Chapter 7
Validation of Effective Therapeutic Targets for ADPKD Using Animal Models

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Abstract Various polycystic kidney disease (PKD) animal models including *Pkd1*- or *Pkd2*-deficient mice have been developed and efficiently utilized to identify novel therapeutic targets as well as elucidate multiple mechanisms of cyst formation in PKD. Based on several successful in vivo studies, preclinical approaches using PKD animal models would shed light on the development of potential therapeutic strategies for PKD. Here, we provide an update on the current evidence obtained by the in vivo evaluation of PKD therapeutic candidates and discuss the effect of therapeutic targets.

Keywords PKD • Animal model • Therapeutic target

7.1 Various Polycystic Kidney Disease (PKD) Animal Models Are Available to Reveal the Biological Functions of PKD-Causing Genes

The initial development of PKD is driven by an increase of cell proliferation. However, depending on the disease progression, dysregulated apoptosis, differentiation, fibrosis, and inflammation can also occur, indicating that PKD is a complex disease induced by defects of multiple signaling pathways. Based on this characteristic of PKD, many research groups have developed PKD mice models to understand the physiological mechanisms of PKD development and screen effective therapeutic targets for curing PKD. Rodent models of PKD share common pathogenic phenotypes, including cyst formation in multiple nephron segments and an increase of cell proliferation, but display different characteristics in the progression

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of cyst formation, life span, and renal cilia phenotypes. In this section, the morphological features and signaling alterations of well-established PKD rodent models are introduced.

7.1.1 *Pkd1 or Pkd2-Targeted Mouse Models*

Mutation of the *PKD1* gene is known as a representative cause of the development of human PKD and the most commonly inherited mutation of *PKD* (Kim et al. 2009). Therefore, *Pkd1*-targeted mice were produced to evaluate the biological function of Pkd1 in vivo. While *Pkd1* constitutive knockout mice show embryonic lethality accompanied by kidney cysts, liver cysts, and abnormal cardiovascular and skeletal development (Boulter et al. 2001), mouse models of the kidney-specific inactivation of *Pkd1* usually survive until birth (Shibazaki et al. 2008). Kidneys conditionally targeted by the *Pkd1* gene show rapid cyst formation from postnatal day 1 (P1) to P14 with an increase of cell proliferation followed by activation of the MAPK/ERK pathway (Shibazaki et al. 2008). Another gene mutated in human PKD is *PKD2*, which causes approximately 15 % of familial autosomal dominant PKD (ADPKD) cases (Kim et al. 2009). To reveal the physiological effect of *Pkd2* inactivation, various PKD mice targeted by *Pkd2* have been generated. The *Pkd2* homozygous knockout mutant mice exhibit embryonic lethality like mice homozygous for *Pkd1* and show body edema, cardiac defects, and cysts of the kidney and pancreas (Kim et al. 2009; Wu et al. 2000). In addition to *Pkd2* constitutive knockout mice, *PKD2* transgenic mice were generated (Park et al. 2009). Histological analysis of these transgenic mice showed that renal cysts originated from a range of nephron segments at 18 months of age (Park et al. 2009). Also, the activation of B-Raf/Mek/Erk signaling was observed in the cystic kidneys of this transgenic mouse model (Park et al. 2009). These polycystin-targeted mice models show that the polycystin proteins play a role in organogenesis during embryonic development and that defects of polycystin induce the cystic kidney phenotype via activation of the MAPK/ERK pathway, leading to an increase of cell proliferation.

7.1.2 *PKD Mouse Models Targeted by IFT-Related Genes*

The first PKD mouse model showing a relationship between ciliary dysfunction and PKD development was the oak ridge polycystic kidney (ORPK) mouse induced by mutation of *Ift88* (Tg737, Polaris), which belongs to the IFT-B complex (Lehman et al. 2008). This model shows a number of abnormal phenotypes induced by ciliary malfunction. It has been reported that renal cysts, hydrocephalus, pancreatic abnormalities, and aberrant patterning of skeletal structure are observed in this model (Cano et al. 2004; Banizs et al. 2005; Ko and Park 2013). Furthermore, a reduction

in the number of ciliated cells and abnormal ciliary structure were observed in the pancreatic and renal cells of ORPK mice (Cano et al. 2004; Pazour et al. 2000).

