

## Polycystic kidney disease and therapeutic approaches

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**Polycystic kidney disease (PKD) is a common genetic disorder in which extensive epithelial-lined cysts develop in the kidneys. In previous studies, abnormalities of polycystin protein and its interacting proteins, as well as primary cilia, have been suggested to play critical roles in the development of renal cysts. However, although several therapeutic targets for PKD have been suggested, no early diagnosis or effective treatments are currently available. Current developments are active for treatment of PKD including inhibitors or antagonists of PPAR- $\gamma$ , TNF- $\alpha$ , CDK and VEGF. These drugs are potential therapeutic targets in PKD, and need to be determined about pathological functions in human PKD. It has recently been reported that the alteration of epigenetic regulation, as well as gene mutations, may affect the pathogenesis of PKD. In this review, we will discuss recent approaches to PKD therapy. It provides important information regarding potential targets for PKD. [BMB reports 2011; 44(6): 359-368]**

### INTRODUCTION

Polycystic kidney disease (PKD) is divided two types: autosomal dominant (AD) and autosomal recessive (AR). Mutations in polycystin-1 (PC-1) and polycystin-2 (PC-2) cause ADPKD; PKD is characterized by numerous cysts and fluid secretions into the lumen in the kidney (1, 2). The main cystoproteins, PC-1 and PC-2, are co-localized in primary cilia and mediate  $Ca^{2+}$  signaling as a mechanosensor (3, 4). Multiple mechanisms have been shown to contribute to PKD, including increased proliferation and apoptosis, in addition to loss of differentiation and polarity (5). PKD causes progressive cyst formation and ultimately results in renal failure. In end-stage renal disease (ESRD) of PKD, many patients depend on hemodialysis to attenuate renal failure or transplantation, but thus far, no suitable treatment has been developed.

Recently, investigators have transferred their focus from the

cause of cyst progression to the search for a target of therapy. The mammalian target of rapamycin (mTOR) pathway is the main target for a PKD therapy, because it is abundantly expressed in cyst-lining epithelial cells in human patients and mouse PKD models (6). Tuberous sclerosis complex 1 and 2 (TSC1 (harmatin) and TSC2 (tuberin)) complex have been shown to regulate mTOR signaling, and directly interact with the C-terminal cytoplasmic tail of PC-1 (6, 7). The mTOR pathway is related to cell growth; thus, mTOR inhibitors such as rapamycin (sirolimus) prevent cystic growth (6, 8, 9). Another crucial target is cAMP, which is stimulated and accumulated in the cystic fluids of PKD patients and affect diverse components of downstream signaling. Furthermore,  $Ca^{2+}$  signaling is disrupted by the mutation of PKD genes and leads to cAMP-MAPK pathway activation in PKD (10). Many cAMP-targeted drugs, such as sorafenib (BAY 43-9006) effects on cell proliferation and cystic growth and B-Raf-targeted drugs will also have to be evaluated (11, 12). Recently, metformin has been shown to reduce cystogenesis via the activation of AMP-activated protein kinase (AMPK) which inhibits two main targets; CFTR and mTOR (13). Therefore, combined therapies for the treatment of PKD still need to be studied.

Many drugs for ADPKD are currently in clinical trial stage, many of which have functional anti-hypertension, anti-proliferation and anti-inflammation effects. Clinical trials are currently underway with angiotensin I-converting enzyme inhibitor (ACEI), angiotensin II-receptor blocker (ARB), V2 vasopressin receptor (VPV2R) antagonist, somatostatin and mTOR inhibitor (14). However, it would appear that one drug alone is not fully capable of attenuating progressive cystic growth; thus, diverse approaches for the treatment of PKD still require further study.

