## Chapter 12

# Epigenetics in ADPKD: Understanding Mechanisms and Discovering Treatment

Xiaogang Li[1,2]

[1]Department of Internal Medicine, [2]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160, USA

**Author for correspondence:** Xiaogang Li, PhD, Department of Internal Medicine, Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160, USA. Email: xli3@kumc.edu

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**Abstract**

Epigenetics is the study of all heritable changes in gene expression and chromatin organization that are caused by mechanisms independent of the DNA sequence itself. Similar to the genetic information found within the sequence of DNA, epigenetic information can also be inherited across generations. Epigenetic gene regulation includes, but is not limited to, DNA methylation and histone modification through acetylation, methylation, ubiquitylation, phosphorylation, or sumoylation. The roles of epigenetic modulation on gene expression and protein function have recently become the focus in autosomal dominant polycystic kidney disease (ADPKD). An interactive picture between

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PKD gene mutations and the epigenome needs to be developed to understand why inherited PKD gene mutations in patients may result in epigenetic changes that increase the progression of renal cyst formation. Recent studies demonstrate that PKD1 mutation increases the expression of epigenetic regulators, including DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone methyltransferases (HMTs) and bromodomain proteins. Conversely, inhibition of epigenetic regulators delays cyst growth in *Pkd1* knockout mouse models, supporting the importance of abnormal epigenetic regulation in ADPKD. One of the exciting findings is that targeting Sirt1, a class III HDAC, with nicotinamide (vitamin B3) delays renal cyst growth and preserves renal function in three *Pkd1* knockout animal models. The hypermethylation of PKD1 gene in gene-body regions implicates that DNA methylation-mediated epigenetic silencing of PKD genes is also a potential mechanism underlying cystogenesis. In this chapter, we will summarize the current knowledge on the role of epigenetics in ADPKD and its translational potential to identify much needed new therapies. We will also discuss the tools to study epigenetic mechanisms in ADPKD and their applications on understanding how epigenetic events intertwine with PKD-associated signaling pathways, including c-Myc, EGFR, HSP90, STAT3/STAT6, AMPK, Wnt/β-catenin, ILK/mTOR, hedgehog, GSK3β and NF-κB/inflammation signaling.

**Keywords:** Epigenetics; DNA methylation; Histone modification; PKD associated signaling pathways; Vitamin B3

## Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common life-threatening genetic disorders and affects approximately 600,000-700,000 people in the United States (1). The hallmark of the disease is the development of fluid-filled cysts in the nephrons of both kidneys, resulting in end-stage kidney disease (ESKD) and requiring painful dialysis. ADPKD are caused by mutations in either PKD1 gene encoding polycystin-1 (PC1), accounting for 85-95% of the cases, or PKD2 gene encoding polycystin-2 (PC2), accounting for the remainder (2). PC1 can form a complex with PC2 which is a calcium-permeable cation channel (3-6). Renal cyst formation can be initiated at all stages of kidney development, which is also associated with renal interstitial inflammation and fibrosis (7, 8). ADPKD patients also develop various extra-renal manifestations including hepatic cysts, intracranial aneurysms and cardiac vascular abnormalities. In addition to the necessity to be finely tuned to the expression of polycystins (9, 10), multiple signaling pathways downstream of PKD gene mutations have been identified in regulating cystic renal epithelial cell proliferation and apoptosis, leading to cyst formation (11-20).

Elucidation of the complex pathways that regulate the expression of polycystins or the signaling pathways downstream of polycystin signaling are critical for achieving a full understanding of ADPKD pathogenesis and for identification of crucial regulatory or structural components that may be useful as therapeutic targets. Apart from the genetic traits of ADPKD, the roles of epigenetics have recently drawn attention of scientific investigation in ADPKD (21-26). This chapter summarizes the current knowledge on the role of epigenetics in ADPKD, and its translational potential to identify much needed new therapies.

**The epigenome and mechanisms of epigenetic regulation**

In mammals, genomic DNA is spun around histone protein cores containing dimers of histones H2A, H2B, H3 and H4 to form chromatin (27). Epigenetic modifications on histone proteins and the DNA wrapped around them result in either loose (euchromatin) or tight (heterochromatin) states of chromatin. Euchromatin allows RNA polymerases and transcriptional factors to bind whereas heterochromatin is associated with transcriptional inactivation (27). Epigenetic marks including DNA methylation, histone post-translational modifications, and noncoding RNAs collectively form the 'epigenome'. The close association between DNA methylation and histone modification is well established (28). Perturbations in the epigenome have been implicated in various pathological conditions including cancer and ADPKD (22, 25, 29).