In addition to the ORPK mouse model, various PKD mouse models induced by the inactivation of IFT-related genes have been developed. One of the PKD mouse models targeting the IFT complex B subunit was induced by the specific inactivation of *Ift20* in renal collecting duct cells (Jonassen et al. 2008). This model shows severe and rapid renal cyst progression with a complete loss of cilia, leading to the alteration of Wnt signaling (Jonassen et al. 2008). In addition to the Ift20-targeted mouse model, Ift25 and Ift27, which belong to the IFT-B complex, are constitutively inactivated in vivo. Interestingly, although Ift25 and Ift27 are subunits of IFT complex B, which is involved in cilia assembly, the inactivation of these two genes was found to have no effect on cilia assembly (Keady et al. 2012; Eguether et al. 2014). In these models, the phenotype of cilia appears normal, but they display multiple developmental defects such as skeletal malfunctions, omphaloceles, and polydactyly as well as an alteration of Hh signaling (Keady et al. 2012; Eguether et al. 2014). Not only IFT complex B, but also a IFT complex A-targeted mouse have been generated. A representative PKD animal model induced by the inactivation of IFT complex A is a mouse with a conditional allele for *Ift140* in the renal collecting duct cells (Jonassen et al. 2012). In general, subunits that belong to IFT complex A are involved in cilia disassembly, so it is conceivable that the inactivation of *Ift140* might induce an increase of cilia length. However, severe shortening or absence of primary cilia was observed in the *Ift140*-deleted renal collecting duct cells with a PKD phenotype (Jonassen et al. 2012). These findings suggest that a normal ciliary function is important for the maintenance of homeostasis in renal epithelial cells and that defects of ciliary structure or function contribute to the development of PKD through an increase of cell proliferation.

7.1.3 Juvenile Cystic Kidney & Congenital Polycystic Kidney Mice

Juvenile cystic kidney (jck) mice are produced by a missense mutation of the *Nek8* gene (Liu et al. 2002). This mouse model shows renal cysts in multiple nephron segments and a life span of approximately 20–25 weeks (Nagao et al. 2012). The protein product of this mutated gene is observed in the entire length of the primary cilia in kidney, and it results in the abnormal localization of polycystins in lengthened primary cilia in the kidneys of jck mice (Sohara et al. 2008; Smith et al. 2006). Interestingly, the kidney phenotype of jck mice displays gender dimorphism in the progression of cyst formation, with a more severe phenotype in male mice because of gonadal hormones (Smith et al. 2006).

The congenital polycystic kidney (cpk) mouse is one of the PKD models with a mutation of the *Cys1* gene encoding cystin protein, which is localized to the primary

cilia (Hou et al. 2002). Most cysts observed in cpk mice are derived from the collecting ducts and proximal tubules and are accompanied by an increased expression of proto-oncogenes and growth factors together with an alteration in the expression of genes associated with cell adhesion (Hou et al. 2002; Ko and Park 2013; Rocco et al. 1992; Nakamura et al. 1993).

7.1.4 Han:SPRD Cy Rat Model

The Han:SPRD Cy rat model is caused by a missense mutation of the *Pkdr1* (also called Cy and Anks6) gene (Nagao et al. 2010). The protein SamCystin that is encoded by the Pkdr1 gene is mainly expressed in the early postnatal kidney and proximal tubules (Nagao et al. 2010). A point mutation of the Pkdr1 gene results in the aberrant expression and mislocalization of SamCystin in this rat model (Nagao et al. 2010). Kidneys of heterozygous mutant rats (Cy/+) show a mild progression of the PKD phenotype compared with homozygous mutant rats (Cy/Cy) (Nagao et al. 2003). In addition, the Han:SPRD Cy rat model displays a gender-specific kidney phenotype. The kidneys of male Cy/+ rats display a more severe renal cystic phenotype compared with that of the female rats, which affects to average life span of both the males and females of the Cy/+ rat model (Nagao et al. 2003).

7.2 Potential Candidate Targets for PKD Treatment

At present, there are no FDA-approved therapies for the treatment of ADPKD. Nevertheless, recent studies have suggested a number of promising targets and molecular pathways related to cystogenesis, providing new insights into potential therapeutic interventions. The main treatment approaches attempted in ADPKD have focused on inhibiting cystic cell proliferation and fluid secretion (Bukanov et al. 2012; Calvet 2008; Yang et al. 2008; Chang and Ong 2012). More currently, inhibition of the renin-angiotensin-aldosterone system, targeting ciliary function, membrane glycosphingolipids, extracellular matrix, and epigenetic restoration, has also been under investigation (Natoli et al. 2010; Elliott et al. 2011; Li 2011). Here, we present a review of candidate ADPKD drugs and current trials according to the drug targets in PKD rodent models, as follows.