Epigenetic regulation is defined as heritable changes in gene expression that are not attributable to any alteration in the DNA sequence. Epigenetic modulations including DNA methylation, histone modification, and gene regulation by micro RNA (miRNA) are importantly associated with several cellular processes and diverse disease states, such as cancers, even under precancerous conditions (15). The nucleosome is composed of a DNA strand surrounding a core histone octamer (16). Complicated chromatin remodeling mechanisms keep DNA accessible to transcriptional factors. Post-translational modifications including acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, ADP-ribosylation, glycosylation, biotinylation and carbonylation occur in the histone

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tails (17). These epigenetic modulations alter gene activity via the regulation of DNA-histone interactions without any alteration of the genetic code (18). miRNAs, which are non-coding small 22-nucleotide RNAs, post-transcriptionally regulate the expression of target mRNAs via the 3' untranslated region (3'UTR) (19, 20). A large number of protein coding genes could be under the control of miRNAs (21, 22). As a consequence, miRNAs play an important role in the regulation of a broad variety of cellular functions, and the deregulation of miRNA expression is frequently associated with a variety of disorders, including cancer (23, 24). Recent studies have demonstrated that epigenome alterations may be involved in renal pathogenesis, including PKD, and the reversible restoration of changed epigenetic factors may improve the potential treatment of renal disease with minimal side effects (25-29).

Many current studies have attempted to devise an effective treatment; however, no preventive treatment for PKD has yet become available. A novel therapeutic approach should continue to be developed in cellular or epigenetic studies, because multiple mechanisms may contribute to PKD. This review focuses on recently described novel therapeutic approaches to the suppression of cystogenesis, specifically from a cellular or epigenetic perspective.

## NOVEL THERAPEUTIC APPROACHES IN PKD

### Therapeutic target: PPAR- $\gamma$

Peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) is a ligand-dependent and nuclear hormone receptor transcriptional factor, which forms a heterodimer with another nuclear receptor, retinoid X receptor (RXR $\alpha$ ) (30). Recent studies have identified PPAR- $\gamma$  as a novel target for the treatment of ADPKD. Specially, the synthetic ligands of PPAR- $\gamma$ , thiazolidinediones (TZDs), are used to suppress cystogenesis and contain pioglitazone and rosiglitazone as PPAR- $\gamma$  agonists (31). In an initial investigation, treatment with the PPAR- $\gamma$  agonist pioglitazone improved the survival of *Pkd1*<sup>-/-</sup> embryos and endothelial functions of adult mice as measured by nitric oxide (NO) concentration (32). The PPAR- $\gamma$  agonist rosiglitazone also affects cystogenesis, which inhibits cell proliferation in ADPKD cyst-lining epithelial cells (31). This study was conducted with the expectation that PPAR- $\gamma$  agonists would attenuate cell proliferation via the checking of cell cycle molecules including PCNA, phosphorylated-Rb, and cyclin D1 and D2 (31). In a recent study, the administration of rosiglitazone was shown to attenuate cyst development and protect renal function via measurements of Blood Urea Nitrogen (BUN) and Creatinine (Cr) in a typical PKD animal model, namely the Han:SPRD rat (33). Additionally, rosiglitazone-treated Cy/+ Han:SPRD rats evidenced longer survival durations than control rats (33). In contrast, Raphael *et al.* reported previously that pioglitazone treatment did not result in any reduction of cystogenesis and proliferation, and only affected survival in PC-Pkd1-KO mice (34).

As interstitial inflammation and fibrosis are relevant mechanisms in the renal function of polycystic kidney, this process progressively leads to renal failure (35). TZDs have been used for the treatment of Type 2 diabetes mellitus (T2DM) because of their good safety during long-term clinical processes for the improvement of renal functions (36). TGF- $\beta$ 1 and MCP-1 expression was suppressed and kidney fibrosis volume and inflammatory cell infiltration were reduced by rosiglitazone treatment in PKD, as was also the case in T2DM disease (33). More recently, the novel PPAR- $\gamma$  agonist known as alpha-aryl-oxy-alpha-methylhydrocinnamic acid derivatives, DH9 also had anti-proliferative effects on PKD (37). Thus, PPAR- $\gamma$  agonists appear to have therapeutic potential in terms of renal function; further research will be required for clinical applications.