DNA methylation as the first identified epigenetic modification has been intensively studied for half a century (30). DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs) which can post-replicatively add methyl groups to the C5 position of cytosines in DNA (31). DNA methylation is usually associated with transcriptional silencing of a number of genes and sequence classes, including tumor suppressor genes, imprinted genes, and genes on the inactive X chromosome (32). Silencing of these sequences is essential for maintaining chromosome stability. DNA methylation is distributed throughout the genome generally at CpG dinucleotides, a cluster of large repetitive sequences (called CpG islands) in regions such as centromeric repeats or at the 5' ends of many genes (33). In humans, 50-70% of all CpGs are methylated, primarily in heterochromatic regions. In vertebrates, there are five known DNMTs with different structure and function. All DNMTs, except DNMT2, have an N-terminal regulatory domain and a C-terminal catalytic domain. The ubiquitously-expressed DNMT1, which displays a strong preference for hemimethylated CpG sites, functions to maintain the DNA methylation patterns established by the DNMT3 subfamily, comprising DNMT3a and DNMT3b, on unmethylated DNA (31, 34, 35)

during DNA replication and DNA repair (36, 37). The cofactor DNMT3L1 stimulates the activity of DNMT3a and DNMT3b (38), but by itself lacks enzymatic activity (39). The fifth member of the DNMT family, DNMT2, has very weak activity toward DNA (40, 41). Promoter DNA methylation is a relatively stable epigenetic modification which represses transcription via interference with transcription factor binding or recruiting repressor complexes consisting of methyl-DNA binding proteins (42), such as methyl-CpG-binding domain proteins (MBDs), UHRF proteins (ubiquitin-like, containing plant homeo domain **[**PHD] and really interesting new gene (RING) finger domains) and zinc finger proteins (43). DNA demethylation occurs mainly by passive mechanisms during development and cell division (44, 45). Aberrant expression of DNMTs and disruption of DNA methylation patterns are closely associated with many forms of cancer. In general, hypermethylation occurs on tumor suppressor genes and hypomethylation occurs on oncogenes (46-48), although the exact mechanisms underlying this link remain elusive.

Histone post-translational modifications as epigenetic marks, including histone lysine acetylation (HKAc), methylation (HKme) and phosphorylation (49), regulate chromatin structure and gene expression (50, 51). Histone acetylation is mediated by histone acetyl transferases (HATs) and is generally associated with relaxed chromatin and active gene expression. In contrast, histone deacetylation is mediated by histone deacetylases (HDACs) and is generally associated with closed chromatin and represses gene expression. On the other hand, histone/lysine methylation is mediated by histone methyltransferases (HMTs) (52) and can be an active or repressive mark depending on the lysine residue modified and the extent of methylation (mono-, di-, or tri-) on different lysine residues. Methylation of lysine residues on histone tails can be erased by histone demethylases (53). Histone modifications can mark and define distinct regulatory regions of the genome, which can serve as docking sites for coactivators, co-repressors, chromatin remodeling proteins, and proteins that bind to modified histones (50, 51, 54). For example, bromodomain proteins, which have an approximately 110 amino acid protein domain called bromodomain, recognize and bind monoacetylated lysine residues on the N-terminal tails of histones (55), whereas chromodomain protein, which has an approximately 40-50 amino acid protein domain called chromodomain (*chromatin organization modifier*), only recognizes and binds methylated histones (56, 57) and appear in the RNA-induced transcriptional silencing complex (58). In general, trimethylation of H3 at lysine 27, namely H3K27me3, is a strong repressor of transcription by attracting chromodomain-containing proteins and HP1 (59, 60).

Noncoding RNAs, including short microRNAs (about 22 nucleotides in length) and long noncoding RNAs (4200 nucleotides long), are also epigenetic marks which work via

epigenetic mechanisms (61-64). The roles of several microRNAs in renal disorders including PKD has recently been studied (65, 66) and will be discussed in next chapter.

## The progression of epigenetic studies in ADPKD

### *DNA methylation and ADPKD*

Aberrant DNA hypermethylation and hypomethylation patterns have been associated with human cancer and other diseases (67) and may play a role in the manifestation, progression and therapy of PKD. It has been reported that *PKD1* is hypermethylated in gene-body regions, and its expression is downregulated in ADPKD (Figure 1), implicating DNA methylation-mediated epigenetic silencing as one of the mechanisms underlying cystogenesis (68). Whether the methylations of the *PKD2* gene and autosomal recessive PKD (ARPKD) genes, as well as the genes of PKD-associated signaling pathways, are changed and contribute to cyst development needs be investigated. In addition, *PKD1* mutations result in the upregulation of DNA methyltransferase 1 (DNMT1) in cystic renal epithelial cells (unpublished data); thus genes downstream of PKD mutations (including ADPKD and ARPKD genes) may also be hypermethylated during cyst formation. Further studies should focus on when and how DNA methylation is altered during cyst development, and whether reversal of DNA methylation variations in the early stages of PKD can delay cyst growth and the progression to ESKD.

