Fundamental insights into autosomal dominant polycystic kidney disease from human-based cell models

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Abstract

Several animal- and human-derived models are used in autosomal dominant polycystic kidney disease (ADPKD) research to gain insight in the disease mechanism. However, a consistent correlation between animal and human ADPKD models is lacking. Therefore, established human-derived models are relevant to affirm research results and translate findings into a clinical set-up. In this review, we give an extensive overview of the existing human-based cell models. We discuss their source (urine, nephrectomy and stem cell), immortalisation procedures, genetic engineering, kidney segmental origin and characterisation with nephron segment markers. We summarise the most studied pathways and lessons learned from these different ADPKD models. Finally, we issue recommendations for the derivation of human-derived cell lines and for experimental set-ups with these cell lines.

Keywords ADPKD · Human cell lines · Calcium · cAMP · mTOR

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent monogenetic disease. Estimated to occur in 2.9–3.3 per 10,000 individuals [1], it leads to end-stage renal disease (ESRD) in half of the cases before the sixth decade of life [2, 3]. Cyst formation in all renal segments and increased total kidney volume (TKV) are the hallmarks of ADPKD. Furthermore, ADPKD can be considered as a multi-organ disorder associated

with the formation of cysts in other organs (like the liver), hypertension and vascular aneurysms.

ADPKD is caused by mutations in the *PKD1* and *PKD2* genes, accounting for approximately 78 and 15% of the affected families [1]. Recently, mutations in other genes (like *GANAB*) have been identified as a cause of ADPKD. *PKD1* and *PKD2* encode for polycystin-1 (PC1) and polycystin-2 (PC2) respectively, which are membrane-spanning proteins. Current knowledge indicates that the polycystins form a protein complex in which PC2 functions as a calcium (Ca^{2+})-permeable ion channel [4]. The cellular function of PC1 remains unclear. Dysfunction of these proteins leads to several cellular changes, including alterations in Ca^{2+} homeostasis, cyclic AMP (cAMP) levels, serine/threonine-protein kinase mTOR (mTOR) activity and overall metabolism, finally resulting in cyst formation [5, 6].

To date, the only available specific treatment for ADPKD patients is tolvaptan, an oral vasopressin receptor-2 (AVPR2) antagonist, which slows down TKV expansion with 50% and kidney function decline by 38% after 3 years [7]. However, poor tolerance and rarely severe liver toxicity have been associated with this therapy. Unfortunately, clinical trials with alternatives have so far been disappointing: somatostatin analogues as well as mTOR inhibitors did not result in a sustained, statistically significant reduction of TKV change [8].

Several hurdles hinder ADPKD research. For example, the large size of the PC1 protein (~460 kDa) complicates

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biochemical research and its function remains largely unknown. In addition, the *PKD1* gene is difficult to sequence or manipulate due to duplications of exons 1–33 in the human genome, leading to six *PKD1* pseudogenes. Functionally, PC1 and PC2 regulate not only each other, but apparently a whole set of other proteins involved in a complex network of intracellular pathways [9]. As such, the cellular consequences of a specific mutation remain difficult to predict. This is illustrated by the extensive range of disease-causing mutations and the difference in the observed phenotypes [1]. Finally, it should be noted that there exists a strong research focus on advanced stages of the disease. Studies of early stage disease development might be more useful to understand ADPKD initiation and progression. Indeed, cyst formation and growth already start in utero and a significant increase of TKV is observed in the first decades of life [10].

In this review, we focus on human-derived cell models. We discuss existing cell lines, the results obtained with these cells concerning PC1 and PC2 localisation, cilia function, proliferation, Ca^{2+} signalling, mTOR metabolism and cyst formation. Comparisons with relevant results obtained from animal models are included.

Human PKD cell models: an overview

Since the first ADPKD patient-derived cell-based research in 1986 [11], a plethora of human-derived cell systems have been used in ADPKD research (Tables 1 and 2). These systems differ by means of cell origin, genetic modification, level of validation and *PKD* mutations (Tables 1 and 2). Most primary cells are not included in these tables, as they are not immortalised and therefore unique. However, valuable results have been obtained from primary cell preparations and are discussed below.

Patient-derived and genetically engineered human cell lines

Cells and cell lines used in ADPKD research are derived from cysts (mainly obtained after nephrectomy) [16, 19, 21, 27–34], from cells shed in urine [22] and from inducible pluripotent (kidney) stem cells (Table 1) [35, 36]. Most cyst-derived cell lines originated from nephrectomised, end-stage kidneys. As such, they represent an advanced disease stage and are probably not suitable to identify initiators of cyst progression. Furthermore, cystic material from patients with *PKD2* mutations is underrepresented in these studies, as in these patients ESRD and nephrectomy are relatively rare. Yet, a significant party of the fundamental ADPKD research field focuses on the loss of *PKD2*. Taken together, only a handful of *PKD1* and *PKD2* mutations have been explored (Table 1). Clearly, results can be influenced by the genetic

donor background and the epithelial origin of control and ADPKD cell lines (see *section below*).

An alternative source of patient-derived cells is urine. Humans shed epithelial cells via their urine, which can be selectively cultured and characterised [22], even in the case of *PKD2*-based ADPKD. Despite these advantages, it is not clear to what extent these cells represent cyst-forming cells. Therefore, further validation of this model is necessary.

Human pluripotent stem cells are another interesting source for ADPKD research [25, 35–37]: both embryonic stem cell lines (hESCs) and induced pluripotent stem cell lines (iPSCs) with patient-specific mutations or artificially edited genes can be of value (see *below* and Tables 1 and 2). An interesting feature of hPSC-derived kidney cell lines (hPSC-KC, which originate from hPSCs and express kidney markers) is their capability to form epiblast spheroids and subsequently tubular organoids [36]. Whether these cells exhibit all features of mature epithelial cells is a matter of debate.

Finally, genetically engineered knockdown (KD) and knockout (KO) cell lines are useful cell models in ADPKD research. Although they do not necessarily represent *PKD1* mutations found in ADPKD patients, they allow investigation of the primary functions of the polycystins and the fundamental pathways involved in ADPKD. For example, *PKD1* and *PKD2* KD mimic the changes in mTOR activity and proliferation observed in animal models and patient cell lines [22, 38]. This phenotype is in agreement with the observation that *PKD1* KD is sufficient to induce cystogenesis in animal models and animal-derived cell lines [39–41]. Recently, the first biallelic *PKD1* and *PKD2* frameshift mutations have been engineered with CRISPR-Cas9 in cell lines derived from hPSC-KCs, showing more cyst formation compared to hPSC-KC controls (Table 2) [36].

Genetic modification for immortalisation and stem cell reprogramming purposes

Patient-derived primary cells are obviously the model of choice, but limitations exist concerning their accessibility, life span and origin. Until now, primary cells used in ADPKD research were derived from patients with advanced stages of disease. This might make it difficult to derive cyst-initiating factors from this source. On the other hand, it is worth realising that a subtle ADPKD cellular phenotype might be overwhelmed or altered because of the immortalisation procedure to create a cell line. A brief comparison between primary cells and cell lines is made in Table 3.

