Tagged Fibrocystin Sheds Its Secrets

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Autosomal recessive polycystic kidney disease (ARPKD), a rare monogenic disorder with an incidence of 1:20,000 live births, is characterized by massive bilateral kidney enlargement due to cystic dilation of the renal tubules. In addition, liver cysts and hepatic fibrosis are also observed in patients with ARPKD. In neonates and infants, ARPKD manifests as severe kidney failure in utero, oligohydramnios, and pulmonary hypoplasia, causing respiratory failure, a major cause of perinatal mortality. ARPKD can also manifest in older children and adolescents. The clinical features include kidney cysts, progressive renal failure, systemic hypertension, and portal hypertension due to hepatic fibrosis. Over 50% of these patients develop end-stage renal disease and require kidney transplantation before the age of 20.

ARPKD is caused by mutations in PKHD1 (polycystic kidney and hepatic disease 1), which encodes a large protein called fibrocystin or polyductin. Fibrocystin is predicted to be a type I membrane protein with a large extracellular N-terminal domain, a single transmembrane segment, and a short cytoplasmic C-terminus. In addition to other places in the cytoplasmic C-terminus.3,4 In addition to other places in the cell, fibrocystin is located on the primary cilium, a hair-like organelle present on the surface of most cells in the body.5 Lack of fibrocystin results in stunted primary cilia.6 These findings indicate that abnormalities of primary cilia may underlie the pathogenesis of ARPKD.7,8

The molecular mechanism by which mutations in PKHD1 produce ARPKD is not known. It has been proposed that mouse Pkhd1 and the human PKHD1 genes undergo extensive differential splicing.3,9 Moreover, at least three of these transcripts are translated into smaller alternative forms of fibrocystin, while numerous other alternative forms remain undiscovered.3,9 Therefore, determining the functional consequences of the pathogenic mutations poses a significant challenge.

In this issue of JASN, Bakeberg et al.10 have revisited the importance of Pkhd1 splicing and fibrocystin processing. Surprisingly, the authors show that splicing is not a major feature of mouse Pkhd1 and that alternative forms of fibrocystin are not present in the mouse kidney. To prove that splicing does not occur, the authors performed long-range overlapping RT-PCRs using primers that amplified exons 1–21, 19–34, 32–52, 48–52, and 48–67. These reactions generated PCR products of predicted size, and their sequences aligned perfectly with the full-length cDNA encoding Pkhd1. If splicing had occurred, due to the exclusion of exons from the transcribed mRNA, PCR products of varying lengths would have been produced.

To determine whether alternative forms of fibrocystin are made, the authors utilized an elegant in vivo approach. They generated mice that harbored a loxP flanked transcriptional STOP cassette in intron–2 and coding-sequences of two SV5–Pk tags in exon–3 of the Pkhd1 gene. As a consequence, Pkhd1 transcription is disrupted and mutant mice develop liver cysts and fibrosis. Consistent with a previous report, the mutant mice do not develop kidney cysts. Only proximal tubule dilation was observed in female mice at older ages. Next, using a cre/loxP recombination strategy, the transcriptional STOP cassette was removed. This resulted in the re-expression of the Pkhd1 gene. Since the coding sequences of SV5-Pk tags were present in exon–3 of the Pkhd1 gene, fibrocystin was tagged with SV5-Pk epitope at its N-terminus. Characterization of these mice demonstrates normal histology of the kidney and liver, indicating that epitope-tagging of fibrocystin does not disrupt its function in vivo. Using antibodies that easily detect the epitope, the authors show that fibrocystin is primarily made as a 500-kD protein.

While the results provide strong evidence against extensive PKHD1 splicing and the presence of alternative forms of fibrocystin, they do not conclusively resolve the issue. It is possible that some of the alternative transcripts are expressed in a spatiotemporal manner; therefore, these transcripts will only be detected at specific stages of development or in specific cell types. Another possibility is that if some of these transcripts are not abundantly expressed, they may elude detection. If transcripts that exclude exon-3 are translated, the encoded protein would not contain the epitope tag and therefore would not be detected. Finally, there are considerable differences in the phenotypes of patients with ARPKD and Pkhd1 null mice.2,11 In contrast to ARPKD patients who develop kidney cysts and renal failure in utero or during childhood, the Pkhd1 null mice display normal kidneys at birth and develop only mild cystic kidney disease at older ages.

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raises the possibility that the mouse *Pkhd1* and the human *PKHD1* genes are processed and function slightly differently.

The study by Bakeberg *et al.* provides important insights and has raised several unanswered questions. The authors have previously shown that the mature cleaved form of fibrocystin is found in exosome-like vesicles (ELVs) isolated from the mouse urine. Exosomes are small extracellular vesicles that are derived from multivesicular bodies. Taking advantage of the easily-detectable tagged-endogenous fibrocystin, the authors confirm their previous findings in the present study. So what is the functional significance of fibrocystin-containing ELVs? Posttranslational processing produces multiple smaller products of the full-length fibrocystin inside the cell and the N-terminal ectodomain is shed out of the cell. Exosomes represent a mechanism by which the processed intracellular fibrocystin is transported out of the cell. In the extracellular space, fibrocystin-containing ELVs rapidly associate with primary cilia. Therefore, ELVs may be the means by which cells communicate with each other.

A novel function ascribed to fibrocystin is maintenance of planar cell polarity (PCP) in the kidney. In addition to apical–basal polarity, cells in multicellular organisms are also polarized along the plane of the tissue, which is referred to as PCP. Coordinated cellular behaviors, convergent extension, and oriented cell division—processes regulated by PCP signaling—result in the establishment and maintenance of PCP in the kidney. PCP signaling ensures that renal tubule diameter remains relatively unchanged during tubule elongation or regeneration after injury. The mechanisms by which cells along the renal tubule communicate to elicit coordinated behaviors and maintain a constant tubular diameter are not well understood. It would be interesting to determine whether urine flow-mediated dispersal of fibrocystin-containing ELVs represents a mechanism of long-range communication between cells of the renal tubule. In tissues such as the *Drosophila melanogaster* wings and eyes, where PCP is also observed, asymmetric expression of core PCP proteins produce a gradient along the proximal–distal plane of the tissue. This is thought to result in the establishment of PCP. Unequal distribution of fibrocystin-containing ELVs along the renal tubule may serve a similar function in the mammalian kidney.

The mouse expressing tagged-endogenous fibrocystin is a valuable reagent with which some of the other questions raised by the study could be addressed. For example, what are the spatial and temporal expression patterns of fibrocystin and what are the consequences of fibrocystin ELVs binding on primary cilia? The ELVs were found to contain proteins that could potentially modulate cilia-regulated and polycystic kidney disease (PKD) relevant signaling pathways. Whether the contents of ELVs affect ciliary signaling in the kidney and impact the pathogenesis of PKD remains to be seen. Finally, what are the other contents of fibrocystin ELVs? In addition to proteins, if these ELVs are also found to contain mRNAs, miRNAs, or other noncoding RNAs, a novel mechanism of intercellular communication in kidney will be uncovered—does the proteomic makeup of fibrocystin ELVs change with kidney development, after kidney injury, during kidney regeneration or in *Pkhd1* null mice?

**DISCLOSURES**

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**REFERENCES**


See related article, “Epitope-Tagged Pkd1 Tracks the Processing, Secretion, and Localization of Fibrocystin,” on pages 2266–2277.