Animal Models for Human Polycystic Kidney Disease

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Abstract: Polycystic kidney disease (PKD) is a hereditary disorder with abnormal cellular proliferation, fluid accumulation in numerous cysts, remodeling of extracellular matrix, inflammation, and fibrosis in the kidney and liver. The two major types of PKD show autosomal dominant (ADPKD) or autosomal recessive inheritance (ARPKD). ADPKD is one of the most common genetic diseases, with an incidence of 1:500–1,000. Approximately 50% of patients with ADPKD develop end-stage renal disease (ESRD) by the age of 60. On the other hand, ARPKD is relatively rare, with an incidence of approximately 1:20,000–40,000. ARPKD is diagnosed early in life, often prenatally. The gene products responsible for ADPKD and ARPKD distribute in primary cilia and are thought to control intercellular Ca\(^{2+}\). Two types of animal model of PKD have been established: spontaneous hereditary models identified by the typical manifestations of PKD and gene-engineered models established by modification of human orthologous genes. Both types of animal models are used to study the mechanism of cystogenesis and efficacy of medical treatments. In PKD progression, critical roles of signaling pathways including MAPK, mTOR, and PPAR-\(\gamma\) have been discovered with these models. Therefore, experimental animal models are indispensable for investigating molecular mechanisms of PKD onset and progression as well as potential therapeutic treatments.

Key words: Ca\(^{2+}\), cilia, gene engineering, PKD, PPAR-\(\gamma\)

Introduction

Polycystic kidney disease (PKD) is characterized by the presence of numerous cysts that originate from the renal tubules. In humans, autosomal dominant PKD (ADPKD) is one of the most common genetic diseases, with an incidence of 1:500–1,000, and is caused by mutations in either \(PKD1\) or \(PKD2\) [15]. About 85% of cases of ADPKD are caused by mutations in \(PKD1\) on chromosome 16, and 15% are caused by mutations in \(PKD2\) on chromosome 4. Autosomal recessive PKD (ARPKD) is also a monogenic disorder, with a lower incidence of 1:20,000–40,000, and is caused by a mutation in the polycystic kidney and hepatic disease 1 (\(PKHD1\)) gene, which encodes fibrocystin/polyductin (FPC) [15, 64]. The gene product of \(PKD1\), polycystin-1 (PC1), is a large receptor-like protein containing a long N-terminal extracellular domain that interacts with the \(PKD2\) gene product, polycystin-2 (PC2), via a short cytoplasmic C-terminus to form a heterodimeric complex [38]. PC1 and PC2 may form a heterotetramer [81]. PC2 is homologous to the transient receptor potential (TRP) family of store-operated calcium (Ca\(^{2+}\)) channels and is thought to function as a nonselective Ca\(^{2+}\) channel [14]. PC2 interacts with the \(PKHD1\) gene product, FPC, as well [67]. The PC complex is reported to localize in the primary cilia and act as a mechanosensor for maintaining the differentiated state of cells in the renal tubular epithelium and hepatic biliary tract [36]. In order to make use of basic research findings in the efforts to progress towards new clinical therapeutic approaches, animal models are imperative precursors for translational stud-
ies. In this review, widely-recognized animal models for human PKD are introduced along with their possible uses in investigating the molecular mechanisms of PKD onset and progression as well as their potential use in establishing therapeutic treatments.

**PKD Animal Models**

There are two major types of PKD animal models: spontaneous hereditary models identified by the typical manifestations of PKD and modified models established by mutation of human orthologous genes. These models are used for the elucidation of molecular-based disease outcomes, determination of endogenous or exogenous factors related to disease progression, and establishment of therapeutic interventions.

Spontaneous hereditary models of PKD

This type of animal model is identified according to symptoms that are typical of human PKD. Therefore, these animals have an obvious PKD phenotype, although the responsible genes are not always orthologous to human genes.

