Tying TAZ and Nek1 into Polycystic Kidney Disease through Polycystin 2 Levels

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doi: 10.1681/ASN.2011030256

Polycystic kidney disease (PKD) is one of the most common genetic diseases in the world and is characterized by chronic renal cystic growth and kidney failure in children and adults. The kidney as well as the liver and pancreas undergo a buildup of fluid-filled cysts. The renal cysts arise from the epithelia of the nephrons and renal collecting system. PKD is usually inherited as an autosomal dominant trait through mutations in either the polycystin 1 (PC1; a transmembrane protein mutated in 85% of the patients) or the polycystin 2 (PC2; a nonselective calcium-permeable cation channel protein) gene.1,2 PC1 and PC2 localize to primary cilia as well as the apical membrane of epithelial cells. Cilium-mediated signaling from PC1 and PC2 is a key determinant of cyst formation,3 although the exact role of planar cell polarity defects in the initiation of cystogenesis remains unclear.2,4

In the story of Goldilocks and the three bears, Goldilocks at each encounter in the bears’ house picks the one that is just right through trial and error. An understanding of PKD is coming to the same conclusion; the balance between PC1 and PC2 needs to be just right, but trial and error is not the method. Modulating the levels of PC2 that function in kidney primary cilia to monitor mechanical forces must be carefully regulated.5 The Benjamin laboratory followed the role of two proteins, Nek1 and TAZ, whose individual loss causes cystic kidneys in mice, and they concluded that finding the right balance is important and that these two proteins are regulated by a negative feedback loop.6

TAZ was originally characterized as a transcriptional co-activator with a PDZ-binding domain.7 Mutations of TAZ result in PKD, emphysema, and partial embryonic lethality in mice,8 and morpholino knockdown causes cystic kidneys in zebrafish.9 Human TAZ is phosphorylated on at least five serine residues (S66, S89, S117, S311, and S314), but only three are discussed. S89 phosphorylation by the LATS2 kinase from the Hippo tumor pathway allows binding to 14-3-3 and cytoplasmic retention.10 S314 phosphorylation by casein kinase 1ε (CK1ε) and S311 phosphorylation by LATS2 both are required for binding to SCFβ-TrCP E3 ubiquitin ligase and degradation of TAZ by ubiquitination.11

The next modulator is Nek1, which is a member of the NimA kinase family. Although NimA was first identified by its role in cell-cycle control in Aspergillus, this kinase family is expanded in organisms with cilia.12 Humans have 11 Nek genes, and several are implicated in ciliary and centrosomal function. Mice with Nek1 mutations develop PKD as well as other cilium-based defects.13 Patients with autosomal recessive short-rib polydactyly syndrome, Majewski type, which is associated with polycystic kidneys, may have causal Nek1 mutations.14 Because deletions of each of these genes result in PKD, whether PC2, Nek1, and TAZ play interrelated roles with respect to development of polycystic kidneys is of great interest.

Previously, the Benjamin laboratory showed that phosphorylation of mouse TAZ on S306 (the equivalent of human S311) and S309 (the equivalent of human S314) is important for PC2 degradation by ubiquitination, and an altered PC1/PC2 ratio results in PKD.9 The Guan laboratory showed that phosphorylation of human TAZ on S311 and S314 is necessary for TAZ destruction by ubiquitination.11 Although TAZ is a transcriptional co-activator, phosphorylation on TAZ S309 has no effect on transcription of PC2 and Nek1.6 Phosphorylation on S309 is reduced when a kinase-dead Nek1 (K33M) gene is introduced, but the level of TAZ protein is unchanged. The catalytically dead construct results in increased PC2 compared with cells expressing the wild-type Nek1. Knockdown of Nek1 with a short hairpin RNA or using kidney cells from the Kat2J mutant mouse results in reduced PC2 protein levels and increased PC2 protein levels. TAZ regulates PC2 degradation through binding to SCFβ-TrCP E3 ubiquitin ligase8 and indirectly through its phosphorylation by Nek1. In TAZ knockout and knockdown cells, both Nek1 and PC2 protein levels increase. Ubiquitination of Nek1 and its loss are observed after overexpression of a GST-TPR construct, whereas the addition of the proteosome inhibitor MG132 or the F-box deletion mutant SCFβ-ΔF prevents the loss of Nek1.

These results suggest an interesting negative feedback loop that regulates the levels of PC2. Nek1 phosphorylates TAZ S309, which allows formation of the TAZ-E3 ligase complex that ubiquinates PC2 to promote its degradation. Phosphorylated TAZ then leads to ubiquitination of Nek1 and its degradation. Loss of Nek1 will result in less E3 ligase-activated TAZ that then will lead to an increase in PC2 and Nek1 levels.

TAZ plays roles in several pathways. In the Hippo pathway, it responds to signals for proliferation and cell death through NF2,
MST1, MST2, LATSI, and LATS2, and mutations in these genes are often associated with a variety of human cancers. Phosphorylated TAZ is retained in the cytoplasm by the action of LATSI2 was thought to be inactive. However, cytoplasmic TAZ is implicated in regulation of PC2 as described already and in inhibiting the canonical Wnt signaling cascade. The Wnt pathway is implicated in PKD, but the role of cilia and Wnt signaling is still murky. It remains hotly debated whether cilia and ciliary proteins affect (or are affected by) the canonical Wnt/beta-catenin signaling pathway because results from different groups are contradictory.

