Targeting Cyst Initiation in ADPKD

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With approximately 12.5 million people worldwide affected by autosomal dominant polycystic kidney disease (ADPKD), one hopes we are now at the beginning of multiple therapeutic breakthroughs. ADPKD is a hereditary disorder caused by a genetic defect in either polycystin-1 (*PKD1*; PC1) or polycystin-2 (*PKD2*; PC2) that leads to progressive kidney cyst formation and enlargement. The resulting high cystic burden in the kidney destroys the normal parenchyma and ultimately results in ESRD for the majority of patients. Although many of the cellular events leading to cystogenesis are not yet identified, the process can be roughly divided into the following stages after somatic inactivation of the remaining normal PKD allele through loss of heterozygosity: Loss of calcium-mediated cellular quiescence leading to an initial proliferative phase of tubule epithelial cells; out-pocketing of the tubule wall and eventual cyst separation from the parent nephron; and continued cell growth, fluid secretion, and progressive expansion of isolated cysts.

Tremendous progress has been made in understanding the various biologic mechanisms responsible for these pathologic stages of cyst formation and growth that range from the identification of the primary cilia as a mechanosensor mediating PC1/PC2 calcium influx to various perturbations of fluid regulation and cell proliferation. Because many proteins associated with these processes have been identified, we have multiple cellular targets to examine for pharmacologic intervention strategies, some of which are already under investigation.

When considering the stages of cystogenesis as described already, it is clear that many small molecule therapeutic agents have focused on cysts that are in the continued growth and expansion phase or the isolated cyst.

There are several promising agents that either are under development or are already in clinical trials to reduce the increase in cyst volume by regulating proliferation, cell growth, or transepithelial secretion and thereby delaying time to ESRD. These include the vasopressin receptor antagonists OPC-31260 and tolvaptan,[1,2] which decrease cAMP production; the somatostatin analog octreotide[3]; antagonists of the Cl⁻ channel cystic fibrosis transmembrane conductance regulator[4,5]; angiotensin-converting enzyme inhibitors[6,7]; and small molecule inhibitors of the basolateral KCa3.1 K⁺ channel.[8] Other candidates include inhibitors of the inflammatory mediator TNF-α,[9] cell growth regulators such as the mTOR antagonist rapamycin[10]; and the antiproliferative cyclin-dependent kinase inhibitor roscovitine.[11]

These potential therapies could be used for adult patients in whom average cystic burden increases at a rate of 5 to 6% per year as reported by the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP).[12] The impact of these types of drugs would be greatest in those with numerous large cysts contributing to increasing renal volume. Although this strategy targeting the isolated, expanding kidney cyst is central to current disease intervention, there is the opportunity for a complementary approach as well: One that targets the origins of cyst formation at the site of the epithelial tubule cell. By focusing on a much earlier stage in disease progression, before a cyst disconnects from its parent nephron, we can begin to question how to prevent the initial proliferative phase stemming from cilial defects.

Although all of the cellular changes and mechanisms involved in initial cyst formation are not yet elucidated, interference with primary cilia formation or its resident PC1/PC2 complex leads to the formation of cysts. For example, murine models with functional or structural defects in the primary cilium, such as *Tg737^orpk*,[13] *cpk*,[14] *inv*,[15] and *kif3a*,[16] all result in kidney cyst development. The cilia of the tubular epithelium, acting as a "flow sensor," are central to calcium signaling and control of the proliferative phenotype of the cell.[17] Because calcium influx as a result of cilial bending maintains cellular quiescence, the loss of this signal initiates the earliest events of cystogenesis. While the PC1/PC2 complex would be the most upstream candidate to restore calcium signaling directly by pharmacologic manipulation, other associated proteins involved in cilia formation, maintenance, and function could be targeted as well.

Recent studies with "adult" inducible animal models of ADPKD demonstrated a delay between the loss of calcium signaling (through *Pkd1* inactivation) and initial cyst formation,[18–20] perhaps indicating that multiple cellular and

extracellular events are required for this process. Examples include changes in adhesion molecule expression, abnormalities in basement membrane composition, and increases in inflammatory mediators that accompany cyst development. It is anticipated, then, that even intermittent PC2-mediated calcium release induced by pharmacologic agents could halt or delay events causing slowly progressive cyst formation.

Triptolide, for example, activates PC2-mediated calcium release, causes cell growth arrest in murine *Pkd1*-null cells, and reduces cyst burden by inhibition of cyst initiation.[21,22] Although these findings result in an exceptional opportunity to examine the potential therapeutic effect of restoration of calcium signaling in PKD, it leaves the door open for the discovery and investigation of additional PC2 agonists as well as other modulators of this signaling process in the kidney. Future therapeutic drugs, for example, could act at the site of the primary cilium to reestablish calcium influx through the PC2 channel directly or target a downstream mediator or associated protein of the PC1/PC2 complex that is directly linked to cell-cycle inhibition. Although all of the components of this PC2-stimulated calcium release pathway are not yet known, new associated members such as the cell-cycle related kinase Nek8[23] have recently been discovered and warrant further investigation as therapeutic targets.

