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Targeting new cellular disease pathways in ADPKD

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited cause of end-stage renal failure. Understanding the molecular and cellular pathogenesis of ADPKD could help to identify new targets for treatment. The classic cellular cystic phenotype includes changes in proliferation, apoptosis, fluid secretion, extracellular matrix and cilia function. Recent research however suggests that the cellular cystic phenotype could be broader and that changes such as altered metabolism, autophagy, inflammation, oxidative stress and epigenetic modification could play important roles in the processes of cyst initiation, cyst growth or disease progression. Here we review these newer cellular pathways, describe evidence for their possible links to cystic pathogenesis or different stages of disease and discuss the options for developing novel treatments.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) results from germline mutations in *PKD1* or *PKD2* [1]. It is the fourth most common cause of end-stage renal disease (ESRD) and accounts for 5-10% of patients on renal replacement therapy globally. The growth of kidney cysts and progressive loss of renal function occurs gradually over many decades. Genotype, other genes, epigenetic modifiers and environmental factors all play a role in determining the rate of disease progression [2].

Despite major advances, the functions of polycystin-1 (PC-1) and polycystin-2 (PC-2 or TRPP2), the proteins encoded by *PKD1* and *PKD2,* are still not completely understood [3, 4]. PC-1 interacts with PC-2, a non-selective calcium channel, to regulate a number of signaling pathways involving cAMP and Ca^{2+} homeostasis [5-8]. Defects in polycystin expression or function result in changes to a number of cellular pathways such as proliferation, apoptosis, fluid secretion, differentiation and cell adhesion [9-14]. Other cellular abnormalities reported include ciliary dysfunction, planar cell polarity, centrosome number and inflammation [15-20].

The past decade has witnessed the first successful translation of results from preclinical studies into clinical trials. Tolvaptan, a vasopressin V2 receptor antagonist, has been approved for ADPKD patients with evidence of more rapid disease progression in several countries [21-23]. Other patients considered at higher risk for disease progression may also warrant treatment. These include those with a *PKD1* truncating mutation and/or increased total kidney volumes for age [24]. However, tolvaptan is only moderately effective, has significant aquaretic side-effects reducing patient tolerability and potential hepatotoxicity mandating more frequent monitoring. Hydration therapy either by water intake or in combination of a low osmolar diet to suppress serum arginine vasopressin (AVP) levels could be an alternative approach although this requires formal testing [25, 26]. Thus additional treatment options which

are safer and more effective would be an advantage.

Table 1 summarises several drugs that are currently undergoing clinical trials. These include somatostatin analogues (inhibiting cAMP), tyrosine kinase inhibitors (targeting proliferation) and other compounds targeting inflammation, oxidative stress, cell metabolism or epigenetic regulation including preclinical studies [27-34]. In this paper, we will focus on the evidence linking these more recent cellular abnormalities to ADPKD pathogenesis, their associated signaling pathways and evaluate their potential as targets for therapeutic development.

Metabolism

An emerging hypothesis is that ADPKD pathogenesis could be causally linked to altered cellular metabolism. A seminal study reported that *Pkd1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) preferentially utilise aerobic glycolysis for energy generation, mimicking the well-known Warburg effect originally described in cancer cells [35]. Glucose deprivation suppressed the high ATP content of *Pkd1*$^{-/-}$ cells and reduced cell proliferation suggesting that metabolic reprogramming could be a new treatment strategy [35]. Several metabolic sensors such as the AMP-activated protein kinase (AMPK), mTOR and Sirtuin 1 (SIRT1) may be involved in mediating this response [36]. These findings have led to a new therapeutic approach of targeting the glycolytic pathway [37, 38]. The administration of a glucose analogue, 2-deoxyglucose (2DG), suppressed aerobic glycolysis and reduced cyst formation in an inducible *Pkd1* model of both early and late disease [39].

A second study has provided support to the concept of metabolic reprogramming by taking a different approach. Mild to moderate calorie restriction (10-40%) reduced cyst progression, fibrosis and inflammation in two slowly progressive orthologous models (*Pkd1*$^{RC/RC}$, *Pkd2*$^{WS25/-}$) through activating AMPK and suppressing the mTOR

pathway [36]. These results confirm earlier studies showing a beneficial effect of metformin in reducing cyst burden through the activation of AMPK and inhibition of mTOR and the Cystic Fibrosis Transmembrane Regulator (CFTR) in two early-onset mouse models of *Pkd1* [40]. Interestingly, these changes were found to be independent of the cAMP pathway and glycolysis [36]. How calorie restriction will impact on disease progression in ADPKD patients remains to be tested [38].

