Wnt Signaling in Polycystic Kidney Disease

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Wnt signaling cascades activate morphogenetic programs that range from cell migration and proliferation to cell fate determination and stem cell renewal. These pathways enable cells to translate environmental cues into the complex cellular programs that are needed to organize tissues and build organs. Wnt signaling is essential for renal development; however, the specific molecular underpinnings involved are poorly understood. Recent research has revealed an unexpected intersection between Wnt signaling and polycystic kidney disease. Some polycystic kidney disease proteins, such as Inversin and Bardet-Biedl syndrome family members, were found to use components of the Wnt signaling cascade to orient cells along a secondary polarity axis within the plane of the epithelium. These spatial cues may be needed to position nascent tubules with a defined geometry.

β-Catenin–Dependent (canonical) Wnt Signaling

Canonical Wnt signaling requires Frizzled (Fz) and LDL-related protein (LRP) co-receptors (Figure 1). Direct binding of Wnt to a cysteine-rich domain has been shown for several Fz receptors, including Drosophila Fz1/Fz2 and mouse Fz8 (reviewed in Cadigan and Liu [1]). Fz induces phosphorylation of Dishevelled (Dsh), which inhibits the APC–Axin–glycogen synthase kinase 3 complex from phosphorylating β-catenin, which translocates to the nucleus and complexes with transcription factors of the LEF/TCF family to activate gene expression.

β-Catenin–Independent (Noncanonical) Wnt Signaling

The noncanonical Wnt signaling pathway is β-catenin independent but shares several components (e.g., Fz, Dsh) with the canonical pathway. The noncanonical pathway has been divided into several distinct branches, including the planar cell polarity (PCP) and the Wnt/calcium pathway (reviewed in Veeman et al. [14]). This review focuses on the PCP pathway that has been best studied in flies but is increasingly recognized to play a critical role in vertebrate organogenesis (reviewed in references 15–17). There are three classes of PCP signaling molecules: The upstream Fat/Dachsous (Ds) PCP proteins, the PCP core proteins, and the downstream PCP effectors (Figure...
2). Ds is expressed in a gradient in the *Drosophila* eye and wing, whereas Fat shows a graded activity and is expressed uniformly in most tissues. These two proteins have therefore been suggested to provide a long-range “upstream” signal for the core PCP proteins (18). By an unknown mechanism, the signal triggers the asymmetric distribution of PCP core proteins that include Fz, Dsh, and Dgo. At least in the *Drosophila* wing, this means that Fz, Dsh, and Dgo move to one side of the cell (distal), whereas Pk and Stbm accumulate at the proximal plasma membrane. Fmi is found on both the anterior and the posterior plasma membrane, engaging in homophilic interactions. PCP effectors such as Inturned (In), Fuzzy (Fy), and RhoA then reorganize the cytoskeleton to ensure proper cell morphogenesis. In some instances (e.g., in the *Drosophila* eye), the morphogenetic processes also involve PCP-dependent gene expression mediated by the c-Jun N-terminal kinase (JNK) family of transcription factors. The resulting effects on tissue architecture can vary, ranging from orientation of wing hairs to ommatidial rotation to regulation of mitotic spindle orientation in the fly. In vertebrates, analogous pathways regulate many aspects of development, including convergent extension and neural tube closure, inner ear development, hair orientation in mammals, and the formation of cilia (15). It has to be pointed out, however, that there are differences between vertebrate and invertebrate cell adhesions, seem to integrate extracellular cues to control morphogenesis and tissue homeostasis (76,77).

Figure 1. Canonical Wnt signaling. Canonical Wnt signaling induces the stabilization of β-catenin. In the absence of Wnt signaling, β-catenin forms a complex with Axin and APC, is phosphorylated by glycogen synthase kinase 3β (GSK-3β) and casein kinase 1 (CK1), and is targeted for proteasomal degradation by the ubiquitin-ligase transductin repeat-containing protein (β-TrCP). Wnt molecules seem to trigger the association of Frizzled (Fz), a seven-membrane–spanning receptor with LDL-related protein 5 (LRP5) or LRP6. This complex formation results in recruitment and phosphorylation of Dishevelled (Dsh), which physically interacts with the C-terminal domain of Fz. Wnt-induced phosphorylation of the C-terminal domain of LRP receptors by GSK-3β and CK1 recruits Axin to the plasma membrane, preventing the degradation of β-catenin; the microtubule actin cross-linking factor 1 (MACF1) is required for translocation of Axin to LRP5/6 (75). Together with transcription factors of the LEF/TCF family, β-catenin activates target genes of the canonical Wnt signaling pathway. Catenins, which can also be released from cadherin-based cell adhesions, seem to integrate extracellular cues to control morphogenesis and tissue homeostasis (76,77).
Renal Development