As the PKD research community continues to fill in the gaps separating cilial mechanosensation and calcium release to cell growth arrest, new targets to inhibit cyst initiation will be identified. It is also of note that hepatic cysts derived from bile ducts are a common extrarenal manifestation of ADPKD because these cells are also responsive to cilial PC2-mediated calcium influx. Because treatment with such drugs as the vasopressin receptor antagonists are ineffective in the liver as a result of a lack of receptor expression, it is plausible that a class of PC2-specific calcium agonists could be therapeutically beneficial to both the kidney and the liver.

Although cyst formation is a continual process throughout adulthood, cysts initiated early in life are the main contributors to adult cystic burden. Once the cyst has disconnected from its originating nephron and begins to increase in size, further destruction of the renal parenchyma occurs by the involvement of inflammation, fibrosis, and ischemia; therefore, targeting the very first events of cyst initiation as early as possible in the course of the disease could have profound benefits in alleviating the total number of mature cysts and the associated changes that accompany renal enlargement.

Because ADPKD progression spans multiple decades, there are many challenges and opportunities to modulate the different stages of this disease. Considering what is known of cyst initiation and growth in experimental models, perhaps a two-tiered treatment strategy can be envisioned whereby initial cyst formation is attenuated through therapies aimed at restoring PC2-mediated calcium signaling and inhibition of cellular proliferation. This would decrease the number of cysts formed at an early age that are ultimately the source of the largest volume increases that lead to ESRD; isolated cysts that have separated from the parent nephron and have begun the process of cyst expansion through fluid secretion can then be treated with agents that target volume regulation in adulthood. Through the use of this dual strategy, a renoprotective effect may be achieved by modulating a range of early and late cellular events associated with the pathogenesis of disease. Even a shift from a *PKD1* phenotype, in which there are a greater number of initial cysts, to a clinically milder course of disease that would mimic the *PKD2* phenotype would be of benefit to many patients with ADPKD.

In summary, the combination of multiple therapeutic agents targeting various aspects of experimental cystic disease, such as restoration of PC2-mediated calcium signaling, inhibition of inflammatory mediators, and reduction of cyst volume and cell growth, may lead to the preservation of kidney function and attenuate the severity of renal cystic burden. Most important, one hopes that combination drug strategies might some day give treatment options to thousands of patients with ADPKD to extend the quality of life for years beyond what is expected today.

DISCLOSURES

None.

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Cross Dendritic Cells Anger T Cells after Kidney Injury

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Nearly 30 yr ago, Stewart Cameron caused a minor revolution by pointing out that simple quantitation of proteinuria was much better at predicting outcome than histopathological diagnosis—then the gold standard prognostic marker.[1] We now know that severe proteinuria does not simply reflect glomerular injury but is itself harmful.[2] Reabsorption of large amounts of protein from the glomerular filtrate induces stress responses in proximal tubular epithelial cells that result in lysosomal instability and can even initiate epithelial-mesenchymal transition.[3] Epithelial cells synthesize chemokines, cytokines, and complement components that recruit inflammatory cells and lymphocytes into the interstitium causing progressive fibrosis. A fascinating paper by Macconi *et al.* from Ariela Benigni's group in the current issue of JASN[4] adds another layer of complexity by showing that albumin reabsorbed from proximal tubules induces the generation of albumin-specific, IFN-γ secreting, CD8 T cells that may contribute to progressive renal injury.

Before discussing the results in detail, it is important to consider how proteins reabsorbed by the proximal tubule are presented to T cells. The interstitium of normal kidneys contain numerous resident monocytic myelocytes, traditionally believed to be macrophages because they express relevant markers such as F4/80 in mice and CD68 in man.[5] It is now known they also express dendritic cell markers and can indeed present antigen—one of the defining features of renal dendritic cells with broadly similar characteristics to dendritic cells in other tissues.[5–10] Studies in mice with fluorescently labeled dendritic cells show essentially all resident myeloid cells are dendritic, reveal their intimate connection with renal tubules through long processes kissing the epithelial cells, and thus are ideally positioned to sample reabsorbed antigens or receive activation signals from them.[6,7] Consequently, renal dendritic cells are the likely sentinals that sample renal antigens and present them to T cells. The endocytosed antigen is processed in one of two ways: either in endocytic compartments to create peptides that are loaded on to MHC class II molecules that