflagellar transporter. IFT proteins are required for cilia formation and maintenance, and IFT88 mutants showed a complete absence of flagella in *Chlamydomonas* (Pazour et al., 2000). Subsequently, localization of *Tg737* to the cilia of renal tubule epithelial cells was demonstrated using the Madin-Darby canine kidney (MDCK) epithelial cell line (Taulman et al., 2001). The primary cilia in the kidney of *Tg737* mutant mice are short and stunted, indicating that primary cilia have an important function in the kidney, and that defects in their assembly lead to PKD (Pazour et al., 2000). The co-localization of polycystins-1 and -2, cystin, and *Tg737* in mouse cortical collecting-ductal cells, further indicated that primary cilia play a key role in normal physiological functions of renal tubule epithelia, and that defects in ciliary function contribute to the pathogenesis of PKD (Yoder et al., 2002).

Although these studies suggested the important connection between cilia and normal kidney architecture, the role of the primary cilium in renal cyst formation remains poorly defined. While mice with insertional mutation of the *Tg737* gene developed renal cysts, *Tg737* null mutations are embryonic lethal, and are noted to have *situs inversus*. In this animal model, it was found that the embryonic node cells lack cilia on their apical surface. The loss of cilia on these cells can cause abnormal nodal flow of morphogens and leads to defects in right-left patterning (nodal flow hypothesis). Polycystin-2, the protein encoded by PKD2, localizes to the primary cilia. PKD2-deficient mice also showed associated defects in left-right asymmetry, further indicating a functional link between primary cilia and polycystin-2 (Pennekamp et al., 2002). Research into *pkd2* function in zebrafish has better elucidated the function of polycystin-2 in cilia. Knockdown of *pkd2* by morpholinos (MO) (Bisgrove et al., 2005), or in mutants (Sun et al., 2004; Schottenfeld et al., 2007), produced phenotypes consistent with a role in cilia function, such as curved tails, pronephric cysts, and edema. Further studies showed that polycystin-2 functions as a calcium-activated intracellular calcium release channel *in vivo*, and that PKD results from the loss of a regulated intracellular calcium release signaling mechanism (Koulen et al., 2002).

Several groups have shown that primary cilia function as mechanosensors, which transmit extracellular mechanical signals into the cell through calcium influx. For example, MDCK

cells respond to bending of the cilium by a micropipette or fluid flow with influx of calcium (Praetorius and Spring, 2001). This calcium influx in response to fluid flow was abolished after removal of the MDCK cell primary cilium by chemical means, indicating that the primary cilium is the flow sensor in MDCK cells (Praetorius and Spring, 2003). Isolated renal collecting ducts from cilia-deficient *orpk* mice also showed blunted flow-sensing (Liu et al., 2005). The identification of polycystin-2 as a calcium channel localized on the primary cilia indicated its important role in the mechanosensory function of the primary cilia. Blocking antibodies against polycystin-2 also abrogated the calcium influx in response to fluid flow (Nauli et al., 2003). Mechanical stimulation of the primary cilia, such as by fluid flow, initiates an intracellular calcium rise, as well as nitric oxide release, and protein modifications.

The cilium has also been shown to be a chemosensor. In addition to being involved in hedgehog signaling as discussed previously, Masyuk et al. (2008) reported that cholangiocyte cilia are chemosensors that detect biliary nucleotides through ciliary P2Y12 receptors and transduce corresponding signals into a cAMP response. Abdul-Majeed et al. (2011) found that dopamine receptor—type 5 (DR5), located on the primary cilia of mouse vascular endothelial cells, can sense systemic dopamine and plays an important role in cilia length and function. Silencing DR5 completely abolished mechano-ciliary function through changes in sensitivity to fluid-shear stress (Abdul-Majeed et al., 2011).