Han:SPRD-Cy rats: The Han:SPRD-Cy (Cy) rat strain was discovered by Kaspareit-Rittinghausen et al.; the original colony was established in Hanover, Germany [18]. Numerous renal cysts are caused by a missense mutation (C to T, R823W) in exon 13 of the Pkd1 (also called Cy, Samd6, or Anks6) gene on rat chromosome 5. Three genotypes, wild-type (+/+), heterozygous (Cy/+), and homozygous (Cy/Cy), have been identified by PCR analysis [6] relying on the inability of the MspI restriction enzyme to recognize and cleave the region containing the missense mutation [6]. Renal cysts are observed in both homozygous (Cy/Cy) and heterozygous (Cy/+), animals. In Cy/Cy animals, expanded renal tubules and initial cysts are observed in the neonatal stage. Rapid disease progression results in a short 3-week life span (Fig. 1A), whereas in Cy/+ animals, disease progression is relatively slow (Fig. 1B), and the average life span is approximately 1.5 years in females and 1 year in males. In animals with the Cy/+ genotype, renal cysts are mainly derived from the proximal tubules in the initial stage [35] (Fig. 1C). Some of them are observed as chimeric cysts with normal and thickened extracellular matrix surrounding chimeric cysts and infiltrated monocytes in the interstitium [10, 35] (Fig. 1D and 1E). Stimulated cellular proliferation with overexpression of certain types of kinases and GTPases in intracellular signaling pathways is apparent in cystic kidney tissues (Fig. 1F and 1I). The Pkd1 (Cy) gene product, SamCystin, is mainly distributed in the proximal tubules in which the initial cysts originate [6, 31]. In Cy/+ rats, SamCystin is overexpressed in the mature kidney after 21 days of age, whereas the protein is underexpressed in immature stages of kidney development compared with the kidney from normal rats [31]. Thus, the abnormal expression of SamCystin may affect the normal structure and function of the proximal tubules. Neudecker et al. developed transgenic rats with Anks6 

Pck rats: Katusyama et al. discovered a PCK rat strain from a Sprague Dawley (SD) outbreeding colony [19, 23]. The responsible gene, Pkhd1, located on rat chromosome 9 is orthologous to the gene affected in human ARPKD [69]. In this rat, polycystic kidney and liver disease results from a frameshift mutation (exon 36 is deleted by a change of A to T in intron 35) in the Pkhd1 gene. The gene product, FPC is located in the claria and expressed in the kidney, liver, and pancreas. The life span of PCK rats is approximately 1.5 years. At 1 year of age, numerous cysts are observed on the surface of the kidney and liver (Fig. 2A). In the kidney, the initial cysts originate from the collecting duct, and the growing cysts diffusely affect whole nephron segments in the end-stage disease (Fig. 2B). In the liver, enlarged bile ducts with fibrosis are observed in the initial stage, and cysts with fibrotic thickening are observed in the later stage (Fig. 2C). In the pancreas, cysts derived from the pancreatic duct are evident (Fig. 2D). Extracellular signal-regulated kinase (ERK) and ribosomal S6 kinase (S6) pathways are involved in stimulated cell proliferation in the kidney (Fig. 2E). In the liver, stimulation of the ERK signaling pathway is involved in cell proliferation in hepatic cysts, and upregulated expression of transforming growth factor-β (TGF-β) may cause fibrosis [78].

