Primary cilia and kidney injury: current research status and future perspectives

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Submitted 10 July 2013; accepted in final form 26 July 2013

Wang S, Dong Z. Primary cilia and kidney injury: current research status and future perspectives. Am J Physiol Renal Physiol 305: F1085–F1098, 2013. First published July 31, 2013; doi:10.1152/ajprenal.00399.2013.—Cilia, membrane-enclosed organelles protruding from the apical side of cells, can be divided into two classes: motile and primary cilia. During the past decades, motile cilia have been intensively studied. However, it was not until the 1990s that people began to realize the importance of primary cilia as cellular-specific sensors, particularly in kidney tubular epithelial cells. Furthermore, accumulating evidence indicates that primary cilia may be involved in the regulation of cell proliferation, differentiation, apoptosis, and planar cell polarity. Many signaling pathways, such as Wnt, Notch, Hedgehog, and mammalian target of rapamycin, have been located to the primary cilia. Thus primary cilia have been regarded as a hub that integrates signals from the extracellular environment. More importantly, dysfunction of this organelle may contribute to the pathogenesis of a large spectrum of human genetic diseases, named ciliopathies. The significance of primary cilia in acquired human diseases such as hypertension and diabetes has gradually drawn attention. Interestingly, recent reports disclosed that cilia length varies during kidney injury, and shortening of cilia enhances the sensitivity of epithelial cells to injury cues. This review briefly summarizes the current status of cilia research and explores the potential mechanisms of cilia-length changes during kidney injury as well as provides some thoughts to allure more insightful ideas and promotes the further study of primary cilia in the context of kidney injury.

primary cilia; kidney injury; IFT; planar cell polarity; ciliopathy

Cilia or flagella (here used interchangeably) contain nine sets of microtubule doublets arranged in a circular pattern with (9+2) or without (9+0) a central pair of microtubule singlets. They are largely membrane-enclosed organelles that project from the apical surface of cells (133, 185, 187). The majority of cells in the human body have either one or multiple cilia. A single or monolocular cilium in one cell is called the primary or nonmotile cilium (9+0), since it is immotile (for cells with primary cilia, see http://www.bowserlab.org/primarycilia/ciliarylist.html), apart from the exceptions such as motile nodal cilia (9+0) and nonmotile olfactory sensory cilia (9+2) (108, 120, 136). However, epithelial cells in some organs, for instance the respiratory tract and reproductive system, harbor multiple cilia (9+2) on the apical side of cells, which can beat upon stimulation, and therefore are named motile cilia although a chemosensory function has been recently suggested (179). Inside the cilium is the microtubule-based axoneme, in connection with bidirectional microtubule motors and associated protein complexes. Outside the axoneme is the enclosed ciliary membrane, which is generally believed to be specific and different from the rest of the plasma membrane. The structure connected to the cilium at the bottom is the basal body, and it is derived from the mother centriole of the centrosome, which provides a docking site for the cilium and transforms into a centriole during mitosis (Fig. 1). The basal body is structurally different from the daughter centriole, owing to its additional distal and subdistal appendages (79).

It has been known that cilia play pivotal roles in embryo development, cell and tissue homeostasis, and human diseases. Although specialized cilia in the retina and kinocilia in the inner ear have been recognized for their specific roles in photoreception and cell polarization, the functions of primary cilia in humans have been obscure for more than a century. It was not until the observation of expression of the polycystin-1 (PC1) homolog Lov-1 in sensory neurons of Caenorhabditis elegans and the generation of Tg737°rpk mice that researchers began to realize the importance of primary cilia, since Lov-1 is required for C. elegans mating and Tg737°rpk mice unexpectedly die of polycystic kidney disease (PKD) shortly after birth. Importantly, primary cilia in the kidney of mice with the Tg737 mutation are stunted (13, 219). These discoveries, for the first time, link the primary cilium to PKD. Later, Nauli et al. (132, 208) found that dysfunctional primary cilia are responsible for cystogenesis in human autosomal dominant (AD) and recessive (AR) PKD. These studies disclosed that primary cilia in the kidney epithelial cells are potentially the mechanosensors to fluid flow (132, 158, 208). During recent years, studies of primary cilia have been expanded to a spectrum of human
genetic diseases, collectively termed the ciliopathies (61, 83), as well as to a few nongenetic disorders such as kidney injury, obesity, hypertension, and diabetes (125, 172) (Table 1). In addition, cilia have been proposed to function in exocytosis (11) in the ciliary pocket of the flagella and kinetoplastid protozoa (66, 126).