Canonical Wnt signaling induces transcriptional changes that direct proliferation, stem cell renewal, and establishment of apical-basolateral cell polarity. Noncanonical Wnt signaling regulates a broad array of morphogenetic cell and tissue functions, including convergent extension and directed cell division. Although it remains unclear how Wnt-directed signaling programs specify cell lineage, patterning, and growth of tissue that compose kidney embryogenesis, genetic evidence clearly establishes a role for Wnt molecules in renal development. The mammalian kidney originates from mesodermal tissue that lies ventral to the paraxial mesoderm and dorsal to intermediate mesoderm (reviewed by Perantoni and by Dressler [23,24]). The intermediate mesoderm forms the pronephric duct, an epithelial tube that extends caudally until it reaches the cloaca. Shortly before it reaches the cloaca, the pronephric duct (primary nephric duct) develops an outgrowth, the ureteric bud (UB), which invades the surrounding metanephric mesenchyme (MM). The basic principle of renal development is the reciprocal interaction between two tissues, the UB and the MM. The transcription factors Pax-2, Pax-8, and Lim-1 control expression of the GDNF receptors c-ret/Gfrα1, allowing the pronephric duct to respond to mesenchymal GDNF. GDNF, regulated by a complex network of positive and negative stimuli (e.g., fibroblast growth factor-7 [FGF-7], FGF-10, retinoid acid), triggers the outgrowth of the UB. GDNF activates at least three signaling pathways that are essential for UB branching: The Ras/extracellular signal–regulated kinase, phosphatidylinositol 3-kinase/AKT, and the PLC-γ pathway. The subsequent branching of the UB is contingent on several transcription factors (Hox11, WT1, Emx-2, and Sall1) that act at least in part through regulation of GDNF expression. A comprehensive review by Costantini (25) summarized the current knowledge of the cellular mechanisms that drive branching morphogenesis of the UB. During mouse embryogenesis, the UB rapidly branches in the MM during embryonic day 11.5 (E11.5) to 15.5, followed by a slower phase of branching shortly before birth. From E15.5 until birth, the UB extensively elongates, giving rise to the long unbranched collecting ducts of the outer medulla (26). A similar elongation process flanked by (rapid) UB branching has been reported for the development of the human kidney. Branching UB cells extend the lumen of the UB while maintaining their polarity (27). Localized cell proliferation seems to transform the rounded UB ampulla to a branched structure, perhaps supported by migration of epithelial cells toward the forming branch poles. Several growth factors are known to regulate growth and branching. FGF-stimulated cell migration was recently described for the branching morphogenesis of the Drosofila air sac (28). GDNF stimulates chemotaxis of cultured tubular epithelial cells in vitro; however, redirecting GDNF expression from the mesenchyme to the UB is sufficient to promote nearly normal kidney development, arguing against a significant role of GDNF in chemotaxis and UB guidance.
Wnt Signaling in Renal Development

