Current status and future perspectives: primary cilia and kidney injury

Shixuan Wang 1 and Zheng Dong 1,2,*

1 Department of Cellular Biology and Anatomy, Medical College of Georgia, Georgia Regents University and Charlie Norwood VA Medical Center, Augusta, GA 30912; 2 Department of Nephrology, The Second Xiangya Hospital, Central South University, Hunan, China

* Correspondence should be sent to:

Zheng Dong, PhD

Department of Nephrology, The Second Xiangya Hospital, Central South University, Hunan, China; Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912. Email: zdong@gru.edu Phone: 706-721-2825 Fax: 706-721-6120
Abstract

Cilia, membrane-enclosed organelles protruding from the apical side of the 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 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 mTOR, 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 in order to allure more insightful ideas and promotes the further study of primary cilia in the context of kidney injury.

Keywords

Primary cilia; Kidney injury; IFT; Planar cell polarity; Ciliopathy.
Introduction

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 mono-cilium in one cell is called the primary or non-motile cilium (9+0) since it is immotile (for cells with primary cilia, see http://www.bowserlab.org/primarycilia/cilialist.html), apart from the exceptions such as motile nodal cilia (9+0) and non-motile 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 is derived from the mother centriole of the centrosome, which provides a docking site for the cilium and transforms to centriole during mitosis (Figure 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) homologue Lov-1 in sensory neurons of
*C. elegans* and the generation of *Tg737orp* mice that researchers began to realize the importance
of primary cilia, since Lov-1 is required for *C. elegans* mating and *Tg737orp* mice unexpectedly
die of polycystic kidney disease (PKD) shortly after birth. Importantly, primary cilia in the
kidney of mice with *Tg737* mutation are stunted (13, 219). These discoveries, for the first time,
link the primary cilia 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 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 for 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 \textit{per se} regulates cilium length (80). During cell proliferation, cilium length fluctuates in parallel with the four phases of the cell cycle (G1, S, G2, and M). In 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 zebrafish 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, \textit{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 mis-orientation of the spindle body during mitosis and hyper-proliferative 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 up-regulated after deflagellation (211). Sharma *et al.* (180) further explored the role of tubulin in mammalian cells and found that soluble cytosolic tubulin regulates cilium 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 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 (100, 101), but does not correlate with ciliary growth in 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 (104). 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 lengthening 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 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 towards the tip of the
axoneme and retrograde transport towards the base of the cilia. Anterograde transport is performed by the heterotrimeric kinesin-II motor protein complex (Kif3a, Kif3b, Kap) and retrograde 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, 121/122b, 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 the IFT88 mutation mice, which demonstrate shortened cilia (153). In comparison to 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 cilium/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 residue of flagella can still regenerate to about half of its 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 cilia, 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: the intrinsic and extrinsic. The intrinsic factors refer to those initiated by any molecules inside the cell and the 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 sub-classified as structural and signaling molecules. Kif3a and Pitchfork are two typical examples for the former molecules. Kinzel 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 cilia 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 the cilium length almost two-fold in three hours by activating adenylyl 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 cillum 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 adenylyl cyclase III (ACIII) and reduction
of cAMP level. Ouabain, the inhibitor of Na-K ATPase, at a concentration of 10nM, promotes
ciliogenesis in an ERK1/2-dependent manner (103). 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 ciliary 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
with 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 cilia in MDCK cells, suggesting that there is a special structure at the base of the cillum preventing certain proteins from entering the cillum (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 cillum 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 pre-ciliary membrane by the first route before the cillum 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 pre-ciliary 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 re-examine 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 co-distribute 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), 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 ten Frizzled genes have been found in humans, and the encoded Wnt proteins comprise a large group of secreted proteins with ~40 kDa in size (31). Based upon the effect pattern, the Wnt signaling pathway can be classified into the canonical and non-canonical types. The canonical signaling pathway exerts its roles via Wnt-Frizzled-Dishevelled-β-catenin-TCF/LEF while the non-canonical signaling pathway functions through either Wnt-Frizzled-Dishevelled-small GTPase-cytoskeleton or Wnt-Frizzled-Dishevelled-IP3-calcium (15).
In the canonical pathway, binding of Wnt proteins to Frizzleds induces phosphorylation of Dishevelled, which prevents β-catenin from phosphorylation by either GSK-3 and/or casein kinase 1. Consequently, stabilized β-catenin triggers downstream effects by translocation to the nucleus and complexes with LEF/TCF (Figure 2). In the non-canonical pathway, instead of β-catenin, Dishevelled transmits its signals to downstream protein targets and then regulates small GTPases such as Rho, Rac, and Cdc42, or IP3/calcium (Figure 2).