Cilia or flagella have been studied using different model systems. In addition to zebrafish, C. elegans, Xenopus laevis, and Tetrahymena, the most popular model systems are Chlamydomonas (http://labs.umassmed.edu/chlamyfp/index.php) and mammals (http://v3.ciliaproteome.org/cgi-bin/index.php). Indeed, a large body of knowledge was obtained studying Chlamydomonas. In this review, we will discuss the current status of studies of primary cilia and focus on the potential roles of cilia in kidney injury. A series of excellent reviews are available for more information about cilia and flagella (7, 122, 130, 205).

**Ciliogenesis and Intraflagellar Transport**

Ciliogenesis generally occurs in differentiated cells and involves a series of steps from cell cycle exit and mother centriole transformation to basal body to axoneme growth and extension. After cells exit the cell cycle, the mother centriole moves to the apical surface of the cell and acquires a series of components necessary for ciliary budding. A microtubule-based axoneme extends from the microtubule of the mature

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**Table 1. Cilia-associated human diseases and genes**

<table>
<thead>
<tr>
<th>Human Diseases</th>
<th>Genes</th>
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<tr>
<td>Alstrom syndrome</td>
<td>ALMS1</td>
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<tr>
<td>Asphyxiating thoracic dystrophy</td>
<td>IFT80, DYNC2H1, TTC21B, WDR19</td>
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<tr>
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<td>BBS1-15</td>
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<td>Joubert syndrome</td>
<td>TMEM67, 138, 216, 231, 237, CC2D2A, ARL13B, NPHP1, NPHP6/CEP290, INPP5E, AH11, RPGRIP1L, CXORF5, TTC21B, KIF7, TCTN1, CEP41</td>
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<td>DNAH1, DNAH11, DNAH5</td>
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<td>Meckel-Gruber syndrome</td>
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<td>Orofaciodigital syndrome 1</td>
<td>OFD1</td>
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<tr>
<td>Polycystic kidney disease</td>
<td>PKD1, PKD2, PKHD1</td>
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<td>Primary ciliary dyskinesia</td>
<td>DNAH1, 2, DNAH5, TXNDC3, DNAH11, KTU, RSHP4A, 9, LRRCS0, CCDC39, 40, 103; DNAAF3, NPHP1, 4, 5, 6; SDCCAG8</td>
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<td>Sensenbrenner syndrome</td>
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<td>TSC1, TSC2</td>
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<td>VHL</td>
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<td>Kidney injury</td>
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<td>Obesity</td>
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centriole/basal body, and new microtubule units are added to the distal tip by a process called intraflagellar transport (IFT) to lengthen the axoneme (68). When a certain point in its length is reached, an axoneme stops growing and starts maintaining. In most cases, the exit of the cell cycle is correlated with ciliogenesis; however, in hTERT-RPE1 cells, cell spatial confinement seems to be a major regulator of ciliogenesis (157). Convincing experiments showed that the cell cycle per se regulates cilium length (80). During cell proliferation, cillum length fluctuates in parallel with the four phases of the cell cycle (G1, S, G2, and M). In the M phase, cilia are resorbed to facilitate cell division while in the G1, S, and early G2 phases cilia can still be observed (22). The molecular mechanisms underlying ciliogenesis during the cell cycle have begun to emerge. Cdc14b phosphatase, an antagonist of Cdk1, is required for both motile and primary ciliogenesis in zebras in a manner independent of fibroblast growth factor (FGF) (38). Aurora A, a mitotic kinase, induces ciliary disassembly in hTERT-RPE cells (161). Cilia-associated proteins, vice versa, have been found to regulate the cell cycle. A typical example is polycystin-2 (PC2), a transmembrane protein responsible for 15% of cases of patients with ADPKD (189). PC2 has been localized to the primary cilia and is known to regulate the cell cycle (154, 198). Polaris, another protein responsible for ciliary assembly, leads to misorientation of the spindle body during mitosis and hyperproliferative kidney cysts if the normal function is disrupted (49).