### Therapeutic target: TNF- $\alpha$

Tumor necrosis factor (TNF) is an inflammatory cytokine and is known as a mediator of cancer-related inflammation; recently, clinical trials of cancer therapy using antagonists have been initiated (38). In PKD, TNF- $\alpha$  was also found to be related to the inflammation process, and were initially detected in the cystic fluids of 54 of 75 (72%) of studied PKD patients (39). Many other cytokines (interleukin (IL)-1, 2, 6, 8, ICAM-1, VCAM-1) are secreted in the fluid of the cystic kidney and may affect growth, inflammation and fibrosis in cases of PKD (39, 40). In a recent study, TNF- $\alpha$  has not only been associated with inflammation, but also appears to perform a crucial function in cystogenesis under both *in vitro* and *in vivo* conditions (41). They focused on FIP-2, a TNF- $\alpha$  induced protein that disrupts the localization of PC-2 in inner medullary collecting duct (IMCD) cells (41). It is interesting to note that TNF- $\alpha$ -treated embryonic kidneys have many more cyst than non-treated embryonic kidneys in *Pkd2*<sup>+/+</sup> and *Pkd2*<sup>+/-</sup> mice using organ culture systems (41). Study results have also confirmed that the TNF- $\alpha$  inhibitor, etanercept, reduced cyst formation for 10 weeks in *Pkd2*<sup>+/-</sup> mice (41). Taken together, TNF- $\alpha$  is a potential target in therapeutic view because it may involve in main mechanism of PKD; cyst progression and inflammatory disorder (42).

### Therapeutic target: CDK

In the cell cycle, cyclin-dependent kinase (CDK) and the cyclin family are primarily involved in the G1/S checkpoint. Bukanov *et al.* previously reported the inhibition of the CDK effect on slowly progressive cystogenesis in *jck* and *cpk* mice models using roscovitine (43). Roscovitine (Seliciclib, CYC202) is a known CDK inhibitor for Cdk2-cyclin E and is in phase II clinical trials as an anti-cancer agent (44). This study showed that treatment with roscovitine resulted in dephosphorylation of Rb and a reduction in cyclin D1 levels, suggesting block G1-S phase in cell cycle in *jck* mice (daily dosing for 5 weeks) (43). Additionally, roscovitine affected apoptosis by checking the levels of caspases 2 and 3, ApaF1 and Bcl-2 (43). In other stud-

ies, Park *et al.* demonstrated that roscovitine was also involved in apoptosis and cellular senescence; this is especially the case in low-dose treatment relative to senescence in renal tubular epithelial (RTE) cells (45). According to the previous report of Belibi and Edelstein (5), PKD requires life-long therapy to prevent side effects, and this approach provides the potential for a PKD therapy using low-dose treatment (45). Furthermore, CDK inhibition results in the downregulation of cAMP and aquaporin 2 (AQP2), main molecules in the pathogenesis of PKD (46).

Recently, roscovitine was used to search for a biomarker in PKD and applied the SELDI-TOF method (47). This study identified a set of 20 urinary and 21 serum biomarkers using chronic or acute treatment of roscovitine in *jck* mice (47). Our results show that CDK inhibition is a novel therapeutic approach for the suppression of cystogenesis, particularly in regard to proliferation and apoptosis.