Cilia and Planar Cell Polarity

We have discussed above the role of cilia as mechano/chemosensors that transmit extracellular flow signal into the cells. Downstream effects of this transmitted signaling pathway include changes in cell differentiation and polarity. Planar cell polarity (PCP), also called tissue polarity, describes the coordinated orientation of cells and cellular structures along an axis within the plane of an epithelial surface (Simons and Mlodzik, 2008). Polarized cellular orientation and migration controlled by PCP are critical for multiple developmental processes. Park et al. (2006) showed that two planar polarity effectors, *inturned* (*int*) and *fuzzy* (*fy*), play a role in cilia formation. Heydeck et al. (2009) further demonstrated that *fy* plays an important role in cilia formation, hedgehog signal transduction, and embryonic development in mice. Strong evidence implicating cilia in PCP came from mice missing cilia through tissue-specific targeting of *Ift88* and *Kif3a*. For example, these animals displayed highly disordered tissue architecture in the inner ear (Jones et al., 2008). However, IFT88 mutant zebrafish, which do not form cilia, had a normal PCP phenotype, indicating that IFT88 plays cilia- and PCP-independent roles in controlling oriented cell division (Borovina and Ciruna, 2013).

The relationship of renal primary cilia, PCP, and PKD was supported by the *inversin* (*Inv*) mouse. *Inv* is encoded by the *inversion of embryo turning* (*Inv*) gene (Mochizuki et al., 1998, Otto et al., 2003), and was discovered due to its role, during mammalian embryonic development, in establishment of left–right asymmetry. *Inv* is a ciliary protein that regulates developmental processes and tissue homeostasis partly through the degradation of Dishevelled (*Dvl*) proteins to coordinate Wnt signaling in planar cell polarity. The *Inv* mutation in mice resulted in both a reversal of left–right axis polarity (*situs inversus*) and

cyst formation in the kidneys and pancreas (Yokoyama et al., 1993; Mochizuki et al., 1998). The human homolog of *Inv* was later found to cause nephronophthisis type 2 (NPHP2) (Otto et al., 2003). *Inv* localizes to the base of primary cilia and is required for the localization of NPHP3 and Nek8/NPHP9 to that region (Shiba et al., 2010).

Another PCP-associated gene, *fat4*, is required for outer medullary collecting duct (OCD) and tubule elongation during kidney development, and its loss results in cystic kidneys in mice (Saburi et al., 2008). *Fat4* localizes to primary cilia and is hypothesized to act in a partially redundant fashion with *Vangl2* during cyst formation. Several other genes that are known to give rise to cystic kidneys have also been shown to cause defects in cilia and PCP signaling, including *Pkd1*, *Bbs4*, *Bbs6*, and *Odf1*.

Another important polarized event is oriented cell division, in which mitotic cells are oriented along the tubular axis during tubule elongation. Orientated cell division dictates the maintenance of constant tubule diameter during tubule lengthening, and defects in this process trigger renal tubule enlargement and cyst formation (Fischer et al., 2006). Cilia also play a role in oriented cell division. Inactivation of KIF3A, the ciliary transport gene, in renal epithelial cells resulted in kidney cyst formation and defects in oriented cell division (Patel et al., 2008). IFT20 mutant mice also lacked cilia and developed renal cysts that were associated with a disoriented axis of cell division (Jonassen et al., 2008). Taken together the cilia, PCP, and oriented cell division data suggest that primary cilia play an important role in transmitting extracellular information, such as urine flow, into the cell, and work through the PCP pathway. The hypothesis, therefore, is that genetic disorders that result in abnormal ciliary sensing of fluid movement lead to loss of PCP, which causes randomized cell division. This, in turn, results in an increased tubule diameter, rather than tubule elongation, leading to cyst formation.

Cilia, Exocyst, and Cdc42

The cilium and the rest of the apical surface are both covered by plasma membrane; however, there is regulated movement of both proteins and lipids into the cilium, and the membrane composition is different in these two areas (Rohatgi and Snell, 2010). There is a diffusion barrier at the base of the cilia membrane. This diffusion barrier involves Septin 2 (SEPT2), a member of the septin family of guanosine triphosphatases, and is essential for retaining ciliary proteins (Hu et al., 2010). Since protein synthesis does not occur in the cilium, the ~700 proteins which are known to reside there (Li et al., 2004) must be transported to and integrated into the cilium. Although the roles that ciliary proteins play in diverse biologic processes are being elucidated (Emmer et al., 2010), we know very little about how these proteins are transported from the sites of synthesis in the endoplasmic reticulum to the cilium.