7.2.1 Cyclic AMP (cAMP)-Dependent Signaling Inhibitors

cAMP is a well-known regulator involved in cyst fluid accumulation (Wallace et al. 2001; Sullivan et al. 1998), and an elevated level of cAMP stimulates the activation of the B-Raf/MEK/ERK pathway in ADPKD (Yamaguchi et al. 2003). It has been

reported that a number of agonists targeting the vasopressin and somatostatin pathways can result in cAMP accumulation (Gattone et al. 2003; Masyuk et al. 2007).

7.2.1.1 Vasopressin V2 Receptor Antagonist

The vasopressin receptor (V2R) on collecting ducts binds to vasopressin and increases cAMP accumulation by activating adenylyl cyclase. The vasopressin V2R antagonists, OPC-31260 and OPC-41061 (tolvaptan), have been shown to reduce renal cAMP and cystogenesis in four rodent models of renal cystic disease (cpk mice, pcy mice, PCK rats, and Pkd2ws25/- mice) (Torres et al. 2004; Wang et al. 2008; Gattone et al. 2003; Gattone et al. 1999). Tolvaptan is effective in the treatment of hypervolemic or euvolemic hyponatremia and congestive heart failure (Irazabal et al. 2011). These promising preclinical results have translated into clinical trials under the Tolvaptan Efficacy and Safety in Management of PKD and Outcomes (TEMPO) 3:4 program (Torres et al. 2011; Torres et al. 2012). The TEMPO 3:4 trial was designed as a 3-year multicenter randomized placebo-controlled trial ($n = 1445$) investigating the progression of changes in total kidney volume (TKV), PKD complications and drug safety. In ADPKD patients who were treated with tolvaptan for 3 years, the rate of TKV increase was reduced by almost 50 % compared with that in the placebo group (2.8 % vs. 5.5 % per year, $p < 0.001$). Tolvaptan was also shown to ameliorate the decline of renal function (Torres et al. 2012). This result is consistent with the findings of a recent study that evaluated the efficacy of tolvaptan in the Japanese sub-population ($n = 177$) (Muto et al. 2015). However, the long-term administration of tolvaptan caused reduced tolerability and significant adverse effects. For example, after the discontinuation of tolvaptan, 8.3 % of patients in the treatment group had severe aquaresis and an elevation of aminotransferase enzyme concentrations, indicating the potential for acute liver failure, and TKV progression continued at the same rate as before therapy (Torres et al. 2012). Overall, tolvaptan is the first pharmacotherapeutic intervention to have been demonstrated to have a therapeutic benefit in ADPKD (Baur and Meaney 2014). At present, the TEMPO 4:4 trial is underway in the USA and tolvaptan has been approved in Europe and Japan for the pharmacological treatment of ADPKD.

7.2.1.2 Somatostatin Analogs

The somatostatin agonist octreotide was shown to be effective in slowing the progression of liver and kidney cystic disease in a small group of ADPKD patients (Ruggenenti et al. 2005) and the PCK rat model (Masyuk et al. 2007). The activation of somatostatin SSTR2 receptor, which is expressed in the kidneys, by octreotide significantly decreased the intracellular level of cAMP, consequently slowing cyst growth and disease progression (Hogan et al. 2010). Ruggenenti et al. demonstrated that octreotide decreased TKV in 12 patients with ADPKD in Italy (Ruggenenti et al. 2005). Although it was a short-term pilot study in a small group,

they observed a reduction in the rate of TKV increase and cyst size with only mild adverse events such as gastrointestinal disorder. In a follow-up paper, the beneficial effect of octreotide was evaluated in a long-term, randomized, placebo-controlled and multicenter trial (Caroli et al. 2013). In this study, adult ADPKD patients (with an estimated glomerular filtration rate > 40 mL/min per 1.73 m^2) were randomly divided into two groups and treated with two 20 mg intramuscular injections of octreotide longacting release (LAR) (n = 40) or 0.9 % (v/v) sodium chloride solution (n = 39) every 28 days for 3 years. As a result, at the 1-year follow-up, mean TKV increased significantly less in the octreotide-LAR group than it did in the placebo group (46.2 mL vs. 143.7 mL, p = 0.032). However, at the 3-year follow-up, the mean TKV was shown to be significantly different between the two treatment groups (220.1 mL vs. 454.3 mL, p = 0.25). This result indicates the probable occurrence of tachyphylaxis due to the downregulation or desensitization of somatostatin receptors (Hogan et al. 2012; Caroli et al. 2013). Notably, the initial short-term reduction in glomerular filtration rate (GFR) showed a correlation with the subsequent decline of GFR in the octreotide-LAR group, suggesting that the participants who had larger initial reductions in GFR appeared to show a slower long-term progression towards renal failure while being treated with somatostatin (Caroli et al. 2013). Overall, somatostatin analogues were shown to be relatively safe and well tolerated in all participants compared with previous ADPKD trials. At present, a follow-up study, the Developing Interventions to Halt Progression of ADPKD 1 (DIPAK1) Study, which was designed to examine the efficacy of another somatostatin analogue, lanreotide, on renal function in ADPKD, has been conducted mostly in Europe (Meijer et al. 2014).