Exiting the cell cycle is just the initial step for ciliogenesis. Microtubule formation and posttranslational modifications are all essential. In *Chlamydomonas*, tubulin levels are significantly upregulated after deflagellation (211). Sharma et al. (180) further explored the role of tubulin in mammalian cells and found that soluble cytosolic tubulin regulates cillum length. Ciliary microtubules, consisting of α- and β-tubulins, can undergo a wide range of posttranslational modifications, including acetylation, glutamylation, glycylation, ubiquitination, methylation, and phosphorylation (93). The former three modifications are unique to the tubulin in cilia and flagella (184). It has been known that acetylation is one characteristic of α-tubulin in the cilia but not in the cytoplasm. Therefore, acetylated α-tubulin is regarded as the standard marker of cilia in cell staining. In *Chlamydomonas*, acetylation of α-tubulin is associated with flagellar growth and resorption (104, 105) but does not correlate with ciliary growth in the sea urchin (191). In *Tetrahymena*, mutated glutamylation of β-tubulin leads to abnormal axonemes and lethality (218). CEP41, an evolutionarily conserved polyglutamylase enzyme, is localized to the basal body and primary cilia, and CEP41 was very recently found to be causative of Joubert syndrome, suggesting that tubulin glutamylation is important in the pathogenesis of human ciliary diseases (102). It seems that mammalian cilia function is not dependent on the polymeric state of tubulin glycylation although monomeric glycylation is likely essential (51). TTLL3, a tubulin glycine ligase, appears important for cilia assembly, and in vivo, glutamic acid and glycine ligase oppose each other probably by competition of shared modification sites of tubulin, because in both *Tetrahymena* and zebrafish, deletion of TTLL3 leads to shortened cilia (217). Ubiquitination and methylation and phosphorylation of tubulin do occur in cilia but also in the cytoplasm. In *Chlamydomonas*, tubulin, IC2, and dynein have been found to be abnormally ubiquitinated and methylated during flagellar resorption, implying that these two posttranslational modifications are likely involved in ciliogenesis (74, 176). In addition to the effect of microtubules on ciliogenesis, microtubule tip-associated proteins such as EB1 and EB3 also regulate ciliogenesis, because depletion of these genes leads to a significant decrement of cilia number in different mammalian cells (177).

In addition to the regulation of ciliogenesis by the cell cycle and microtubules, actin dynamics is associated with ciliogenesis. Cytochalasin D and jasplakinolide are two reagents that disrupt polymerization of actin filaments, both of which facilitate the shortening of the primary cilia in different types of cultured cells (20, 180). Bershteyn et al. (20) showed that MIM (Missing-in-Metastasis), an actin-regulatory protein, is required for ciliogenesis at the basal body of mesenchymal cells, and they proposed that MIM promotes ciliogenesis by antagonizing phosphorylation of cortactin. It has been known that cortactin is a monomeric protein and plays an important role in promoting polymerization and rearrangement of the actin cytoskeleton (46). ACTR3, an interaction protein of cortactin identified by functional screening, has been shown to increase the length of primary cilia (89). Filamin A, an actin-binding protein, has been reported to be crucial in ciliogenesis and positioning of the basal body (3), and meckelin, an interaction protein of filamin A, regulates ciliogenesis possibly by affecting the distribution of cytoplasmic stress fibers (47).

IFT has been proven to be an indispensable process for ciliogenesis and is well conserved evolutionally from *C. elegans* to *Chlamydomonas*, and to mammals. Kozminski et al. (97) first described IFT as the bidirectional movement of particles along the axoneme of flagella. Later, the same group found that the IFT process is dependent on a protein called FLA10 (96). Kinesin-II, the homolog of FLA10 in mammals, was identified to be important for both motile and primary cilia (110, 127). IFT is a two-parallel process of anterograde transport toward the tip of the axoneme and retrograde transport toward the base of the cilia. Anterograde transport is performed by the heterotrimeric kinesin-II motor protein complex (Kif3a, Kif3b, Kap), and retrograde transport is facilitated by the motor protein cytoplasmic dynein. Thus far, it has been known that IFT particles contain at least 20 polypeptides which are divided into complex A (IFT43, 121/122h, 122/122a, 139, 140, 144) and B (IFT20, 22, 25, 27, 46, 52, 54, 57/55, 70, 74/72, 80, 81, 88, 172) (40, 62, 81, 145). Proteins in complex A and B are distinct in functions because mutations of complex A proteins generally do not affect cilia assembly while mutations of complex B proteins do (162). The typical example for complex B proteins is IFT88 mutation mice, which demonstrate shortened cilia (153). Compared with complex B proteins, mutation of the complex A protein IFT140 causes PKD but does not completely prevent cilia assembly (84).