To derive stable cell lines, several immortalisation protocols have been used, of which the effect of both the oncogene and the used (viral) carrier have to be considered (Table 1). Often (retroviral or adenoviral assisted) genomic insertion of SV40 large T antigen (SV40 LT) and SV40 LT antigen mutants is used [12, 16]. This dominant oncogene originating

Table 1 Overview of patient-derived cell lines and controls

Cell lines	Source	Mutation	Immortalisation	Validation	Cyst formation	Origin	Reference
WT/TS 1 until 19 [12] With mostly used: WT 9-7 [12, 13]	30 individual renal cysts < 11 ADPKD nephrectomies		AD-SV40 TS or WT LT	<ul style="list-style-type: none"> – All cultured cells: pancytokeratin, vimentin (+) [12] • For D/P determination: <ul style="list-style-type: none"> – DBA/LTA/PNA/CD13 staining [12, 13] – Growth: depends on immortalisation – Response to PTH and AVP tested – mRNA for <i>CFTR</i>, <i>SNN1A</i>, <i>SLC9A3</i>, <i>AQP1</i>, <i>ATP6V1B1</i>, <i>EGFR</i> – Relatively well-developed junctional complexes (<i>EM</i>) [12] – Difference in electrical resistance between ADPKD and RCTE cell types [12] – Additionally in WT 9-7: cilia [13] 			Loghman-Adham et al. [12] Further subcloned distal variants: Nauli et al. [13]
WT 9-12 [12, 13]	From cyst no. 7 of patient 9	<i>PKD1</i> p.Q2556*, apparently heterozygous; probably non-pathogenic p.A1302S in 2nd allele				P	
RCTE [12, 13]	From cyst no. 12 of patient 9	<i>PKD1</i> p.Q2556*, apparently homozygous (c.7877C>T)				P D*	
	Normal human renal cortical tubular epithelial cells purchased from clonetics	Healthy, not genotyped				P D*	
OX938 C6/C7/- C8	From 3 individual cysts from an ADPKD nephrectomy (C6/C7/C8)	<i>PKD1</i> c.7000_7001insGCTGGCG [14]	RV_SV40 TS LT + RV_hTERT				Streets et al. [15]; Parker et al. [16]
OX161	Pooled cysts from an ADPKD nephrectomy (for persistent infection) [15]	<i>PKD1</i> p.E1537* + somatic c.402del		<ul style="list-style-type: none"> – NCAD at 33 °C/37 °C (<i>ICC</i>) [16]; ~ compared to control [15] – IgPKD (Ab against PKD region) did not disrupt cell adhesion, compared to ctrl [15] 	Form cysts, no comparison to ctrl [17]		
SKI-001	Pooled cysts from an ADPKD nephrectomy	<i>PKD1</i> IVS43-1G>A					
UCL93/3	From <i>normal</i> kidney tissue of removed carcinoma [15]	Healthy, not genotyped					
RFH	From <i>normal</i> kidney removed 'for clinical indications'	Healthy, not genotyped					
SKI-002	Pooled cysts from an ADPKD nephrectomy	<i>PKD1</i> IVS25-3C>G + somatic c.2312_2324del					Streets et al. [18]
PKD 10-27-98- [19]	Multiple superficial cortical cysts from an ADPKD nephrectomy	<i>PKD1</i> p.L2433del + IVS31-38C>G, and IVS44 + 22delG					Xu et al. [19, 20]
PKD 3/14/00 [19]	Multiple superficial cortical cysts from an ADPKD nephrectomy			<ul style="list-style-type: none"> – LTL (+) – Cilia: ADPKD cells < NK cells (<i>ICC</i>) 			

Table 1 (continued)

Cell lines	Source	Mutation	Immortalisation	Validation	Cyst formation	Origin	Reference
NK 6-1-99 [19]	From cadaveric kidney	Healthy, not genotyped					
NK 11-7-02 [19]	From kidney tissue removed for renal cell carcinoma	Healthy, not genotyped					
NK57M03 [19, 20]	From kidney tissue removed for renal cell carcinoma	No mutations in <i>PKD1/2</i>	RV_hTERT [20]	– Additionally sorted by [20] for LTL+ and DBA+. – Cilia: – DBA+ < LTL+. – LTA+ PKD _{TERT} < LTL+ NK _{TERT}		P; D [20]	
NK _{TERT} Q4004X	From pooled cysts from an ADPKD nephrectomy (57 years, male)	<i>PKD1</i> p.Q4004*; apparently heterozygous	RV_hTERT			P; D [20]	Xu et al. [20]
PKD _{TERT} -T Q4004X			RV_hTERT (different packaging cell line for production compared to [20])	– Sorted for LTL+ – Proliferation: ~ – Cell volume: ↑ (NS) – Size cells: ↑ (<i>EM</i>), no quantification – Cilia: ↓ (<i>EM</i>), no quantification – Form both low-resistance monolayers – (+) for TJP1, vimentin, CK-24, AQP1, NBC1; (–) for NHE-3 (<i>ICC</i>)	Form cysts in growth factor reduced matrigel, NHPTK do not	P	Herbert et al. [21]
NHPTK	From unsuitable kidney for transplant (53 years, male)		RV_SV40 TS LT + RV_hTERT	– Sorted for CD13 • Compared to 34.8; 1.5; 14.4; 33.5 ctrls: – mTOR activity: ↑ – <i>AQP1</i> and <i>ABCB1</i> RNA expression (RT-PCR)		P	Mekahli et al. [22]
10.032	From the urine of an ADPKD patient (37 years, female)	<i>PKD1</i> c.2674dup					
10.055	From the urine of an ADPKD patient (48 years, male)	<i>PKD1</i> c.12138 + 3_12138 + 6del					
10.056	From the urine of an ADPKD patient (13 years, female)	<i>PKD1</i> c.6574_6580del					
10.057	From the urine of an ADPKD patient (41 years, female)	<i>PKD1</i> c.6574_6580del					
10.064	From the urine of an ADPKD patient (43 years, male)	<i>PKD1</i> c.10167 + 24_10167 + 42del					
10.066	From the urine of an ADPKD patient (17 years, female)	<i>PKD1</i> c.10500-1G>A					
10.068	From the urine of an ADPKD patient (14 years, male)	<i>PKD1</i> c.3425_3428del					
10.061	From the urine of an ADPKD patient (16 years, male)	<i>PKD2</i> p.R361*					
10.062	From the urine of an ADPKD patient (16 years, female)	<i>PKD2</i> p.R361*					
11.164	From the urine of an ADPKD patient (17 years, female)	<i>PKD2</i> p.Q630*		– Sorted for CD13 • Compared to 34.8; 1.5; 14.4; 33.5 ctrls: – mTOR activity: ~			
34.8	From healthy urine donors (4–13 years)	Healthy, not genotyped	RV_SV40 TS LT + RV_hTERT	– Expression of CD13, TJP1, AQP1 and MDR4		P	Wilmer et al. [23, 24]