### *Histone deacetylases (HDACs) in ADPKD*

Evidence generated to date indicates that HDACs are important regulators of ADPKD (21-26). Depending on the sequence similarity and cofactor interactions, HDACs are classified into four classes: Class I HDACs (HDAC1, 2, 3 and 8), which are nuclear enzymes and can be widely expressed in different tissue types (69); Class II HDACs (HDAC4, 5, 6, 7, 9 and 10) and class IV HDAC (HDAC11), which are predominantly located within the cytoplasm and can be expressed in a tissue-specific manner (69); and Class III HDACs, which are called sirtuin family proteins (SIRT1–8) with different subcellular localizations, substrate specificities and functions (70). HDACs are able to deacetylate histones or non-histone substrates, for example, transcriptional factors, to either regulate the expression of the PKD1 gene or genes and proteins involved in regulating cystic renal epithelial cell proliferation and apoptosis (Figures 1 and 2) (11, 21). Pharmacological inhibition of HDACs delays cyst growth and preserve renal function in *Pkd1* (21-26) and *Pkd2* mutant mice (71), respectively, implicating the potential clinical application of HDAC inhibitors on ADPKD treatment.

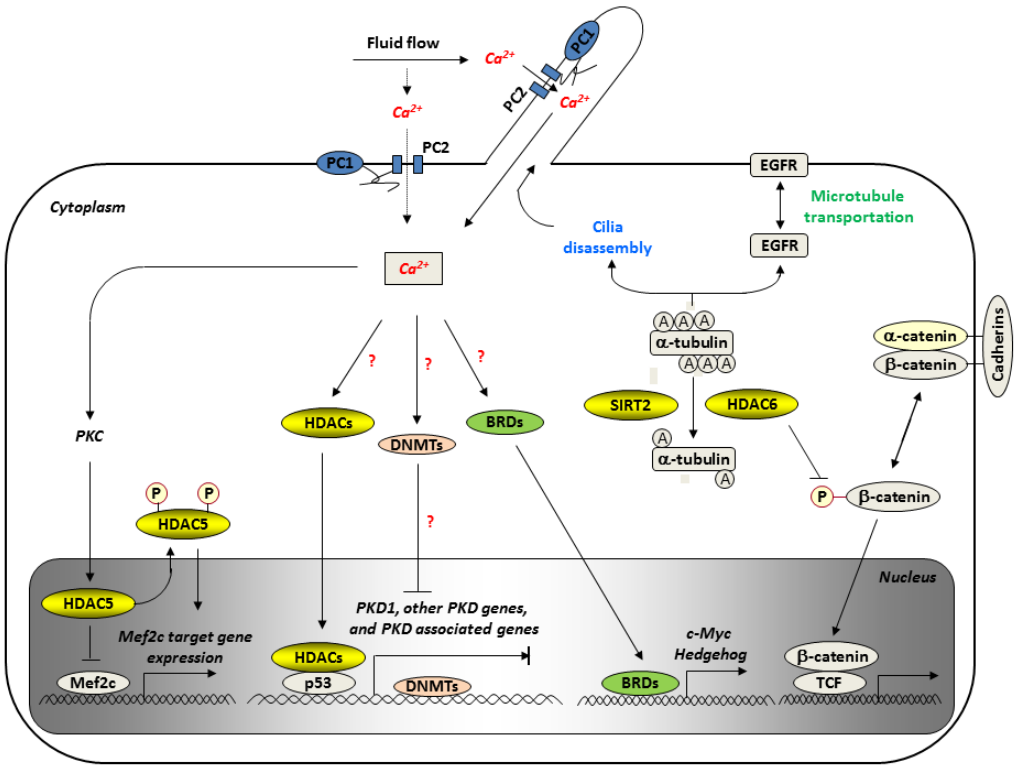


**Figure 1.** The roles of histone deacetylases (HDACs), Bromodomain proteins (BRDs) and DNA methyl transferases (DNMTs) in renal epithelial cells. In this schematic diagram, we depicted the roles of HDACs in regulating PKD1 gene expression and PKD associated signaling, including that i) HDAC5 is the target of fluid flow-induced calcium signal in renal epithelial cells; ii) HDAC6 and SIRT2 regulate cilia disassembly through deacetylation of α-tubulin during the normal cell cycle; iii) HDAC6 regulates epidermal growth factor receptor (EGFR) trafficking through deacetylation of α-tubulin; and iv) HDAC6 either alone or with EGF regulates β-catenin nuclear translocation. We also indicate the potential roles of DNMTs in regulating the transcription of PKD1 gene, other PKD genes and PKD-associated genes. The roles of BRDs in regulating the transcription of c-Myc and the components of Hedgehog signaling are also included. The involvement of calcium signaling in these processes is possible but is uncertain.

*HDACs are involved in repression of the expression of PKD1 gene*

The expression of polycystins is required to be finely tuned to prevent cyst formation (9, 10). The PKD1 gene promoter contains a hybrid p53-Sp1-binding motif which has been shown to be bound by p53 *in vivo*. Binding of p53 to the promoter of PKD1 gene decreases

its expression. This process is also regulated by HDACs since a pan-HDACs inhibitor trichostatin A (TSA) could attenuate p53-induced repression of the PKD1 expression (72). Although we propose a model that polycystin signaling activates p53 (73), which in turn, in cooperation with HDACs, controls PKD1 gene expression (Figure 1), however, the role of p53 in regulating mutant PKD1 gene expression needs be further investigated. In addition, the HDAC(s) involved in p53-mediated repression of PKD1 gene is unknown. HDAC1 interacts with p53 and Sp1, which suggests that it may be involved in p53-mediated repression of PKD1 through deacetylation of p53 (74, 75).