### Cilia Maintenance

Once cilia are established, the next step is to maintain them. This process is also performed by IFT because no protein synthesis machinery is found in the cilia. Thus almost all ciliary components are synthesized in the cell body (167). Two models for cillum/flagellum length control have been described (7, 99, 117). The first one is the limiting-precursor model based on the hypothesis that the quantity of precursors or building...
blocks in one cell is limited (99). Obviously, this model cannot explain why after flagella are severed, the residues of the flagella can still regenerate to about half of their normal length (167). Although there might be reserved building blocks, the question is where they are inside the cell. Marshall et al. (117) proposed the balance point model in which it was postulated that there is continuous tubulin unit assembly and disassembly at the tip of the cilium, and which one (assembly or disassembly) predominates depends upon a set point. This means when cilia are shorter than the set point, assembly will exceed the disassembly, and when cilia are longer than the set point, disassembly will be more predominant. The intriguing question then is what determines the balance point.

Many factors (physical, chemical, and biological) have been found to modulate cilium length. The regulators of cilium length can be divided into two classes: intrinsic and extrinsic. Intrinsic factors refer to those initiated by any molecules inside the cell, and extrinsic factors point to those from the extracellular environment, while extrinsic factors most likely regulate cilium length by affecting the intrinsic ones. The intrinsic factors can be subclassified as structural and signaling molecules. Kif3a and Pitchfork are two typical examples of the former molecules. Kintel et al. (90) found that Pitchfork regulates primary cilium disassembly, probably through activating Aurora A. Haploinsufficiency of Pitchfork leads to left-right asymmetry, heart failure, and more importantly, node cilium duplication phenotype. Among the cilium-signaling molecules, calcium and cAMP are two key players in determining cilium length. Besschetnova et al. (21) reported that forskolin and gadolinium can increase cilium length almost twofold in 3 h by activating adenyl cyclase and decreasing intracellular calcium and subsequent PKA activation in mIMCD3, MEK, and bone mesenchymal cells. Three kinase members of the NIMA family (Nek1, 4, 8) have been known to regulate cilium length. Interestingly, loss of Nek1 shortens cilia in mice whereas loss of Nek8 results in excessively long cilia (39, 188, 195).

Researchers have used different approaches to treat cultured cells or animals and then defined the effect on cilium length. Deflection of the primary cilium by fluid shear stress can shorten its length and consequently ameliorate mechanosensitivity, which coincides with the observations seen with mutated ADPKD gene products, PC1 or PC2 (123, 132). Miyoshi et al. (123) showed that lithium elongates primary cilia in the mouse brain and in cultured NIH3T3 and neuronal cells. Simultaneously, Ou et al. (143) independently found that lithium can elongate the primary cilium length in FLS cells, rat PC12 cells, and human astrocytes and suggested that lithium elongates cilium length partially by the inhibition of adenyl cyclase III (ACIII) and reduction of the cAMP level. Ouabain, the inhibitor of Na-K-ATPase, at a concentration of 10 nM, promotes ciliogenesis in an ERK1/2-dependent manner (101). Based on the observation that primary cilium length is increased in osteoarthritis, Wann and Knight (209) tested the effect of interleukin-1 (IL-1) on cilium length and found that fibroblasts and chondrocytes exhibited a significant increment in cilium length after incubation with IL-1. They further identified that this elongation depended upon protein kinase A.

If axoneme structure maintenance is the physical basis for cellular function, ciliary membrane proteins are essential for many signaling pathways. It has been known that a number of receptors and channels are located on the ciliary membrane, including PC1, PC2, and fibrocystin/polyductin (FPC), somatostatin receptor 3, serotonin receptor 5, platelet-derived growth factor receptor-α (PDGFRα), and components of Wnt and Hedgehog and Notch signaling pathways (1, 70, 77, 124, 140, 210). One key question is how these receptors and channels arrive at the cilary membrane.