#### Therapeutic target: VEGF

The blood supply needs to provide oxygen and some nutrients for expansion of the epithelium; this process is called 'angiogenesis' in the cells (48). Vascular endothelial growth factor (VEGF) is a key molecule in angiogenesis and interacts with two types of receptor 1 and 2 (VEGFR-1 and VEGFR-2) (49). In PKD, angiogenesis was observed around the cyst via angiography, and VEGFR-2 is abundantly expressed in cyst cells relative to VEGFR-1 (48). Tao *et al.* reported that VEGFR-1 and VEGFR-2 ribozyme treatment inhibits proliferation and cystogenesis, and improves renal function in Han:SPRD (male, heterozygous(Cy/+)) (50). VEGFR-1 and VEGFR-2 ribozymes specifically targeted to the mRNA of VEGFR-1 and VEGFR-2 and administrated using a subcutaneous osmotic pump (30 mg per kg per day) to protect against nuclease degradation under *in vivo* condition (50). In another study, the VEGF receptor inhibitor, SU-5416, reduced cystic development of the liver, but did not affect renal cysts in *Pkd* (WS25<sup>-/-</sup>) mice (51). Early disruption of VEGFR-2 with antibody (DC101) results in renal cyst formation in CD1 mice (52). These results indicate that VEGF signaling is quite complex because of VEGF's effects on normal or abnormal tubule development.

VEGF/VEGFR is regulated by hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), therefore VEGF was detectable in epithelial cells which also corresponds to HIF-1 $\alpha$  expression in the Han:SPRD rat model (53). In human ADPKD kidneys, some angiogenic-related factors, such as glucose-transport (Glut-1), endoglin and VEGF, were abundantly expressed (53). Hypervascularity mechanism-related HIF and VEGF may affect the progression of PKD. Specifically, VEGF/VEGFR signaling may play a role in cell proliferation (50) and fluid secretion (48) in cases of PKD. As a result, VEGF signaling represents a therapeutic target in PKD.

#### Other approaches to therapeutic targets of PKD

It has been determined that inhibitors or antagonists of PPAR- $\gamma$ ,

TNF- $\alpha$ , CDK and VEGF are anti-cancer agents, but also may slow cystogenesis and improve renal function in PKD. Recently, many investigators are currently investigating novel approaches to therapy using natural compounds. Curcumin (diferuloylmethane), a natural product derived from the plant *Curcuma longa*, was shown to inhibit MDCK cyst development (62%) and forskolin-promoted cell proliferation in MDCK cells, and also attenuated cystogenesis in embryonic kidney organ cultures (54). Additionally, curcumin was tested *in vivo* and was shown to reduce cystogenesis via an alteration of STAT3 activation and proliferation in a iKSP-Pkd1del mouse model (55). This study indicated that curcumin might be a potential compound for the safe and effective therapy of PKD (54, 55). In addition, in traditional Chinese medicine, triptolide has also been suggested to attenuate cyst formation by restoring Ca<sup>2+</sup> signaling in a *Pkd1*<sup>-/-</sup> mice model (56).

In another study, a novel target-related glycosphingolipid metabolism was approached using the glucosylceramide (GlcCer) synthase inhibitor, Genz-123346 (57). GlcCer and lactosylceramide (LacCer) have already been shown to be elevated by approximately 2-3 fold in ADPKD patients, and may stimulate proliferation via the activation of p44 MAPK (58). Blockade of GlcCer accumulation attenuated cystogenesis via cell cycle arrest and inhibition of the Akt-mTOR pathway in *Pkd1* conditional knockout mice, *jck* and *pcy* mice (57). These novel compounds also need to be assessed in terms of their relationship with PKD.

#### EPIGENETIC THERAPY IN PKD

##### Histone acetylation and HDACs in PKD

**The regulatory mechanism of histone acetylation:** The nucleosome consists of DNA wrapped around an octamer of two core histones such as H2A, H2B, H3 and H4. Most importantly, the acetylation of lysine residues on the N-terminal tails of H3 and H4, which is mediated by histone acetyl transferases (HATs) is related to the open structure of chromatin. However, the acetyl groups of lysine residues can be removed by histone deacetylases (HDACs), which leads to closed chromatin structure and transcriptional inactivation (59). Deacetylation has been identified as the first step in transcriptional repression (60). HDACs are classified into classes I-IV (27). The class I HDACs include HDAC1, HDAC2, HDAC3 and HDAC8, which are ubiquitously expressed and localized within the nucleus (61, 62). The class II HDACs include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10, which are expressed in a tissue-specific manner and in both the nucleus and cytoplasm (63). Class IV HDAC has only one member, HDAC11, and the Class III HDACs are NAD<sup>+</sup>-dependent deacetylases (27).