Pcy mice: The pcy mouse was originally discovered in the KK strain by Takahashi et al. [55]. It has nephrophihtis caused by a missense mutation (T1841G) in
Fig. 1. Characteristics of the kidney in Cy rats. (A) Hematoxylin and eosin (HE)-stained kidneys of 7-day-old +/+ (wild-type) (a, d), Cy/+ (b, e), and Cy/Cy (c, f) rats. The ratio of total kidney weight to body weight is approximately 2-fold higher in Cy/+ rats and 6-fold higher in Cy/Cy rats compared with +/+ (wild-type) rats. At 7 days of age, expanded renal tubules are observed microscopically in Cy/+ rats. In Cy/Cy rats, enlarged kidneys with numerous cysts are observed at the same age. Bars=1 mm (a–c) and 100 µm (d–f). (B) Macroscopic photographs of kidneys from +/+ (wild-type) (a) and Cy/+ (b) rats at 8 months of age. Numerous renal cysts are obvious in the kidney. In contrast, no cysts are observed in the liver (photo not shown). (C–H) Histological findings of the Cy/+ rat kidney. By Periodic acid-Schiff (PAS) staining, expansion of the renal tubules (C) and thickening of the extracellular matrix (D) are shown in the kidneys of 3-week-old Cy/+ rats. Chimeric renal tubules with normal and cystic transformed epithelia in the kidneys of 5-week-old Cy/+ rats (E). In the cystic transformed epithelium, thickening of the extracellular matrix (E: asterisks) and phosphorylated ERK (pERK) expression (F: arrow) are observed. After staining with Sirius Red, the red areas (#) indicate fibrosis in the kidney of a 6-week-old Cy/+ rat (G, kidney of a +/+ (wild-type) rat; H, polycystic kidney of a Cy/+ rat). Bars=50 µm (C and D) and 100 µm (E–H). (I) Overexpressed cell signaling proteins in the Cy rat kidney. In the kidneys of 12-week-old Cy/+ rats, the abundance, activity, or abundance and activity of Src, Rap-1, B-Raf, ERK, and AKT are upregulated compared with the +/+ (wild-type) rat kidney (n=2). These kinases and GTPases in cell signaling pathways are thought to be involved in accelerated cell proliferation.
Fig. 2. Characteristics of the liver and kidney in PCK rats. (A) Macroscopic photographs of the liver (a), kidney (c), and HE-stained liver (b) and kidney (d) from a PCK rat at 12 months of age. Numerous diffuse cysts are obvious in both the kidney and liver. Bar=1 mm (b and d). (B) HE-stained kidney from PCK rats. The cysts are initially derived from collecting ducts and develop in whole nephron segments in later stages (HE-stained kidney; a, 5 weeks; b, 10 weeks; c, 15 weeks; and d, 25 weeks). Bar=1 mm. (C) Sirius Red-stained liver from PCK rats. Congenital hepatic fibrosis (CHF) is observed in the liver. The cysts are derived from bile ducts, and fibrosis is marked from 6 days of age (Sirius Red-stained liver; a, 1 day; b, 6 days; c, 12 days; and d, 21 days). Bar=100 μm. (D) HE-stained pancreas from PCK rats. In the pancreas, the cysts are derived from the pancreatic duct (HE-stained pancreases at 1 year of age; a, trivial; b, mild; c, moderate; and d, severe). Bar=100 μm. (E) Overexpressed cell-signaling proteins in the PCK rat kidney. In the kidneys of 14-week-old PCK rats, the abundance, activity, or abundance and activity of Src, Rap-1, B-Raf, ERK, and AKT are upregulated compared with that in the normal SD rat kidney. These kinases and GTPases in cell-signaling pathways are thought to be involved in accelerated cell proliferation.
Fig. 3. Characteristics of the kidney in pcy and jck mice. (A) Computed tomographic (CT) images (a, whole body; b, kidneys) of a contrast medium-injected pcy mouse and macroscopic photograph (c) of a pcy mouse kidney at 30 weeks of age. (B) HE- and PAS-stained kidneys of a wild-type mouse (a and b) and a pcy mouse (c–d). Numerous renal cysts are obvious in the HE- (c, low magnification; e, high magnification) and PAS-stained (d, low magnification; f, high magnification) kidney of the pcy mouse. Bars=100 μm (a–f). (C) Upregulated ERK activity in 26-week-old pcy mice. Expression of the activated form of ERK (pERK) is upregulated in the pcy mouse kidney compared with that in the normal mouse kidney. (D) HE-stained kidneys of 4-, 10-, and 20-week-old wild-type (a, 4 weeks; b, 10 weeks; and c, 20 weeks) and jck (d, 4 weeks; e, 10 weeks; and f, 20 weeks) mice. The ratios of total kidney weight to body weight (K/B%) are approximately 5- to 10-fold higher in jck mice at 20 weeks of age compared with wild-type mice. Microscopically, a few cysts are observed at 4 weeks of age, and enlarged kidneys with numerous cysts are observed at 10 weeks of age in jck mice. Bar=1 mm. (E) Histological findings in the jck mouse kidney. Initial renal cysts (a: HE staining) are shown in the kidney of a 4-week-old jck mouse. At the same age, Ki67 (b) and pERK (c) are obviously expressed in the initial renal cysts and normal-shaped renal tubules. At 10 weeks of age, larger cysts are observed (d: HE staining). In epithelial cells with cysts, expression of cell proliferation markers Ki67 (e) and pERK (f) is obvious. Bar=100 μm. (F) Upregulated ERK activity in 10-week-old jck mice. ERK activity in the kidneys of jck mice is upregulated compared with that in a normal mouse kidney.
the gene orthologous to human Nphp3 [40]. The gene product, nephrocystin-3, is expressed in the primary cilia of epithelial cells in the kidney, pancreas, heart, and liver. The lifespan of pcy mice with either the DBA/2 or ICR strain background is approximately 40 weeks. Numerous cysts are observed on the kidney surface at 30 weeks of age (Fig. 3A). Enlarged renal tubules are observed at embryonic day 15, and growing cysts are evident in the corticomedullary junction at 3 weeks of age. Initially, cysts are derived from distal tubules, and whole nephron segments become diffusely occupied by cysts by 30 weeks of age, often with the occurrence of end-stage renal disease (ESRD). In cystic epithelia, stimulated cellular proliferation with upregulation of the ERK signaling pathway is observed (Fig. 3C) [39]. The longer 2-year life span reported in pcy mice with the C57BL/6 strain background suggests that modifier genes may influence the rate of disease progression [29]. Although retinal degeneration and hepatic fibrosis are observed in human patients with nephronophthisis, these phenomena are not observed in the pcy mouse strain.