Table 1 (continued)

Cell lines	Source	Mutation	Immortalisation	Validation	Cyst formation	Origin	Reference
14.4							
33.5							
ADPKD 1 iPSC - OX3502	From the skin of an ADPKD patient (60, male, cysts)	<i>PKD1</i> p.C39Y heterozygous; during culturing: loss of heterozygosity	RV with <i>POU5F1</i> , <i>SOX2</i> , <i>KLF4</i> and <i>MYC</i> **	– Alkaline phosphatase activity, albumin uptake and functional assays vs. <i>ATCC CRL-2522 and Invitrogen C-013-5C</i> ; – Proliferation, apoptosis and ciliogenesis: ~			Freedman et al. [25]
ADPKD 2 iPSC - OX3503	From the skin of an ADPKD patient (44, male, cysts)	<i>PKD1</i> p.R2051P heterozygous					
ADPKD 3 iPSC - OX3504	From the skin of an ADPKD patient (62, female, cysts)	<i>PKD1</i> p.E1929* heterozygous					

(+): positive; (–): negative; ~: similar; *Ab*: antibody; *ABCB1*: encoding for multidrug resistance protein 1 (P-glycoprotein 1); *MDR4*: ABC transporter B family member 4; *AD-SV40*: origin-defective (ori-) SV40 DNA cloned into an adenovirus vector in place of early regions 1a and 1b; *ctrl*: control; *AQP1*: encoding for aquaporin-1 (AQP1); *ATP6V1B1*: encoding for V-type proton ATPase subunit B, kidney isoform (H⁺-ATPase); *AVP*: vasopressin; *CD13*: aminopeptidase N; *CFTR*: cystic fibrosis transmembrane conductance regulator; *CK-24*: keratin, type I cytoskeletal 24 (cytokeratin-24); *D*: distal; *DBA*: *Dolichos biflorus* agglutinin; *EGFR*: encoding for epidermal growth factor receptor; *EM*: electron microscopy; *hTERT*: human telomerase reverse transcriptase; *ICC*: immunocytochemistry; *iPSC*: induced pluripotent stem cells; *KLF4*: Krueppel-like factor 4; *LTL*: *Lotus tetragonolobus* lectin; *mTOR*: serine/threonine-protein kinase mTOR; *MYC*: Myc proto-oncogene protein (c-Myc); *NBC1*: electrogenic sodium bicarbonate cotransporter 1; *NCAD*: cadherin-2 (N-cadherin); *P*: proximal; *POU5F1*: encoding for POU domain, class 5, transcription factor 1 (Oct-4); *PTH*: parathyroid hormone; *RV*: retroviral; *SLC9A3*: encoding for sodium/hydrogen exchanger 3 (NHE-3); *SNVIA*: encoding for amiloride-sensitive sodium channel subunit alpha (ENaC); *SOX2*: transcription factor SOX-2; *SV40 LT*: large T antigen from simian virus 40; *TJPI*: tight junction protein ZO-1; *TS*: temperature sensitive; *WB*: Western blotting analysis; *WT*: wild type; *XTT*: XTT proliferation assay

*Subcloned, distal and proximal variant

**Genes used for the reprogramming of fibroblasts to stem cells

Table 2 Overview of human-based PKD cell models, non-patient-derived

Cell line	Method	Result	Immortalisation	Validation	Cyst formation	Origin	Reference
34.8 <i>PKD1</i> <i>KD</i>	Lentiviral vectors expressing miRNA-based shRNA	PC1 reduction	RV, SV40 TS LT + RV_ hTERT	– See 34.8 in Table 1 – KD confirmed by WB		P	Mekahli et al. [22]
34.8 <i>PKD2</i> <i>KD</i>		PC2 reduction					
34.8 <i>PKD1</i> + <i>PKD2</i> <i>KD</i>		PC1 + PC2 reduction					
34.8 control	Lentiviral vectors expressing miRNA based shRNA against DS Red	Normal PC1 and PC2 expression					
hPSC-KCs	= derived from H9 hESCs			– (+) for binding to LTL, (+) for PODXL, TJP1, ECAD, β CAT in different structures after differentiation into tubular organoids – Tight junctions, microvilli (<i>EM</i>)	Tubular structures Tubular structures + cyst-like structures Tubular structures + cyst-like structures	Several segments, form organoids	Freedman et al. [26]
hPSC-KCs <i>PKD1</i> <i>KO</i>	CRISPR	<i>PKD1</i> c.1194del Biallelic truncation					
hPSC-KCs <i>PKD2</i> <i>KO</i>	CRISPR	<i>PKD2</i> c.276del Biallelic truncation					

(+): positive; (–): negative; ~: similar; β CAT: catenin beta-1; D: distal origin; ECAD: cadherin-1; EM: electron microscopy; hESC: human embryonic stem cell; hPSC-KC: human pluripotent stem-cell-derived kidney cells; hTERT: human telomerase reverse transcriptase; KD: knockout; KO: knockdown; LTL: *Lotus tetragonolobus* lectin; miRNA: micro RNA; P: proximal origin; PC1: polycystin-1; PC2: polycystin-2; PODXL: podocalyxin; RV: retroviral; shRNA: short hairpin RNA; SV40 LT: large T antigen from simian virus 40; TJP1: tight junction protein ZO-1; TS: temperature sensitive; WB: Western blotting analysis

Table 3 Brief comparison of primary cells and cell line characteristics

	Primary cells	Cell lines, stem cells*
Isolation from	Nephrectomy (cysts) (e.g., [27, 29, 42–44])	Nephrectomy (cysts), urine [22], skin*/blood*/embryonic* [25, 35, 36]
Accessibility	Limited	Unlimited
Life span	Short (4–6 passages) [27]	Longer, but should preferentially be used between a defined passage window
Alterations	Due to culturing conditions	Due to culturing conditions Due to immortalisation [45, 46]
Nephron origin	Often unknown or minimally validated (e.g., [27, 29, 42–44]) Typically mixed population	Validation can be performed more in depth (see Table 1) Can be subcloned (see Table 1)
Disease stage	Mostly end-stage (e.g., [27, 29, 42–44])	Both early and end-stage possible, according to the cell type
Germline mutation	Often not determined, but possible [19]	Mostly determined (see Table 1)

