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Third-Hit Signaling in Renal Cyst Formation

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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 intralumenal 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).

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.

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DISCLOSURES

None.

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*[3]—may need to occur, which then leads to proliferation and cyst growth. Ischemic[7,14,15] and nephrotoxic injury[16] 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 activation of signaling pathways (mammalian target of rapamycin [mTOR]), protein expression (kidney injury molecule 1), and tissue abnormalities (fibrogenesis).[17]

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 polaris. It was previously shown that this loss does not result in renal cyst formation until approximately 6 months later.[5] One week after the polaris gene was eliminated, unilateral nephrectomy was performed. Normally, this leads to compensatory hypertrophy of the remaining kidney involving an increase in the size of the tubule epithelial cells but very little cell proliferation. In kidneys lacking cilia, however, this treatment led to induction of proliferation and massive cystic disease by 3 months.[10]

These new results suggest that compensatory hypertrophy is another, *third hit* leading to renal cyst growth in addition to ischemic and nephrotoxic injury. Interestingly, the mTOR pathway is activated in renal epithelial cells in all of these conditions: After injury, during hypertrophy, and in virtually all forms of PKD.[17–19] Treatment with the mTOR inhibitor rapamycin strongly inhibits compensatory hypertrophy and proliferation during injury repair and in PKD.[17,18] Furthermore, primary cilia and fluid flow down-regulate mTOR activity in renal epithelial cells.[20]

A possible model emerging from these studies is that cilia and polycystins are required to *turn off* hypertrophic and proliferative signaling in renal epithelial cells after they have completed their response to stress-related insults. In the same way, cilia and polycystins may be required to *turn off* proliferation after renal maturation is complete, around day P13 in the mouse. Once cilia and polycystins have done their job, they seem no longer required to suppress proliferation on a day-to-day basis as long as the kidney does not experience any new stress.

Tubule epithelial cells lacking functional cilia as a result of genetic disease or manipulation seem hypersensitive to growth factor signaling that occurs after nephrectomy or injury. Many open questions remain about the molecular mechanisms that connect primary cilia and the regulation of proliferation. In particular, what is the role of polycystins, mechanosensation, calcium signaling, growth factors, and the immune system?

The induction of cyst growth in hypertrophic and injured kidneys could explain the rapid decline in renal function in the late stages of ADPKD. Progressive cyst growth leads to increased injury of normal tissue and increased functional impairment to which the kidneys may attempt to respond with more hypertrophy and repair, but this should make matters only worse because it should induce accelerated cyst growth until renal destruction spirals out of control. Although there is some evidence that family history predicts renal dysfunction from cystic disease,[21] this notion of a *third hit* could also explain the enormous heterogeneity of phenotypes even within the same family with ADPKD. Untoward environmental factors may lead to subclinical renal injuries that trigger bursts of cyst growth that accelerate disease progression.

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