A significant number of proteins destined for the ciliary compartment are likely transported in Golgi-derived vesicles to the base of the cilium, where the ciliary proteins are delivered and associate with IFT particles. However, of the IFT proteins located in the cilia, only IFT20 has been shown, to date, to also be located in the Golgi complex and function in the delivery of ciliary proteins from the Golgi to the cilium (Follit et al., 2006). At the ciliary

base, proteins containing specific ciliary targeting motifs are allowed access through the zone defined by the transition fibers (Follit et al., 2010). Lateral movement and recycling of proteins from the apical membrane to the cilium involving the BBSome (Nachury et al., 2007) are also possible routes of entry into the cilium. Following entry into the ciliary compartment, these proteins, along with inactive cytoplasmic dynein 2, are transported in an anterograde fashion along the axoneme by kinesin II-mediated IFT. At the ciliary tip, IFT particles and ciliary turnover products (e.g., inactive receptors) are remodeled, kinesin-II is inactivated, and cytoplasmic dynein 2 is activated. Ciliary turnover products are, in turn, transported in a retrograde fashion along the ciliary axonemes by cytoplasmic dynein 2 for recycling or degradation in the cytoplasm.

Trafficking of proteins from the cytosol and Golgi to the primary cilium is regulated by polarized vesicle trafficking. The primary cilium, the Golgi, and the nucleus are consistently found in proximity to each other (Poole et al., 1997), but the functional links between these organelles are not known. The exocyst, originally identified in the yeast, *Saccharomyces cerevisiae* (Novick et al., 1980), is a highly conserved 750-kD eight-protein complex known for the targeting and docking of vesicles carrying membrane proteins to the plasma membrane (Lipschutz and Mostov, 2002), and is composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (also known as EXOC1–8) (Hsu et al., 1996). Notably, in addition to being found near the tight junction, exocyst proteins were localized to the primary cilium in kidney cells (Rogers et al., 2004; Zuo et al., 2009). Of the exocyst components, Sec10 and Sec15 are most vesicle-proximal, and Sec15 directly binds Sec4 (Rab8 in mammals), a Rab GTPase found on the surface of transport vesicles. Sec10 then acts as a “linker” by binding Sec15 (which is attached to the vesicle) to the other exocyst components at the membrane (Guo et al., 1999). It was shown that knockdown of exocyst Sec10 in MDCK cells abrogated ciliogenesis, while Sec10 overexpression enhanced ciliogenesis (Zuo et al., 2009) (Fig. 2). Furthermore, Sec10 knockdown decreased the levels of other exocyst components (and IFT88) and caused abnormal cystogenesis when the cells were grown in a collagen matrix (Zuo et al., 2009). *In vivo*, knockdown in zebrafish using antisense morpholinos of *sec10* and *pkd2* phenocopied each other, with a curly tail up, small eyes, and glomerular edema. Furthermore, small amounts of *sec10* and *pkd2* morpholinos, which alone had no effect, together had a severe effect. This is called genetic synergy, and suggests that *sec10* and *pkd2* act in the same pathway (Fig. 3) (Fogelgren et al., 2011). From these findings, together with their known role in trafficking proteins to the plasma membrane (Grindstaff et al., 1998; Lipschutz et al., 2000, 2003; Moskalenko et al., 2002), Sec10 and the exocyst are thought to be required to build primary cilia by targeting and docking vesicles carrying ciliary proteins.

Small guanosine triphosphatases (GTPases) are versatile temporal and spatial regulators of virtually all cellular processes including signal transduction, cytoskeleton dynamics, and membrane trafficking. Rab GTPases are the largest group of the Ras superfamily of small GTPases. The exocyst interacts with Rab GTPases as described above (Zhang et al., 2004; Wu et al., 2005). The Cdc42 protein (Johnson et al., 1987) is a member of the Rho GTPase subfamily of the Ras superfamily, and acts as a molecular switch to regulate many cellular processes (Johnson and Pringle, 1990). Like all GTPases, Cdc42 can exist in two states, a

