Kidney Medicine

Narrative Review

MicroRNAs and Polycystic Kidney Disease

Dantong Li and Liangzhong Sun

Important advances have been made regarding the diagnosis and management of polycystic kidney diseases. Care of patients with polycystic kidney diseases has moved beyond supportive care for complications and chronic kidney disease to new potentially disease-modifying therapies. Recently, the role of noncoding RNAs, in particular microRNAs, has been described in polycystic kidney diseases. microRNAs are involved in the regulation of gene expression, in which *PKD1*, *PKD2*, and other genes that contribute to the pathogenesis of polycystic kidney diseases are considerable participants. Seminal studies have highlighted the potential importance of microRNAs as new therapeutic targets and innovative diagnostic and/or prognostic biomarkers. Furthermore, an anti–miR-17 drug has advanced through preclinical autosomal dominant polycystic disease studies, and an anti–miR-21 drug has already cleared a phase 1 clinical trial. Most probably, new drugs in the microRNA research field will be yielded as a result of ongoing and planned therapeutic trials. To provide a foundation for understanding microRNA functions as a disease-modifying therapeutic drug in novel targeted therapies, in this narrative review we present an overview of the current knowledge of microRNAs in the pathogenesis of polycystic kidney diseases.

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INTRODUCTION

After the first discovery of small nuclear RNAs as possible players in the excision of introns in early 1980s, abundant discoveries of other classes joined the ranks of noncoding RNAs (ncRNAs).[1,2] The ncRNA genes produce transcripts that function directly as structural, catalytic, or regulatory RNAs, rather than express messenger RNAs (mRNAs) that encode proteins.[3] Remarkable work from the past decade has altered our perception of ncRNAs from "junk" transcriptional products to functional regulatory molecules that mediate cellular processes, including post-transcriptional modifications and signal transduction.[4] ncRNAs are classified according to their length, localization, and/or function into long ncRNAs, microRNAs (miRNAs), small interfering RNAs, small nucleolar RNAs, small nuclear RNAs, and PIWI (P-element induced wimpy testis in Drosophila)–interacting RNAs.[5] Collectively, apart from long ncRNAs comprising various RNA species longer than 200 nucleotides,[6] all the others are known as small ncRNAs. Among all the small ncRNAs, miRNA is the most thoroughly studied.

Polycystic kidney diseases (PKDs) are common causes of kidney failure and represent a heterogeneous group of kidney disorders defined by the presence of distinctive fluid-filled epithelial sacs in the kidneys.[7] Autosomal recessive PKD (ARPKD) is the most common type of PKD in children, in which kidney cysts are noted as part of the phenotypes of many clinical syndromes with associated anomalies. However, autosomal dominant PKD (ADPKD) is most common in adults.

Previous analyses have successfully identified the causative genes of some PKDs. Mutations in *PKD1* are the cause of almost 80% of cases of ADPKD, whereas ~15% of cases can be attributed to mutations in *PKD2*, and the remaining 5% to 10% of cases are either genetically unresolved or result from rare mutations in other loci.[7] As one of the rare mutations, hepatocyte nuclear factor 1β (*HNF1β*), which is required for the initiation of nephrogenesis and nephron segmentation in the embryonic kidney, upregulates the expression of multiple *PKD*-associated genes, including *PKHD1* and *PKD2*.[8] Of particular note, *PKHD1* is a large gene encoding fibrocystin and is regarded as the primary defect in ARPKD.[9]

In recent years, research regarding the detrimental role of turbulent posttranscriptional regulation in the kidney cystic phenotypes has emerged.[8] miRNAs have been shown to play critical roles in defining DNA methylation patterns, including imprinting as well as chromatin remodeling, thus substantially affecting epigenetic signaling.[10] Therefore, it is important to understand the mechanisms through which miRNA-mediated post-transcriptional regulation is involved in kidney cystic formation. Understanding the role of miRNAs will provide a foundation for future medical interventions and may also identify diagnostic and prognostic biomarkers that could lead to better health care for patients. In this review, we provide an overview of miRNAs and their involvement in PKDs.