Finally, a microarray study in an inducible *Pkd1* knockout mouse (deletion P5-P9) identified transcriptional changes in genes involved in metabolic pathways as a key feature in this severe early-onset model [41]. The authors postulated that a key transcription factor, hepatocyte nuclear factor-4α (HNF4α), could be a major disease modifier; indeed *Pkd1/Hnf4α* double mutants had significantly more severe cystic kidneys [41]; a follow-on study reported that lipid metabolism and fatty acid oxidation (but not glycolysis) were dysregulated in these mice [42]. Significantly, a modest dietary reduction (25%) in lipid content resulted in a small but significant improvement in disease progression in both models [42]. Overall, these data indicate that altered cellular glucose and/or lipid metabolism are signature features of the ADPKD phenotype, may contribute to disease pathogenesis and could potentially be modified through changes in diet or lifestyle.

Autophagy

Autophagy (macroautophagy) is a dynamic cellular process that recycles intracellular proteins and organelles through the highly regulated lysosomal degradation pathway during development or in response to cellular stresses such as nutrient deprivation, endoplasmic reticulum stress and hypoxia [43]. It maintains mitochondrial function and energy homeostasis to meet the elevated metabolic demands of cell proliferation in pathological conditions such as cancer [44]. A connection

between autophagy and PKD has been proposed since cyst development and growth involves tubular proliferation and apoptosis, processes modulated by autophagy [45]. Autophagy is also tightly regulated by mTOR, AMPK and hypoxia-inducible factors (HIFs) in response to changes in nutritional status and cellular metabolism [46] indicating a significant crosstalk with metabolic status.

Research evidence suggests that autophagy may play a role in modifying the course of ADPKD. However, there are reports of both decreased and increased autophagy in different model systems. Markers of autophagy (LC3-II and beclin-1) and HIF-1α expression were found to be increased in late stage *cpk* and Han:SPRD cystic kidneys [47]. Conversely, inefficient autophagy upon glucose deprivation resulted in increased apoptosis in murine *Pkd1* cells suggesting that *Pkd1* deficiency was associated with impaired autophagy [35]. Indeed, insufficient autophagic flux and inadequate autophagosome formation have also been reported in a zebrafish *pkd1a* mutant and in *Pkd1* mutant kidney cells [48]. How impaired autophagy in tubular epithelia leads to cyst formation or expansion remains unclear. Interestingly, both impaired autophagy and enhanced autophagy may promote tumor growth depending on its tissue of origin [49]. Recent studies have shown that autophagy regulates cilia length by removing key proteins involved in ciliogenesis [50] and that the primary cilium may regulate epithelial cell volume through flow-dependent autophagy in an mTOR and PC2 dependent manner [51]. Alterations in cilia structure or cell volume could potentially modify the cystic cellular phenotype through mTOR and/or Ca^{2+} dependent pathways.

In zebrafish *pkd1a* fish, the activation of autophagy using mTOR-dependent (rapamycin) or mTOR-independent compounds (carbamazepine, minoxidil) attenuated cyst formation [48]. Another study reported that phospholipase D inhibitors induced autophagosome formation in human ADPKD cystic cells [52]. Whether autophagy is a

crucial signal which initiates cyst formation or is a secondary feature of cyst expansion is presently unclear; it could play different roles in the early and late stages of disease. However, it should be possible to test whether altering autophagy is beneficial or harmful at different stages of disease in animal models since libraries of autophagy enhancers and suppressors are readily available [46].

Inflammation and innate immunity

Serum markers of inflammation and oxidative stress are elevated in ADPKD patients with preserved kidney function [53, 54]. Microarray analysis of ADPKD kidneys revealed up-regulation of genes associated with immune and inflammatory responses [55, 56]. Urinary levels of the lipopolysaccharide receptor CD14, macrophage chemoattractant protein-1 (MCP1) and macrophage migration inhibitory factor (MIF) were elevated in ADPKD patients and demonstrated a positive correlation with either total kidney volume (TKV) or change in TKV [57, 58]; correlations for CD14 were stronger in males than females [57]. Inflammation correlates not only with the renal phenotype but also with vascular changes. In one study, increased arterial stiffness and pulse wave velocity correlated positively with elevated serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentrations [59].