Jck mice: Jck (juvenile cystic kidney) mice were isolated from a transgenic line by Atala et al. in 1993, but the effects are unrelated to the transgene [2]. The jck gene, also called Nek8 or Nphp9, is located on mouse chromosome 11 [17]. The mutated gene product is found in the primary cilia and affects normal expression of the PKD1 and PKD2 gene products, PC1 and PC2 [50]. The lifespan of jck mice is approximately 20–25 weeks. The initial renal cysts are observed at 4 weeks of age, and the cysts are obvious at 10 weeks of age (Fig. 3D). Expression of activated ERK is shown in proliferative cells in the cystic epithelia (Fig. 3E and 3F) [49].

Other models: The cpk (congenital polycystic kidney) and bpk (BALB/c polycystic kidney) mice are other spontaneous models of PKD. Cpk mice have renal cysts derived from the proximal tubules and collecting ducts by mutation of the Cys1 gene located on mouse chromosome 12 [16]. The gene product, cystin, is located in the primary cilia of renal epithelial cells with polaris, which is known to be a gene product of TgN737Rpw, in orpk mice [76]. The bpk mice have cysts derived from the collecting ducts in the kidney and from bile ducts in the liver caused by mutation of the Bicc1 gene located on mouse chromosome 10 [9]. The Bicc1 gene product is also located in the primary cilia. It is intriguing that various gene products in the spontaneous hereditary models are located in the primary cilia.

Gene-modified models generated by mutation of human orthologous genes

Transgenic mice: Transgenic mouse models with increased expression of the human orthologous PKD1 gene have important roles for understanding the mechanism of cystogenesis in human disease because expression of the PKD1 gene product, PC1, is thought to be increased in patients with ADPKD. After the identification of the entire sequence of the PKD1 gene in 1995 [59, 60], several groups tried to make transgenic animals. For example, development of renal cysts was observed in the kidney and liver in transgenic mice in which a P1-derived artificial chromosome (PAC) for PKD1 and TSC2 was used [43]. Recently, Thivierge et al. reported a novel transgenic mouse model produced with a bacterial artificial chromosome (BAC) [61]. In this model, the expression of the PKD1 gene is increased in tissues including the kidney, liver, and heart, and overexpression of the gene product, PC1, is observed in renal cysts. In addition, hepatic cysts and fibrosis and cardiac and vascular abnormalities are shown as in human ADPKD.