MICRORNAS

The Interplay Between miRNAs and mRNAs

More than 1,000 different miRNAs are encoded by the human genome. Within the genome, miRNA coding sequences are either present in intergenic DNA regions as independent transcriptional units or located inside introns or even exons of protein-coding genes.[11] Initially transcribed by RNA polymerase II, the primary miRNA folds back on itself to form at least 1 distinctive hairpin structure. This structure is cleaved by a heterotrimeric complex containing 1 molecule of the Drosha endonuclease and 2

molecules of its partner protein DGCR8[12] to release a pre-miRNA. The pre-miRNA hairpin is then exported to the cytoplasm in which the hairpin is cleaved by Dicer, another endonuclease, to produce an miRNA duplex. One strand of the miRNA duplex, the mature miRNA, is loaded into the guide-strand channel of an Argonaute protein to form a silencing complex, whereas the other strand is degraded. Within the free silencing complex, miRNA nucleotides 2 to 5 are poised to initially interact with target mRNAs. This pairing usually extends to nucleotide 7 or 8 of the miRNA and occasionally is more extensive. If pairing is extensive enough, the target mRNA can be sliced, whereas if it is not, the target mRNA can undergo other types of repression (Fig 1).[13]

Gene targets of miRNAs in the pathogenesis of PKDs include the *PKD* genes and other genes associated with cystogenesis or kidney fibrosis (Table 1[14-36]). Accumulating evidence indicates that reduced polycystin (PC) dosage encoded by *PKD* genes promotes cyst growth. A mouse model mimicking the hypomorphic $PKD1^{R3277C}$ allele showed the association of the functional PC1 dosage to disease severity.[37] Patients with digenic ADPKD with mutations in both *PKD1* and *PKD2* had more severe disease phenotypes than monogenic family members.[38] Taken together, these findings from both mouse model and clinical data supported the hypothesis that the kidney phenotypes and disease severity in patients with PKDs were PC dosage dependent. Moreover, concomitant reduction in the expression of multiple *PKD* genes could promote cyst formation.

The Role of the miR-17∼92 Cluster

The full regulatory reach of miRNAs in PKDs is yet to be defined, despite having been thoroughly studied in PKD. In a *Kif3a*-KO (knockout) mice model, Ksp/cre-mediated inactivation of *Kif3a* resulted in the loss of primary cilia from renal tubules and the formation of kidney cysts. miRNA microarray analysis revealed that the miR-17∼92 cluster was notably upregulated in *Kif3a*-KO kidneys, whereas in the same research, double-knockout mice (*Kif3a*-miR-17∼92-KO) showed reduced cyst growth compared with single *Kif3a*-KO mice. Furthermore, in other forms of PKD, expression of the miR-17∼92 cluster was uniformly increased in cystic kidneys. These models vary from Pkhd1/cre;$Pkd2^{F/F}$ mice (F/F, a type of conditional knockout mice via a Cre-lox recombination system), an orthologous model of ADPKD, $Pkhd1^{-/-}$ mice, an orthologous model of ARPKD, to *Pkhd1*/cre;$Hnf\text{-}1\beta^{F/F}$ mice, an orthologous model of kidney cysts and diabetes, compared with their respective controls.[14] An in vivo anti-miR screening was performed to identify the miRNA drug targets within the miR-17∼92 cluster. They revealed that after treatment with the anti–miR-17 drug, multiple indexes regarding either kidney function, kidney injury markers, or cyst proliferation indicated dramatic improvement in the mice model. They also speculated that anti–miR-17 treatment attenuated cyst growth by improving cyst metabolism and inhibiting cyst-associated inflammation. Pivotal evidence hardwired to *Pparα* and *Ppargc1α* expression, proteins in the mammalian target of rapamycin (mTOR) pathway, expression of cytokines Tgf2, Ifng, Ccl5, Ccl22, IL6, and Mip2, as well as expression of *Arg1* and *Mrc1*, was provided.[15,39]