Three working models have been suggested for ciliary membrane protein trafficking (130). The simplest one is that membrane proteins are transported into the nearby area of the cilium and then fused with the ciliary membrane as occurs with the plasma membrane proteins because the ciliary and plasma membranes are topologically continuous and the ciliary axoneme is connected to the cytoskeleton of the cell body. However, data from a number of experiments do not support this model. For instance, expressed glycosylphosphatidylinositol-fluorescent protein (GPI-FP) is transported to the apical side of the plasma membrane but excluded from an area around the base of the primary cilium in Madin-Darby canine kidney (MDCK) cells, suggesting that there is a special structure at the base of the cilium preventing certain proteins from entering the cilium (206). This observation is further supported by ultrastructural analysis of the ciliary base (173). The second model and probably the most recognized one, is that vesicles containing membrane proteins are transported to the base of the cilium and then, together with the ciliary membrane docking proteins, gradually move to the ciliary membrane (168). This model has been supported by a number of experiments (146, 155). The third model is that membrane proteins first fuse with the plasma membrane and then the proteins move to the ciliary membrane laterally. This model originates from an observation in the Snell lab (78). Additional evidence supporting this model is Smo trafficking to the ciliary membrane (121). Indeed, considering the complexity at the ciliogenesis stage, all these three possibilities may be feasible. The route that the transported protein takes to the ciliary membrane depends on the property of the protein and the stage of ciliogenesis. For instance, at the very early stage, some ciliary membrane proteins may be transported to the preciliary membrane by the first route before the cilium starts to protrude, but disappear from the cilia of well-differentiated cells. At the late stage of ciliogenesis, proteins are transported to the ciliary membrane by the second and third models. Thus proteins on the preciliary membrane may not necessarily be the same ones present after full differentiation of the cell.

**Cilia-Associated Signaling Pathways**

In the past, many studies, particularly on ligand-receptor signaling pathways, have not focused on the cilia. Currently, cilia have been proven to be a hub involved in many signaling pathways relevant to development and human diseases. Thus it is necessary to reexamine these signaling molecules and determine whether and how they function in terms of cilia.

**Calcium signaling.** The association between cilia and calcium has been noticed for decades but has been mainly focused on motile cilia. However, people did not know that calcium can enter into the cell through the primary ciliary membrane of renal tubular epithelial cells until PC2 was localized on this organelle (132, 154). Nauli et al. (132) found that PC1 and PC2 codistribute on the primary cilia where they regulate calcium
signaling through PC2 upon fluid flow. Furthermore, PC2 can regulate calcium signaling through genetic or biochemical interactions with other molecules such as FPC (208), the inositol 1,4,5-trisphosphate receptor (107), CAML (131), and CAMK-II (170). It has been known that PC2 can form a channel protein complex with TRPC1/TRPV4 on the primary cilia (10). Because depletion of TRPV4 abolishes flow-induced calcium transients, TRPV4 was considered to be an essential component of the renal ciliary mechanosensor (94). Clearly, the presence of cilia is required for calcium signaling in ARPKD collecting duct cells (183).

**Wnt signaling.** The Wnt signaling pathway is best known for its significant roles in embryonic development and cancer (15). Nineteen WNT and 10 Frizzled genes have been found in humans, and the encoded Wnt proteins comprise a large group of secreted molecules, i.e., Sonic Hedgehog, Indian hedgehog, and Desert hedgehog. The best studied is Sonic Hedgehog, which was proven to be essential in cell proliferation and embryonic development. Basically, Hedgehog proteins consist of three kinds of secreted molecules, i.e., Sonic Hedgehog, Indian Hedgehog, and Desert Hedgehog. The best studied is Sonic Hedgehog signaling. Sonic Hedgehog binds to the Hedgehog receptor Patched, which relieves the downstream inhibition of Smo. This then activates the Gli transcription factor. Subsequently, activated Gli trafficking to the nucleus regulates the transcription of Hedgehog target genes.

