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Connecting the dots toward a polycystic kidney disease therapy

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Abstract

Understanding the complex interactions between the various pathways disrupted in polycystic kidney and liver disease is essential to identify and optimize therapies for these disorders. Studies published in the past year have demonstrated a functional interaction between the main proteins implicated in these diseases and identified novel therapeutic approaches.

The enlarged kidneys and liver characteristic of polycystic kidney disease (PKD) have for centuries attracted attention. Pathologists in the 19th century debated whether cysts are developmental or the result of tubular obstruction or excessive epithelial cell proliferation. Microdissection, electron microscopy and physiology studies carried out during the 20th century showed that cysts in patients with autosomal dominant PKD (ADPKD) detach from renal tubules and grow as blind sacs by a process that requires cell proliferation, net fluid secretion and remodeling of extracellular matrix. After the *PKD1* and *PKD2* genes and their encoded proteins, polycystin-1 and polycystin-2, were identified in 1994 and 1996, respectively, research in this area greatly accelerated. In the following years, genes mutated in autosomal recessive PKD (*PKHD1* encoding fibrocystin) and in autosomal dominant polycystic liver disease (*PRKCSH* encoding glucosidase 2 subunit β, and *SEC63* encoding translocation protein SEC63 homolog) were identified. Polycystin-1, polycystin-2 and fibrocystin are membrane glycoproteins, whereas glucosidase 2 subunit β and SEC63 are endoplasmic reticulum resident proteins needed for translocation and folding of integral membrane proteins and secreted proteins. Polycystin-1, polycystin-2 and fibrocystin have numerous interacting partners and their disruption affects multiple signaling pathways. Despite the multiplicity of genes and signaling pathways involved in PKD, interventions affecting many diverse targets have been effective in animal models of PKD, which indicates the presence of connections between the various pathways. Understanding these complex interactions will be essential to effectively treat PKD.

Fedeles *et al.* used mouse mutants to show that the main proteins implicated in ADPKD, autosomal recessive PKD and autosomal dominant polycystic liver disease functionally interact.[1] Loss of glucosidase 2 subunit β or SEC63 reduces polycystin-1 and, to a lesser

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Competing interests

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extent, polycystin-2 expression, blocks polycystin-1 trafficking to primary cilia, causes renal and hepatic cysts, worsens cystic disease in heterozygous *Pkd1*$^{+/-}$ and *Pkd2*$^{+/-}$ mice and induces renal cystogenesis in *Pkhd1*$^{del4/del4}$ mice. Transgenic overexpression of polycystin-1, but not polycystin-2, rescues the renal and hepatic phenotype of tissue-selective *Prkcsh*-knockout or *Sec63*-knockout mice and the renal phenotype of *Pkhd1*$^{del4/del4}$ mice. Polycystin-1 is the rate-limiting component in the polycystin-1/polycystin-2 complex as its level of expression determines the severity of the cystic phenotype, but some functional polycystin-2 is essential for polycystin-1 to exert this effect. Immunocytochemical analysis revealed that the collecting duct is the segment most susceptible to the cystogenic effect of reduced polycystin-1 dosage. Proteasome inhibition increases polycystin-1 levels and attenuates cystic disease in *Prkcsh*-knockout models, thus offering a conceptual therapeutic approach to autosomal dominant polycystic liver disease and possibly ADPKD.

The central role of cyclic AMP in PKD, and the ability to hormonally modulate cyclic AMP in a cell-specific manner, enables targeting of an important cystogenic pathway with relative safety. Among hormonal systems that affect renal cyclic AMP is the vasopressin–V2 receptor axis. This target is attractive because V2 receptors are mostly restricted to the thick ascending limb and collecting ducts, which are main sites of cystogenesis where vasopressin is the major agonist of cyclic AMP. These sites are continuously subjected to tonic vasopressin action. Circulating vasopressin levels are increased in ADPKD and V2 receptors are overexpressed in polycystic kidneys. Pharmacological and genetic inhibition of vasopressin or V2 receptor expression has been shown to be effective in rodents. Reif *et al.* examined the effects of tolvaptan on human ADPKD cyst epithelial cells.[2] Low concentrations inhibited vasopressin-induced cyclic AMP production, cell proliferation, chloride secretion and cyst growth in collagen matrices. Other analyses showed that tolvaptan administration for 1 week reduced total kidney volume by 3.1% in patients with ADPKD, and that 3 years of tolvaptan therapy reduced kidney growth by 70% compared to historical controls (1.7% versus 5.8% increase per year, respectively).[3] Changes in kidney volume and in estimated glomerular filtration rate were significantly and negatively correlated. This finding supports the use of kidney volume as a biomarker to monitor ADPKD progression. Further evaluation of tolvaptan for the treatment of patients with ADPKD awaits the conclusion of a randomized, double-blind clinical trial (NCT00428948) in 2012.