Further studies have corroborated the efficacy of a short oligonucleotide inhibitor of miR-17. Currently on a partial clinical research hold by the US Food and Drug Administration, RGLS4326 was discovered due to its optimal pharmaceutical properties. This inhibitor preferentially distributes to collecting duct–derived cysts, displaces miR-17 from translationally active polysomes, and de-represses multiple miR-17 mRNA targets including *PKD1* and *PKD2*, thus attenuating cystic cell growth.[40]

miR-20b-5p and miR-106a-5p are 2 members of the miR-17 family. The RNA analysis from kidneys in $PDK2^{f/f}$:HoxB7-Cre mice or from mouse cell lines such as inner medullary collecting ducts with a knockdown of *PKD2* suggested downregulation of miR-20b-5p and miR-106a-5p. It was speculated that this downregulation was associated with increased levels of Klf12,[41] a DNA-binding transcriptional regulator with diverse functions in a multitude of cellular processes including proliferation, differentiation, and inflammation.[42] This may signify a potential downstream target of the miR-17 family and pave a way for prospective in-depth research.

The Role of miR-199

miR-199 has been implicated in a large variety of cellular and developmental mechanisms, including the development and progression of various cancers, cardiomyocytes protection, and skeletal formation.[43] In a rat model of ADPKD heterozygous (Cy/+), together with human ADPKD cell lines OX161 and WT9-12, a dramatical upregulation of miR-199a-5p level was identified, whereas the transfection of miR-199a-5p inhibitor suppressed proliferation of cystic cells and induced cell apoptosis in a reasonable degree. Moreover, miR-199a-5p might exert this effect through targeting *CDKN1C*/p57,[16] a negative regulator of cell proliferation by inhibiting G1 cyclin-dependent kinases.[44]

The Role of miR-200

ADPKD cystic epithelial cells display an undifferentiated phenotype and undergo epithelial to mesenchymal transition changes as part of their phenotype.[45] Dysregulation of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) is characteristic of epithelial to mesenchymal transition,[46] which implicates a role of miR-200 in the pathogenesis of ADPKD. The transcription factors *Zeb1* and *Zeb2* are repressors of *E-cadherin* gene expression and are known miR-200 targets in transforming growth factor β–associated kidney fibrosis.[47] However, in a Dicer mutant mouse model,