GTP-bound, active state, and a GDP-bound, inactive state. The cycling of Cdc42 between these states is controlled by two sets of proteins: guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Cdc42 is an important regulator of exocytosis in yeast (Wu et al., 2010). In cell culture, Cdc42 biochemically interacts with Sec10, and the two co-localize at the primary cilium. Expression of dominant negative Cdc42 and shRNA-mediated knockdown of both Cdc42 and Tuba, a Cdc42 GEF, inhibit ciliogenesis and result in MAPK pathway activation (Zuo et al., 2011). In zebrafish, Cdc42 knockdown was recently shown to phenocopy many aspects of sec10 (and pkd2) knockdown. In mice, kidney-specific knockout of Cdc42 resulted in renal failure within weeks of birth. Histology revealed cystogenesis in distal tubules and collecting ducts, decreased ciliogenesis in cyst cells, increased tubular cell proliferation, increased apoptosis, increased fibrosis, and MAPK activation, consistent with a nephronophthisis phenotype (Choi et al., 2013) (Fig. 4). In fact, a family with Joubert syndrome, a nephronophthisis form of PKD, had a mutation in the exocyst (Dixon-Salazar et al., 2012). Indeed, of the 1969 proteins found in the mouse photoreceptor sensor cilium, there are 96 small GTPases, GEFs, and GAPs (Liu et al., 2007). Taken together, these results suggest that Cdc42 localizes the exocyst to primary cilia, whereupon the exocyst targets and docks vesicles carrying ciliary proteins. Abnormalities in this pathway result in deranged ciliogenesis and PKD (Fig. 5).

CONCLUSIONS

After having been discovered more than 300 years ago and having been “ignored” for the vast majority of that time, primary cilia have caught the attention of many investigators in recent years because of research connecting primary cilia to PKD. Cilia extend from the surface of most eukaryotic cells, so structural or functional defects can cause multisystemic disorders, and this apparently disparate group of human diseases was recently given a common name, “ciliopathies.” Among this group, PKD is by far the most common. Cilia are present on the apical surface of renal tubule epithelial cells, and function as mechanoand chemosensors that sense and transmit extracellular signals, such as urine flow, into the cell, and maintain normal renal epithelial planar cell polarity and oriented cell division. Defects in cilia structure and/or function cause PKD. Protein trafficking and IFT play very important roles in normal ciliogenesis, and the exocyst complex and its regulatory GTPases appear to be key players in this process. Although we now have considerable insight into the molecular and cellular basis of ciliopathies such as PKD, many questions remain. For instance, the mechanisms connecting mechanosensing components at the cilium and the downstream signal cascades are poorly understood. Further studies are clearly needed to better understand how cilia-related effects cause cyst formation to devise rational treatments for human PKD, diseases for which no approved treatments currently exist.

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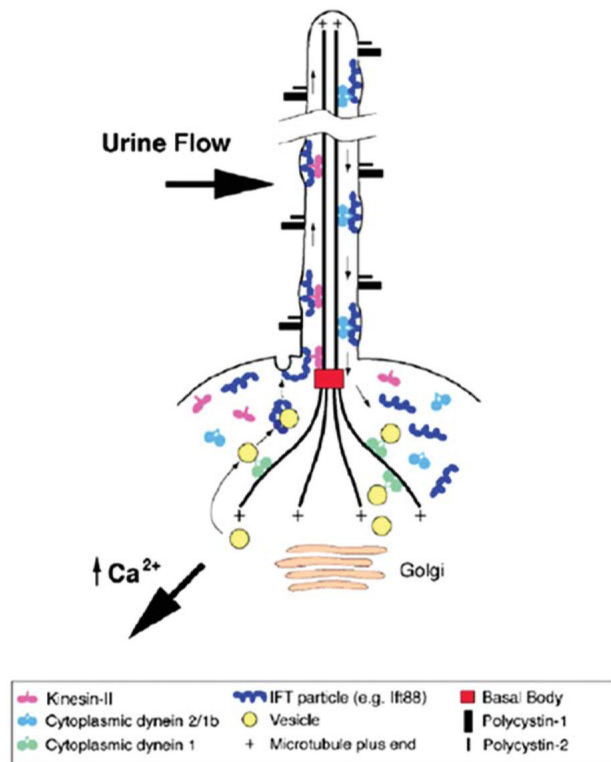
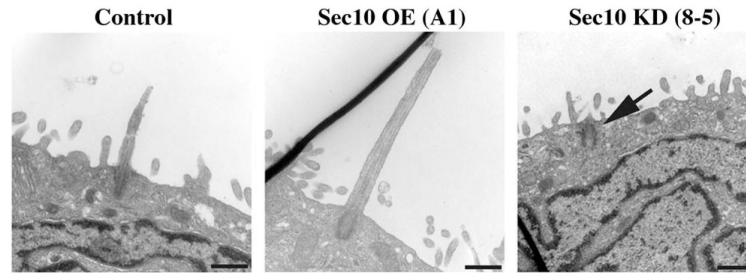


FIGURE 1.