*For the specific case of hPSCs/iPSCs

from simian virus, its interference with the p53 and Rb-family of tumour suppressors is well established [45]. Hence, SV40 LT itself might be cystogenic [47]. This can be avoided by immortalising with the temperature-sensitive SV40_{tsA58}(U19) or SV40_{tsA209} (SV40 TS) mutants [23, 48, 49]. These cells do not express the large T antigen at non-permissive temperatures (37–39 °C) enabling differentiation. A drawback is that cultivation occurs at 33 °C in order to retain large T antigen expression, which slows down metabolic processes and cell growth. Another disadvantage is the influence of SV40 LT on cell growth: growth of PKD-cell lines was mainly dependent upon the applied immortalisation process and not upon their origin [12]. In each of these approaches, senescence can occur, which has been circumvented by the additional presence of a retroviral vector that expresses the catalytic subunit of the human telomerase reverse transcriptase (hTERT) (Table 1) [16, 22]. Some groups also managed to immortalise cells using solely a retroviral construct expressing hTERT [20, 21]. hTERT is believed to interfere to a lesser extent with cellular processes compared to SV40 LT, in particular considering JAK/STAT, Myc and Src pathways [19, 21, 31]. Nonetheless, hTERT-mediated immortalisation will also affect proliferation, for example through the Wnt and NF-κB pathways [46]. Not only is the particular oncogene of importance, but also the vector backbone and titers: for example, MLV-based γ-retroviral vectors can already have oncogenic effects by integrating nearby promotor and transcription start sites [50]. Furthermore, it has been shown that by reprogramming cells using retroviral vectors (illustrated by the iPSCs in Table 1), additional mutations occur

that influence cellular features [25]. Thus, when comparing cell lines, the immortalisation process is an important feature to consider.

The necessity of a meticulous characterisation

The detection of several markers indicates the origin from a specific nephron segment. Despite their frequent use in nephrology research, the specificity of these markers is rarely absolute and ADPKD cyst-derived cells seem to show signs of dedifferentiation, including downregulation of several nephron segment markers [9]. Furthermore, dedifferentiation also occurs through immortalisation and cell culturing. For example, both control NHPTK and ADPKD Q4004X_{TERT} cell lines (Table 1) express vimentin, a marker for mesenchymal and progenitor cells, which is upregulated by hTERT [21, 51].

Detection of cytokeratin and cell-adhesion molecules is often used to confirm epithelial origin of cell lines. However, not every epithelial marker is expressed abundantly in every kidney segment. E-cadherin for example, which is used to follow epithelial mesenchymal transition (EMT), is not (or only weakly) detected in proximal tubules [52].

Several markers have been used to define proximal tubular origin of cells (Fig. 1). However, it should be considered that some of these markers are not exclusively expressed in this segment. Aquaporin-1 (AQP1) and sodium-hydrogen exchanger 3 (NHE3) are also highly expressed in the descending limb of the loop of Henle [53, 59]. Also, multidrug resistance protein 1 (MDR1, P-glycoprotein 1 or CD243, encoded by *ABCB1*) and multidrug resistance-associated protein 4 (MRP4, encoded by *ABCC4*) are (weakly) detected in other nephron segments [60–62].

Binding to *Lotus tetragonolobus* lectin (LTL) and detection of membrane alanyl aminopeptidase (CD13, encoded by *ANPEP*) and Neprilysin (CD10, encoded by *MME*) are often exploited for sorting and detection of proximal tubule cells [22, 23, 63] (Fig. 1). Nevertheless, LTL also binds to cells of the descending loop of Henle and moderately to the collecting duct [64]. Furthermore, cell lines tend to lose expression of CD13 over time and both CD13 and CD10 are used as fibroblast and vascular endothelial cell markers [65–67].

As cyst formation is argued to start in the collecting ducts [68], obtaining these cells is of large interest for ADPKD research. To this end, cells positive for binding to the lectin dolichos bifloris agglutinin (DBA) or peanut agglutinin (PNA) are collected (Table 1). Yet, cells originating from the loop of Henle and distal convoluted tubules can also bind to these lectins [69]. Discrimination can be made by staining for the solute carrier family 12 member 1 (the kidney specific Na-K-Cl-symporter, NKCC2, encoded by *SLC12A1*) and solute carrier family 12 member 3 (the Na-Cl cotransporter, NCC, encoded by *SLC12A3*), which are markers for the loop of

Fig. 1 Markers for nephron segments. Depicted are commonly used markers for epithelial cells originating from the proximal tubule (dark blue), collecting ducts (dark grey), loop of Henle (green) and distal tubule (light blue). Markers also (weakly) staining other segments are depicted at the respective segment in a lighter colour. AQP1: aquaporin-1; NHE3: the sodium–hydrogen antiporter 3 (encoded by *SLC9A3*); NBC1: electrogenic sodium bicarbonate cotransporter 1 (encoded by *SLC4A4*); NaPi-2a: sodium-dependent phosphate transport protein 2A (encoded by *SLC34A1*) [53–55]; OCT-2: solute carrier family 22 member 2 (also known as Organic cation transporter 2, encoded by *SLC22A2*); OAT1: solute carrier family 22 member 6 (encoded by

SLC22A6); OAT3: solute carrier family 22 member 8 (encoded by *SLC22A8*); OAT4: solute carrier family 22 member 11 [56, 57]; GGT1: glutathione hydrolase 1 proenzyme (CD224) [58]; BCRP: ATP-binding cassette sub-family G member 2; LTL: *Lotus tetragonolobus* Lectin; CD13: membrane alanyl aminopeptidase; CD10: Neprilysin; NKCC2: solute carrier family 12 member 1 (or kidney-specific Na-K-Cl-symporter, encoded by *SLC12A1*); NCC: solute carrier family 12 member 3 (or Na-Cl cotransporter, encoded by *SLC12A3*); PMCA: plasma membrane calcium-transporting ATPase; NCX: sodium/calcium exchanger; EnACB: amiloride-sensitive sodium channel subunit beta; AQP2: aquaporin-2

Henle and distal convoluted tubules [70]. Several other commonly used markers also stain multiple segments (Fig. 1).

In conclusion, it is advised to use an extensive panel of (positive and negative) markers in order to determine the origin of a cell line. Considering the putative dedifferentiation of ADPKD cystic cell lines, this approach clearly has limitations considering the determination of the segmental origin of cystic cells.

Lessons learned

A plethora of pathways is altered in the described ADPKD cell models [71]. Furthermore, recent papers have investigated the transcriptome in human ADPKD cyst-derived cell lines,

producing a set of data for further research [31]. In this review, we will focus on the influence of ADPKD on cilia function, proliferation, Ca^{2+} signalling, cAMP and mTOR metabolism, as studied in human-based cell models.