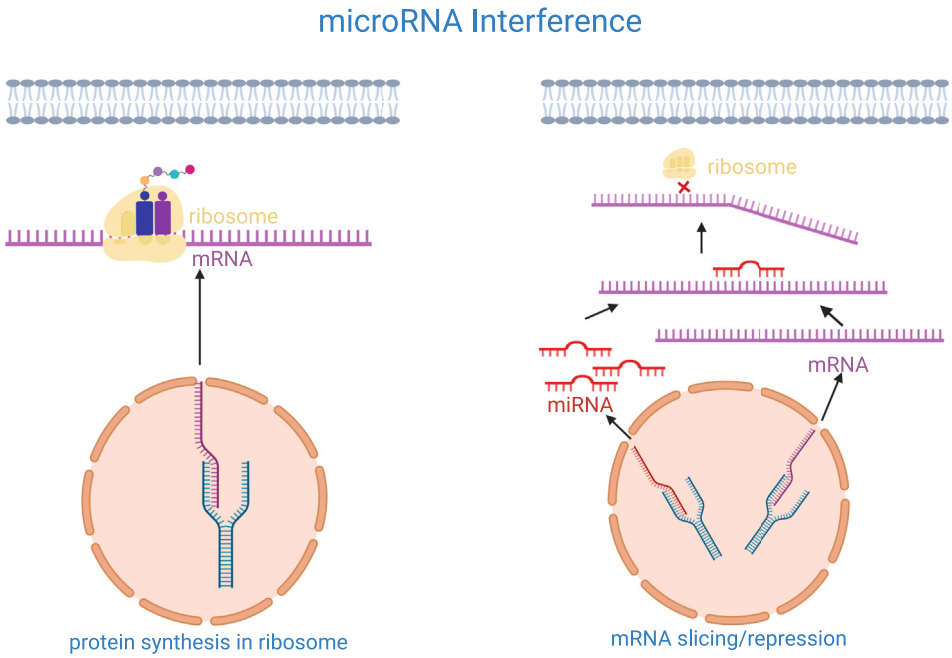

Figure 1. microRNA (miRNA) interference. Mature miRNA binds to a specific target sequence within a messenger RNA (mRNA), leading to mRNA slicing or repression (right) instead of conventional protein synthesis in ribosome (left).

cyst formation was driven directly by posttranscriptional repression of *Pkd1* through base-pairing with miR-200, rather than *Zeb1*- or *Zeb2*-dependent mechanisms.[17] And not coincidently, an independent study showed that miR-429, an miR-200 family member, physically interacted with Pkd1 3′ untranslated region in vivo.[48] Taken together, miR-200 possesses an indelible place in ADPKD, either through the process of epithelial to mesenchymal transition or direct effect on *Pkd1*. Or perhaps there is overlap between these 2 mechanisms, which is worth exploration.

The Role of miR-9-5p

In previous studies, miR-9-5p was shown to act as an miRNA with antifibrotic potential in the lung, peritoneum, and skin.[49,50] In a unilateral ureteral obstruction model, miR-9-5p exerted a protective effect in kidney fibrosis and provided data supporting a crucial role for metabolic reprogramming mediating this action. Overexpression of miR-9-5p prevented tubular epithelial renal cell dedifferentiation and reprogramed fibrosis-related metabolic derangement, with increased levels of fatty acid oxidation and oxidative phosphorylation.[51] The gene *Elovl6* is involved in elongation of long-chain fatty acids.[52] In a *Pkd1*$^{+/-}$;Hoxb7-Cre mouse model, bioinformatic analysis predicted an *Elov16*-miR-9-5p correlation in the embryonic development of cysts in ADPKD.[18] In ADPKD, expansion of cysts and loss of kidney function are associated with progressive fibrosis.[53] Although straightforward evidence for miR-9-5p as an antifibrotic factor in ADPKD is yet to be discovered, some fascinating results may be anticipated in the future.

The miRNAs and Altered Signaling

The cysts in kidneys form in a small percentage (~1%) of nephrons and eventually detach when the amount of functional PC1 or PC2 falls below a critical threshold level. The PC proteins modulate several signaling pathways, such as cyclic adenosine monophosphate (cAMP), mTOR and Wnt,[7] and consequently lead to various cellular changes. These alterations include apical-basal polarity, planar cell polarity, increased extracellular matrix production, and cellular metabolism, in conjunction with essential cellular functions such as fluid transport, proliferation, apoptosis, cell adhesion, and differentiation.[54-56]

The cAMP-CREB-miR-21-PDCD4 Axis

cAMP signaling has been shown to play a key role in kidney cyst growth, with evidence showing that cAMP levels were increased in ADPKD and inhibition of this pathway slowed cyst growth in both mice and humans.[57] A study derived from kidney samples of patients with ADPKD and orthologous mice models of PKD illustrated that cAMP signaling transactivated miR-21 promoter and promoted miR-21 expression.[19] cAMP activates protein kinase A, which then phosphorylates cAMP response element binding protein (CREB) and promotes its transcriptional activity.[58] Bioinformatic analysis has shown that the miR-21 promoter contains 4 conserved CREB binding sites, suggesting that cAMP signaling regulates miR-21 expression. On the contrary, genetic deletion of miR-21 was shown to reduce cyst burden and kidney injury and improve survival of an orthologous mice model of ADPKD. In addition, RNA sequencing analysis and