*HDAC5 is the target of fluid flow-induced calcium signal in renal epithelial cells*

HDAC5, a class II HDAC, was identified as one of the targets of polycystin-dependent fluid stress sensing in renal epithelial cells by microarray analysis (76). Fluid flow stimulation of polarized renal epithelial monolayers results in calcium influx into the cells to activate protein kinase C (PKC). PKC then directly or indirectly phosphorylates HDAC5 at two 14-3-3 binding sites, leading to the translocation of HDAC5 from the nucleus to the cytosol (Figure 1) (77). Nuclear export of HDAC5 releases its inhibition on MEF2C-based transcription (78, 79). Heterozygous knockout of HDAC5 or inhibition of HDAC5 activity with TSA delayed cyst growth in *Pkd2*$^{-/-}$ mouse embryos, a result that supports an epistatic relationship between *Pkd2* and HDAC5 (76). In addition, treatment with TSA also delayed cyst growth in kidneys from *Pkd1*$^{-/-}$ embryonic mice (23). Furthermore, treatment with valproic acid (VPA), a class I HDAC specific inhibitor, slowed cyst growth and the decline of kidney function in *Pkd1* conditional knockout mice (71). These results suggest that class I and II HDACs are the potential therapeutic targets for the treatment of ADPKD.

*HDAC6 regulates epidermal growth factor receptor (EGFR) endocytic trafficking and degradation as well as β-catenin nuclear localization in renal epithelial cells*

HDAC6, a microtubule-associated α-tubulin deacetylase, demonstrates increased expression and activity in *Pkd1* mutant renal epithelial cells (24). The epidermal growth factor-EGF receptor (EGF-EGFR) axis has a documented role in the expansion of renal cysts (80). Inhibition of HDAC6 with TSA or tubacin, a specific HDAC6 inhibitor, increased α-tubulin acetylation and decreased the expression of EGFR in *Pkd1* mutant renal epithelial cells. HDAC6, through deacetylation of α-tubulin, affects the stability of microtubule, which further regulates EGFR intracellular trafficking and degradation along microtubules in normal and mutant renal epithelial cells (Figure 1) for the following reasons: 1) targeting HDAC6 with pharmacological inhibitor not only increased EGFR endocytic trafficking but also normalized the localization of EGFR from apical to basolateral of the cystic epithelial cells in *Pkd1* conditional knockout mouse

kidneys; and 2) treatment with nocodazole, which depolymerized microtubules, decreased the degradation of EGFR and EGFR endocytic trafficking from early endosomes to later endosomes in *Pkd1* mutant renal epithelial cells stimulated with EGF. In addition, HDAC6 is through deacetylation of β-catenin at lysine 49, a site often mutated in cancers, to increase the nuclear localization of β-catenin induced by EGF (81, 82). Inhibition of HDAC6 not only blocks EGF-induced β-catenin nuclear localization but also decreases c-Myc expression, leading to decrease epithelial cell proliferation. In addition, HDAC6 forms complex with HSP90 and MIF (83, 84), the two recent identified PKD associated signaling (7, 11). These results suggest targeting HDAC6 may be a potential therapeutic approach for polycystic kidney disease.

*SIRT1 regulates cyst development through deacetylation of Rb and p53 in ADPKD*

SIRT1, a member of class III HDACs, targets both histone and nonhistone proteins. SIRT1-mediated histone deacetylation, including histones H1K26, H3K9 and H4K16, is necessary to form heterochromatin and to silence the transcription (85). SIRT1 also deacetylates non-histone proteins, including the retinoblastoma (Rb) protein, E2F1, p53, nuclear factor-kappaB (NF-κB), FOXO1, FOXO3, c-Myc, β-catenin, heat shock protein 90 (HSP90), and Smad7 to potentially regulate cell proliferation and apoptosis (86-89). SIRT1 can remove an acetyl group from acetylated lysine residues of histone and non-histone proteins to generate lysine, 2′-O-acetyl-ADP-ribose (OAADPr), and nicotinamide which is also a noncompetitive inhibitor of SIRT1 (90, 91).

SIRT1 was upregulated in embryonic and postnatal *Pkd1* mutant mouse renal epithelial cells and tissues, partially through c-Myc and tumor necrosis factor-α (TNF-α) signaling (Figure 2) (22). SIRT1 deletion delayed cyst formation and normalized kidney function in a *Pkd1* mutant mouse model (22). SIRT1 regulates cystic renal epithelial cell proliferation and apoptosis through deacetylation and increased phosphorylation of Rb (at residue S780) which becomes inactive, in turn enabling transcription of genes that mediate entry into the S-phase of the cell cycle (89) and through deacetylation of p53 (22), an important tumor suppressor protein, at residue K382, respectively. Inhibition of SIRT1 with nicotinamide or EX527, a specific SIRT1 inhibitor, decreased proliferation and increased apoptosis of cystic epithelial cells. Targeting SIRT1 with nicotinamide delayed cyst growth, decreased kidney weight to body weight (KW/BW) ratio and decreased blood urea nitrogen (BUN) levels in *Pkd1* knockout mouse models (22). This study provides strong evidence that nicotinamide is a particularly attractive candidate for treatment of PKD.