PC1 and PC2 localisation and expression

Most cystic- and urine-derived ADPKD cell lines show absent or decreased levels of full-length PC1 compared to controls (Table 4), while a few ADPKD-derived cell lines show similar global PC1 expression levels as healthy controls [12, 16, 19–22, 25]. On the other hand, all tested human-derived *PKD1* cell lines seem to have little or no PC1 in primary cilia (see section “Cilia function and ADPKD”) and at the plasma membrane [12, 21]. In the majority of *PKD1*-mutated cell lines, a ciliary PC2 reduction

Table 4 PC1 and PC2 expression and localisation

Cell lines	Mutation	PC1 total (WB/ RT-PCR/ICC)	PC1 PM/ER (ICC)	PC1 Cilia (ICC)	PC2 total (WB/RT- PCR/ICC)	PC2 PM/ER (ICC)	PC1 Cilia (ICC)	Compared to	Reference
WT/TS 1-19 [12]		(+) (RT-PCR/ ICC)							Loghman-Adham et al. [12]
With mostly used:									Further subcloned distal variants: Nauli et al. [13]
WT 9-7 [12, 13]	<i>PKD1</i> p.Q2556*; apparently heterozygous	↓ (WB) [13]						RCTE	
WT 9-12 [12, 13]	<i>PKD1</i> p.Q2556*; apparently homozygous	FL: (−) (WB) [13]		(−) [13]	~ (WB) [13]		↓ [13]		
OX938 C6/ C7/C8	<i>PKD1</i> c.7000_ 7001insGCTGGCG [14]	(−) (WB)			~ (WB)			UCL93/3	Streets et al. [15]; Parker et al. [16]
OX161	<i>PKD1</i> p.E1537* + somatic c.402delG	↓ (WB) [15, 16]			~ (WB) [15, 16]				
SKI-001	<i>PKD1</i> IVS43-1G>A	↓ (WB)			~ (WB)				
PKD 10-27-98 [19]	<i>PKD1</i> p.L2433del + IVS31-38C>G and IVS44 + 22delG	~ (WB); FL ↓		(−)	~ (WB)		In 30%	NK	Xu et al. [19, 20]
PKD 3/14/00 [19]									
Q4004X PKD TERT [20]	<i>PKD1</i> p.Q4004* heterozygous	~ (WB) ≠ fragments in exosomes [21]	↓ @ PM [21]	(−) [19]	↑ (WB); ≠ splice variants [21]		Almost (−) [20]	NK57M03 [20] NHPTK [21]	Xu et al. [20] Herbert et al. [21]
10.032	<i>PKD1</i> c.2674dup	↓ (WB)			~ (WB)			34.8	Mekahli et al. [22]
10.055	<i>PKD1</i> c.12138 + 3_ 12138 + 6del							10.5	
10.056	<i>PKD1</i> c.6574_6580del							14.4	
10.057	<i>PKD1</i> c.6574_6580del							33.5	
10.064	<i>PKD1</i> c.10167 + 24_ 10167 + 42del								
10.066	<i>PKD1</i> c.10500-1G>A								
10.068	<i>PKD1</i> c.3425_3428del								
10.061	<i>PKD2</i> p.R361*				↓ (WB)				
10.062	<i>PKD2</i> p.R361*								
11.164	<i>PKD2</i> p.Q630*								
ADPKD 1 iPSC - OX3502	<i>PKD1</i> p.C39Y - loss of heterozygosity	~ (WB)	Antibody too aspecific		~ (WB)	~	↓; rescue by functional PC1 ↑	ATCC CRL-2522 and Invitrogen C-013-5C:	Freedman et al. [25]
ADPKD 2 iPSC - OX3503	<i>PKD1</i> p.R2051P heterozygous								
ADPKD 3 iPSC - OX3504	<i>PKD1</i> p. E1929* heterozygous	↓, additional band at 250 kDa (WB)	Antibody too aspecific		~ (WB)				
34.8 <i>PKD1</i> KD	Lentiviral vectors expressing	↓ (almost (−), WB)			~ (WB)			34.8 control	Mekahli et al. [22, 38]
34.8 <i>PKD2</i> KD	miRNA-based shRNA	(+) (WB)			(−) (WB)				
34.8 <i>PKD1</i> + <i>PKD2</i> KD		(−) (WB)			(−) (WB)				

Table 4 (continued)

Cell lines	Mutation	PC1 total (WB/ RT-PCR/ICC)	PC1 PM/ER (ICC)	PC1 Cilia (ICC)	PC2 total (WB/RT- PCR/ICC)	PC2 PM/ER (ICC)	PC1 Cilia (ICC)	Compared to	Reference
hPSC-KCs <i>PKD1</i> KO	<i>PKD1</i> c.1194del CRISPR Biallelic truncation	PC1 _{FL} : (-) (WB)						hPSC-KCs	Freedman et al. [26]
hPSC-KCs <i>PKD2</i> KO	<i>PKD2</i> c.276del CRISPR Biallelic truncation				(-) (WB)				

(+): positive; (-): negative; ~: similar; ↓: decrease; ↑: increase; ≠: distinct; *D*: distal origin; *EM*: electron microscopy; *ER*: endoplasmic reticulum; *FL*: full length; *hESC*: human embryonic stem cell; *hPSC-KC*: human pluripotent stem-cell-derived kidney cells; *KD*: knockdown; *KO*: knockout; *miRNA*: micro RNA; *P*: proximal origin; *PC1*: polycystin-1; *PC2*: polycystin-2; *PM*: plasma membrane; *shRNA*: short hairpin RNA; *WB*: Western blotting analysis

was observed, despite no differences in global PC2 expression (see section “Cilia function and ADPKD”) [12, 19, 25].

Cilia function and ADPKD

The primary cilium, an organelle involved in mechano- and chemosensing, harbours several proteins encoded by genes commonly mutated in renal disease [72]. ADPKD is considered a ‘ciliopathy’ as PC1 and PC2 are at least partially located in the primary cilium [73]. Several observations in animal models and human-derived cell lines point towards a contribution of an altered ciliary response in ADPKD progression [30, 72, 74, 75]: (1) an altered position of the basal body, required for signalling in *Pkd1* KO mice; (2) reduced cyst formation in ADPKD mice models lacking cilia; and (3) reduced Ca²⁺-response upon fluid-shear stress in human- and mice-derived ADPKD models [13].

As mentioned above, ADPKD is associated with lower ciliary PC1 and PC2 expression: in several cell lines (WT 9-12, WT 9-7, PKD 10-27-98, Q4004X PKD_{TERT}) irrespective of their segmental origin, mutation, zygosity and global PC1 expression, no PC1 could be detected in cilia [12, 19–21]. PC2 expression in cilia was reduced in all reported human ADPKD-derived cell lines: from undetectable in cilia to a 70% reduction in cells with detectable PC2 [12, 19–21, 25]. Ciliary PC2 levels were restored after backcomplementation with a functional *PKD1* construct in hepatoblasts derived from ADPKD iPSC [25]. These findings were confirmed in several mammalian cell lines, where overexpression of PC1 influenced PC2 localisation in primary cilia [4, 25, 76]. In contrast, in a homozygous *Pkd1* KO model, PC2 was still detectable in the primary cilium [77]. In conclusion, *PKD1* mutations seem to affect PC1 and PC2 localisation in the cilium, irrespective of immortalisation, origin, mutation and zygosity of cultured human ADPKD-derived cell lines. Finally, the cell line harbouring the truncating p.Q4004* mutation, as well as cells with p.L2433del (Table 1), had shorter cilia compared to the respective controls [19, 21]. This is in contrast to *Pkd1*^{del34/del34} mice harbouring normal ciliary length [30].