As early as 2003, the Hedgehog signaling pathway was connected to the cilia. Huangfu et al. (76) performed genetic screening and identified that Wimple, Polaris, and Kif3a are required for Hedgehog signaling in mice, and the Wimple gene was shown to be IFT172 (67). Subsequent experiments done by many groups showed that key Hedgehog components (Patched1, Smo, Gli, and Sufu) are all enriched in the cilia and/or basal body (44, 72, 166) (Fig. 3). In the absence of Hedgehog molecules, Gli proteins are inhibited by the cytoplasmic form of SuFu (Suppressor of Fused). With Hedgehog stimulation, the Gli-SuFu protein complex is quickly recruited to the cilia proximal to Smo and Gli proteins are released to enter into the nucleus to activate certain genes (199). Furthermore, Sufu controls the protein levels of Gli by antagonizing the activity of Spop, a conserved Gli-degrading factor. Interestingly, regulation of Gli by SuFu is cilia independent (36). In mammalian cells, Smo translocation to the primary cilia is necessary for Smo-dependent signaling. With knockdown of Arrestin, it was found that Smo failed to traffic to the primary cilia and Smo-dependent activation of Gli was prevented, suggesting that Arrestin plays a significant role in Hedgehog signaling (95). Very recently, IFT25 was also shown to be essential for movement of Hedgehog components (88). Interestingly, the IFT80 trap mouse exhibits short rib polydactyly syndrome and abnormality of Hedgehog signaling without malformation of cilia (165).

![Fig. 2. Wnt signaling pathway in the primary cilia. Binding of Wnt to Frizzled3 stabilizes β-catenin, which translocates to the nucleus and activates target genes with TCF/LEF by the canonical pathway. Instead of β-catenin, Dishevelled2 transmits signals to small GTPase or inositol 1,4,5-trisphosphate (IP3) receptor by the noncanonical pathway, which regulates the cytoskeleton and calcium respectively.](http://ajprenal.physiology.org/)
SuFu and Spop are all involved in Gli trafficking and stability. The activation of active form of Gli, which enters the nucleus to regulate target genes, is facilitated by binding to Patched1, Smo is activated and subsequently facilitates the formation of cilia. After Hedgehog signaling, Smo is suppressed by an unclear mechanism. It has been reported that primary cilia regulate mTORC1 activity by Lkb1 (25). Indeed, the most intensive studies about primary cilia and mTOR in the kidney field lie in the PKD-associated proteins (212). It was found that the loss of primary cilia is involved in cystogenesis and kidney hypertrophy signaling (17), and PC1 suppresses mTOR activity through regulation of Tuberin localization (50). Furthermore, mTOR inhibitors are effective for the treatment of PKD in animal models (109). Very recently, CCDC28B, a Bardet-Biedl syndrome-related protein, was reported to interact with SIN1 and modulate mTORC2 function (32).

Others. The evidence from the zebrafish study raised the possibility that FGF signaling regulates cilium length, since knockdown of Fgfr1 causes short cilia in Kupffer’s vesicles (134). Christensen et al. (37) summarized the relationship of primary cilia and receptor tyrosine kinases. PDGFRα has previously been located to the primary cilia, and in growth-arrested fibroblasts primary cilium coordinate PDGFRα-mediated cell migration (174). Furthermore, NHE1 is required for cell migration stimulated with PDGFRα (175).

**Role of Cilia in Cell Polarity and Planar Cell Polarity**

Recent studies have shown that cilia play important roles in cell and planar cell polarity (PCP) and left-right asymmetry (73). In epithelial cells, there are three major cell polarity protein complexes, i.e., Polarity protein (Par), Crumbs, and Scribble (29). The former two complexes define the apical polarity of the cell, and the latter one functions at the basolateral surface. Pars have been recognized as the fundamental players in animal cell polarity, in coordination with atypical protein kinase C (PKC) and CDC42. Fan et al. (55) reported that Par3, Par6, Crumbs3, atypical PKCδ, and 14-3-3δ are all localized in the primary cilium of MDCK and IMCD3 cells by immunostaining and GFP-tagged target protein expression. They also found that Crumbs3, atypical PKCδ, and 14-3-3δ are all required for ciliogenesis. Furthermore, they showed that Crumbs3 regulates ciliogenesis by an interaction with Importin β (54). These findings provided convincing evidence of cellular polarity roles in ciliogenesis.