Several groups have reported that experimental approaches which inhibit or reduce inflammation could have a beneficial effect both on cyst burden and disease progression in preclinical models. Macrophage-derived soluble factors may directly stimulate epithelial cell proliferation and cyst growth since macrophage depletion in an early-onset *Pkd1* model improved kidney function and reduced cyst burden[60]. Similarly, the administration of TNF-α induced cyst formation in *Pkd2* heterozygous kidneys *ex-vivo* and *in vivo*, an effect blocked by the TNF-α inhibitor, etanercept [30]. It has also been reported that MIF, a key regulator of the TNF-α pathway, had a pathogenic role in

three *Pkd1* mouse models; MIF also activated ERK, mTOR and glycolysis in cystic cells [31]. Thus targeting MIF using a mAb or small molecule (isoxazolines) could potentially reduce macrophage recruitment and cyst formation [31, 61]. A natural compound, triptolide, was shown to suppress cystogenesis in several *Pkd1* mouse models and can restore cytosolic Ca^{2+} release in *Pkd1* null cells possibly by acting as a PC2 agonist [62-64]. However, triptolide also has well-known anti-inflammatory effects through inhibiting nuclear factor kappa B (NF-kB) transactivation [65]. The results of a Phase 3 clinical trial of triptolide in ADPKD (NCT02115659) are awaited. Finally, another natural compound, resveratrol, was reported to reduce cyst formation in the Han:SPRD rat and in a zebrafish *pkd2* model through blocking the NF-kB pathway [32]. Changes in cell proliferation were accompanied by a reduction in pro-inflammatory chemokines and macrophage infiltration.

Neutrophil gelatinase-associated lipocalin (Ngal) is involved in the innate immune response to bacterial infection through regulating intracellular iron concentrations [66]. Increases in urinary Ngal have been reported to correlate strongly with GFR in ADPKD suggesting that it could be a sensitive disease biomarker [58]. Recent studies have however suggested a protective rather than a pathogenic role for Ngal in ADPKD. Kidney-specific (*Ksp-Cdh16*) transgenic overexpression of Ngal in a *Pkd1* hypomorph model ($Pkd1^{L3/L3}$) prolonged survival, reduced cystic size and interstitial fibrosis [67] confirming earlier results using adenoviral delivery of exogenous Ngal in a different *Pkd1* model (*Ksp*-Cre) [68].

Oxidative stress

Microarray network analysis in a *Pkd1* model has correlated oxidative stress as one of the transcriptional pathways associated with disease severity [41]. Heterozygous *Pkd1* and *Pkd2* tissues were more sensitive to ischaemia-reperfusion injury suggesting

an increased sensitivity to oxidative stress either due to increased oxidant production and/or decreased antioxidant protection [69-71]. Both mechanisms are possible. Evidence of increased oxidative stress and decreased mRNA expression of several antioxidant enzymes have been reported in non-orthologous models (*cpk* mice, Han:SPRD rats) indicating that reduced protection could contribute to disease severity [72]. However, it has not yet been shown that anti-oxidants can reduce disease severity in ADPKD. An increase in basal oxygen reactive species has been reported to underlie the reduced endothelium-dependent arterial dilatation observed in a murine ADPKD model [71] suggesting that this could be a more general phenomenon. Consistent with this, serum markers of oxidative stress (8-isoprostane, PGF2α, oxidised LDL) were reported to be higher in ADPKD patients with preserved kidney function (eGFR >60ml/min/1.73m^2) and correlated significantly with elevated levels of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase (NOS) [73, 74]. ADMA is metabolised by demethylarginine dimethyl aminohydrolase (DDAH) which is sensitive to inactivation by oxidation of a reactive cysteine residue in the active site: reduced breakdown of ADMA due to oxidative stress could account for these findings.

Increased oxidative stress could lead to cellular injury or lipid peroxidation through the production of highly reactive oxygen free radicals including peroxynitrite, a product of superoxide and NO [71] resulting in tissue inflammation (see above) and the release of intracellular mediators such as ATP. ADPKD cells have been shown to have higher ATP content and the encapsulated cysts create closed microenvironments for autocrine purinergic signaling though P2X and P2Y receptors [75]. Purinergic receptors are known to play a key role in inflammation, apoptosis and fluid secretion [76-78]. Inhibition of the P2X7 receptors has been shown to reduce cyst formation in *pkd*2 zebrafish morphants [79]. These promising results require further confirmation in

mammalian ADPKD models. The reported beneficial effects of pravastatin on kidney growth in children and young adult (8-22 years) [80] could have been mediated through suppression of inflammation and oxidative stress [81, 82].