Structure of primary cilia. The cilium protrudes from the apical surface of the cell. The axoneme, or cilium core, grows out from the basal body and is covered by ciliary membrane. The axoneme is composed of nine doublet microtubules nucleated directly from the basal body. Intraflagellar transport (IFT) particles transport proteins to the distal end of the cilium. Kinesin-II is the microtubule motor responsible for anterograde IFT. Retrograde transport utilizes dynein. Polycystin-2 is a calcium channel located on the primary cilium, where it interacts with polycystin-1.

**FIGURE 2.**

Sec10 knockdown inhibits, while Sec10 overexpression enhances, primary ciliogenesis. We grew control, Sec10 overexpressing (Sec10 OE), and Sec10 knockdown (KD) cells on Transwell filters for 14 days. The cells were fixed in glutaraldehyde and electron microscopy (EM) was performed (Phillips XL20 SEM). Confirming the results by IF and scanning EM (SEM) (data not shown here), by transmission EM (TEM) primary cilia were longer in Sec10 OE cells compared to control cells and were rarely identified in Sec10 KD cells. Bar =0.5 μm . (Zuo et al., 2009) (Reproduced with permission from Molecular Biology of the Cell).

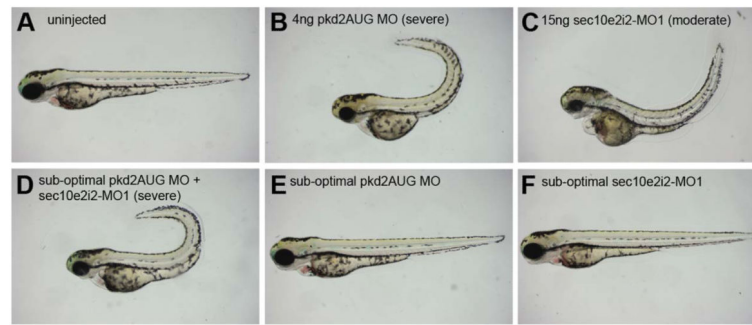
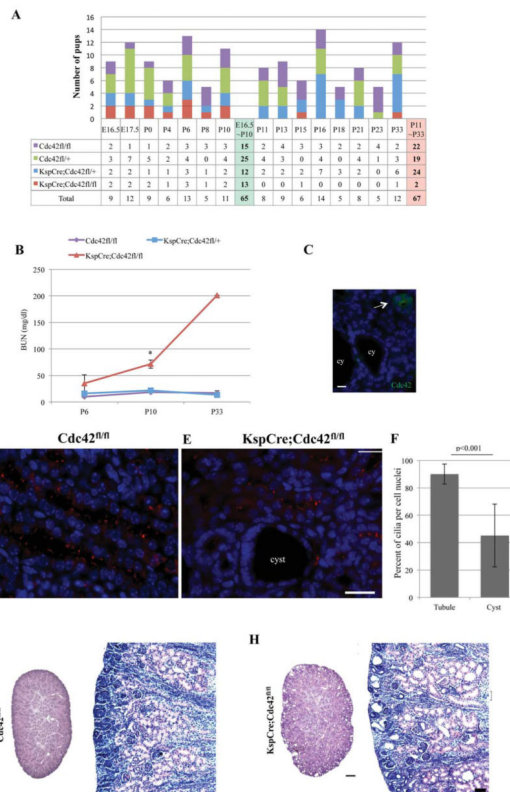
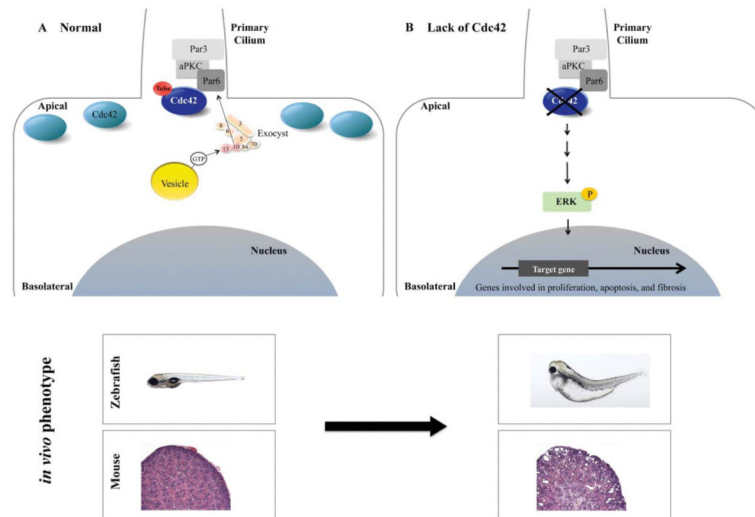


FIGURE 3.