Gene targeting mice: Pkd1 Gene targeting mice. Gene targeting models are produced by deletion of human orthologous PKD genes, PKD1 and PKD2 for ADPKD and PKHD1 for ARPKD, respectively. Particularly, because PKD1 is the responsible gene in 85% of ADPKD cases, quite a few Pkd1 knockout mice have been established with null or deleted exons (2–6, 2–11, 33 or 43–45) [21, 24, 25, 28]. Slow or no disease progression is observed in heterozygous Pkd1 targeting mice. Interestingly, in heterozygous Pkd1Δdek-6+/− gene targeting mice, Pkd1 haploinsufficiency is observed, and it causes a syndrome of inappropriate antidiuresis [1]. On the other hand, almost all types of homozygous Pkd1 targeting mice are lethal in the infant period [21, 24, 25, 28]. Various devices are used for establishing appropriate PKD models. For example, in a model obtained by conditional knockout using Pkd1-Cre/loxP and Aqp2-Cre systems with the principal cells of the collecting duct, the progression of PKD is rapid, and the mean life span is relatively short (8.2 weeks) [44]. Furthermore, in the transgenic knockdown mouse developed by microRNA-based conditional RNAi against Pkd1, numerous renal cysts are observed with fibrosis and increased cell proliferation [66].

The time period of inactivation of the Pkd1 gene in conditional knockout mice may influence the progression of PKD. In fact, Piontek et al. reported that inactivation
of Pkd1 before postnatal day 13 caused severe PKD within 3 weeks, whereas inactivation at day 14 or later resulted in the formation of renal cysts only after 5 months [42]. Takakura et al. have made similar observations in Pkd1 conditional knockout mice as well [56]. Therefore, the kidney seems to possess a critical checkpoint for determining the severity of PKD progression.

Pkd2 gene targeting mice. Several gene targeting mice for Pkd2 gene have been reported. In particular, the Pkd2<sup>WS25/−</sup> mouse with an unstable allele is one of the important models used for drug treatment studies because it has cysts in both the kidney and liver, as in human ADPKD patients [73]. It was established by crossbreeding of Pkd2<sup>+/−</sup> and Pkd2<sup>WS25/+</sup>. In Pkd2<sup>WS25/−</sup> mice, the weight (% of body weight) of the kidney and liver is 2-fold heavier compared with that in wild-type mice [52]. The rates of proliferation and apoptosis in epithelial cells of either renal cysts or cystic cholangiocyes are upregulated compared with those in normal tissues [52].

Pkhdl gene targeting mice. In homozygous Pkhdl gene targeting mice produced with a BAC, renal cysts derived from the collecting ducts and dilatation of the intrahepatic bile ducts are seen in adulthood, which is similar to the symptoms of the Pck rat, a spontaneous ARPKD model with a mutation in the rat gene orthologous to the human ARPKD gene [72].

**Signaling Pathways Related to PKD Progression in Animal Models**

In PKD, kidney enlargement is caused by stimulated cellular proliferation of the tubule epithelia, leading to the formation of numerous fluid-filled cysts with extensive loss of functional nephrons, increased inflammation, and interstitial fibrosis. It is thought that these phenomena are related to the alteration of cell signaling pathways.