Epigenetic modification

Experimental evidence of a role for epigenetic modification of gene expression and/or protein function in ADPKD has started to emerge from animal models with a major focus on the role of histone acetylation. This modifies gene expression through a direct effect on chromatin structure including the recruitment of accessory factors such as bromodomain-extraterminal (BRD BET) proteins which bind to the acetylated lysines on histone tails. An early clue for a role of histone acetylation in PKD pathogenesis came from a focused chemical modifier screen in zebrafish *Pkd2* mutants and morphants [33]. In this study, the pan-HDAC (histone deacetylase) inhibitor, trichostatin A (TSA) and a Class I HDAC, valproic acid (VPA) were effective in reversing body curvature, laterality and reducing cyst formation. Furthermore, VPA was able to reduce cyst growth in a kidney-specific (*Pkhd1*-Cre) *Pkd1* model. It was also reported recently that tubacin, an inhibitor of HDAC6 (Class II HDAC), improved disease and kidney function in an inducible *Pkd1* conditional model (*iPax8*-Cre) by reducing cAMP levels, proliferation and CFTR-mediated chloride secretion [83]. The Class III HDAC, SIRT1, was shown to have a similar role in promoting cyst growth in three other murine models of *Pkd1* [84]. A Phase 2 trial with nicotinamide, a pan-sirtuin inhibitor, in ADPKD, is currently in progress (NCT02558595). Finally, JQ1, an inhibitor of BET BRD proteins delayed cyst growth in two *Pkd1* mouse models, probably by targeting Brd4 [34].

Concluding remarks and future directions

The last decade has seen promising results emerging from a number of preclinical studies and the first clinical trials for ADPKD. The first treatment shown to slow renal disease progression in ADPKD has become available although more effective treatments with fewer side-effects are needed. The identification of new cellular pathways linked to ADPKD such as enhanced aerobic glycolysis, altered cellular lipid and glucose metabolism, autophagy, inflammation signaling, oxidative stress and epigenetic modification point to alternative approaches for therapeutic intervention (Figure 1, Table 1). It seems likely that a combinatory and/or serial approach targeting different signaling and cellular pathways at different stages of disease will be required for the effective and lifelong treatment of ADPKD.

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Conflict of interest

Results presented in this paper have not been published previously in whole or part, except in abstract format. ACMO has received consultancy fees from Otsuka and Mironid and grant funding from Otsuka and ONO.

Figure legend

Figure 1. New cellular disease pathways implicated in the pathogenesis of ADPKD

Mutations in *PKD1* or *PKD2* trigger multiple cellular events, such as enhanced aerobic glycolysis, inflammation, oxidative stress, autophagy impairment and epigenetic modification. In addition to cell proliferation, apoptosis, fluid secretion and cilia dysfunction, these cellular events contribute to the full blown cystic phenotype The different stages of ADPKD (cyst initiation, cyst growth, disease progression and renal fibrosis) are likely to require different therapeutic approaches. The figure was produced using illustrations adapted from Servier Medical Art. AMPK, AMP-activated protein kinase; ATP, adenosine 5'-triphosphate; BRD BET, bromodomain-extraterminal; 2DG, 2-deoxyglucose; HDAC, histone deacetylase; HIFα, hypoxia-inducible factor-α; HNF4α, hepatocyte nuclear factor-4α; LC3, microtubule-associated protein light chain 3; MIF, migration inhibitory factor; mTOR, mammalian target of rapamycin; NF-kB, nuclear factor kappa B; PLD, phospholipase D; ROS, reactive oxidative species; SIRT1, Sirtuin 1; TNF-α, tumor necrosis factor-α.

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Table 1. Recent clinical trials and preclinical studies targeting specific signaling pathways or mediators in ADPKD

Drug	Signalling pathway or mediator	Development Status	Placebo controlled	Age at inclusion (yrs)	CKD Stage, eGFR or TKV at inclusion	Clinical Outcomes or Animal models	References or ClinicalTrials.gov identifier
Curcumin	mTOR, STAT3	Phase 4	Yes	6-25	CKD 1-2 (≥80ml/min/1.73m^2)	FMD, PWV CRP, IL-6, urine 8-iso-PGF2α, 8-OHdG, TKV	NCT02494141
Lanreotide	cAMP	Phase 3	No	18-60	CKD 3 (30-60ml/min/1.73m^2)	TKV, eGFR	NCT01616927
Octreotide-LAR	cAMP	Phase 3	Yes	>18	CKD 3-4 (15-40ml/min/1.73m^2 MDRD)	TKV, mGFR	NCT01377246
Triptolide	PC2, NF-kB	Phase 3	Yes	40-75	CKD 1-2 (≥60ml/min/1.73m^2; ΔTKV >6% pa)	TKV, eGFR	NCT02115659
Bosutinib	Src tyrosine kinase	Phase 2	Yes	18-50	CKD 1-2 (≥60ml/min/1.73m^2; TKV ≥750ml)	TKV, eGFR	NCT01233869
Niacinamide	SIRT1	Phase 2	Yes	18-60	CKD 1-3 (>50ml/min/1.73m^2)	TKV, eGFR, pain score, urine MCP-1	NCT02558595