The first demonstration that Wnt are involved in mesenchymal-to-epithelial transition came from Wnt4-deficient mice. These mice experience renal agenesis because they cannot undergo mesenchymal-to-epithelial transition (31). Later it was shown that another Wnt, Wnt9b, functions even earlier than Wnt4. Wnt9b is expressed in the UB; it is essential for the early inductive response of the MM, and causes condensation of mesenchymal cells (32). Wnt4 is not required for the condensation of mesenchymal cells, but is essential for their subsequent conversion to a polarized epithelium. It is thought that these Wnt act via the canonical Wnt pathways. This is supported by the fact that lithium, which inhibits glycogen synthase kinase-3 (GSK3), blocks canonical but facilitates noncanonical Wnt signaling (43, 44). Inversin targets cytoplasmic Dsh for ubiquitin-dependent degradation and blocks canonical Wnt signaling. However, membrane-associated Dsh is spared, and both Inversin and Dsh co-localize to the plasma membrane of polarized tubular epithelial cells. Inversin is required for C/E movements during Xenopus gastrulation, suggesting that Inversin facilitates noncanonical Wnt signaling by interacting with membrane-associated Dsh. Containing N-terminal ankryrin repeats, Inversin shares its domain architecture with Diversin, the mammalian homologue of Dgo. Diversin blocks canonical but facilitates noncanonical Wnt signaling (45, 46), and both Diversin and Dgo interact with Pk and Stbm, providing evidence for a PCP multiprotein complex (15, 43, 46). Knockdown of Inversin in zebrafish causes pronephric cysts. This phenotype is rescued by Diversin (43), further supporting a role for noncanonical PCP signaling during normal renal development. Additional evidence for this hypothesis comes from the genetic interaction between Bardet-Biedl syndrome (BBS) proteins and the PCP core protein Vangl2. BBS is a rare pleiotropic disorder that is characterized by multiple symptoms, including obesity, mental retardation, retinal degeneration, and cystic kidneys (reviewed in Blacque and Leroux [47]). BBS1, BBS4, and BBS6 (Δ−/−) mice display abnormalities that are consistent with abnormal PCP signaling, including incomplete neural tube closure and disrupted cochlear stereociliary bundles; these phenotypic changes are enhanced by Vangl2 mutations (48). Although BBS members belong to a diverse group of proteins, they localize to the cilium/basal body complex (CBC) and seem to participate in microtubule-dependent intracellular trafficking. BBS4 interacts with p150Glucl, a subunit of dynactin that links dynactin with the dynein motor protein, and mutations of all BBS proteins seem to affect the dynein-driven, microtubular transport (49). Although it has been hypothesized that BBS proteins target PCP proteins to the epithelial nephrons (37). Because Wnts can activate JNK in renal tissues (38), PCP-dependent JNK activation may be necessary to maintain the integrity of the developing nephron.

Wnt Signaling in PKD

Efforts to uncover the pathogenesis of PKD have provided novel insight into the cellular mechanisms that are required for normal renal embryogenesis. Ectopic activation of the Wnt signaling cascade is associated with PKD. For example, targeting of constitutively active β-catenin to the kidney of transgenic mice results in severe polycystic disease that affects all segments of the nephron (39). A similar phenotype ensues when the degradation of β-catenin is prevented by deletion of APC (40). Abnormal β-catenin levels have also been reported in KIF3A-deficient tubulat epithelial cells, suggesting that mislocalization of the polycystin complex and/or the absence of cilia results in dysregulation of β-catenin–dependent signaling (41). Whereas canonical Wnt signaling seems mandatory for early renal development, persistent β-catenin signaling seems to trigger cyst formation at later developmental stages. Inversin, a protein that is mutated in nephronophthisis type II (reviewed by Hildebrandt and Otto [42]), seems to facilitate a switch from canonical to noncanonical Wnt signaling (43, 44). Inversin targets cytoplasmic Dsh for ubiquitin-dependent degradation and blocks canonical Wnt signaling. However, membrane-associated Dsh is spared, and both Inversin and Dsh co-localize to the plasma membrane of polarized tubular epithelial cells. Inversin is required for C/E movements during Xenopus gastrulation, suggesting that Inversin facilitates noncanonical Wnt signaling by interacting with membrane-associated Dsh. Containing N-terminal ankryrin repeats, Inversin shares its domain architecture with Diversin, the mammalian homologue of Dgo. Diversin blocks canonical but facilitates noncanonical Wnt signaling (45, 46), and both Diversin and Dgo interact with Pk and Stbm, providing evidence for a PCP multiprotein complex (15, 43, 46). Knockdown of Inversin in zebrafish causes pronephric cysts. This phenotype is rescued by Diversin (43), further supporting a role for noncanonical PCP signaling during normal renal development. Additional evidence for this hypothesis comes from the genetic interaction between Bardet-Biedl syndrome (BBS) proteins and the PCP core protein Vangl2. BBS is a rare pleiotropic disorder that is characterized by multiple symptoms, including obesity, mental retardation, retinal degeneration, and cystic kidneys (reviewed in Blacque and Leroux [47]). BBS1, BBS4, and BBS6 (Δ−/−) mice display abnormalities that are consistent with abnormal PCP signaling, including incomplete neural tube closure and disrupted cochlear stereociliary bundles; these phenotypic changes are enhanced by Vangl2 mutations (48). Although BBS members belong to a diverse group of proteins, they localize to the cilium/basal body complex (CBC) and seem to participate in microtubule-dependent intracellular trafficking. BBS4 interacts with p150Glucl, a subunit of dynactin that links dynactin with the dynein motor protein, and mutations of all BBS proteins seem to affect the dynein-driven, microtubular transport (49). Although it has been hypothesized that BBS proteins target PCP proteins to the
Oriented Cell Division during Renal Development