These findings correlate with a distinct shear stress response observed in several human-derived ADPKD cystic cell lines. WT 9-7 and 9-12 cells (p.Q2556*, Table 1) show no cytosolic Ca²⁺-response upon fluid-shear stress, unlike control renal cortical tubular epithelial cells (RCTE) cells [13, 30]. Similar observations were made in primary PKD cells and a retroviral hTERT-immortalised PKD cell line (p.L2433del, Table 1) [13, 19, 20].

Proliferation in ADPKD

Although it is generally accepted that cystogenic cells lacking functional polycystins *in/ex vivo* exhibit uncontrolled proliferation, results are less clear *in vitro*. Overexpression of heterologous genes, dominant negative constructs and observations in cells derived from KO mice hint to an inverse relationship between functional PC1 dosage and proliferation. For instance, Mekahli et al. observed that *PKD1* KD cell lines grow faster compared to control cell lines [22]. In contrast, most tested primary cells derived from ADPKD cysts showed no difference in serum-induced proliferation compared to cells derived from kidneys unsuited for transplantation [21, 27]. It has to be pointed out that the segmental origin of these cells and their genotype are not clearly defined. Furthermore, animal studies indicate that hyperproliferation mainly occurs in early stages of disease, and less in large cysts of the failing kidney [78]. Additionally, ADPKD-derived iPSCs and the Q4004X_{TERT} cell line showed no difference in (serum-induced) proliferation rate, compared to appropriate control cell lines [21, 25]. ADPKD WT 9-12 cells also showed no (serum-induced) increased proliferation compared to normal kidney HK-2 cells [79]. However, the latter cells were differently immortalised, which could have influenced these results. Indeed, Loghman-Adham et al. demonstrate that the growth rate of patient-derived cell lines seems mainly dependent on the type of immortalisation: WT 9-12 cell lines immortalised with Adv-SV40 LT grow almost twice as fast as TS 9-12 cell lines immortalised with the temperature-sensitive variant [12].

Nonetheless, differences in proliferation rates were observed in response to several stimuli: the cyst-derived OX161, OX938 C8 and SKI-001 ADPKD cell lines (Table 1) grew faster compared to UCL93, RFH and CL-11 control cell lines (Table 1) when stimulated with insulin-like growth factor-1 (IGF-1), dbcAMP or cAMP agonists like forskolin and 3-isobutyl-1-methylxanthine (IBMX) (see *section below*) [16]. Primary ADPKD-derived cells showed increased proliferation (and apoptosis) under stimulation with membrane-permeable cAMP analogues, cAMP agonists, ouabain, vasopressin (AVP) and various other growth-stimulating compounds [43, 80, 81].

Aberrant Ca^{2+} signalling in ADPKD

From different ADPKD models, it is clear that cellular Ca^{2+} signalling is altered. Indeed, PC2 is a non-selective Ca^{2+} -permeable channel [82] and PC1 might regulate several Ca^{2+} -permeable ion channels (see below and [83]), including PC2 [4]. Mutations in PC1 and PC2 or lack of the respective proteins can therefore result in aberrant Ca^{2+} responses induced by various chemical or mechanical stimuli. For example, some primary and immortalised ADPKD cyst-derived cells displayed almost no Ca^{2+} signal compared to normal kidney cells in response to fluid flow, though the role of PC1 and PC2 in this process remains unclear [13, 19]. It should be noted that the type of mutation in PC1 likely matters. A mutation that only affects ciliary PC1 localisation might imply that PC1 is still functioning at other subcellular locations. Specific mutations could influence PC1 expression at the plasma membrane and/or the ER and subsequently alter Ca^{2+} signalling through regulation of PM- or ER-resident PC2 or other polycystin-interacting Ca^{2+} channels. Examples of such channels are transient receptor potential channels TRPC1, 3, 4 and 7 [84–86]; the L-type Ca^{2+} channels [87]; inositol 1,4,5-trisphosphate receptors [38, 88–91]; and ryanodine receptors [92].

In addition to the alterations in stimulated Ca^{2+} signals, basal Ca^{2+} homeostasis is often altered in ADPKD models, as was the case in the *PKDI*^{+/-} mouse model [93]. Primary cultures of cells derived from three ADPKD kidneys displayed ca. 20 nM lower basal cytosolic $[\text{Ca}^{2+}]$ compared to cells from three normal kidneys [94]. Interestingly, a similar difference of 20 nM was twice observed in cystic and non-cystic cells of the same kidneys that were removed from patients due to abdominal pain, despite normal renal function [94]. However, in another study, basal cytosolic $[\text{Ca}^{2+}]$ was similar in an immortalised renal cortical tubular and ADPKD cyst-lining epithelial cell line as well as in normal-looking tubules and cystic tubules from three different ADPKD kidneys [13]. The reason for this discrepancy is unclear, as is the functional significance of a 20-nM reduction in basal $[\text{Ca}^{2+}]$. Interestingly, ER $[\text{Ca}^{2+}]$ is also lowered in primary cyst-lining

epithelial cells from an ADPKD patient with the PC1 p.L2433del mutation (PKD 10-27-98, Table 1) [19]. The reason for the observed changes remain unclear and could involve (a combination of) alterations in Ca^{2+} -pump activity [93], Ca^{2+} -buffering capacity, Ca^{2+} leak rates [38, 95] and intracellular Ca^{2+} release mechanisms [38, 84, 86–92].

How much these alterations in Ca^{2+} signalling may contribute to ADPKD disease progression remains a major question. Since PC2 is primarily a Ca^{2+} channel, it is suggested that the loss of ciliary Ca^{2+} responses is the primary trigger, leading to increased cAMP levels, increased cell proliferation and finally cystogenesis (discussed more in detail below) [96].

In conclusion, it seems clear that altered Ca^{2+} homeostasis, dynamics and machinery is an attribute of cyst-forming cells in ADPKD. However, many details are lacking regarding the exact players in the process and the consequences of their malfunctioning.

A distinct cAMP responsiveness in ADPKD

Increased cAMP is another hallmark of ADPKD and might be the direct cause of proliferation and anion secretion [5]. The first available ADPKD treatment, the AVPR2 antagonist tolvaptan, blocks cAMP increase (in response to AVP) in collecting ducts and thereby highlights the key role of cAMP in cyst growth.

Upon addition of cAMP-generating agonists like IBMX, prostaglandin E2 (PGE2) or forskolin, enhanced proliferation in primary cystic cells and cell lines derived from nephrectomies was observed [16, 81, 97–99], indicating a phenotypic switch to a cAMP-stimulatory proliferative response. These compounds also stimulated fluid secretion [100] and cyst formation of cyst-derived cells in a 3D matrix (see below) [28, 81].

Cystic fluid itself can stimulate the production of cAMP and subsequent proliferation in both animal-derived cell lines and human-derived primary cells [98, 101–103]. From this cystic fluid, a lipid compound was identified in 15 out of 18 patients as being a ‘forskolin-like’ molecule [104]. However, the production site of this molecule remains unclear.