Nicotinamide (also known as niacinamide) is a water-soluble amide derivative of nicotinic acid, which represents a major form of vitamin B3. Nicotinamide has been

studied in humans for several decades and has an excellent safety profile when given at adult doses of no more than 3 g/day (92). Nicotinamide has been used in humans at doses ranging from 1 g/m² to 6 g daily as an anti-oxidant, anti-inflammatory and immune modulator in many disease conditions, including type I diabetes mellitus, schizophrenia, Alzheimer's disease, neurodegenerative disorders, hyperphosphatemia in dialysis patients, and as a radiosensitizer in cancer. Because nicotinamide is a safe and inexpensive dietary nutritional supplement that does not require FDA approval, two early phase clinical trials are ongoing at the University of Kansas Medical Center (KUMC) to test the effect of niacinamide in humans with ADPKD (Clinical Trials.gov. Identifier: NCT02140814). Once it can be proven to be safe and effective in PKD patients, it could be used to treat patients immediately.

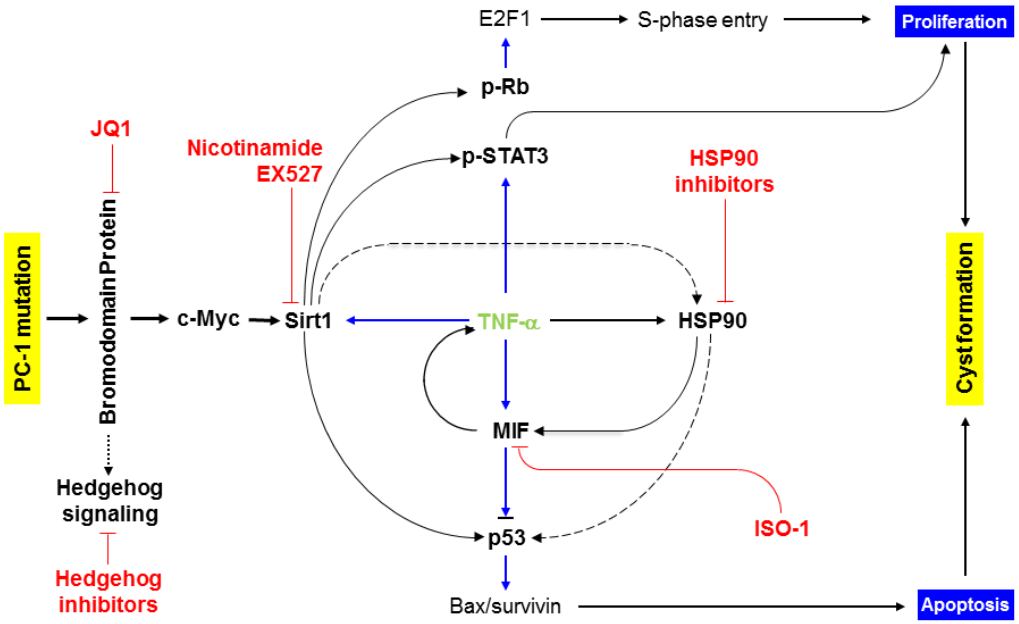


**Figure 2.** The relationship between epigenetic regulators and PKD-associated signaling. In this schematic diagram, we depicted the interplays of epigenetic regulators, Sirt1 and Bromodomain protein, with several already known PKD-associated signaling pathways, including tumor necrosis factor-α (TNF-α signaling, c-Myc signaling, STAT3 signaling, heat shock protein 90 (HSP90) signaling, macrophage migration inhibitory factor (MIF) signaling and Hedgehog signaling. The inhibitors of these pathways are marked in red. Solid lines indicate the already known connections among these signaling pathways in cystic renal epithelial cells, whereas the dashed lines indicate the potential connections of these signaling pathways in cystic renal epithelial cells based on the studies in other cell types.