During proliferation of tubular epithelial cells and elongation of the tubular segments, the dividing cells precisely align along the anterior-posterior axis of the growing nephron (50). This exact alignment is partially lost in animal models of PKD, and cell divisions are randomly oriented. Oriented cell division is one of the outputs of PCP signaling and mandatory to orient tissue in the plane of an epithelial cell layer (51,52). Randomized cell divisions in PKD suggest that proteins that are mutated in PKD contribute to the orientation of the dividing axis. Because most PKD proteins localize to the CBC, it seems obvious to assume that the CBC plays a crucial role in oriented cell division. However, most Drosophila cells lack cilia, and deletion of the centrioles does not impede development of the fruit fly. Centriole-deficient flies reach maturity and succumb to defects in mechanosensation as a result of a lack of cilia on sensory neurons (53,54), suggesting that centrioles, at least in the fly, orient the microtubular cytoskeleton that is required for ciliogenesis but are not essential to deliver other microtubule-dependent cargo molecules to their subcellular destinations. These findings also emphasize the importance of positional cues from cell–cell and cell–matrix contacts such as the adherens junction and focal adhesions. In fact, recent data suggest that the orientation of cell division in mammalian cells is dominated by cell adhesion and traction forces that are applied during interphase (reviewed in Thery and Bornens [55]). In cultured cells, adhesive properties during interphase seem to encode the information that controls the orientation of subsequent cell divisions. Similarly, the site of neurite formation is determined by the orientation of the spindle poles and therefore is specified before the mitosis of the mother neuron. Because inhibition of actin polymerization inhibits neurite outgrowth, reorganization of the actin cytoskeleton seems to precede the organization of the microtubular cytoskeleton. The sequential specification of the actin cytoskeleton followed by the orientation of microtubules is supported by the function of the PCP effectors In and Fy. Deletion of either In or Fy causes defects in ciliogenesis in Xenopus embryos in combination with PCP and Hedgehog signaling abnormalities (56), linking both pathways to ciliogenesis. On the subcellular level, the defect seems to be a disordered apical cytoskeleton that fails to align the ciliary microtubules. Although the CBC complex may provide spatial cues to control and/or stabilize the localization of PCP proteins, signaling upstream of the core PCP proteins involving the actin cytoskeleton seems to control ciliogenesis. Because the polarized transport of Fz requires microtubular arrays along the apical plane of Drosophila wing epithelium (57), it will be interesting to see how the “upstream” PCP proteins instruct In and Fy to control microtubule-dependent ciliogenesis without affecting the microtubular transport of core PCP proteins.