Lower cytosolic Ca^{2+} levels could contribute to a distinct cAMP response in PKD-derived cell lines. Ca^{2+} channel blockers verapamil and nifedipine increased the cell proliferation rate in both cystic and non-cystic cells obtained from different regions from the same kidneys, especially when combined with cAMP increase [94]. Compounds increasing basal cytosolic $[\text{Ca}^{2+}]$, such as the L-type Ca^{2+} channel agonist Bay K8644 and the Ca^{2+} ionophore A23187, induced opposite effects. In addition, Bay K8644 inhibited forskolin-induced cyst formation in cystic cells. This is in line with results in non-orthologous rodent models, where increased or decreased cystic disease was observed upon administration of verapamil or the calcimimetic R-568, respectively [105,

[106]. Interestingly, an increase of [cAMP] alone already increased proliferation in cystic cells but reduced proliferation in non-cystic cells, suggesting that the difference in basal cytosolic $[Ca^{2+}]$ in cystic versus non-cystic cells determines whether cAMP stimulates or inhibits proliferation [94]. However, it cannot be excluded that other alterations in cystic cells may also play a role in establishing the proliferative and cystogenic characteristics of cystic cells in ADPKD.

While several rodent models of cystic disease show highly increased levels of cAMP in the kidney and urine [107], basal cAMP levels in human-derived cells are seldom reported. Pinto et al. observed higher basal cAMP levels in cystic-derived primary cells compared to normal human kidney cells (1.1 ± 0.1 vs. 0.7 ± 0.1 pmol/monolayer), measured with a cAMP enzyme immunoassay. They attribute these observations to a (weak) upregulation of Ca^{2+} -regulated adenylate cyclases (AC5 and AC6) and a downregulation of Ca^{2+} -regulated phosphodiesterases [43, 108]. Furthermore, adenylate cyclase activity itself could be induced by Gi-signalling inhibition [5].

High levels of circulating AVP have been associated with ADPKD [107]. Furthermore, AVPR2 mRNA levels were 1.4-fold higher in primary cystic-derived cells compared to normal kidney controls [108]. AVP and its more stable AVPR2-specific analogue desmopressin (DDAVP) were able to increase cellular cAMP levels and enhance proliferation in primary cystic cells and cell lines, but not in controls [16, 44, 81, 97], which was inhibited by pre-treatment with tolcapten, but not in control kidney cells [44]. These results are in line with the results observed in the non-orthologous PCK rat and orthologous *PKD1* (*Pkd1^{RC/RC}*) and *PKD2* (*Pkd2^{WS25/-}*) ADPKD mouse models [107]. Remarkably, Pinto et al. observed a lower response upon AVP addition in cystic-derived cells compared to normal human kidney cells [108]. In contrast, Loghman-Adham et al. did not observe a cAMP response to similar concentrations of AVP in a cell line of distal origin (WT 10-7, Table 1), while control RCTE did mildly respond [12]. Interestingly, cystic cells of proximal origin (WT 9-7, Table 1) did not only show a response to parathyroid hormone (PTH), a known stimulator of proximal tubular cells, but also to AVP, which did not stimulate control cell lines of tubular origin [12].

In conclusion, cAMP-mediated signalling seems to be different in PKD-derived cystic cells and cell lines compared to controls. Furthermore, several factors do contribute to higher cAMP levels observed in diseased kidneys and cystic-derived cells. The mechanism remains, however, unclear and might be heterogeneous.

Altered mTOR metabolism in ADPKD

Increased mTOR activity has been observed in ADPKD cell and animal models [6]. Accordingly, mTOR inhibitors

showed a reduction of cyst growth and prevented kidney function loss in several rodent models. Unfortunately, this strategy was unsuccessful in clinical trials [109–111]. Apart from methodological issues with these studies, translation between mouse and human kidney is not self-evident. For example, some of these mice models develop cysts due to alterations in non-orthologous genes of *PKD1* or *PKD2*. Furthermore, in most orthologous models, a conditional homozygous knock-out of *pkd1* results into a fast progressing disease with a fast simultaneous development of multiple cysts. This in contrast to human ADPKD, characterised by a development over decades [72]. Thirdly, an upregulated mTOR activity was only detected in cell lines derived from patients with mutations in *PKD1* or a *PKD1* KD and not in cell lines with affected PC2 expression [22]. Finally, typical compounds that inhibit mTOR activity are notoriously unspecific.

It was also shown that rapamycin and metformin could reduce mTOR activity synergistically in *PKD1* KD cell lines, which could be useful for further trials. A reduction in mTOR activity was also observed upon cAMP and mTOR signalling inhibition by A3-receptor antagonist CI-IB-MECA together with rapamycin in WT 9-7 and WT 9-12 cell lines (Table 1) [112]. However, reduction of proliferation was only observed after sequential administration. The latter results suggest that inhibition of mTOR activity alone might not be sufficient to inhibit proliferation. Additionally, berberine slows down proliferation of the same cell lines, by also inhibiting ERK and S6 phosphorylation [113].

In conclusion, the contribution of a reduction of mTOR on ADPKD should be evaluated more in depth, and synergistic treatments targeting multiple pathways involved in ADPKD might be more effective. Furthermore, genotyping might be necessary for future clinical trials, as only *PKD1* KD or mutant cell lines showed an altered mTOR metabolism [22].

In vitro cyst formation in ADPKD: a valuable tool for drug development?

As the main goal in battling ADPKD is to find therapies preventing cyst formation, the development of a reproducible cystogenesis assay seems crucial. Several cyst lining-derived primary cells and cell lines have been shown to form cysts, while controls did not, or to a lesser extent (Table 1) [21, 34, 81]. 8-Br-cAMP, forskolin, IBMX, transferrin, dexamethasone, insulin and triiodothyronine all induced cyst growth and/or fluid excretion in cultured cysts from primary ADPKD-derived cells [28, 81]. Tolcapten or a STAT5 inhibitor resulted in decreased cyst growth in vitro with primary cells and the OX161 cell line, respectively [17, 44]. Interestingly, also miR-17 downregulation was found to reduce cyst growth originating from cell lines (and in animal models), indicating that such an assay is useful to find novel

approaches to inhibit cyst formation [32]. Unfortunately, no comparison with control cell lines was included in these experiments. Moreover, some non-ADPKD-derived cells tend to form cysts in vitro.

However, the studied cell lines were derived from end-stage kidneys, and the specific pathways that contribute to cyst initiation remain unclear. Therefore, it would be of prime interest to study cell signalling and the effect of various compounds in ‘early stage’ cell models or genetically engineered models as for example organoids from ADPKD *PKDI*^{−/−} hPSCs, which were described to form cyst-like structures alongside tubular organoids, while controls did not [26, 36]. Moreover, this model suggested that this cystic phenotype is not fully penetrant, as cyst formation was only observed in a minority of kidney organoids. These results are in line with the in vivo situation. Hence, they form a proof of concept for the use of kidney organoids as valid models.