*SIRT2 and HDAC6 regulate ciliogenesis and SIRT2 contributes to abnormal centrosome amplification caused by loss of polycystin-1*

The localization of PC1 and PC2 to the primary cilia has led to development of the ''primary cilia'' hypothesis for PKD, in that the abnormalities in primary cilia structure and function in tubular epithelia contribute to cyst initiation and development. The primary cilium is a microtubule-based organelle that originates from the one of the two basal bodies (centrioles) that form the core of the centrosome in quiescent cells. For cell division, the primary cilium has to be disassembled to liberate one of the captive centrioles of the centrosome which directs assembly of the bipolar spindle during mitosis (93, 94). Thus, cilia may passively affect the cell cycle. It has been demonstrated that HDAC6 and SIRT2, another member of class III HDACs, regulate the stability of microtubules through deacetylation of α-tubulin and regulate disassembly of cilia during the normal cell cycle (Figure 1) (26, 95). The fact that SIRT2 and HDAC6 are able to form a complex and α-tubulin binds to the SIRT2-HDAC6 complex *in vitro* (96, 97) suggest that SIRT2 and HDAC6 may regulate α-tubulin deacetylation and cilial size together. However, inhibition of either SIRT2 or HDAC6 alone is sufficient to induce hyperacetylation of α-tubulin and block cilial disassembly (26, 96) suggesting that SIRT2 and HDAC6 can regulate ciliogenesis independently. These results may explain why knockout of HDAC6 in mice does not cause hyperstable microtubules or persistent cilia (26) since SIRT2 may compensate for the loss of HDAC6 in knockout cells and organs. SIRT2-mediated α-tubulin deacetylation is able to regulate chromosomal segregation during mitosis to ensure normal cell division through affecting mitotic structures including the centrosome, mitotic spindle and midbody (98, 99). SIRT2 was upregulated in *Pkd1* knockdown mouse inner medullary collecting duct (IMCD3) cells and *Pkd1* knockout mouse kidney cells. This was responsible for the aberrant centrosome amplification and polyploidy induced by loss of PC1 (26). However, the role of SIRT2 in renal cyst development remains to be determined.

*A BET bromodomain protein, Brd4, in ADPKD*

Bromodomain proteins specifically bind to acetylated lysine residues on histone tails through bromodomains to regulate gene expression (55). Recently, we reported that a BET bromodomain (BRD) protein, Brd4, is a novel epigenetic regulator of ADPKD and a novel client protein of HSP90 (100). Brd4 was upregulated in *Pkd1* mutant mouse renal epithelial cells and tissues, which might be partially mediated by the chaperone activity of HSP90, leading the cells to escape proteasomal degradation. Targeting Brd4 with JQ1, a selective small-molecular inhibitor of BET bromodomain protein(s) (100), slowed cyst growth and kidney enlargement in two early stage *Pkd1* mutant mouse strains. Brd4 regulates the expression of c-Myc and p21, which further affects the phosphorylation of Rb and Rb mediated S-phase entry to regulate cystic renal epithelial cell proliferation (100). Our study

not only addresses how c-Myc is upregulated in PKD but also provides a rationale for targeting Brd4 with JQ1 as a potential epigenetic therapy in ADPKD. In addition, the association of Brd4 and HSP90 in ADPKD may also be a general mechanism for the upregulation of Brd4 in cancer cells.

## Other potential functions of epigenetics in PKD

### *Epigenetic mechanisms in renal inflammation*

Interstitial inflammation has been consistently reported in human and animal models of PKD. Whether renal inflammation is one of the primary factors for cyst initiation or a consequence of renal cyst formation is uncertain. However, its role in promoting cyst growth is supported by the findings that depletion of macrophages in kidneys of *Pkd1* conditional knockout mice and *cpk* mice, which develop renal cysts via the disruption of cystin (a cilia-associated protein), caused a significantly lower cystic index, reduced proliferation of cyst-lining cells, and improved renal function (7, 101). Inflammation in cystic kidneys is characterized by increased release of proinflammatory cytokines/chemokines, such as TNF-α, interleukins (ILs), and monocyte chemoattractant protein-1 (MCP-1), by tubular and endothelial cells, as well as dendritic cells and infiltrating leukocytes/monocytes (7, 20, 102). In recent years, epigenetic mechanisms have been shown to have a role in renal inflammation in kidney disease (102). Changes in histone acetylation and methylation were observed at inflammatory genes, including TNF-α and Ccl2/MCP-1 in various models of acute kidney injury (AKI) (103, 104). It has been found that endoplasmic reticulum stress can increase the levels of SET7, a histone methyltransferase, leading to increased histone 3 lysine 4 (H3K4) methylation at the Ccl2/MCP-1 promoter and its upregulation in the kidneys from diabetic db/db mice (105). Changes in histone 3 lysine 9 (H3K9) acetylation at inflammatory gene promoters had also been observed in diabetic mice models (106-108). In addition, the deacetylase Sirt2 also played a proinflammatory role in lipopolysaccharide-induced acute kidney injury by induction of NF-κB activation and chemokine production in proximal tubular epithelial cells. Deletion of Sirt2 in mice delayed renal function decline and was protective against LPS induced infiltration of neutrophils and macrophages, and acute tubular injury (109). Together, these studies suggest that epigenetic mechanism may be involved in renal inflammation in PKD.