7.2.2 Mammalian Target of Rapamycin (mTOR) Inhibitors

mTOR is a serine/threonine kinase that is involved in the promotion of cell proliferation and cell division as well as transcription and protein synthesis. Intriguingly, the mTOR signaling pathway is abnormally upregulated in the cyst-lining epithelial cells of ADPKD mouse models, possibly due to a loss of regulation by PC1 (Wahl et al. 2006; Wander et al. 2011). Rapamycin, also known as sirolimus, inhibits mTOR's kinase activity by binding to FK506-binding protein (Sabers et al. 1995). In preclinical studies, mTOR inhibitors including sirolimus and everolimus were shown to be highly effective in decreasing renal cystogenesis and improving kidney function in several rodent models of ADPKD (Shillingford et al. 2006; 2010; Wahl et al. 2006). However, two key randomized, Phase II trials of the studies evaluating mTOR pathway inhibition have failed to demonstrate the therapeutic efficacy of either drug on either TKV or estimated glomerular filtration rate (Torres et al. 2010; Serra et al. 2010; Walz et al. 2010). In addition, both studies showed that treatment with mTOR inhibitors led to therapy-specific side effects including immunosuppression, diarrhea, acne, and mucositis as well as being limited by an inadequate degree of mTOR inhibition (Watnick and Germino 2010). Therefore, it should be

considered that the doses of these drugs that were shown to significantly reduce cyst growth in several rodent models were high (approximately 10-fold higher than the doses used in clinical trials) (Novalic et al. 2012). Efforts to overcome the systemic toxicity of mTOR inhibitors are also being made to enhance the drug specificity to the kidney. One possible approach is using folate-conjugated drugs as candidates for kidney-specific targeting, because folate receptors are overexpressed in the apical membranes of proximal tubule cells. In fact, a recent study demonstrated that treatment with folate-conjugated rapamycin (0.3 mol/kg per day) effectively reduced renal cyst development and preserved renal function without adverse events in the bpk mouse model (Shillingford et al. 2012).

7.2.3 Statins (HMG CoA Reductase Inhibitors)

Statins are widely used to reduce cholesterol in clinical settings by inhibiting the enzyme HMG-CoA reductase. Statins were also shown to decrease renal cystogenesis and improve renal function in the Han:SPRD rat model (Gile et al. 1995; Zafar et al. 2007). Recently, it has been reported that 110 young adults with ADPKD were randomly assigned to treatment with pravastatin or placebo for 3 years to determine the effect of pravastatin in ADPKD. Significant effects on the primary outcomes were shown with a significant decrease in the rate of TKV increase over the study period (pravastatin: 23 % vs. placebo: 31 %, $p = 0.02$) (Cadnapaphornchai et al. 2014). However, it is difficult to determine the efficacy of pravastatin because there were no significant changes of renal function or urinary protein excretion between the pravastatin and placebo treated groups in a randomized clinical trial of 49 adults with ADPKD for 2 years (Fassett et al. 2010).

7.3 Other Pre-Clinical Trials That Have Attempted to Identify the Potential Therapeutic Targets of ADPKD

Other therapeutic strategies targeting cell proliferation have been investigated, including direct inhibition of the cell proliferation-regulating proteins that are involved in the Raf/MEK/ERK signaling pathway. Sorafenib, a non-selective Raf inhibitor that finally reduces ERK activation, completely inhibited in vitro cyst growth in human ADPKD cystic cells cultured within a three dimensional collagen gel (Yamaguchi et al. 2010). However, unexpectedly, the administration of sorafenib to *Pkd2* conditional knockout mice promotes liver cyst growth (Spirli et al. 2012). Another group reported that a different small molecule Raf inhibitor (PXL5568) retarded cyst expansion without an improvement in renal function in the Han:SPRD rat model (Buchholz et al. 2011). In addition, PD184352 of MEK inhibitors reduced cyst development and disease progression in the pcy mouse model, but UO126,

which is another MEK inhibitor (Omori et al. 2006), was not shown to significantly alter cyst growth in the Pkd1 conditional knockout mouse model (Shibazaki et al. 2008). For another trial, metformin, an AMP-activated protein kinase (AMPK) activator, repressed cyst growth of MDCK cells cultured in vitro in collagen gels and in vivo *Pkd1* conditional knockout mice by activating AMPK and suppressing mTOR and CFTR (Takiar et al. 2011). Furthermore, alterations of glycosphingolipid metabolism with increased GlcCer may have an important role in promoting cyst development. Inhibition of the synthesis of GlcCer blocked cell cycle progression and proliferation by repressing the Akt/mTOR pathway in ADPKD mouse models (Natoli et al. 2010).