HDACs affect a variety of cellular processes, including apoptosis, cell cycle regulation, and cell proliferation by regulating the deacetylation of both histone and nonhistone proteins and ultimately altering DNA-protein binding affinity (15).

Each HDAC regulates different and specific transcription factors. For example, HDAC1 deacetylates p53, HDAC2 removes the acetyl group from the glucocorticoid receptor, and HDAC3 mediates the deacetylation of myocyte enhancer factor 2 (MEF2) transcription factor (27). Additionally, HDAC6 targets the deacetylation of  $\alpha$ -tubulin and heat shock protein 90 (HSP90) and finally affects their cellular functions (64-66).

**The functional role of HDACs in PKD:** The results of recent studies have demonstrated that the acetylation of histone lysine residues might be correlated with PKD and CKD pathogenesis (27, 28, 67-69). The *PKD1* gene is the most critical factor in ADPKD pathogenesis (1). It has been recently reported that the transcriptional level of the *Pkd1* gene could be regulated by several transcription factors, including p53 (67). p53 null mice evidenced higher levels of *Pkd1* mRNA expression than wild-type littermates. Additionally, *Pkd1*<sup>-/-</sup> cells evidence lower p53 expression levels than wild-type cells (70). It has been demonstrated that p53 functions as a negative regulator of *Pkd1* gene transcription by binding to the *Pkd1* gene promoter *in vivo*. Importantly, trichostatin A (TSA), a general inhibitor of class I and II HDACs, has been shown to abolish the p53-mediated repression of *Pkd1* gene expression in *Pkd1* mutant mice (67, 68). This indicates that p53 negatively controls *Pkd1* gene expression in cooperation with HDACs and may contribute to deregulated *Pkd1* expression and cyst formation (67). Moreover, HDAC1 is responsible for p53 deacetylation, and may result in the p53-induced repression of *PKD1* gene transcription (27, 69). Either treatment with HDAC1 inhibitors such as TSA and valproic acid (VPA) or *hdac1* knockdown results in a reduction in kidney cyst formation in *Pkd2* knockdown fish, as well as *Pkd1* mutant mice (68).

Another important point is that HDAC5 is associated with fluid flow-induced calcium signaling in kidney epithelial cells (27). Fluid flow stimulation of polarized epithelial cells induces activation of the PC-2 calcium channel and increases calcium influx within the cells. Increased intracellular calcium levels result in the activation of protein kinase C, which, in turn, induces phosphorylation and the nuclear export of HDAC5 (71). Although the mechanisms of fluid flow sensing in kidney epithelial cells remain unclear, HDAC5 may be responsible for the fluid flow mechanical pathway. Xia *et al.* noted a significant reduction in cyst formation in *Pkd2*<sup>-/-</sup>*Hdac5*<sup>+/-</sup> embryos relative to *Pkd2*<sup>-/-</sup>*Hdac5*<sup>+/+</sup> embryos (72).

Additionally, HDAC6 has a potential role in PKD pathogenesis. It is associated with ciliar disassembly via the deacetylation of  $\alpha$ -tubulin during the normal cell cycle (73). HDAC6 overexpression in *Pkd1* mutant mouse embryonic renal epithelial cells is consistent with the critical role of HDAC6 in cillogenesis and cyst formation (27). HDAC6 regulates Wnt signaling via the deacetylation and nuclear translocation of  $\beta$ -catenin. Abnormal activation of Wnt/ $\beta$ -catenin, which is a feature of ADPKD, induces the translocation of  $\beta$ -catenin into the nucleus and the activation of target genes such as the

*c-myc* oncogene (74). Recently, HDAC6 has been shown to suppress epidermal growth factor receptor (EGFR) endocytosis and degradation via regulation of  $\alpha$ -tubulin acetylation. EGFR has been shown to stimulate renal cyst formation in murine PKD and human ADPKD and ARPKD (27, 64). These data indicate that HDAC6 is closely associated with EGFR activity and cyst formation.

