Polycystic Kidney Disease: New Knowledge and Future Promises

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Abstract

Polycystic kidney disease (PKD) is one of the most common genetic kidney diseases, characterized by the formation of fluid-filled renal cysts, which eventually leads to end-stage renal disease. Despite several decades of investigation, the explicit molecular and cellular mechanisms underpinning renal cyst formation have been until recently unresolved, severely hampering the development of effective therapeutic approaches. Currently, most PKD therapies have been developed for limiting disease complications, such as hypertension. Although Tolvaptan has been approved for treating PKD in few countries, the associated hepatic toxicity remains a major concern. In this Review, we will discuss the most recent advances in PKD research, covering aspects ranging from newly identified genetic/epigenetic causes, increment in mechanistic interpretation, novel therapeutic targets, to the promises offered by the emerging stem cell technology.

Introduction

Polycystic kidney disease (PKD) is constituted by three major forms: autosomal dominant (ADPKD), autosomal recessive (ARPKD), and syndromic (such as Joubert, Meckel and Bardet Biedle syndromes) PKD, in which renal and liver cyst formation accounts for most of the morbidity [1]. ADPKD is usually adult-onset with an estimated occurrence of 1:500-1:1,000. Clinical studies estimate that mutations in *PKD1* (encoding Polycystin1) and *PKD2* (encoding Polycystin2) account for approximately 78% and 13% of ADPKD cases respectively, with the remaining 9% cases genetically unsolved [2]. ARPKD, mostly caused by *PHKD1* mutations, has an occurrence of 1:20,000, and often manifests *in utero* or in the perinatal period [2].

PKD has long been categorized as a ciliopathy, because most PKD-related loci are targeted to ciliary-centrosome complex. There has been a growing consensus on the possible factors contributing to cyst growth (Figure 1). Polycystin1 (PC1) and Polycystin2 (PC2) appear to constitute the foundation of cystic pathway. Genetic mutations within the central PKD machinery produce functionally defective proteins, leading to decreased intracellular Ca^{2+} and increased cyclic adenosine monophosphate (cAMP) levels, the two cellular hallmarks of PKD [2]. These early events remodel multiple pathways, including mTOR, LKB1-AMPK, JAK-STAT, Ras-MAPK, EGFR, PKA and WNT, the combinatory effects of which result in aberrant cell proliferation/apoptosis, increased trans-epithelial fluid secretion, faulty extracellular matrix (ECM) turnover, and metabolic reprogramming [3,4]. The recent advances in understanding the basis of PKD have both substantiated and challenged the existing theory of cyst formation. Here, we discuss these latest PKD studies, and the future directions that may lead to better understanding and novel therapeutics for the treatment of PKD.

New genetic/epigenetic causes

Whole exome sequencing (WES) has greatly facilitated the identification of novel genes associated with PKD. These novel genes encode proteins that interact with PC1 and PC2 in some way, and are important for maturation, trafficking, localization, and function of PKD proteins. These studies reiterated the importance of the central PKD machinery and highlighted novel regulators of Polycystin, which could serve as potential therapeutic targets. As one of the examples, *PKHD1* has long been considered as the only ARPKD gene until a recent landmark study described mutations in *DZIP1L* that encodes DAZ interacting protein 1-like in ARPKD patients [5*]. As a centriole protein, DZIP1L interacts with Septin2 to maintain the periciliary diffusion barrier at the ciliary transition zone [5*]. Loss of *DZIP1L* leads to bulged cilia, reduced ciliogenesis, as well as compromised ciliary translocation of PC1 and PC2 [5*,6]. Readers interested in other ciliary genes are referred to several excellent reviews on this topic [1,7-9].