Knockdown of sec10 phenocopies loss of pkd2. (A–F) Gross phenotypes of zebrafish embryos at 3 dpf, lateral view, 4× magnification. Uninjected embryo (A), 4ng pkd2MO embryo with a severe curly tail up, small eyes, and glomerular edema (B), 15ng sec10MO embryo with a moderate curly tail up, small eyes and glomerular edema (C). A synergistic genetic interaction was observed upon co-injection of suboptimal doses of 0.25/2ng pkd2 MO+ 1 7.5ng sec10MO (D)—which do not result in curly tail up when injected alone (E, F). This suggests that exocyst sec10 and pkd2, one of two genes which are mutated in ADPKD, act in the same pathway. (Reproduced with permission from PLoS Genetics).

**FIGURE 4.**

Lack of *Cdc42* in kidney tubule cells leads to an early postnatal death due to lack of cilia and PKD. *KspCre;Cdc42^{fl/+}* mice (*KspCadherin* is expressed in distal tubules and collecting ducts) were mated to *Cdc42^{fl/fl}* mice, and all four possible genotypes were readily identified by PCR (data not shown here). (A) Of 67 pups tested from P11-P33, there were 22 *Cdc42^{fl/fl}*, 19 *Cdc42^{fl/+}*, 24 *KspCre;Cdc42^{fl/+}* mice, with one dead (mostly eaten) homozygous *Cdc42* knockout (*KspCre;Cdc42^{fl/fl}*) mouse found at P15, and one long-surviving homozygous *Cdc42* knockout (*KspCre;Cdc42^{fl/fl}*) mouse that was euthanized at P33 due to failure to thrive, growth restriction, and renal failure. (B) By linear regression modeling, BUN levels in *Cdc42* kidney-specific knockout mice were significantly higher than in littermate control *KspCre;Cdc42^{fl/+}* and *Cdc42^{fl/fl}* mice at P10 ($p = 0.035$). (C) By immunofluorescence, *Cdc42* is not seen in the cysts (cy), but is seen in the normal-appearing tubules of *Cdc42* kidney-specific knockout mice (arrow). Bar = 10 μm . (D-F) Immunostaining of acetylated alpha tubulin (red) in kidneys from control (D) and *Cdc42* kidney-specific knockout mice (E) at P4, shows lack of cilia in cells surrounding cysts. (F) Quantification from kidney tubule cell-specific *Cdc42* knockout mice shows a significant decrease in the number of cilia seen per cell in cysts (which presumably are deficient in *Cdc42*, as no cysts were seen in control mice) compared to normal-appearing tubules, which most likely express *Cdc42*. Bar = 20 μm . (G, H) Hematoxylin/eosin stained sections of kidneys from control (G) and *Cdc42* kidney-specific knockout mice (H) at P4 (left) and P6 (right). Bar for kidneys on left in G, H = 200 μm . Bar for kidney sections on right in G, H = 20 μm . (Reproduced with permission from the Journal of American Society of Nephrology).

**FIGURE 5.**

Model for the role of Cdc42 and the exocyst in delivery of ciliary proteins. **(A)** Data support a model in which the exocyst complex is localized to the primary cilium by Cdc42, which is located all along the apical surface, but is activated at the primary cilium by Tuba, a Cdc42 GEF. The exocyst is then stabilized by Sec10 binding directly to the Par complex via Par6. Once the exocyst complex is stabilized at the nascent primary cilium, it then targets and docks vesicles carrying ciliary proteins, by interacting with a small GTPase, Rab8, found on vesicles. **(B)** When Cdc42 (or the exocyst or the PAR complex) is lacking in a cell, the exocyst no longer localizes to primary cilia and increased cell proliferation, apoptosis, and fibrosis occur, with the result being ciliary phenotypes (hydrocephalus, small eyes, tail curvature, and edema in zebrafish; and PKD in mice). (Reproduced with permission from the Journal of American Society of Nephrology).