**The potential therapeutic insights of HDAC inhibitors on PKD:** Previous studies have demonstrated a close association between the overexpression of HDACs and tumorigenesis (75, 76). The administration of HDAC inhibitors can repress cell proliferation and induce differentiation. In PKD, the progression of cyst formation is also characterized by an increased cell proliferation rate (77). Multiple HDAC inhibitors have either been approved by the Food and Drug Administration (FDA) or tested in clinical trials (27, 68). In the field of cancer biology, a combination therapy of HDAC inhibitors and other drugs against growth factor signaling may prove more effective than treatment alone (59). Similarly, such a combination therapy may also constitute an effective therapy for the repression of cyst formation.

Several previous studies have shown that the class I HDAC inhibitor, VPA, and the class II HDAC inhibitor, TSA, suppress cyst formation and retard renal failure in *Pkd1* conditional knockout mice and *Pkd2* knockout mice, respectively (67-69, 72). Therefore, the class I HDACs perform an important function in PKD pathogenesis, and HDAC inhibitors may prove to be promising drug candidates for a treatment for PKD (27).

#### microRNAs in PKD

**Biogenesis and mechanism of microRNAs:** The biogenesis of miRNA is a multi-step event generated by the RNase III type enzyme from an endogenous transcript containing a local hairpin structure (78). Long primary miRNAs (pri-miRNAs) are transcribed by RNA polymerase II or RNA polymerase III and cleaved by the nuclear RNase III enzyme, Drosha, into shorter precursor miRNAs (pre-miRNA) (79). The pre-miRNAs are transported from the nucleus to the cytoplasm via exportin-5 and then cleaved by the cytoplasmic RNase III, Dicer, into the mature miRNA duplex 20-25 nucleotides (79, 80). One of the mature double-stranded miRNAs is incorporated into a RNA-induced silencing complex (RISC) and guides the complex to its target mRNAs (81).

miRNAs bound to RISC can downregulate protein levels via two alternative pathways. When base pairing between miRNA and its target mRNA is nearly perfect, the target mRNA is degraded by the RISC component argonaute 2. More commonly, if the miRNA has imperfect complementarity with a matching sequence in the 3'UTR of its target mRNA, translational repression is the result. In animals, miRNAs commonly repress translational activity by binding to the 3'UTR of their target mRNAs (79).

However, it remains unclear how miRNA is generated and

processed. Most miRNA genes are located in the introns of host genes and endogenously generated. Although it is predicted that the expression of these miRNAs may be regulated with that of the host genes, this remains to be clearly elucidated (82). Some miRNA genes are located close to each other in the genome and include miRNA clusters; other miRNA genes are encoded for by multiple copies of genes (83). In addition, the major strand of a mature miRNA is generally found at a much higher level than the minor strand, which is denoted by an asterisk (\*) at the end of the name. The major and minor strands of an miRNA may be co-expressed, but mostly target different genes (84).

**The role of microRNAs in PKD:** It has been previously reported that the profiles of miRNA expression evidence differential patterns in the kidneys of humans and mice (85, 86). For example, several miRNAs have been shown to be specific in the human kidney as compared with other organs, including miR-192, miR-194, miR-204, miR-215, miR-216, miR-146 and miR-886. Moreover, miR-196a-b, miR-10a-b, miR-872 and miR-200a are all enriched in kidney tissue (85, 86). The downregulation of these miRNAs in the kidney may result in upregulation of target proteins relevant to normal kidney function. Additionally, miRNA expression occurs in different patterns depending on the localization of the kidney. In fact, the different functions are related to renal cortical and medullary aspects. miR-192 is markedly more highly expressed in the renal cortex than in the medulla, thus implying that it may be related with sodium transport in renal epithelial cells (84, 87). Sun *et al.* determined that Ets-1, a key transcription factor associated with kidney development and function, has a conserved putative binding site for miR-192 and miR194-2 (86, 88). This implies that the loss of miR-192 in kidney tissue may result in renal defects, such as PKD progression.