Metformin and peroxisome proliferator-activated receptor (PPAR)-γ agonists are widely used to treat type 2 diabetes mellitus. Takiar *et al.* found that metformin stimulates the energy-sensing molecule AMP-activated protein kinase (AMPK), inhibits the activities of AMPK-dependent cystic fibrosis transmembrane conductance regulator (CFTR) and mammalian target of rapamycin in Madin–Darby canine kidney renal epithelial cells, and attenuates cyclic AMP-dependent growth of Madin–Darby canine kidney cysts in collagen matrices and of cysts in metanephric organ explants, and cystogenesis in constitutive and inducible *Pkd1*-knockout mice.[4] Yoshihara *et al.* and Blazer-Yost *et al.* found that the PPAR-γ agonist pioglitazone inhibits renal and hepatic cystogenesis in PCK rats by possibly complementary mechanisms through the inactivation of mitogen-activated protein kinase 3

and mammalian target of rapamycin,[5] and inhibition of CFTR synthesis and cyclic AMP-activated chloride secretion.[6] Because metformin and PPAR-γ agonists exert salutary effects by affecting the same pathways through different mechanisms (such as phosphorylation and inhibition of CFTR by metformin and inhibition of CFTR synthesis by pioglitazone), clinical trials of metformin and PPAR-γ agonist combinations in early ADPKD should be considered to exploit their possible synergism.

Evidence accumulated over the past two decades points to the importance of inflammation in PKD. Karihaloo *et al.* hypothesized that macrophage infiltration contributes to the proliferation of cyst-lining cells and PKD progression.[7] This premise was based on work showing that macrophages homing to the kidney after ischemia–reperfusion undergo a transition from classically activated, proinflammatory cells to alternatively activated cells that promote epithelial cell proliferation. The investigators found that *Pkd1*-null cells secrete large amounts of the macrophage chemoattractant C-C motif chemokine 2 (also known as monocyte chemotactic protein 1) and C-X-C motif chemokine 16. Kidneys from conditional *Pkd1*-knockout mice and the $Pkd2^{WS25/-}$ mouse model of PKD2 exhibit a 10-fold increase in the number of macrophages (most with an alternatively activated phenotype and aligned along cyst walls). Macrophage depletion by intraperitoneal liposomal clodronate administration inhibits epithelial cell proliferation and cyst growth and improves renal function.[7] How the loss of polycystin expression leads to increased cytokine production remains to be determined. Approaches that inhibit the expression or action of homing and proliferation signals provide novel strategies for treating PKD.

Three studies have shown marked upregulation of the signal transducer and transcription activator (STAT)3 in patients with ADPKD and in rodent PKD models.[8–10] In the STAT3 signaling pathway, activation of various cell surface growth factor receptors and cytokine receptors induces specific tyrosine phosphorylation of the receptors, which creates docking sites for latent cytoplasmic STAT3. STAT3 is then phosphorylated at tyrosine 705 by intrinsic tyrosine kinase activity of the activated growth factor receptors or by cytokine receptor-associated Janus kinase. The trigger for phosphorylation and activation of STAT3 in PKD is uncertain. Talbot *et al.* showed that phosphorylated STAT3 is highly expressed in cyst-lining cells and normal- appearing tubules and interstitial cells in proximity to cysts.[8] This finding suggests that diffusible factors such as cytokines and growth factors are involved in the activation of STAT3. Phosphorylated STAT3 homodimers translocate to the nucleus and bind to promoter elements of genes that regulate cell differentiation, proliferation, apoptosis and angiogenesis. Because STAT3 is critical during development (deletion of *STAT3* leads to embryonic lethality), but is dispensable postnatally in conditional knockouts, targeting STAT3 might be well tolerated in patients with PKD. Takakura *et al.* screened a small-molecule library using a cell-based functional assay and found that pyrimethamine, a drug used to treat malaria and toxoplasmosis, inhibits STAT3 signaling.[9] Pyrimethamine and S3I-201, an inhibitor of STAT3 homodimer complex formation, suppressed epithelial cell proliferation and cystogenesis in an inducible *Pkd1*-knockout mouse model without toxic effects. In another study, Leonhard *et al.* found that curcumin, a compound with anti-inflammatory and antiproliferative properties, reduced STAT3 activation, attenuated cell proliferation and cystogenesis and delayed renal failure

