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Editorial Comments

Molecular pathogenesis of ADPKD and development of targeted therapeutic options

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disease characterized by formation and progressive enlargement of cysts in kidneys, liver and other organs, leading to end stage renal disease by the fifth decade [1]. Mutations in the PKD1 gene encoding polycystin-1 are responsible for ~85% of ADPKD cases, while mutations in the PKD2 gene cause ~15% of ADPKD cases with a less severe phenotype. Autosomal recessive polycystic kidney disease (ARPKD) affects newborns and results from mutations in the PKHD1 gene encoding fibrocystin [2]. Regardless of the genetic defect underlying PKD, cystic epithelia seem to display common abnormalities. Cellular mechanisms of cystogenesis have been studied for decades and suggest that cystic epithelia are characterized by a secretory phenotype with increased proliferation, apoptosis, loss of cellular differentiation and polarity. Considerable progress towards understanding the molecular pathogenesis of cyst formation has been made since the cloning of the PKD1 and PKD2 genes as well as other cystogenes responsible for multiple forms of PKD in animal models. Based on the enhanced understanding of the signalling pathways involved in cystogenesis, novel therapeutic approaches targeting mTOR activity, and cAMP activated signalling pathways are being tested in the clinic [3]. Also, recent studies highlight the importance of the primary cilium as a common trigger of cystic diseases [4]. The molecular link between cilia, mechanosensation and the cell cycle can now be explored for novel targeted therapeutic intervention in PKD. Here we focus on reviewing recent advances in understanding the molecular pathways of cystogenesis and therapeutic approaches to interfere with these pathways in preclinical and clinical trials.

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Increased proliferation and apoptosis in PKD

A number of studies have demonstrated an important role for EGF/TGF alpha/EGFR in promoting cystic epithelial proliferation. In human PKDs and multiple, though not all, animal models of PKD, EGFR was found to be overexpressed and mislocalized to the apical membranes of cystic epithelial cells [5]. In addition, overexpression of TGF alpha in transgenic animals resulted in renal cyst formation [6]. It has been shown that inhibition of EGFR tyrosine kinase activity significantly attenuated cystogenesis in mouse models of PKD, including *bpk* and *orpk*, as well as in the Han:SPRD rat model [7,8]. All of these models are characterized by overexpression and mislocation of EGFR into the apical membranes. No efficacy, however, was detected in the PCK rat, possibly because this model does not display the characteristic expression pattern for EGFR in cysts [9].

Apoptosis has been shown to be essential for cystogenesis. Thus, formation of cysts in 3D collagen cultures *in vitro* by MDCK cells proceeds with increased apoptosis [10]. In addition, deletion of the anti-apoptotic Bcl-2 and AP-2β genes and overexpression of the pro-apoptotic c-myc in mice results in renal cyst formation [11]. To evaluate the effect of inhibition of apoptosis on PKD progression, a caspase inhibitor was tested in the Han:SPRD rat model [12]. This study demonstrated inhibition of cystic disease and attenuation of the loss of renal function through decrease of both apoptosis and proliferation in cystic and non-cystic tubules.

cAMP activated pathways

Polycystic kidneys are characterized by increased levels of cAMP [13–15]. An activated cAMP pathway

is responsible for both increased proliferation and fluid secretion seen in cystic epithelia. It has been shown that in normal renal epithelial cells, cAMP blocks proliferation through inhibition of the Ras/Raf/MEK/ERK pathway [13]. In contrast, cAMP induces proliferation of ADPKD-derived cystic cells through activation of the B-Raf/MEK/ERK pathway. This switch to the cAMP growth-stimulated phenotype of cystic cells is mediated by decreased intracellular calcium levels [Ca2+]i [16]. Polycystin-2 has been shown to assemble with polycystin-1 and function as a cation channel to control [Ca2+]i homeostasis [17]. Mutated polycystins disrupt Ca2+ signalling, leading to an abnormal cAMP-mediated proliferation of cystic cells. The importance of this pathway in PKD is highlighted by the successful experimental therapy in PKD animals with MEK inhibitor. Omori *et al.* demonstrated that MEK inhibition in the *pcy* model of PKD (NPHP3 mutation) resulted in the effective block of cystogenesis and preservation of renal function [18]. Thus, inhibitors of the B-Raf/MEK/ERK pathway may hold promise for PKD therapy.