It is noteworthy that several newly identified PKD genes are associated with endoplasmic reticulum (ER)-mediated protein homeostasis. One such gene is *GANAB*, which encodes the glucosidase IIα subunit (GIIα), a resident ER enzyme involved in asparagine (N)-linked glycosylation, which may participate in maturation and quality control of transmembrane glycoproteins. *GANAB* mutations were identified in patients that present with ADPKD or the related autosomal-dominant polycystic liver disease (ADPLD) [10-12]. *GANAB*$^{-/-}$ cells showed defects in maturation, surface and ciliary localization of PC1 and PC2 [10]. Interestingly, in *GANAB*$^{-/-}$ cells, either introduction of wild-type GIIα or targeting of defective polyglutamylation restored PC2 localization, thus proposing a possible role of GANAB in cystogenesis [13]. WES also identified pathogenic mutations of *DNAJB11* in ADPKD. DNAJB11, a co-factor of BiP co-chaperone, has extensive functional overlap with GANAB in the maintenance of ER protein homeostasis. Thus, loss of *DNAJB11* results in defective maturation and trafficking of PC1 and UMOD [14*]. In addition, Cabezas *et al.* showed that a promoter mutation (c.-167G>T) in *PMM2*, which encodes, Phosphomannomutase 2, a key enzyme in N-glycosylation, may contribute to renal cyst formation through alteration of tissue-specific chromatin loop formation and transcriptional activity. While coding mutations in *PMM2* cause a congenital disorder of glycosylation with severe neurological symptoms, patients with this promoter mutation present with hyperinsulinemic hypoglycemia and congenital PKD (HIPKD) [15].

Epigenetic regulation represents another layer of regulation on the dose of gene products. Genome-wide methylation analysis described that hypermethylation of the functional copy of *PKD1* in ADPKD patients resulted in haploinsufficiency of *PKD1* gene products [16]. In ADPKD patients, significant epigenetic silencing was also seen in genes implicated in cellular transport, adhesion, and cell differentiation pathways [16]. A recent study demonstrated global hypothemylation in ADPKD cortical kidney tissues using single nucleotide resolution methylome profiling [17]. Increased methylation at the 3' end of *PKD1* gene body was also observed, although *PKD1* mRNA expression remained unchanged [17]. Single cell sequencing approaches may help to elucidate more subtle cell-level changes that correlate with disease onset and progression. Further evaluation of epigenetic and transcriptomic changes in PKD patients or disease models may reveal novel modifiers of cystogenesis that could serve as therapeutic

targets. For example, Li *et al.* identified lysine methyltransferase SMYD2 as an epigenetic modifier that facilitates PKD pathogenesis in *PKD1* knockout mice via modulation of the methylation status of p53, p65, STAT3, and histones [18].

Old pathways, new interpretation

New findings have both supported and challenged the cilia-centered hypothesis, in which primary cilia function as a mechanosensor to regulate intracellular Ca^{2+} concentration via PC1 and PC2 complex-mediated Ca^{2+} currents. Kim *et al.* provided supportive evidence that WNTs bind to the extracellular domain of PC1 and induce Ca^{2+} currents in a PC2-dependent manner [19]. However, work by the Clapham group showed that PKD1L1 and PKD2L1, instead of PC1 and PC2, form a heteromeric ion channel to control ciliary Ca^{2+} concentration without altering global cytoplasmic Ca^{2+} concentration, in a flow-insensitive manner [20,21]. Recently, the Clapham group demonstrated a complete lack of mechanically induced Ca^{2+} increase in primary cilia, further challenging the ciliary hypothesis [22]. Future study may need to consider combinatory modes of Ca^{2+} channel sensing and activation in addition to mechanosensation or ligand binding. Furthermore, it has been intensively discussed whether primary cilia safeguard against or facilitate cystogenesis. Disruption of ciliary intraflagellar transport (IFT) leads to renal cyst formation in non-orthologous mouse models [23]. On the contrary, ablation of primary cilia [24], or induction of cilia disassembly via Aurora-A activation [25], reduces cyst growth in *PKD1 and PKD2* mutant mice.

It is not until recently had we started to be aware of the negative feedback loop between primary cilia and autophagy. Defective ciliary IFT compromises nutrient deprivation-induced autophagosome biogenesis [26,27], while autophagy negatively regulates ciliogenesis, possibly through the autophagic degradation of OFD1, which promotes ciliogenesis [26,27]. Since impaired autophagic flux was observed in kidneys of ADPKD patients [26], autophagy activation may offer a promising strategy for cyst management [28].