Table 1. Summary of microRNAs Involved in PKD

microRNA	Gene Target	Model	Phenotype	Reference
miR-17∼92	*Pkd1, Pkd2, Pkhd1, Hnf-1β*	*Kif3a*-KO mouse	PKD	14
miR-17	*Pparα, Ppargc1a*	KspCre/*Pkd1*$^{F/RC}$ mouse	ADPKD	15
miR-199a-5p	*CDKN1C*	Han:SPRD rat	ADPKD	16
miR-200	*Pkd1*, *Hnf-1β*	mIMCD3 cell, Ksp/cre; Dicer $^{F/F}$ mouse	Hydronephrosis, kidney cysts, interstitial fibrosis	17
miR-9-5p, miR-17-5p	*Elovl6*	*Pkd1*$^{+/-}$;Hoxb7-Cre mouse	ADPKD	18
miR-9-5p, miR-15a-5p, miR-223-3p, miR-181a-5p	*Scd1*	*Pkd1*$^{+/-}$;Hoxb7-Cre mouse	ADPKD	18
miR-21	*PDCD4*	*Pkd2*-KO mouse	ADPKD	19
miR-15a	*Cdc25A*	PCK rat	ARPKD	20
miR-15a	*Cdc25A*	Human cholangiocytes lining liver cysts	ADPKD, ARPKD, congenital hepatic fibrosis	20
miR-9a-5p	*SCNN1B*	PCK rat	ARPKD	21
miR-146b, miR-503, miR-214, miR-31, miR-34a, miR-199a-5p, miR-132	*Fn1*	*Pkd/Mhm* rat	ADPKD	22
miR-30a-5p	*Adam22, Cpeb3, Hdac9*	*Pkd1*$^{-/-}$mouse	ADPKD	23
miR-200a	*Calcr, Cpeb3, Pitx2*	*Pkd1*$^{-/-}$ mouse	ADPKD	23
miR-10a	*Calcr, Grap2, Ltbp1*	*Pkd1*$^{-/-}$ mouse	ADPKD	23
miR-126-5p	*Edil3, F2rl2, Fgf10, Mysm1*	*Pkd1*$^{-/-}$ mouse	ADPKD	23
miR-182	*Edil3, Hdac9, Sox6*	*Pkd1*$^{-/-}$ mouse	ADPKD	23
miR-488	*Fgfr3*	*Pkd1*$^{-/-}$ mouse	ADPKD	23
miR-204	*Hdac9, P2rx7*	*Pkd1*$^{-/-}$ mouse	ADPKD	23
miR-96	*Sox6*	*Pkd1*$^{-/-}$ mouse	ADPKD	23
miR-25-3p	*ATG14*	*Pkd1*$^{flox/-}$;Ksp-Cre mouse	ADPKD	24
miR-214	*TLR4*	mIMCD3 cell, *Pkd2*$^{fl/fl}$ mouse	ADPKD	25
miR-21	*Pparα, MPV17L, Reck*	UUO mouse	Kidney injury	26
miR-192	*ZEB2*	MDCK cell	Cyst growth	27
miR-194	*CDH2*	MDCK cell	Cyst growth	27
miR-501-5p	*PTEN, TSC1*	Human cystic (9.7 and 9.12) kidney cell lines	ADPKD	28
miR-182-5p	*Wasf2, Dock1, Itga4*	*mIMCD cell, Pkd1*$^{fl/f}$:HoxB7-cre mouse	ADPKD	29
miR-181a	*BCL2*	PKD lymphocyte	PKD	30
miR-193b-3p	*ErbB4*	Human cystic (OX161, OX938, SKI-001, and SKI-002) epithelial cell, Cre;*Pkd1*$^{del2-11,lox}$ mouse	ADPKD	31
miR-20a	*Pkd1*	Dicer$^{fl/fl}$;KspCre+ mouse	Hydronephrosis, renal failure	32
miR-27a	*PKIα*	*Bicc1*$^{+/-}$ mouse	ADPKD	33
miR-365-1	*PKHD1*	HEK293T cell line	—	34
miR-17	*Pkd2*	HEK293T cell line	—	35
miR-15a	*Cdc25A*	PCK rat	PCLD	36