Several lines of evidence have revealed that the Wnt signaling pathway is coupled to cilia. Probably the earliest report about cilia and Wnt is from the study of Inversin, mutations of which lead to both NPHP and situs inversus (142). It has been identified that several Wnt signaling members (Frizzled3, Dishevelled2, adenomatous polyposis coli (APC), β-catenin, GSK-3β, Vangl2) are located to the cilia, suggesting that both canonical and non-canonical Wnt signaling cascades can occur in the ciliary area (113, 148, 169, 196). Mice with knockout of a few ciliary proteins (Kif3a, Tg737, BBS1, 4, 6) showed abnormal β-catenin level and the dysfunctional canonical Wnt responses (45, 64, 110). The PCP effector proteins Inturned and Fuzzy function importantly in cilia formation and orientation and apical actin assembly (147, 222). All these experiments point to the association of cilia and Wnt signaling. However, it is unknown why the IFT88 mutation in zebrafish displays no cilia but normal Wnt signaling (75).

**Hedgehog signaling**

Hedgehog signaling was originally identified by Nusslein-Volhard and Wieschaus and later was proven to be essential in cell proliferation and embryo development (137). 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 cilium. 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). 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 cilium-
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 traffick
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, IFT80 trap mouse exhibits
short rib polydactyly syndrome and abnormality of hedgehog signaling without malformation of
cilia (165).

Notch signaling

Notch signaling is best known for cell-cell communications. In mammals Notch refers to four
different kinds of receptors, from Notch1 to Notch4. The Notch receptor is a single
transmembrane protein containing a large extracellular portion associated with calcium, a single
membrane pass, and a short intracellular fragment. Notch protein can undergo a series of cleavages upon binding to its ligands. One of the cleaved forms enters into the nucleus to regulate gene expression. It has been reported that the Notch signaling pathway regulates left-right asymmetry by cilium length control and plays a key role in cilium length adjustment (98, 111). Lopes et al. (111) found that in deltaD zebrafish mutants, the cilium length in Kupffer’s vesicle is decreased but can be restored by Foxj1a, while cilium length increases if Notch signaling is overactivated. In Tg737 and Kif3a mutant embryos, there are defects of Notch signaling and cell differentiation that can be rescued by activated Notch. Furthermore, Notch receptors are located in cilia. All these findings suggest the roles of primary cilia in the nexus of signaling, proliferation, and differentiation (53). Apart from the primary cilia, Notch signaling regulates multiple cilia formation and controls the balance of ciliated and secretory cell fates in developing airways (116, 197).