The successful targeting of cystogenic pathways of the cAMP and vasopressin axis using vasopressin V2 receptor (VPV2R) inhibitors showed efficacy in several PKD animal models [19]. Tolvaptan (human VPV2R antagonist, Otsuka Pharmaceutical) treatment yielded significant lowering of cAMP levels in PCK rat kidneys and inhibition of renal cystogenesis and fibrosis. Tolvaptan has entered human clinical trials in ADPKD, and phase II results have shown that it is well tolerated, with thirst and polyuria as side effects [14,20]. Interestingly, a recent study showed that effective reduction of plasma arginine vasopressin levels through increased water intake decreased VPV2R expression and slowed PKD progression in PCK rats [21]. An alternative approach to interfere with an abnormal cAMP signalling in PKD was shown through the use of somatostatin. Somatostatin is thought to inhibit cAMP-stimulated fluid secretion. A recent study with long-acting somatostatin showed significant inhibition of renal volume expansion in ADPKD patients over a 6-month period of therapy [22], and several clinical trials with somatostatin are on their way.

mTOR activation

Several studies proposed a functional link between polycystin-1 and tuberin, which causes the tuberous sclerosis complex (TSC) when mutated [23,24]. It has been shown that polycystin-1 interacts with tuberin and regulates the activity of mTOR, which is important for controlling protein synthesis and regulation of cellular differentiation [23]. The mutations in polycystin-1 would result in constitutive activation of mTOR. Increased mTOR activity was detected in cyst-lining ADPKD epithelia, as well as in cystic epithelia of the *bpk* and *orpk-rescue* animal models of PKD. Rapamycin treatment (mTOR inhibitor) of the *bpk* and *orpk-rescue* mouse models resulted in effective inhibition of cystic disease and preservation of kidney function. This effect was reportedly due to selective induction of apoptosis in cystic cells [23]. Similar results were previously observed in a separate study with rapamycin performed in Han:SPRD rats [25]. Importantly, a small retrospective study showed reduction in volumes of cystic kidneys of ADPKD patients receiving rapamycin after renal transplantation [23]. Several clinical trials are currently underway to test efficacy of rapamycin (sirolimus) and everolimus in ADPKD.

Cilia and cell cycle disruption

Polycystins and other cystoproteins responsible for human and animal forms of PKD were recently found in a novel subcellular location—primary cilium [4]. These findings suggested that ciliary dysfunction might be a common abnormality causing cystic transformation [26]. The primary (non-motile) cilium is a microtubule containing organelle that grows out of basal bodies/centrosomes and protrudes from the apical membrane of an epithelial cell (Figure 1). Basal bodies/centrioles of the cilia act as mitotic spindle pole organizers during cell division, connecting ciliogenesis with cell cycle regulation. When cells enter the cell cycle, cilia are disassembled. Disruption of proteins associated with cilia or basal bodies could lead to dysregulation of the cell cycle and proliferation, resulting in cystic disease. Polycystin-1 was found to directly regulate the cell cycle by inhibiting CDK2 (cyclin dependent kinase) activity through up-regulation of the CDK inhibitor $p21^{waf1}$, arresting cells in G0/G1 phase (Figure 1) [27]. It is likely that dysregulation between cilia and cell cycle progression occurs early in cystogenesis, therefore, therapeutic targeting of this pathway through CDK inhibition seems an attractive approach. Roscovitine (Seliciclib, CYC202), being a potent inhibitor of CDK2/cyclin E, CDK7/cyclin H, CDK9/cyclin T1 and CDK5/p35-p25, was chosen for this trial [28,29]. This study resulted in a robust arrest of PKD in the *jck* mouse model of slowly progressive disease and the *cpk* mouse model of aggressive PKD [29]. Importantly, continuous daily administration of the drug was not required to achieve efficacy, due to long lasting durability of treatment effect. These data point to an important potential clinical benefit for the life-long therapy that ADPKD will likely require. Roscovitine was active against cysts originating from different segments of the nephron, a desirable feature for a potential drug against ADPKD, where cysts are formed in multiple parts of the nephron. Mode of action studies reveal that roscovitine inhibits cystogenesis through a combination effect of cell cycle blockade, transcriptional inhibition and apoptotic arrest. Roscovitine also affected Raf/MEK/ERK signalling, which regulates cyclin D1 expression [29].