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; HEK, human embryonic kidney cell line; KO, knockout; MDCK, Madin-Darby canine kidney; mIMCD, mouse inner-medullary collecting duct; PCK, a model of polycystic kidney disease and liver disease that developed spontaneously in the rat strain Crj:CD/SD, whose causative gene is *PKHD1*; PCLD, polycystic liver disease; PKD, polycystic kidney disease; Han:SPRD, the causative gene of this strain rat is *Pkdr1*; UUO, unilateral ureteral obstruction.

additional in vivo assays showed that miR-21 inhibits apoptosis of cyst epithelial cells, likely through direct repression of its target gene programmed cell death 4 (PDCD4)[19] (Fig 2).

Wnt/β-Catenin-c-Myc-miR-17∼92 Axis

The Myc proto-oncogene coordinates the transcription of a multitude of genes required for normal cellular growth and proliferation.[59] Important studies have revealed the

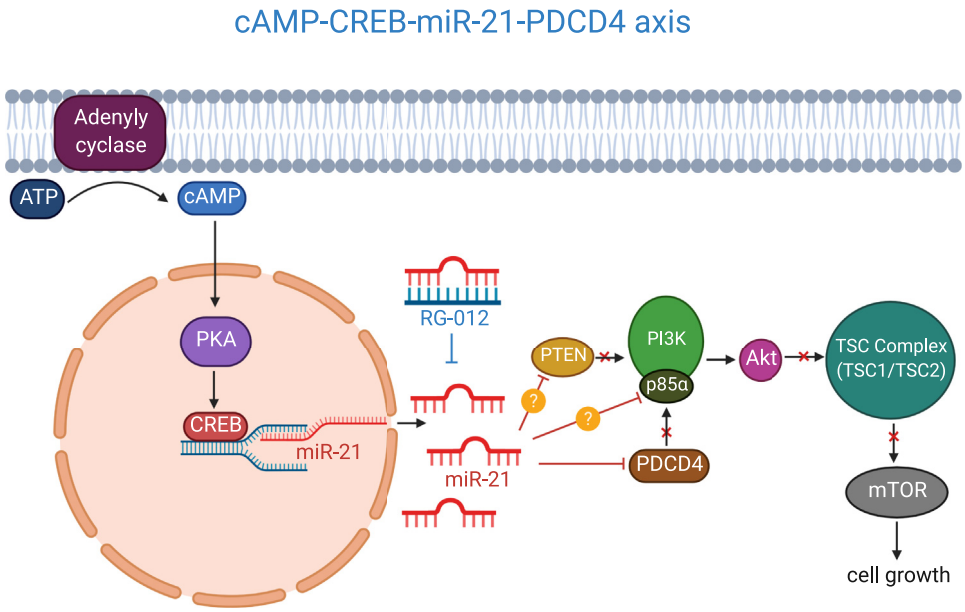

Figure 2. Cyclic adenosine monophosphate (cAMP)-cAMP response element binding (CREB)-miR-21- programmed cell death protein 4 (PDCD4) axis. In cystic kidney cells, elevation of phosphorylated CREB protein through activation of protein kinase A (PKA) has pleiotropic activities. The microRNA-21 (miR-21) gene is identified as a target of this protein, for which the binding site is within the proximal promoter, thus conferring the transcription of miR-21. Phosphatase and tensin homolog (PTEN) is known as a negative regulator of the PTEN/phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, whereas PDCD4 is recognized to inactivate PI3K/Akt signaling to regulate cell growth. Class IA PI3Ks are heterodimeric enzymes containing a p110 catalytic and a p85 regulatory subunit, for which the free monomer variant, p85α, may act as a negative regulator of PI3K signaling so as to reduce PI3K/Akt activation. As an oncogenic miRNA, miR-21 has been demonstrated to target multiple genes in the mTOR pathway to induce cell growth in tumor cells, including PTEN, PDCD4, and p85α. However, in cystic kidney cells, only the role of PDCD4 in the mTOR pathway is revealed to date. RG-012 is a single-stranded chemically modified oligonucleotide that binds to and inhibits the function of miR-21 for the treatment of Alport syndrome. The finding that miR-21 inhibits apoptosis of cyst epithelial cells, likely through direct repression of its target gene PDCD4, makes the manipulation of miR-21 expression by miR-21 inhibitors such as RG-012 a possible therapeutic approach for polycystic kidney diseases. Abbreviations: ATP, adenosine triphosphate; TSC, tuberous sclerosis complex.