Communication of Extracellular Cues to the Centrosome

The cilium originates from one of the two centrioles that form the centrosome and microtubule-organizing center (MTOC) in most cells. In tubular epithelial cells, the mother centriole moves to the apical membrane of polarized cells, transforms into the basal body, and nucleates the microtubular skeleton of the cilium. How cilia are inserted into a predefined region of the apical membrane is not well understood. Because the orientation of the mitotic spindle requires cues from cadherin- and integrin-based signaling, it is tempting to speculate that similar cues position the basal body. Recent findings demonstrate how focal adhesion proteins may communicate with the centrosome (58). HEF1, a multidomain scaffold protein that shares with p130Cas an amino-terminal SH3 domain, localizes to integrin-based focal adhesions, where it interacts with focal adhesion kinase (Figure 3). During the G2/M phase of the cell cycle, however, HEF1 translocates to the centrosome, where it activates Aurora A, a protein kinase that promotes activation of cyclinB/Cdk1, and transitions through G2. Another protein that shuttles between focal adhesions and the centrosome is the GRK-interactor 1–associated kinase p21-activated kinase, which binds and phosphorylates Aurora A. It is interesting that nephrocystin, a protein that is mutated in nephronophthisis type I, shares with HEF1 an amino-terminal SH3 domain and interacts with several focal adhesion components, including p130Cas, tensin, and Pyk2 (59). Nephrocystin contains two phosphorylatable acidic clusters that flank the SH3 domain. Interaction of the amino-terminal acidic cluster with the connector protein phosphaturin acidic cluster sorting protein 1 seems to recruit nephrocystin to the CBC (60), thereby providing another link between focal adhesions and the centrosome. It is interesting that nephrocystin also interacts with Inversin (61). Although, it has not been tested whether nephrocystin affects the function of Inversin in PCP signaling, it is tempting to speculate that the Inversin/nephrocystin complex could link PCP signaling to focal adhesions and organization of the actin cytoskeleton.

Intersection between Apico-Basal and Planar Cell Polarity

At this point, there is no unifying theme in ciliogenesis. One problem but perhaps also an advantage is that cilia are studied in many tissues and species; the underlying mechanisms of cilia formation likely vary significantly between various organisms. Most of what is known about ciliogenesis stems from studies in Chlamydomonas, a unicellular organism (62). However, assembly and maintenance of cilia in multicellular organisms may require additional cues. For example, kidney epithelial cells do not form cilia until they are embedded in a confluent monolayer and fully polarized (Figure 4). The evolutionary conserved PAR complex, converts initial polarity cues into the establishment of apical and basolateral membrane domain (reviewed in Suzuki and Ohno [63]). The PAR complex consists of three essential compo-
components: Par6, one of three Ras-related Rho-GTPases that control microtubule–cortex interactions and coordinate the growth of cortical microtubules (64,65); Par3, a PDZ domain–containing scaffold protein that interacts with the anterograde intraflagellar transport motor protein kinesin-2, (66); and αPKC. Because the future basal body has to travel to the apical plasma membrane (Figure 4), it is perhaps not surprising that the PAR polarity complex plays a crucial role in the ciliogenesis of MDCK cells (66). Recent data demonstrate that the tumor suppressor gene product pVHL binds Par6 and controls the direction of microtubule formation (67). Because VHL mutations lead to defective ciliogenesis and premalignant kidney cysts, it is conceivable that pVHL mediates the PAR-dependent contributions to ciliogenesis. The signal for cilia formation after polarization of epithelial cell layers remains unclear. Conceptually, the PAR complex needs to establish an apical membrane before the mother centriole can find its correct location to form the basal body and nucleate the cilium. Hence, any interference with the specification of the apical membrane should result in defective ciliogenesis. However, the precise positioning of the basal body, for example, and the angle of the cilium relative to the apical membrane may require additional cues. Therefore, it has been postulated that the PCP signaling cassette can provide such a fine tuning of the ciliary complex (56). For example, Inversin mutations do not disrupt ciliogenesis but rather affect the normal tilting of the cilium on the ventral node, a structure that establishes normal left–right asymmetry during mouse development (68). Recently, a molecular link between the PAR complex and PCP signaling was established by demonstrating that apical Fz can be phosphorylated and regulated by αPKC (69). Fz, in turn, triggers phosphorylation of ddboraya, a mediator of noncanonical Wnt signaling that regulates ciliogenesis and left–right patterning in zebrafish (70). However, PCP components also affect the PAR complex and apico-basolateral polarity. For example, Dsh together with the tumor suppressor product of lethal giant larvae, a negative regulator of the PAR complex, are required for normal apical-basal polarity in the Xenopus ectoderm (71). It is intriguing that overexpression of Par3/Bazooka, a protein that is essential for the apico-basolateral polarity of the Drosophila embryo, causes PCP defects of the Drosophila wing without affecting the apico-basolateral polarity (72). These findings suggest that the PAR proteins display tissue- and development-specific functions. In ciliated epithelia, the PAR proteins might therefore interact
Figure 4. Relationship between centrosome and cellular polarity of tubular epithelial cells. (A) The centrosome, consisting of the mother and daughter centrioles, migrates to the apical membrane (83). (B) Cadherin-mediated cell–cell contacts reorganize the actin cytoskeleton. Although the centrosome (microtubule-organizing center [MTOC]) organizes the microtubular cytoskeleton in migrating cells (e.g., fibroblasts), the centrosome organizes few microtubules of tubular epithelial cells during interphase. Most microtubules are ordered in either longitudinal arrays along the apical-basal axis of the cell with their plus ends toward the apical membrane or in a network parallel to the apical membrane (84). (C) The cadherin-based adherens junctions (AJ) facilitate recruitment of the Par6–Par3–atypical protein kinase C (PAR) complex. The PAR complex initiates apical-basolateral polarization and recruits the CRB complex (Drosophila: Crumbs) to the apical membrane (tight junction [TJ]). Pals1 (Drosophila: Discs Lost [Dlt]), a membrane-associated guanylate kinase (MAGUK)/PDZ domain–containing scaffold protein, binds to the carboxy-terminal CRB and to the PDZ domain of Par6. The basolateral membrane is controlled by a complex that consists of Scribble, a leucine-rich PDZ protein, the MAGUK protein mDlg (Drosophila: Discs Large [Dlg]), and the WD40-repeat protein mLgl (Drosophila: Lethal Giant Larvae [Lgl]). The CRB/PAR complex excludes the Lgl/Dgl/Scribble complex from the apical membrane, whereas the Lgl/Dgl/Scribble complex prevents the accumulation of the PAR complex at the basolateral membrane (85,86). Dsh is required for Lgl-mediated specification of the basolateral membrane and establishment of apical-basolateral polarity in Xenopus (71). Whereas an actomyosin-based cortical process is necessary to localize the PAR complex to tight junctions, Par3 can also interact with KIF3A. This interaction couples the PAR complex to kinesin II–powered, microtubular transport processes that are required for ciliogenesis (66) as well as for axon specification in developing hippocampal neurons (87,88).
with components of the PCP pathway to regulate cooperatively cell polarity, ciliogenesis, and ciliary function.