### *Epigenetic mechanisms in renal fibrosis*

Severe interstitial fibrosis has also been associated with sustained enlargement of fluid-filled cysts in PKD. However, the mechanism for the development of interstitial fibrosis in

PKD remains elusive. It has been found that treatment of aberrant histone acetylation in experimental kidney fibrosis with TSA attenuates intra-renal inflammation and tubulointerstitial fibrosis in mice (110, 111). Administration of MS-275, a selective Class I HDAC inhibitor, and tubacin, a specific HDAC6 inhibitor, ameliorated fibrosis through inhibition of transforming growth factor (TGF)-β signaling, which is up-regulated in tissue fibrosis of several organs and causes fibroblast activation (112). Similarly, administration of vorinostat, the first FDA-approved HDAC inhibitor for clinical application, ameliorated diabetes-associated renal fibrosis in an animal model through the normalization of EGFR-mediated signaling (113). Other Class I and II HDAC inhibitors like phenylbutyrate and valproic acid could also be of benefit to experimental renal fibrosis (114-116).

In addition, it has been shown that DNMT1 is induced in experimental renal fibrosis (112) and Dnmt1 heterozygous knockout mice show ameliorated aberrant promoter methylation and reduction of renal tubulointerstitial fibrogenesis (112). RASAL1, a negative regulator of Ras signaling, is transcriptional repressed due to its hypermethylation in experimental kidney injury, acute renal damage and chronic progressive fibrosis (112). Exposure to TGF-β further inhibited the expression of Rasal1 by promoting DNA methylation at its promoter via DNMT1, leading to Ras activation and increased fibrosis in fibroblasts, which can even be persistent after TGF-β is removed (112). Since only DNMT1 but no other member of the DNMT family is altered in kidney fibrosis, it suggests a predominant role of DNMT1 mediated DNA methylation in context of chronic progressive kidney disease. Together, the roles of HDACs and DNMT1 in PKD-associated interstitial fibrosis need be investigated.

*Epigenetic mechanisms in hypertension*

Mutations of PKD1 and PKD2 result not only in renal, hepatic and pancreatic cyst formation but also in cardiovascular complications characterized by an increased incidence of cardiac valve abnormalities and left ventricular hypertrophy (117-121). It has been suggested that ADPKD associated cardiovascular complications result from renal cyst growth induced cardiovascular hypertension, which occurs in patients at an earlier age than that in the general population even before any substantial reduction in renal function, and is associated with a rapid progression toward renal failure (122-125). It has been found that the mineralocorticoid aldosterone via upregulation of the tubular epithelial sodium channel (ENaC) regulates the disorders of Na+ transport, reabsorption, and excretion in the renal collecting duct, leading to abnormal blood pressure in humans (126). Under basal conditions, the transcription of ENaC subunit alpha (ENaCα) can be repressed by Dot1a, a lysine methyltransferase, mediated the methylation of histone 3 lysine 79 (H3K79) on the ENaCα promoter, keeping it constrained but poised for activation by aldosterone and other stimuli. Hypertension induced by high-salt diet is also associated with epigenetic mechanism

mediated upregulation of angiotensin I converting enzyme (ACE1), which has an important role in hypertension by activating the renin–angiotensin system, via increases in activation marks (H3KAc and H3K4me) and decreases in repressive mark (H3K9me2) at the promoter of ACE1 (127). Study of epigenetics in ADPKD-associated hypertension and its potential heritability is clearly warranted. Furthermore, the fact that cardiac hypertrophy also occurs in young ADPKD patients with normal blood pressure and renal function (128, 129) suggests that cardiac dysfunction in ADPKD patients does not develop solely in response to hypertension and/or renal failure. Additional epigenetic or environmental factors may be required and more investigations are anticipated.

*Epigenetic mechanisms in regulating ADPKD associated signaling pathways*

In addition to the well-documented PKD gene mutations that have been associated with cyst development, considerable attention is being focused on the participation of epigenetic events on the regulation of transcriptional and/or translational activities of PKD-associated signaling pathways, including HSP90 (11), STAT3/STAT6 (12-14), AMPK (15), Wnt/β-catenin (16), GSK3β (130), ILK/mTOR (8, 17), hedgehog (18), MIF (7) and NF-κB/inflammation signaling (19, 20). Knockout of Thm1 in mice resulted in renal cyst formation and the upregulation of the components of hedgehog signaling, including Gli1 and Gli2 (131). Knockout of *Pkd1* also induced the abnormal upregulation of Gli1 and Gli2 (131), which suggested that abnormal regulation of hedgehog signaling contributes to cyst development. A recent study found that the expression of Gli1 and Gli2 could be regulated by the bromodomain protein, Brd4, an epigenetic regulator that binds to acetylated histone tails, in NIH3T3 and mouse embryonic fibroblast (MEF) cells (132). Brd4 may regulate the expression of Gli1 and Gli2 through binding to the promoters of these genes in Thm1 and *Pkd1* mutant renal epithelial cells. Additionally, the HSP90, MIF, GSK3β and Wnt-β-catenin signaling pathways have been associated with epigenetic regulators (83, 133). It is highly possible that epigenetic event is involved in regulation gene expression and protein function of most, if not all, of PKD associated signaling pathways. Thus, determining the nature of epigenetic modifications and extent to which they occur on PKD-associated genes, and establishing how epigenetic events intertwine with PKD associated signaling pathways is highly significant for our understanding of the pathogenesis of PKD and can be achieved with the advance of epigenetic tools.