Meanwhile, recent studies are gradually elucidating the complex regulatory network underpinning the defective cell proliferation and apoptosis occuring in PKD [29-32]. It was also revealed that multiple adenylyl cyclases (ACs) are implicated in the upregulation of intracellular cAMP. Loss or inhibition of ACs, in most cases, effectively ameliorates renal cyst formation in orthologous PKD and PLD mouse models [33-36]. Furthermore, last three years have witnessed an outbreak on the revelation of PKD protein/protein complex structure [37,38,39*,40,41]. A comprehensive structural analysis will provide a perfect means to bridge the knowledge gap in between genetic mutations and functional defects, as well as disease presentation in PKD.

Novel therapeutic target --- metabolic reprogramming in PKD

Over the last few years, accumulating evidence indicates that ADPKD shares many similarities with solid tumors despite overarching differences in disease presentation [42]. Among these, metabolic reprogramming has emerged as a novel hallmark of PKD [43]. In cancer, glucose metabolism is shifted from TCA cycle to glycolysis to meet high energy demand. In the context of PKD, it is similarly important for the cells to produce sufficient energy to sustain two essential cellular cystic phenotypes, proliferation and trans-epithelial fluid transportation.

In 2013, Rowe and colleagues demonstrated for the first time that *PKD1* mutations lead to dependence on enhanced glycolysis in ADPKD, via simultaneous upregulation of mTORC1 and inhibition of LKB1-AMPK signaling [44,45]. A follow up study further illustrates that PKD progression can be ameliorated upon chronic treatment of low doses of 2DG, which restores the levels of phosphorylated AMPK without obvious toxicity in the brain and heart [46]. Likewise, food restriction, an alternative approach to modulate energy metabolism, ameliorates PKD-related disease phenotypes, similarly through activating LKB1-AMPK as well as inhibiting mTOR pathway, without affecting the cellular levels of cAMP and SIRTUIN [46,47].

Consistent with increased glycolysis, reduction in lipid metabolism and oxidative phosphorylation (OXPHOS) was observed in PKD. $PKD1^{-/-}$ mice display intrinsic dysfunction in fatty acid oxidation (FAO) [48]. Decreasing dietary lipid intake improves some of PKD-related phenotypes in $PKD1^{-/-}$ mice [48]. Hajarnis *et al.* reported that in renal cysts of mouse and human ADPKD, reduction of OXPHOS and FAO may be attributed to miR-17-medicated suppression of *Ppar*α [49*]. Anti-miR-17 treatment attenuates cyst growth in two PKD mouse models ($PKD1^{RC/RC}$, and $Nphp3^{pcy/pcy}$), as well as an *in vitro* human cell culture model of PKD, thus offering a promising therapeutic approach [49*].

Aberrant glutamine metabolism has recently been described in both murine ARPKD and ADPKD models. It is plausible that a glutamine-dependent TCA shunt towards 2-hydroxyglutarate (2-HG) production serves to fuel cell growth and proliferation during cystogenesis [50]. Flowers and colleagues further demonstrated that both *LKB1* and *PKD1* mutant mice display glutamine-dependent ureteric growth and branching morphogenesis, which can be rescued by adding either non-essential amino acids or by reducing glutathione [51*]. Inhibition of glutamine metabolism greatly retards cyst progression in both orthologous and non-orthologous PKD mouse models *in vivo* [51*,52*].

There have been accumulating efforts to address the correlation between metabolic reprogramming with the PKD machinery. There is evidence that lack of PC1 expression may mimic a low oxygen environment, leading to decreased ER-mediated Ca^{2+} release [53]. Subsequently, defective Ca^{2+} signaling results in oxidative stress through reduced PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α) expression and increased cAMP-PKA signaling [54]. Clinical studies have identified oxidative stress early in ADPKD, which can be reduced by administering antioxidants [54]. Several recent studies proposed that Bicc1 (Bicaudal C1), a non-classic PKD protein, is implicated in multiple metabolic pathways, including mTOR and gluconeogenesis [29,55]. It is conceivable that metabolic rewiring may be a common phenotype shared by various types of cystic kidney diseases. We are only just starting to shed new lights on the roles of metabolic reprogramming in PKD (Figure 2). It will be exciting if we could re-purpose drugs that are currently used to target cancer or metabolic diseases for the treatment of PKD [56].