influence of the Myc proto-oncogene family in the transcriptional regulation of miR-17∼92.[60] A study found that increased expression of c-Myc correlated with the expression of primary miR-17 in *Kif3a*-KO kidneys, *Pkhd1*/cre;*Pkd2*$^{F/F}$ kidneys, and *Pkhd1*/cre;*HNF1β*$^{F/F}$ kidneys compared with their respective controls.[14] Further studies have shown that c-Myc binds to the promoter of the miR-17∼92 cluster[61] and activates its transcription, which is in line with the previous study and might suggest upregulation of miR-17∼92 in the transcriptional level depending on c-Myc in *PKD2* and *HNF1β* mutant mice. Myc is activated by various mitogenic signals such as serum stimulation or Wnt, Shh, and EGF (via the MAPK/ERK pathway). Interestingly, a previous study confirmed that canonical wnt/β-catenin signaling was upregulated and c-Myc expression was increased in cystic kidneys from *Kif3a*-KO mice.[62] These results collectively implicated a potential insight into the Wnt/β-catenin-c-Myc-miR-17∼92 axis in the pathogenesis of PKDs (Fig 3).

miR-21-mTOR Axis

Both the cAMP-CREB-miR-21-*PDCD4* axis and Wnt/β-catenin-c-Myc-miR-17∼92 axis underlie a role of the signaling pathways in the generation of miRNAs. Surprisingly, as PKD progresses, the miRNA alterations in the kidneys can conversely disrupt the network of signaling pathways. Of note, in another experimental setting relevant to miR-21, it has been demonstrated that miR-21 targets multiple genes in the mTOR pathway, including *PTEN*, *p85α*, and *PDCD4*. These bonds later correlated with enhanced cell proliferation and survival, reduced apoptosis, and autophagy.[63] Two small retrospective studies suggest that mTOR inhibitor can be effective in limiting kidney and/or liver enlargement. Through their inhibition of vascular remodeling, angiogenesis, and fibrogenesis, mTOR inhibitors may attenuate nephroangiosclerosis, cyst growth, and interstitial fibrosis, potentially benefitting PKDs at multiple levels.[64] Although a more explicit association between miR-21 and mTOR pathway in cystogenesis is yet to be found, abundant results from numerous carcinoma-associated models