mTOR signaling

mTOR is the mammalian target of rapamycin identified in 1994 by Sabatini et al. (171). mTOR is a serine/threonine protein kinase that is responsible for a number of cellular functions such as cell growth, proliferation, and survival. By binding to different protein partners, mTOR forms a rapamycin-sensitive (mTORC1) and –insensitive (mTORC2) complexes. mTORC1 regulates transcription and protein synthesis through downstream S6 kinase and 4EBP1 and mTORC2 exerts its roles by Akt, Rac1, protein kinase C, and cytoskeleton (114). The detailed subcellular localization of mTOR family members is largely unclear except that Hamartin (TSC1) was localized to the basal body of the primary cilia (71). It was reported that primary cilia regulate mTORC1 activity by Lkb1 (25). Indeed, the most intensive studies about primary cilia and mTOR in kidney field lie in the PKD-associated proteins (212). It was found that 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 cillum 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 cilia 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 PKCξ, and 14-3-3η are all localized in the primary cilia of MDCK and IMCD3 cells by immunostaining and GFP-tagged target protein expression. They also found that Crumbs3, atypical PKCξ, and 14-3-3η 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, 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 rat and HNF1β-deficient mice, Fischer et al (60) found a distorted mitotic orientation prior to 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 precystic 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. Please note that Nishio et al. (135) did not find that precystic tubular cells in Pkd1 and Pkd2 knockout mice lost oriented 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 non-canonical Wnt signaling (182). The researchers found that Inversin degrades cytoplasmic Dishevelled to inhibit the canonical Wnt pathway and is necessary for convergent extension in *Xenopus laevis* embryos which is regulated by non-canonical 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 the relationship between kidney injury and cystogenesis in murine Pkd1 model, Takakura et al. (192) found that inactivation of Pkd1 in adult kidney increased cellular susceptibility to ischemic injury, which promoted cystogenesis although the cilium length in Pkd1 mutant mice was unknown. The contribution of kidney injury to cystogenesis was also confirmed in a Pkd1 haploinsufficiency model by Bastos et al. (14) and in Pkd2 heterozygous kidneys in which more neutrophils and macrophages were detected, followed by interstitial inflammation and fibrosis (159). Zhou et al. (224) further explored the mechanism of cystogenesis underlying kidney injury by use of Cys1^{cpk/cpk} mice. They found that induction of heme oxygenase (HO) ameliorated both kidney injury and cystogenesis while inhibition of HO enhanced cystogenesis. Furthermore, component 3 strongly correlated with cystogenesis. All these findings confirmed that kidney injury does play a certain role in cystogenesis, although insightful detailed mechanisms are not fully clear.
Since cilium length regulation and kidney injury bear some common characteristics in cell proliferation, differentiation, and cell death, it would be intriguing to investigate the functional roles of cilia in injured kidneys. Verghese et al. (202, 203) have studied cilium length of kidney tubular epithelial cells in both humans and mice. After ischemia-reperfusion kidney injury in mice, the average length of renal cilia in the proximal tubule decreased at day 1 and 2 (~3 µm) as compared to the control (~4 µm). During the kidney repair stage at day 4 and 7, the average length of cilia increased in both proximal (~6 µm day 7) and distal tubule/collecting duct (~5.5 µm day 7). In a unilateral ureteral obstruction model, at day 8 cilium length in the distal tubule/collecting duct was also lengthened. Thus, it was proposed that cilia may play important roles in sensing environmental cues caused by injury and in the repair process for re-establishing 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 two-fold longer cilia one week 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 up-regulated (16, 52). The function of HIF-1α in kidney injury-associated cilia length change is yet to be determined.

The mechanisms for cilia 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-κB, 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 cilia 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 coordinately regulate cilia 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 one 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 cyst growth. These two studies suggest that the STAT signaling pathway may be involved in cilia 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. But the question remains as to how short cilia 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 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 ciliopathy 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 cilia (82). With regard to cilia and kidney injury, the key question is the role of primary cilia during kidney injury and recovery stages. Once the detailed mechanisms are determined, we can design different strategies to interfere in 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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References


Figure legends

Figure 1. Left, anti-acetylated tubulin stained primary cilia of human proximal tubular epithelial cells (HK-2). DAPI, 4',6-diamidino-2-phenylindole. Right, basic structure of primary cilia.

Figure 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 IP3 receptor by the non-canonical pathway, which regulates the cytoskeleton and calcium respectively.

Figure 3. Hedgehog signaling pathway in the primary cilia. In the absence of Hedgehog, Smo is suppressed by an unclear mechanism. After Hedgehog binding to Patched1, Smo is activated and subsequently facilitates the formation of active form of Gli, which enters the nucleus to regulate target genes. SuFu and Spop are all involved in Gli trafficking and stability.
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Figure 1
Figure 2

Cilium

Ciliary membrane

Dishevelled2

Vangl2

Frizzled3

APC

GSK-3β

β-catenin

small GTPase

Plasma membrane

Basal body

ER

IP3R

Ca

Cytoskeleton

TCF/LEF

β-catenin

Nucleus