Conclusions

Although the cilium seems to serve as platform for several signaling cascades such as the Hedgehog pathway (reviewed in Zariwala et al. and Badano et al. [73,74]), it is unclear which components of the Wnt signaling cascades require an intact cilium. Clearly, in Drosophila, most cells lack a cilium; therefore, canonical and noncanonical Wnt signaling during Drosophila development have to function independent of this organelle. Ciliary bending can augment Inversin expression, suggesting that flow-induced signaling modulates Wnt signaling. It is interesting that the synchronized elongation of collecting ducts at approximately E15.5 coincides with the occurrence of the first cyst formation in several animal models of PKD. Thus, the initiation of glomerular filtration and/or tubular fluid secretion may modulate the shaping of the nephron by using Inversin to switch from canonical to noncanonical Wnt signaling. This raises the intriguing possibility that tubular flow might act as an instructive factor to shift the developmental needs of the kidney from cell fate decisions toward tissue morphogenesis. Clearly, this hypothesis requires a lot more experimental support. To study these questions in more depth, it would be important to know more about the potential readouts of PCP signaling during renal development. Are there changes in PCP core protein localizations that are induced by flow? Is the spindle orientation in dividing renal epithelial cells influenced by flow? The requirement for PCP signaling may vary during development and in the adult kidney. Whereas PCP/ciliary signaling may be absolutely mandatory during tubular maturation to orient proliferating cells along the axis of the nephron, mature tubules may have little need for PCP/ciliary signaling. Therefore, depletion of PCP proteins may have little effect if the insult occurs after the tubules have reached their final geometry. It is interesting that several PKD proteins are expressed only at low levels in the adult kidney but are upregulated after tubular injury and tissue loss, suggesting that they may be needed once again to orient the cells of a regenerating tubule.

Disclosures

None.

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