Renal cysts are mainly caused by the dysregulation of cell proliferation followed by imbalanced calcium influx as well as the malfunction of the PC1-PC2 protein complex. A large majority of pre-clinical trials have been focused on cAMP signaling, vasopressin-V2R, and the signaling pathways centered on the mTOR or MAP kinases. However, accumulating evidence has suggested that ADPKD progression seems to be influenced by the accumulated factors inside the cysts released by cyst-lining epithelia (Ye et al. 1992; Gardner et al. 1991). Fluids secretion is mainly accelerated by abnormal chloride efflux into the cyst cavity via cystic fibrosis transmembrane conductance regulator (CFTR) or other specific transporters (Miranda et al. 2013). Approaches targeting CFTR or its regulating mechanisms using natural compounds have been attempted in several pre-clinical trials (Yuajit and Chatsudthipong 2016). Among them, steviol, a natural compound firstly isolated from the plant Stevia rebaudiana, effectively slowed down cyst development in ADPKD mice. The rodent model used was *Pkd1*$^{f/f}$: *Pkhd1-cre*, in which *Pkd1* is conditionally knocked-out only in kidney tubular cells, leading to ADPKD. Treatment with steviol delayed the growth of renal cysts in *Pkd1*$^{f/f}$: *Pkhd1-cre* with enhanced renal function as indicated by reduced blood urea nitrogen and creatine levels. The specific mechanisms by which steviol inhibits disease progression were found to be mediated by the CFTR signaling pathway followed by the activation of AMPK. A lowered expression of CFTR subsequently inhibited fluids secretion as well as cell proliferation in steviol-injected mice, and it finally attenuated the disease phenotypes (Yuajit et al. 2014). Some other trials have targeted inflammation or fibrosis, which commonly accompany the progression of ADPKD. Among the tested interventions, Angiotensin-converting enzyme inhibitors have effectively ameliorated the renal cysts development and improved renal function in Han:SPRD rats (Keith et al. 1994; Zafar et al. 2007). Angiotensin essentially stimulates the production of pro-inflammatory factors as well as cell proliferation; therefore, inhibition of its synthesis reasonably led to an alleviation of the disease. The other drug that was evaluated for targeting inflammation in ADPKD was pyrrolidine dithiocarbamate, which has both anti-inflammatory and anti-proliferative effects. Treatment with this agent clearly resulted in a decreased TKV of male Lewis polycystic kidney rats, thereby reducing the ratio of kidney weight to total body weight by about 25 %. However, no changes in cell proliferation, interstitial inflammation, and fibrosis occurred, leading to no effects on renal function (Ta et al. 2014). Apoptosis-regulating mechanisms have been also suggested as another potential target.

Inhibition of caspase-3 activity via treatment with IDN-8050 for 5 weeks resulted in reduced kidney enlargement as well as cysts volume density by 44 % and 29 %, respectively, in Han: SPRD rat model. It led to enhanced renal function with down-regulation both of cell proliferation and apoptosis. Those therapeutic effects have been observed with only three-hour treatment with the same drug as well, which means that long-term administration is not necessarily required for effective alleviation of the disease phenotype (Tao et al. 2005a; b). The indirect effect of targeting apoptosis to inhibit PKD progression has been also been tested by injection of CDK inhibitor roscovitine into cpk and jck mice. Mice treated with roscovitine showed delayed renal cyst development with blockade of cell cycle as well as apoptosis (Bukanov et al. 2006). Besides, a pre-clinical study using lovastin has revealed its therapeutic effects on the metabolic distributions in Han: SPRD rats. Lovastin is a lipid-lowering therapeutic medication and its use in the treatment of the Han:SPRD rat model led to the alleviation of renal cysts with enhanced renal function as well as metabolic alterations (Klawitter et al. 2013). Finally, dietary modulation has been recently suggested as another novel potential therapeutic option for ADPKD. Interestingly, food restriction effectively delayed ADPKD progression with a reduction in the volume of renal cysts, interstitial inflammation, and fibrosis. These changes were mediated by regulating mTOR and AMPK activities (Warner et al. 2015). In these regards, targeting additional disease-stimulating factors other than the main mechanisms that initiate the disease could be another strategy to identify novel therapeutic targets for ADPKD.

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