To date, there is little information available regarding the functions of miRNAs in the context of PKD. However, there are several evidences to correlate between ADPKD pathogenesis such as cystogenesis and abnormal miRNA expression patterns. Lee *et al.* previously demonstrated that miR-15a expression levels were reduced in cholangiocytes derived from a rat model of PKD via *in situ* hybridization. miR-15a is known to be a regulator of Cdc25A associated with the cell cycle. The downregulation of miR-15a induces an increase in cell proliferation and promotes *in vitro* cystogenesis via the targeting of Cdc25A (89).

Pandey *et al.* identified global transcriptional reprogramming during the progression of PKD using the *Pkd1*<sup>-/-</sup> model. They suggested that several miRNAs, including miR-10a, miR-30a-5p, miR-96, miR-126-5p, miR-182, miR-200a, miR-204, miR-429 and miR-488 were differentially expressed and may be associated with renal cyst formation and growth via the regulation of signaling pathways including Wnt, calcium, TGF- $\beta$  and MAPK in PKD (90). Indeed, the *PKD1* gene has two putative binding sites for miR-200b/c and miR-429 as well as miR-

17-5p, miR-20, miR-93, miR-106 and miR-519 according to *in silico* analysis by Target Scan (<http://www.targetscan.org/>) (91).

Importantly, the *PKD2* gene, a key regulator of PKD development, contains a binding site for miR-17, and they directly interact with each other in the *PKD2* 3'UTR under *in vitro* conditions. Reduced PC-2 levels targeted by miR-17 induced cell proliferation in a human embryonic kidney cell line (HEK293T) (92). In further study, Tran *et al.* demonstrated that the RNA-binding protein bicaudal (Bicc1) modulated PC-2 expression in the kidney by antagonizing miR-17 activity. They proposed that the PKD phenotype of Bicc knockout mice is attributable to the dysregulation of the miR-17 family, which post-transcriptionally regulates *Pkd2* mRNA expression (93).

Interstitial inflammation and fibrosis are two of the characteristics for progression in all forms of PKD (35). In particular, miR-192 regulates TGF- $\beta$ /Smad3-induced renal fibrosis under both *in vitro* and *in vivo* conditions (94). The expression of miR-192 is increased by TGF- $\beta$  and mediates TGF- $\beta$ -induced collagen genes in mouse mesangial cells (MCs) by reducing the expression of its target, ZEB2 (95, 96). In addition, the expression of miR-192 and miR-200 family members can be attenuated by TGF- $\beta$ , which in turn induces epithelial-to-mesenchymal transition (EMT). The miR-200 family members are known to regulate EMT in several cancer and other kidney-derived epithelial cell lines by upregulating ZEB1 and ZEB2 to suppress E-cadherin (97-99). Shigeyoshi *et al.* determined previously that miR-200 family members, especially miR-200b, were upregulated in unilateral ureter obstruction (UUO), which is a general model of EMT of tubular cells (100). As the blockade of EMT attenuates renal fibrosis, the miR-200 family members might be novel therapeutic targets in fibrosis-related renal disease. Thus, even though no direct correlation between PKD and either miR-192 or miR-200 family may exist, the relevant data suggest that they may be closely connected to EMT and renal fibrosis.