In general, kidney organoids form an interesting starting point to model ciliopathies. Recently, Forbes et al. managed to generate kidney organoids from iPSCs derived from a nephronophthisis-related ciliopathy patient with compound-heterozygous mutations in *IFT140* [114]. Cilia were shorter

in organoid tubules derived from patient iPSCs, compared to iPSCs where the germline mutation was corrected using CRISPR-Cas9 technology.

Conclusion

Human-based ADPKD cell models could be valuable tools in ADPKD research to increase the understanding of the molecular mechanisms behind the disease and for the development of assays for drug discovery. Nevertheless, several concerns have to be taken into account (Fig. 2): First, most published cell models are derived from end-stage kidneys, which do not reflect disease initiation. The inclusion of cells derived from patients exhibiting signs of early disease or genetically engineered cell lines is crucial. Second, comparison of different human-derived cell lines requires identical immortalisation procedures, similar oncogene expression levels and similar segmental origin. Additionally, epithelial cells tend to lose or change a fraction of their characteristics when kept in culture. Therefore, thorough characterisation, inclusion of adequate controls and evaluation of multiple human-derived models are mandatory to obtain reliable results.

Fig. 2 Considerations and strategies for experiments with human-derived cell lines, concerning genetics and epigenetics, transformation, epithelial and segmental origin, culturing conditions and the observed phenotype. = similar + positive; − negative

	CONSIDERATIONS	STRATEGY
genetics and epigenetics	<ul style="list-style-type: none"> ○ background ○ PKD mutations ○ gender/age ○ end stage disease 	<ul style="list-style-type: none"> ○ multiple donors ○ mutation sequencing ○ matched controls ○ early stage disease
transformation	<ul style="list-style-type: none"> ○ oncogene/reprogramming gene: interference with ≠ pathways ○ integrating vector: effect integration sites 	<ul style="list-style-type: none"> ○ same oncogene ○ similar expression levels ○ inactivate/flox out oncogene ○ same vector backbone
nephron segment and epithelial origin	<ul style="list-style-type: none"> ○ ≠ kidney segments with ≠ properties ○ non-epithelial origin 	<ul style="list-style-type: none"> ○ identification using a panel of +/- markers
culturing conditions	<ul style="list-style-type: none"> ○ plastic, media, coating, matrix, ... ○ spontaneous cyst formation 3D 	<ul style="list-style-type: none"> ○ experimental conditions in parallel ○ take along appropriate non-cystic controls
phenotype	<ul style="list-style-type: none"> ○ specific effect of mutation 	<ul style="list-style-type: none"> ○ introduce/correct mutation using gene editing

Characterisation should minimally include sequencing to confirm a disease-causing mutation, the detection of a panel of markers for epithelial origin and comparison of expression levels of the introduced oncogene or reprogramming gene. Determination of the zygosity of the mutation would also be of interest. An overview of recommendations for the generation and use of ADPKD-derived cell lines is given in Fig. 2.

Yet, these cell-based models allow new experimental approaches. For instance, backcomplementing *PKD1/PKD2* KO or KD kidney cell lines with *PKD1/PKD2* containing known patient mutations or the direct introduction of precise patient mutations and corrections using gene editing would be valuable strategies to study the mechanisms of disease in ADPKD. This latter technology would also enable to edit mutations in modifier genes to identify pathways contributing to cyst formation. The effect of PC1 dosage could be further studied by differential *PKD1* KD (or *PKD1* backcomplementation) resulting in distinct PC1 expression levels. Finally, as cysts are multicellular structures, 3D-culturing and kidney-based organoids offer new perspectives for ADPKD research, particularly by allowing the development of a cyst-forming assay. These assays can be used for in vitro drug screening, as shown with tolvaptan for example [44]. Furthermore, these assays can contribute to a thorough understanding of the contribution of specific pathways or mutations to cyst formation, especially in combination with gene editing [36, 114].

In conclusion, this review demonstrates the importance of a thorough validation and an appropriate experimental design when using human-derived cell models for ADPKD research (Fig. 2). Nonetheless, when appropriately exploited, these models can be of great value, (complementary with animal studies) to give fundamental insights into ADPKD. Considering that the key hallmark of ADPKD, cyst formation by a fraction of epithelial cells, is still incompletely understood, the development of reliable patient-specific cell models remains crucial to the field.

Key summary points

1. Human-derived cell models are an essential tool to study ADPKD.
2. Results in cell lines derived from patients can be influenced by the mutation, the disease stage, the immortalisation procedure, nephron origin and patient background.
3. Cyst-initiating factors can be studied using human-derived cell lines with *PKD1/PKD2* KO/KD and non-cystic patient-derived cell lines.
4. Alterations in mTOR activity, cAMP response and Ca^{2+} homeostasis can be observed in cyst-derived cells and cell lines.
5. In vitro cyst formation could be a valuable screening tool.

Multiple choice questions (answers are provided following the reference list)

1. Which cell line is not a published human ADPKD-derived cell model until now?
 - a) Cell lines from ADPKD patients obtained from urine
 - b) Cell lines from ADPKD patients obtained from nephrectomies
 - c) Cell lines from ADPKD patients obtained from skin, reprogrammed to hPSCs
 - d) Cell lines with ADPKD patient-derived *PKD2* mutations, inserted using CRISPR
 - e) Cell lines with *PKD2* knockout or knockdown
2. Which statement is true about the immortalisation procedure?
 - a) If cells are immortalised using SV40 LT, it does not matter with which vector they were immortalised.
 - b) SV40 LT can stimulate proliferation through suppression of the tumour suppressor gene p53 (amongst interference with other pathways).
 - c) Cells immortalised with SV40 LT will never become senescent.
 - d) Immortalisation with hTERT alone does not affect cell properties.
 - e) HK-2 cells and RCTEs are immortalised with the same constructs and, hence, are interchangeable.
3. Which markers are expected on cell lines from proximal tubular origin?
 - a) AQP1, ECAD, MDR1, AQP2
 - b) AQP1, NCAD, MDR1, AQP2
 - c) AQP1, NCAD, MDR1, NCC
 - d) AQP2, ECAD, MDR1, NHE3
 - e) AQP1, NCAD, MDR1, NHE3
4. What has not been described in ADPKD patient-derived cells?
 - a) Decreased ciliary $[\text{Ca}^{2+}]$ -influx upon shear stress in several cystic-derived cell lines
 - b) A decrease in [cAMP] upon tolvaptan administration in several cystic-derived cell lines
 - c) An increase in mTOR activity in human proximal tubular-derived cell lines with *PKD2* KD
 - d) cAMP-generating agonists stimulate proliferation in several cystic-derived cell lines
 - e) Full-length PC1 expression is decreased in several cystic-derived cell lines

5. Which statement is not true about in vitro cyst formation?

- IBMX slows down cyst growth of the OX161 cell line.
- STAT5 inhibitors slow down cyst growth of the OX161 cell line.
- Tolvapatan slows down cyst growth of primary cystic cells.
- Forskolin stimulates cyst growth of cystic primary cells.
- miR-17 downregulation reduces cyst growth of cyst-derived primary cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Answers

1. d; 2. b; 3. e; 4. c; 5. a