Unlike single-cell polarity, PCP involves a complex coordination of a group of cells. The best studied organism is *Drosophila*, from which many signaling pathways, such as Wnt, Hedgehog, Notch, and small GTPases, were elucidated and found to regulate PCP (41, 57, 58, 87). Recent work regarding cilia and PCP in cystic kidney diseases has drawn much attention (60, 113, 150). By using Pck rats and HNF1α-null mice, Fischer et al. (60) found a distorted mitotic orientation before the onset of cystogenesis in the kidney and suggested that PCP is responsible for PKD. This discovery was further confirmed by two other groups (113, 150). In one study, Patel et al. (150) examined tubular regeneration in Kif3a mutant mice by inducing acute kidney injury and found that the loss of cilia does not promote cell proliferation but causes aberrant PCP in the precryptic tubules. They concluded that primary cilia are essential for the maintenance of PCP, and cystic kidney disease is exacerbated by acute kidney injury.
Another study by Luyten et al. (113) is in line with the observation of aberrant regulation of PCP in PKD. It is worth noting that Nishio et al. (135) did not find that precystic tubular cells in Pkd1 and Pkd2 knockout mice lost all orientation division but found that distorted orientation of cells in Pkhd1 mice occurred, which did not develop into kidney cysts. We do not know exactly what causes this difference, but different animal models are one factor to be considered.

Inversin, a protein mutated in nephronophthisis type II, has been regarded to play a molecular switch role between canonical and noncanonical Wnt signaling (182). The researchers found that inversin degrades cytoplasmic Dishevelled to inhibit the canonical Wnt pathway and is necessary for convergent extension in X. laevis embryos, which is regulated by noncanonical Wnt signaling. Furthermore, in Xenopus, inversin suppresses Dishevelled-induced axis duplication. Ross et al. (169) recently knocked out BBS protein in mice and found disrupted cochlear stereociliary bundles. They further provided evidence showing the genetic interaction between the PCP gene Vangl2 and the BBS genes. All these findings suggest that cilia are involved in PCP signaling. In a special form of PCP, i.e., left-right asymmetry, nodal cilia have been studied in detail (9, 69). Many proteins have been localized to the nodal cilia, and mutant mice exhibit left-right patterning defects (120). Interestingly, in mice with mutations of a few of the PCP genes, such as Fz3/6, Vangl1, Vangl2, and Dvl1/2, ciliogenesis seems normal (85, 190).

### Cilia and Kidney Injury

The earliest report linking kidney injury and cilia is, to our knowledge, the study by Verghese et al. (203). By studying kidney injury in mice, the average length of renal cilia in the proximal tubule decreased at days 1 and 2 (~3 μm) compared with the control (~4 μm). During the kidney repair stage at days 4 and 7, the average length of cilia increased in both proximal (~6 μm day 7) and distal tubule/collection duct (~5.5 μm day 7). In a unilateral ureteral obstruction model, at day 8 cilia length in the distal tubule/collection duct was also lengthened. Thus it was proposed that cilia may play important roles in the regulation and repair of kidney injury. Verghese et al. (202, 203) have studied cilia length of kidney tubular epithelial cells in both humans and mice. After ischemia-reperfusion injury in mice, the average length of renal cilia in the proximal tubule decreased at days 1 and 2 (~3 μm) compared with the control (~4 μm). During the kidney repair stage at days 4 and 7, the average length of cilia increased in both proximal (~6 μm day 7) and distal tubule/collection duct (~5.5 μm day 7).