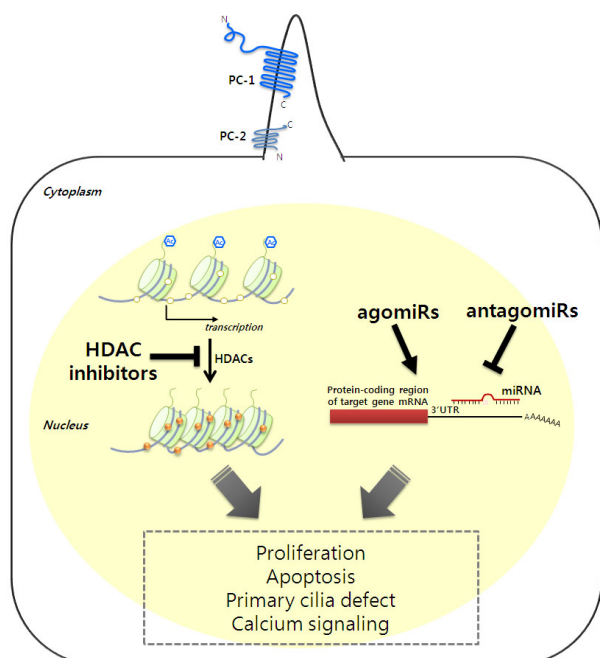
**microRNAs as therapeutic targets of PKD:** In previous studies, it has been shown that miRNA expression patterns may potentially be used as biomarkers for several diseases, including cancer (101). In the kidney, miRNAs are essential for renal development and homeostasis, and their deregulation induces and exacerbates renal diseases including PKD, diabetic nephropathy, acute kidney injury and renal carcinoma (102). Thus far, research into miRNA in kidney diseases, particularly PKD, is in its infancy. Recently, several lines of evidence and the results of previous studies suggest that miRNA may be tightly correlated with the pathogenesis of PKD (26, 29, 93, 102-104). Since abnormal miRNA expression has been implicated in several renal diseases, miRNA biomarkers for the detection of renal diseases such as PKD may provide advantages in terms of early diagnosis. Therefore, further study will be necessary to identify valuable miRNA markers in the early detection of PKD.

The miRNA-based therapeutic technique has been suggested

to either inhibit the activity or restore the repressive functions of miRNAs. Knockdown of miRNA activity can be carried out via the delivery of antagomiRs such as cholesterol-coupled 2'-O-methyl-modified oligoribonucleotides or antisense-locked nucleic acid (LNA-anti-miR). For example, Krutzfeldt et al. previously demonstrated that the silencing of miR-192 expression could be conducted using anti-miR-192 in several organs, including the kidney (105). Conversely, in the case of down-regulated miRNA-induced disease development, the most viable therapeutic approach is the restoration of miRNA activity. This can be accomplished by the administration of miRNA precursors with structures similar to that of endogenous miRNA (106). This report proposes another area of potential miRNA-based therapy for PKD.

## CONCLUSION

Although many studies have suggested beneficial effects for PKD, treatment for PKD has been limited. The pathological functions of novel drugs including nature products remain to be determined. Furthermore, several studies suggested the therapeutic possibilities of epigenetic drugs in PKD. HDAC inhibitors, agomiRs and antagomiRs may regulate their target gene transcriptions and ultimately PKD pathogenesis including proliferation, apoptosis, calcium signaling and primary cilia assembly (Fig. 1). Therefore, even though only few evidences of



**Fig. 1.** The potential effect of epigenetic drugs such as HDAC inhibitor, agomiRs and antagomiRs on human PKD pathogenesis.

epigenetic mechanisms in PKD, further understanding the epigenetic factors regarding to PKD may provide novel mechanisms important for prevention and treatment of PKD.

## Abbreviation

cAMP, cyclic AMP; CFTR, cystic fibrosis transmembrane conductance regulator; CKD, chronic kidney disease; ICAM-1, inter-cellular adhesion molecule 1; MAPK, mitogen activated protein kinase; MCP-1, monocyte chemotactic protein-1; MDCK, Madin-Darby canine kidney; PCNA, proliferating cell nuclear antigen; Rb, retinoblastoma; SELDI-TOF, surface enhanced laser desorption/ionization time-of-flight mass spectrometry; STAT3, signal transducers and activators of transcription protein; TGF- $\beta$ , transforming growth factor  $\beta$ ; VCAM-1, vascular cell adhesion protein 1.

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