from 105 to 119 days in an inducible *Pkd1*-knockout model.[10] As curcumin has poor bioavailability, however, novel analogues with improved pharmacological profiles might be more effective than curcumin itself.

In summary, insights into the complex network of signaling pathways disrupted in PKD have increasingly led to the identification of potential therapies, some of which are currently used for other indications. Those compounds best supported by preclinical studies and with the desired pharmacological profile should be prioritized for clinical trials.

Acknowledgments

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References

1. Fedeles SV, et al. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. Nat Genet. 2011; 43:639–647. [PubMed: 21685914]
2. Reif GA, et al. Tolvaptan inhibits ERK-dependent cell proliferation, Cl^- secretion, and *in vitro* cyst growth of human ADPKD cells stimulated by vasopressin. Am J Physiol Renal Physiol. 2011; 301:F1005–F1013. [PubMed: 21816754]
3. Higashihara E, et al. for the TEMPOFormula and 156-05-002 Study Investigators. Tolvaptan in autosomal dominant polycystic kidney disease: three years' experience. Clin J Am Soc Nephrol. 2011; 6:2499–2507. [PubMed: 21903984]
4. Takiar V, et al. Activating AMP-activated protein kinase (AMPK) slows renal cystogenesis. Proc Natl Acad Sci USA. 2011; 108:2462–2467. [PubMed: 21262823]
5. Yoshihara D, et al. PPAR-γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. Am J Physiol Renal Physiol. 2011; 300:F465–F474. [PubMed: 21147840]
6. Blazer-Yost BL, et al. Pioglitazone attenuates cystic burden in the PCK rodent model of polycystic kidney disease. PPAR Res. 2010; 2010:274376. [PubMed: 21052534]
7. Karihaloo A, et al. Macrophages promote cyst growth in polycystic kidney disease. J Am Soc Nephrol. 2011; 22:1809–1814. [PubMed: 21921140]
8. Talbot JJ, et al. Polycystin-1 regulates STAT activity by a dual mechanism. Proc Natl Acad Sci USA. 2011; 108:7985–7990. [PubMed: 21518865]
9. Takakura A, et al. Pyrimethamine inhibits adult polycystic kidney disease by modulating STAT signaling pathways. Hum Mol Genet. 2011; 20:4143–4154. [PubMed: 21821671]
10. Leonhard WN, et al. Curcumin inhibits cystogenesis by simultaneous interference of multiple signaling pathways: *in vivo* evidence from a *Pkd1*-deletion model. Am J Physiol Renal Physiol. 2011; 300:F1193–F1202. [PubMed: 21345977]

Key advances

- The main proteins implicated in polycystic kidney disease (PKD)—polycystin-1, polycystin-2 and fibrocystin—and in autosomal dominant polycystic liver disease (glucosidase 2 subunit β and SEC63) functionally interact[1]
- The vasopressin V2 receptor antagonist, tolvaptan, inhibits cystogenesis *in vitro*,[2] and reduced the volume of polycystic kidneys after 1 week of treatment, and slowed the growth of polycystic kidneys in a 3-year, open-label study of patients with PKD[3]
- Two insulin-sensitizing drugs used to treat type 2 diabetes mellitus, metformin and a peroxisome proliferator-activated receptor-γ agonist, inhibit cyst growth in rodent models of PKD by different and possibly complementary mechanisms[4–6]
- Macrophage infiltration contributes to the proliferation of cyst-lining cells and PKD progression[7]
- STAT3, a transcription factor that is essential during development but dispensable postnatally, is a novel therapeutic target in PKD[8–10]