Metformin	AMPK	Phase 2	Yes	18-60	CKD 1-3 (>50ml/min/1.73m^2)	QoL, TKV, TLV, eGFR	NCT02656017
Pioglitazone	PPAR-γ	Phase 2	Yes	18-55	CKD 1-3 (>50ml/min/1.73m^2)	TKV, bone marrow fat	NCT02697617
Tesevatinib (KD019)	Receptor Tyrosine kinases	Phase 1/2	No	22-62	CKD 3 (≥35ml/min/1.73m^2; htTKV≥1000ml/m)	TKV, eGFR	NCT01559363
Etanercept	TNF-α	Preclinical				*Pkd2* mice	[30]
Isoxezolines	MIF	Preclinical				*Pkd1* mice	[31]
Resveratrol	NF-kB	Preclinical				Han:SPRD (Cy/+) rats	[32]
Valproic acid	HDAC	Preclinical				*Pkd1* mice	[33]
JQ1	Brd4	Preclinical				*Pkd1* mice	[34]

AMPK, AMP-activated protein kinase; Brd4, bromodomain containing protein 4; cAMP, cyclic AMP; CKD Staging by CKD-EPI equation unless otherwise stated; CRP, C-reactive protein; FMD, flow mediated dilation; GFR, glomerular filtration rate; HDAC, histone deacetylase; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein–1; MIF, migration inhibitory factor; mTOR, mammalian target of rapamycin; NF-kB, nuclear factor kappa B; PC2, polycystin-2; PPAR-γ, peroxisome proliferator-activated receptor; PWV, pulse wave velocity; QOL, Quality of life; SIRT1, Sirtuin 1; STAT3, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α; TKV, total kidney volume; TLV, total liver volume; 8-iso-PGF2a, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy 2 deoxyguanosine.

PKD1/PKD2 deficiency

Cyst initiation

Warburg effect

Chemokines

Autophagy complex

Oxidant production

Histone acetylation

Cyst growth

AMPK
mTOR
HNF1β

TNFα
MIF
NF-kB

AMPK
HIFα
mTOR

ROS
ATP

HDACs
BET BRD

Metabolism

Inflammation

Autophagy impairment

Oxidative stress

Epigenetic control

Disease progression

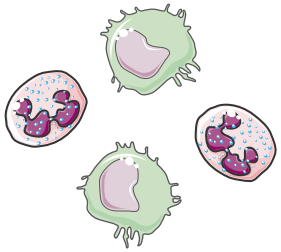
Glucose

Pyruvate

Lactate

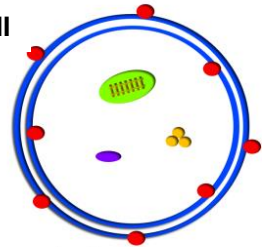
ATP

Aerobic glycolysis



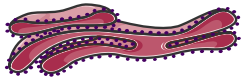
Inflammatory cells

LC3-II



Autophagosome

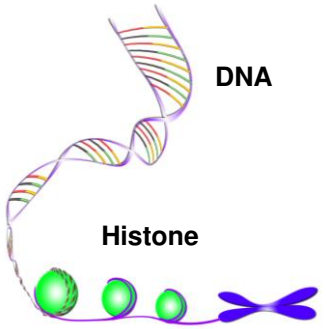
ROS



Endoplasmic reticulum

DNA

Histone



Chromosome

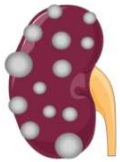
Etanercept
Isoxazolines
Triptolide
Resveratrol

Rapamycin
Carbamazepine
Minoxidil
PLD inhibitors

Pravastatin
P2X inhibitors

Metformin
2DG
Calorie restriction
Lipid reduction

Trichostatin A
Valproic acid
Nicotinamide
JQ1



Polycystic kidney