Stem cell-based PKD model

Over the last few years, tremendous efforts have been made to coax human pluripotent stem cells (PSCs), including both embryonic stem cells (ESCs) and induced PSCs (iPSCs), into three-Dimensional (3D) organoids, which hold great promise in regenerative medicine [57]. Earlier studies successfully differentiated human PSCs into kidney progenitors that are capable of further maturating into 3D kidney structures upon organ co-culture [58,59]. More recently, several groups have successfully established protocols for differentiating human PSCs into complex 3D kidney organoids that are comprised of nephron-like structures patterned along a proximal-to-distal axis [60-64].

This approach offers unprecedented opportunities for studying human kidney development, disease pathogenesis, and to test drug toxicity and efficacy in a patient-specific manner (Figure 3). Morizani *et al.* provided proof-of-concept that in human PSC-derived kidney organoids, renal toxins damage specific nephron segments that are known to be susceptible to the tested drugs [61]. Meanwhile, the possibility of growing kidney organoids in a high-throughput manner greatly increases the feasibility and amenability in performing drug screening [65]. ADPKD patient iPSCs have been employed to study the cellular behavior of PC1 and PC2 upon differentiation into hepatic cells [66]. Freedman and colleagues have taken one step further by using CRISPR-Cas9 gene editing tools to generate *PKD1*$^{-/-}$ and *PKD2*$^{-/-}$ human PSCs that, upon differentiation into kidney organoids, form cysts spontaneously [67]. A recent study by the Freedman group has demonstrated remarkable cyst formation upon interference with the microenvironment of kidney organoids [68*]. The removal of adherent cues expedites cystogenesis in *PKD1*$^{-/-}$ and *PKD2*$^{-/-}$ kidney organoids [68*]. It is beyond any doubt that human PSC-derived kidney organoid platform will soon be widely utilized to model various aspects of human PKD pathogenesis.

Future perspectives

The progress made during the last five years has been remarkable, offering new insights as well as raising questions on our current understanding of PKD. Technological advances always lead to knowledge explosion. Single cell sequencing approaches may be harnessed to decipher the genetic/epigenetic regulations underpinning the sporadic cyst origin of PKD. In the foreseeable future, structure elucidation of PKD proteins (wildtype and mutants) will sketch a map that accurately correlates genetic lesions with the respective functional defects.

The discovery of metabolic reprogramming as a novel hallmark and therapeutic target of PKD has been encouraging. Several clinical trials are already in the pipeline. In many diseases, metabolic reprogramming represents a secondary effect and a a result, it may not be sufficient to cure PKD by modulating metabolism alone. It will be critical to dissect the interactions between metabolic reprogramming and the core PKD machinery, including the PC1 and PC2 complex as well as the primary cilia.

The discrepancy of findings between different studies strongly indicates the importance of model selection and validation. To this point, patient iPSC-derived kidney organoids offer both promises and challenges in our efforts towards personalized medicine. The current major concern is that most human PSC derivatives are fetus-like and immature. Recent single cell studies have also revealed missing cell types, as well as inter-organoid variation [69,70]. There is an urgent need

to consistently generate kidney organoids that harbor the desired cellular components with correlative functionality. A cross-consultation between directional differentiation [71] and organ-on-a-chip technology [63,72] may bring the current kidney organoid platform to the next level.

Conflict of interest statement

The authors declare no conflict of interest.

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Figure legends

Figure 1 Mechanisms underpinning polycystic kidney disease pathogenesis.

Schematic drawing that summarizes our current understanding of PKD pathogenesis from genetic causes to dysregulated pathways and cellular phenotypic outputs. Light blue squares indicate recent discoveries and arrows indicate upregulation or downregulation of the indicated pathway.

Figure 2 Current schematic of metabolic reprogramming in PKD.

In PKD, an increase in glycolysis, fatty acid synthesis, and glutamine metabolism is accompanied with a decrease in oxidative phosphorylation and fatty acid oxidation.

Figure 3 Proposed workflow for using stem cell-based models to study PKD.

Skin biopsies derived from PKD patients or healthy individuals are reprogrammed into iPSCs. CRISPR-Cas9 gene editing tools are employed to either correct the defective gene, or to introduce a genetic lesion that is associated with PKD. Kidney organoids derived from patient-specific iPSCs will be utilized for studying disease mechanisms or drug screening.

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