It is important to note that roscovitine treatment affected downstream pathways of cystogenesis,

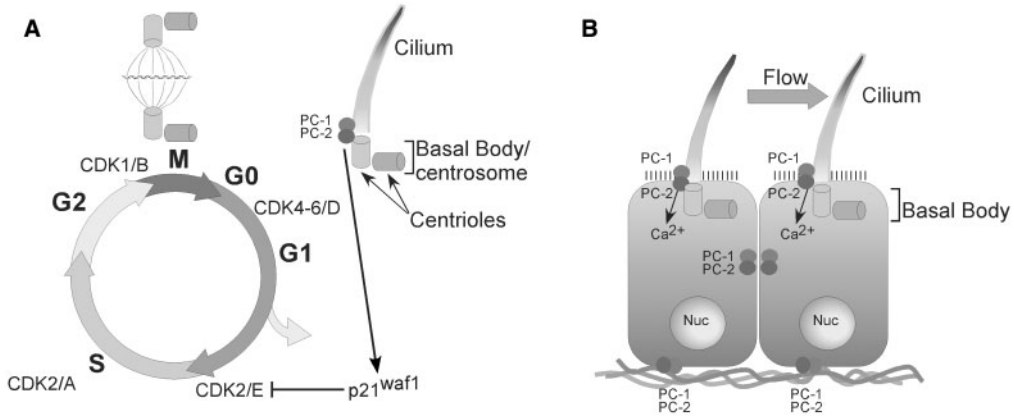

Fig. 1. Cilia and the cell cycle are coordinately regulated. Panel **A** depicts stages of the cell cycle controlled by CDK/cyclins and cilia/centrosome formation. The primary cilium forms in fully differentiated cells in G0 phase of the cell cycle. Primary cilium extends from the basal body formed by the centrosome. Centrosome consists of two centrioles: mother centriole (shown as blue cylinder) and daughter centriole (shown as green cylinder). As cells enter the cell cycle, the cilium is resorbed and the centrosome can now serve as a centre for mitotic spindle formation during mitosis (M). The centrosome is required for several cell cycle transitions: G1to S phase and G2 to M phase. Panel **B** shows terminally differentiated tubular epithelial cells. Polycystins (PC-1, red circle and PC-2, blue circle) are found in multiple subcellular locations of tubular epithelial cells: cell-matrix contacts, intercellular membranes and primary cilium. Polycystins are expressed in the basal body/centrosome of the cilium and thought to mediate ciliary mechanosensation in response to tubular flow through Ca^{2+} influx. Polycystins and other cystoproteins may, therefore, connect mechanosensory function of the cilium to the centrosome which, in turn, anchors signal transduction pathways and controls cell-cycle progression. Experimental evidence suggests that PC-1 functions to regulate the cell cycle by inhibiting CDK2 activity through up-regulation of $p21^{waf1}$, arresting cells in G0/G1 phase (as shown on the bottom of panel A).

probably because it targets early events in molecular pathogenesis. Thus, a significant reduction in cAMP levels was detected in treated kidneys along with down-regulation of aquaporin-2 expression [30]. Seliciclib, an orally bioavailable small molecule, is currently in clinical trials as a cancer drug candidate [31]. Unlike first-generation CDK inhibitors with multiple off-target activities, Seliciclib was designed to be a highly specific CDK inhibitor. Several treatment schedules were evaluated in clinical trials to determine its safety, tolerability and pharmacokinetic properties. It was shown that Seliciclib can be administered safely without side effects seen in modern cancer therapeutics. Adverse events included transient elevations in serum creatinine, hypokalaemia and reversable elevations in liver enzymes [31].

Beneficial effects of CDK inhibition were also described in other renal proliferative diseases such as collapsing glomerulopathy, mesangial proliferative glomerulonephritis, crescentic glomerulonephritis and lupus nephritis [32]. It is likely that such therapy will promote restoration of cell cycle quiescence, terminal differentiation and preservation of renal function.

Conclusions

Significant progress has been made over the last few years towards a greater understanding of the molecular pathogenesis of PKDs. These advances have already brought several potential therapies designed to interfere with specific disease pathways. It seems likely that new therapeutic targets will be coming shortly from studies focusing on other cystogenic cascades such as Wnt signalling, regulation of [Ca2+]i homeostasis and fluid secretion. The availability of reliable magnetic resonance imaging techniques to accurately measure PKD progression in a relatively short period of time opens opportunities for clinical trials of potential therapies that showed promise in pre-clinical testing. Ultimately, successful treatment for PKD may involve a combination therapy targeting several key pathways of cystogenesis.

Conflict of interest statement. None declared.

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Mannose-binding lectin and the kidney

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Introduction

The complement system is a key component of innate immunity that plays a major role in host defense against invading pathogens. This is effectuated by a direct attack of pathogens, by mediating inflammation and opsonin-dependent phagocytosis, and by induction and amplification of adaptive immunity. In recent years, it has become increasingly clear that the complement system also plays an important role in