### Table 2. Cilia formation and maintenance-associated factors

<table>
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<tr>
<th>Factors (References)</th>
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- ACIII: AKAP12; CEP135: CEP135; Dcn: Dishevelled; Dvl: Dishevelled; Fz: Frizzled; Klp: Kinesin-like protein; Nek: Nekat; RPI: Regulator of motility 1; Tct: Tectonic; TCTN: Tectonic; TTIIL3: TTB1; VDAC: Voltage-dependent anion-selective channel.
roles in sensing environmental cues caused by injury and in the repair process for reestablishing a new epithelial layer of differentiated cells. The finding that cilium length was lengthened in the recovery stage was further confirmed in human renal transplants suffering from acute tubular necrosis. By using series biopsies of human renal transplants, it was found that acute tubular necrosis caused more than twofold longer cilium 1 wk after kidney injury, and normalization of cilium length occurred at a late stage. These results indicate that cilium length could be a clinically relevant indicator of kidney injury and repair in patients with kidney transplantation. To further investigate the mechanisms, cultured MDCK cells were treated with bovine serum albumin, cobalt chloride, and tumor necrosis factor-α. Cilium length was only increased in cells treated with cobalt chloride. Because cobalt chloride is a chemical inducer of hypoxia-inducible factor 1α (HIF-1α), HIF-1α may be a regulator of cilium length following renal injury (204). However, data from Lutz and Burk (112) using renal-derived cells did not support this hypothesis. Other indirect evidence is from a study in murine models of PKD and normal ischemic kidneys, in which HIF-1α is upregulated (16, 52). The function of HIF-1α in kidney injury-associated cilium length change is yet to be determined.

The mechanisms for cilium length regulation are probably not the same during early and late phases of kidney injury: cilia retract in the injury phase, while they elongate in the repair phase and then gradually return to normal. It is not surprising that at the early stage of kidney injury, cilia are shortened especially after exposure to different sorts of toxic substances such as ochratoxin A and cisplatin although the mechanism for cilia resorption remains unsolved (163, 207). Many signaling pathways, such as MAPK, p53, reactive oxygen species, NF-kB, AMPK, mTOR, and Lkb1, and many key molecules, such as ATP, interleukins, TNF-α and -β, toll-like receptor 2 and 4, heme oxygenase, and heat shock proteins, are all involved in kidney injury and should be the candidates responsible for cilium resorption. Indeed, some of them have been found to regulate primary cilia (Table 2). For instance, it has been reported that heat shock protein 90 and HDAC6 coordinate regulation cilium resorption in response to extracellular stress (160). In addition, urine flow blockage, augmentation of extracellular pressure in the urogenital tract, cell death, and de-differentiation may also be involved in cilia shortening although which is the earliest event is unknown. Takakura et al. (193) reported the sustained activation of STAT3 in ischemic-injured and uninjured Pkd1 knockout polycystic kidneys and in human ADPKD kidneys, and Olsan et al. (138) found the role of STAT6 in renal cystogenesis. These two studies suggest that the STAT signaling pathway may be involved in cilium length regulation upon kidney injury. One interesting report is from the study of kidney injury in Kif3a knockout mice, in which PCP was abnormal before cystogenesis (150). We know that these Kif3a knockout mice harbor very short cilia in the kidney epithelial cells and that Wnt signaling plays a significant role in PCP. However, the question remains as to how short cilium affect PCP through cell polarity and PCP molecules. The elongation of cilia during tubular cell regeneration may not only increase the ability of cilia to sense extracellular cues but may also facilitate the secretion of metabolic wastes from cilia, which are generated during the kidney injury stage. Indeed, cilia have been regarded as a secretary organelle (11). We (207) and others (2)

very recently found that Erk1/2 regulates cilia length in renal tubular cells and endothelial cells. The relationship between cilia and many more kidney injury-associated molecules needs to be further elucidated.

Conclusions and Perspectives

Taken together, we conclude that the primary cilium is an important organelle responsible for integrative signaling from outside cues to normal physiological functions of cells. Mutations of ciliary proteins cause different kinds of human diseases displaying a diversity of clinical features. Probably a larger spectrum of human ciliopathic diseases is related to primary cilia than expected originally.

However, a large number of questions remain to be answered. One basic question is why a majority of, but not all, cells grow cilia. Although different approaches have been used to study the composition of cilia or flagella in different model organisms (8, 12, 23, 141, 152), we have just begun to know the composition of primary cilium (82). With regard to cilia and kidney injury, the key question is the role of primary cilium during kidney injury and recovery stages. Once the detailed mechanisms are determined, we can design different strategies to interfere with the process of kidney injury recovery in animal models by regulating cilia-associated signaling pathways. It is expected that studies on cilia and kidney injury will shed light on identifying novel mechanisms that can be translated into clinical treatment.

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