Receptor tyrosine kinase (RTK) and cyclic AMP (cAMP) signaling pathways

In the PKD kidney, the RTK pathway appears to have a major role in cell proliferation. Activation of RTK with sequential stimulation of mitogen-activated protein kinase (MAPK) components, Raf, MEK, and ERK increases the number of cyst-lining cells in the kidneys of Cy rats (Fig. 1I) [33, 35], PCK rats (Fig. 2E) [32], pcy mice (Fig. 3C) [39], and jck mice (Fig. 3F) [49]. In the Cy/+ rat model, a Raf kinase inhibitor reduced cell proliferation in the cystic epithelia [7]. An inhibitor of MEK ameliorated renal cystic disease in pcy mice and was associated with decreased downstream ERK activity [39]. In the kidney tissue of the Cy/+ rat and two mouse PKD models, orpk and bpk, overexpression and activation of EGFR are observed, and treatment with tyrosine kinase inhibitors ameliorated PKD progression [46, 54, 62]. On the other hand, activation of the cAMP signaling cascade through the adenylyl cyclase-activating receptor is known to stimulate MAPK as well. In fact, decreased intracellular cAMP achieved through reduction of plasma levels of vasopressin by increasing water intake or blocking the cAMP-mediated cellular effect of vasopressin with an arginine vasopressin V2-receptor antagonist ameliorated PKD progression in PCK rats [13, 68], Pkd2<sup>−/tm1Som</sup> mice [63], and pcy mice [13]. These findings suggest that blocking of the MAPK pathway activated by cAMP is also important for ameliorating PKD progression. On the basis of the results from animal models, a phase III clinical trial of a vasopressin V2-receptor antagonist is being performed in ADPKD patients. In addition, in order to avoid elevation of endogenous vasopressin levels, physicians recommend that early-stage patients who do not have concerns about reduced renal function drink water continuously.

Other pathways are also known to be related to MAPK signaling. For example, Src kinase is activated by EGFR and G protein-coupled receptors. The non-RTK activity of Src is upregulated in the polycystic kidney and/or liver in BPK mice and PCK rats and in the polycystic kidney of Pkd1<sup>+/−</sup> mice, and inhibition of Src ameliorates disease progression [12, 53]. Therefore, the inhibition of kinases in the accelerated MAPK pathway may have therapeutic potential to ameliorate disease progression in PKD patients.

**Mammalian target of rapamycin (mTOR) signaling pathway**

mTOR regulates cell proliferation by upstream signals from a serine-threonine kinase, AKT (also called Protein Kinase B). The mTOR signaling pathway is upregulated in the kidneys of Cy rats [58], PCK rats [45], Pkd2<sup>WS25/−</sup> mice [79], and Pkd2<sup>WS25/−</sup> mice [51]. Several inhibitors of mTOR are known to suppress disease progression in these models [51, 58, 79].

mTOR kinase forms at least two distinct complexes, mTOR complex 1 (mTORC1), which phosphorylates S6
protein, and mTOR complex 2 (mTORC2), which activate AKT by the phosphorylation of Ser 473. Sirolimus, a major mTOR inhibitor, suppressed the activity of either mTORC1 or mTORC2 in male Cy/+ rats; on the other hand, there were no effects against mTORC2 in females [3]. Although two clinical trials of mTOR inhibitor did not show a significant effect on disease progression in ADPKD patients [48, 65], the efficacy in relation to severe renal insufficiency in ADPKD is currently under investigation.

**Intracellular Ca2+**

It is thought that PC2 interacts with PC1 and FPC to regulate Ca2+ influx [14, 67]. Therefore, intracellular Ca2+ may have a key role in causing the characteristic cellular phenotype of PKD. In fact, in primary culture cells obtained from the kidneys of PKD1 knockout heterozygous mice, the concentration of intracellular Ca2+ is decreased to approximately half of that in cells from wild-type mice [1]. In Cy/+ rats, decrease of intracellular Ca2+ by treatment with L-type calcium channel blocker (CCB) accelerates PKD progression with increased ERK activity in the cystic cells [33]. After these preclinical findings were reported, a careful clinical study was performed, and the results suggested that CCB should be avoided in ADPKD patients unless CCB treatment for resistant hypertension is necessary [27].

**Other signaling cascades**

Peroxisome proliferator-activated receptor (PPAR)-γ: Activation of PPAR-γ has a crucial role in growth inhibition, cell cycle arrest, induction of apoptosis, fibrosis, and inflammation in cancer cells [5, 20, 75]. PPAR-γ agonists inhibit cell proliferation by downregulation of ERK and/or mTOR signaling pathways and decrease inflammation and fibrosis by downregulation of TGF-β [34, 77, 78]. Therefore, PPAR-γ may have therapeutic value in ameliorating PKD progression.