## Tools to study epigenetic mechanisms in ADPKD

The importance of epigenetic alterations in ADPKD and in regulating ADPKD-associated signaling pathways is increasingly being appreciated. Epigenetics research has been spurred

by the technological breakthroughs in next generation sequencing (NGS) and advances in epigenomics platforms and data analysis tools that have aided in detecting epigenetic modifications such as histone modifications and DNA methylation, chromatin structure (open or condensed), as well as long-range interaction of enhancers in transcription regulation. Utility of these approaches to detect epigenetic changes at PKD genes and at PKD associated candidate genes should provide us an opportunity to gain new insights into the pathologies of PKD and uncover targets for novel epigenetic therapies.

The fact that *PKD1* is hypermethylated in gene-body regions, and its expression is downregulated in ADPKD (68) suggests that other ADPKD or ARPKD genes and genes downstream of PKD mutations may also be methylated. This can be determined by DNA methylation analysis. This mode of analysis, including Methylation Specific PCR, Bisulfite Sequencing, Bisulfite Pyrosequencing, and Genome Wide Methylation Analysis, is based on the treatment of genomic DNA with sodium bisulfite. Sodium bisulfite only deaminates cytosine but not 5-methylcytosine into uracil, which can be identified by sequencing to determine the DNA methylation status (134). During PCR and sequencing, uracil hydrogen bonds to adenine which will then hydrogen bond to thymine. Therefore, the unmethylated cytosines will become thymines and methylated cytosines will remain cytosines in the amplified sequence. DNA methylation analysis with genome-wide quantification of sodium bisulfite conversion–based cytosine method can be performed by NGS or the widely used Infinium Human Methylation 450K Bead-chips Assay (San Diego, CA), which is especially for large-scale clinical projects. In comparing with affinity-based methods, including methylated DNA immunoprecipitation-sequencing (MeDIP-seq) or methyl-CpG binding domain (MBD) protein-enriched genome sequencing (MBD-seq), Bisulfite Sequencing (bisulfite-seq) provides better resolution and genome wide coverage but it is more expensive and it involves more complex bioinformatics analysis.

Whole transcriptome profiling by NGS (for coding and noncoding genes) and epigenome-wide association studies, such as chromatin immunoprecipitation (ChIP) and ChIP-sequencing (ChIP-seq) analyses which combine immunoisolation of epigenetic marks and NGS, have been developed. These new tools can yield information on genome-scale dynamic changes and will help to identify novel epigenetic regulators and transcription factors involved in the expression of PKD genes or genes associated with PKD as well as novel downstream targets. A major advantage of NGS-based studies such as RNA-seq, ChIP-seq, bisulfite-seq, and others is that these unbiased approaches provide genome-wide and quantitative information unlike microarrays. However, before performing these studies, the more expensive costs and the complicated data analyses need to be considered. We believe that with advance in new and cheap technologies, epigenome association studies should be performed more frequently in experimental and clinical studies in PKD.

## Perspectives and conclusions

Increasing evidence suggests a critical role for epigenetic modifications, including DNA methylation and histone/lysine deacetylation in ADPKD (21-26) (68). Studies have explored the potential beneficial effects of HDAC inhibitors in animal models of ADPKD. However, as the specificity and the mechanism of action of these inhibitors are not fully clear, more work, except for nicotinamide (vitamin B3), is needed before these inhibitors can be evaluated in humans. In order to screen novel HDAC inhibitors that could delay cyst growth in PKD mouse models, a zebrafish model was recently used and generated very promising results (71). Due to other epigenetic regulators, including HMTs and DNMTs, being also potentially involved in regulating cystogenesis, this novel screening approach may help to discover additional epigenetic modulators specific to PKD. In addition, we may test the epigenetic drugs that target histone-modifying enzymes in cancer treatment (135, 136) for preclinical treatment in PKD animal models. The links between epigenetic mechanisms and PKD associated signaling pathways have encouraged investigators to think about dual therapies, such as combining HDAC inhibitors with metformin or mTOR inhibitor in PKD treatment. It may be advantageous to sensitize cells by epigenetic therapy followed by treatment with chemotherapy, which targets PKD associated signaling pathways, including HSP90 (11), STAT3/STAT6 (12-14), AMPK (15), Wnt/β-catenin (16), GSK3β (130), ILK/mTOR (8, 17), hedgehog (18), NF-κB/inflammation signaling (19, 20). In summary, epigenetics is clearly an exciting emerging field in basic and clinical studies of ADPKD, and the development of 'epigenetic therapies', specifically HDACs, have shown promising effects for PKD treatment. To investigate epigenetic mechanisms underlying cystogenesis is an exciting challenge but it may lead to a better understanding of cyst development and direct new therapeutic strategies of ADPKD. However, several roadblocks and challenges should also be overcome, including low specificity/selectivity of inhibitors of epigenetic regulators and unwanted side effects.

## Conflict of interest

The author declares that he has no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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