Ocbina et al. showed that mouse embryos lacking proteins of the anterograde ciliary transport (IFT) machinery show no change in Wnt target gene expression. However, several groups found that ciliary/basal body proteins may restrain Wnt signaling. Loss of the IFT/Golgi protein, Ift20, in the kidney causes PKD and results in an increase in nuclear beta-catenin as well as increased expression of several Wnt target genes. Chibby, a basal body protein, prevents nuclear entry of beta-catenin and thus inhibits Wnt signaling as suggested for IFT20.

Future work on TAZ and its causal relationship to PKD may want to consider the effects of the Wnt pathway on renal development and PC2 levels. Varelas et al. showed that phosphorylation of TAZ S89 and its cytoplasmic localization result in its binding to Dishevelled (DVL2), which is likely to prevent DVL2 phosphorylation by CK1\(\delta\)/\(e\), which prevents Wnt-induced transcriptional responses. TAZ would result in increased assembly of the destruction complex that contains Axin, adenomatous polyposis coli, CK1\(\delta\)/\(e\), and GSK3. In the absence of TAZ, disassembly of this complex would occur. Thus, S89 phosphorylation could have two effects on the TAZ-Nek1 negative feedback loop. Reduced availability of CK1\(\delta\)/\(e\) may reduce the amount of S314 TAZ phospho-degron and recruiting the SCFbeta-TrCP E3 ligase.

Alterations in GSK3 levels may also influence cilia. In the unicellular alga *Chlamydomonas*, inhibition of GSK3 results in elongated flagella. Less GSK3 could result in longer cilia, and more GSK3 could result in short cilia; each could alter PC2/PC1 ratios. TAZ, through its many partners, is a key regulator; more GSK3 could result in short cilia; each could alter PC2/beta-catenin signaling as suggested for IFT20.

ACKNOWLEDGMENTS

This work is supported by funds from the National Institutes of Health (GM-032843) and National Institutes of Health American Recovery and Reinvestment Act funds (GM-032843-S1) to S.K.D. We thank the members of the Dutcher laboratory and Gary Stormo for helpful comments.

DISCLOSURES

None.

REFERENCES

During the past decade, primary cilia and the associated centrosomes have moved to center stage in investigations to understand the molecular mechanisms that lead to renal cyst growth in polycystic kidney disease (PKD) and other so-called ciliopathies.1–3 Renal tubule epithelial cells possess exactly one primary cilium that protrudes into the tubule lumen. These mechanosensors bend in response to intraluminal fluid flow and trigger a calcium signal. Numerous cilia-associated proteins have been identified, and mutations in many of them lead to proliferation of tubule epithelial cells and renal cystic disease.4 These moieties include the polycystins, which are affected in autosomal dominant PKD (ADPKD).5

The consensus among many investigators has been that the loss of function of renal cilia somehow leads to aberrant proliferation of tubule cells. However, it is unknown what the actual purpose of renal cilia is and why flow sensing of fluid movement should have anything to do with the regulation of proliferation in the essentially nonproliferative adult kidney.

Several groups around the same time made a surprising observation using inducible-gene null mouse models; the elimination of polycystins in mature kidneys—or even of primary cilia altogether—had no apparent immediate consequence on the kidneys for months. Whereas disruption of polycystins or cilia in embryonic or early postnatal mice led to rapid, massive renal cyst growth, the same disruption in fully grown kidneys led to cyst growth only after a lag of several months.5–9 Therefore, polycystins and primary cilia seem to regulate proliferation and cyst growth in the developing and growing kidney but are dispensable for the minute-to-minute operation of healthy adult kidneys.

How, then, does one explain the renal cyst growth in ADPKD that is thought to involve numerous somatic second-hit mutations that presumably occur during adulthood in individual tubule cells? This loss of heterozygosity mechanism involves the inherited first-hit germline mutation in a polycystin gene, followed by later somatic second-hit mutations in the remaining polycystin allele, leading to the growth of genotypically heterogeneous clonal cysts. If polycystins and cilia were indeed dispensable in adult kidneys, then a second-hit mutation should be inconsequential.

Recent results from several groups, including an article in this issue of JASN,10 provide important insights to explain these puzzling findings. The bottom line is that the simple loss of polycystins or cilia in mature kidneys indeed does not always lead to immediate renal cyst formation. Although gene dose or epistasis may play a role,11–13 another event—which has logically been termed a third hit—may need to occur, which then leads to proliferation and cyst growth. Ischemic7,14,15 and nephrotoxic injury16 have been identified recently as important stress events providing a third hit.

In these latter experiments, polycystin 1 or a protein required for cilia formation, Kif3a, was eliminated in adult animals by inducible gene knockout. Subsequent renal injury led to cyst growth instead of the normal tissue regeneration and resolution of injury. Collectively, these findings suggest that polycystin 1 and cilia may not have major functions in the healthy adult kidney but are required to orchestrate the orderly execution of tissue regeneration in response to renal injury. They seem to be especially involved in the inhibition of proliferation once tubules have been repaired because tubule cells seem to keep going to form cysts in the absence of cilia or polycystin. Therefore, PKD could be regarded as a disease facilitated by unexpected or inappropriate continuous activation of an innate renal epithelial repair program. This notion is consistent with the fact that renal repair and PKD exhibit numerous similarities with regard to the renal injury, fibrosis, and progression in PKD.

The article in this issue of JASN adds another important piece to the puzzle.10 Similar to the previous work described, the investigators eliminated renal cilia in adult mice by gene knockout of the intraflagellar transport protein polars. It was previously shown that this loss does not result in renal cyst formation until approximately 6 months later.5