Monocyte chemoattractant protein-1 (MCP-1): MCP-1 is one of the factors that activate inflammation. Because urinary MCP-1 is increased in ADPKD patients and overexpression of MCP-1 is observed in the kidney of Cy/+ rats [10, 80], it seems to be a potential biomarker for disease severity [26, 80]. Interestingly, PPAR-γ agonists decrease the expression of MCP-1 in Cy/+ rats [11] with a concomitant reduction in disease progression.

Retinoid X receptor (RXR): Recently, we reported that the RXR-mediated pathway is upregulated in the Cy rat model [22]. This finding suggests that the regulation of this pathway may control PKD progression through cell proliferation mechanisms in this model.

**Androgen receptor**: Progression of PKD in male Cy/+ rats and jck mice is more severe than that in females. One of the factors that causes this increased severity is thought to be androgen, a male hormone [30, 49]. Androgen stimulates PKD progression via the androgen receptor with the activation of downstream MAPK pathways in males or testosterone-treated females after ovariectomy [30, 49]. Careful hormone therapy may be required in male PKD patients.

**Signal transducers and activators of transcription 3 (STAT3):** STAT3 is a key signaling component in pathways activated by growth-factor receptors. Activated STAT3 makes dimers and stimulates cell proliferation and has anti-apoptotic activity. Recently, it was reported that STAT3 activation is upregulated in the cystic epithelial cells of the PKD1 knockout mouse model, and STAT3 inhibitors, pyrimethamine and S31-201, ameliorate PKD progression with downregulated expression of STAT3 and Bcl-x and decreased activity of cyclin D and c-Myc [57]. Because pyrimethamine is already used clinically as an antimalarial drug, STAT3 inhibitors potentially have therapeutic value in ADPKD patients in the near future.

**Cyclooxygenase-derived prostanoids**: Cyclooxygenase-2 (COX-2) and its metabolites, including prostanoids, are thought to be involved in the inflammatory response. Activated COX-2 was observed in the kidney of Cy/+ rats, and inhibition of this enzyme resulted in reduced fibrosis and cyst expansion as well as amelioration of inflammation, oxidative injury, and cell proliferation, possibly by decreased production of thromboxane B2 and prostaglandin E2 [47]. Because COX-2-selective inhibitors are widely used as nonsteroidal anti-inflammatory drugs (NSAIDs), clinical application in PKD patients may be considered.

**“Two-Hit” Theory and “Third-Hit” Theory**

Currently, the “two-hit” theory is widely advocated to explain the initial stage of disease progression in PKD. Namely, a germ-line mutation in one allele and a somatic mutation or insufficient expression in another allele of either of the PKD genes causes cystogenesis [41, 70, 74]. It is thought that the variability in progression
of PKD depends on the timing of the somatic mutation or insufficient expression of the PKD gene in each renal cell. On the other hand, Weimbs et al. recently proposed a “third-hit” theory of PKD progression [4, 56, 71], in which unknown gene modification and/or environmental factors cause the “third-hit” and may accelerate disease progression. Because genetic background and environmental factors (e.g., food, water, temperature, humidity, lighting, and microorganisms) are uniformly controlled and disease progression is fairly invariable between individuals, the animal models of PKD are thought to be useful to investigate the “third-hit” mechanism.

Conclusions

So far, neither gene therapy nor antibody therapy is potentially realistic because PKD gene products are very large in molecular size and complicated in structure, and they exist in the membranes of various types of cellular organelles. Therefore, drug treatments have been aimed at regulation and alteration of known cell-signaling pathways in cystogenesis. At present, certain classes of drugs have been transitioned to clinical trials on the basis of the results from animal experiments. Therefore, there is no question that disease models are essential for the development of novel therapeutic treatments. Recently, a breakthrough study has reported that restoration of the normal Pkd1 allele (+/repaired+) from the heterozygous Pkd1 deletion (+/−) occurred by spontaneous mitotic recombination in induced pluripotent stem cells (iPSCs) obtained from a Pkd1+/− knockout mouse [8]. This finding suggests that research on genetic correction may enable new strategies of regenerative medicine. Thus, various approaches to curing PKD have now been revealed by the animal models of this genetic disorder.

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