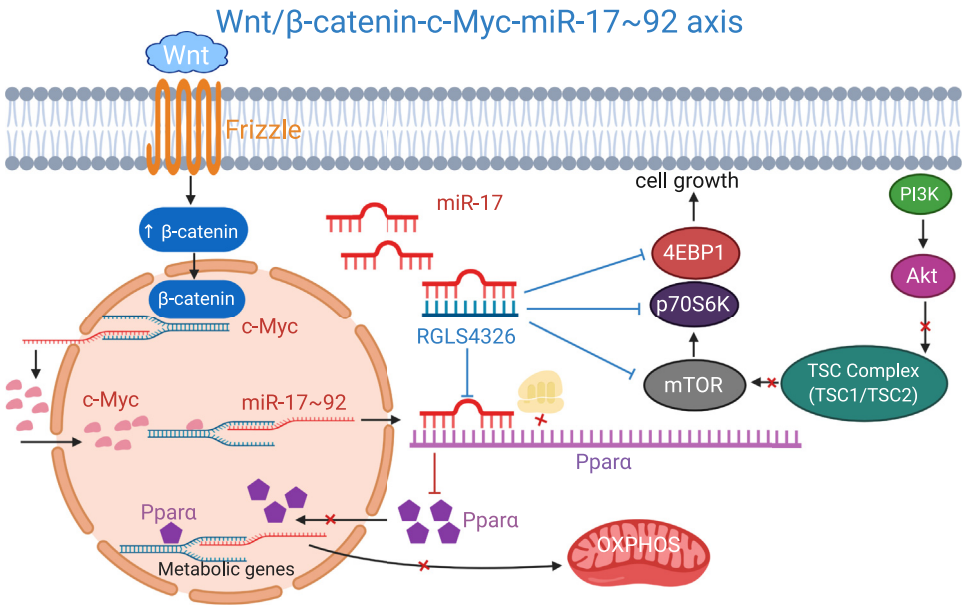

Figure 3. Wnt/β-catenin-c-Myc-miR-17∼92 axis. In cystic kidney cells, β-catenin escapes from degradation in the "Wnt signal-on" state. Free cytoplasmic β-catenin translocates to the nucleus and activates the transcription of target genes including c-Myc. c-Myc protein translocates into the nucleus and binds to the promoter of the microRNA-17∼92 (miR-17∼92) cluster, leading to its transcription. In the cytoplasm, mature miR-17 binds to messenger RNA of peroxisome proliferator-activated receptor-α (Pparα), a factor known to regulate the expression of key metabolic genes, which in turn causes reduced Pparα expression and then restrains mitochondrial oxidative phosphorylation (OXPHOS) metabolism. Mammalian target of rapamycin (mTOR) activity is modulated by a number of positive and negative regulators, such as the recruiting of Akt through active phosphoinositide 3-kinase (PI3K), which inhibits tuberous sclerosis complex (TSC)1/TSC2, thus subsequently removing the inhibitory effect of TSC complex on mTOR and eventually activating mTOR. mTOR initiates its influence on many downstream proteins, including p70S6K and 4EBP1, resulting in cell growth. Treatment with RGLS4326 (a novel oligonucleotide designed to inhibit miR-17) remodels the gene expression pattern and is associated with upregulation of mitochondrial metabolism and suppression of the mTOR pathway because Pparα is released from repression and expression of mTOR, phosphorylated forms of p70S6K, and 4EBP1 is decreased. These ultimately contribute to impaired cyst growth.

exemplified the miR-21–modulated mTOR pathway.[65-67] These results in tumorigenesis may pave a way for the miR21-mTOR axis in PKDs owing to their mutual pathophysiologic mechanisms regarding cell proliferation and altered apoptosis.

The miRNAs and Mitochondrial Metabolism

Several main themes have emerged regarding how miRNAs may promote PKD progression. Apart from the most well-known idea that the miRNAs can regulate a network consisting of PKD genes, the miRNA-mediated rewiring of mitochondrial metabolism is being progressively unraveled. Cyst expansion in ADPKD seems to be accompanied by changes in cellular metabolism; namely, an increase in glucose consumption through aerobic glycolysis and impaired fatty acid oxidation.[68-70] Recently, a study concerning preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 in the treatment of PKD underlay RGLS4326 treatment was associated with normalization of metabolism pathways (eg, peroxisome proliferator-activated receptor-α [PPARα]/retinoid X receptor α [RXRα]).[40] Consistent with this, a previous study observed that c-Myc upregulated miR-17∼92 in cystic kidneys, which in turn aggravated cyst growth by inhibiting oxidative phosphorylation and directly repressing the expression of Pparα.[15] This finding subtly confirms the assumption about the Wnt/β-catenin-c-Myc-miR-17∼92 axis discussed and extrapolates this conception to a metabolic layer (Fig 3). Moreover, in a study treating $Pkd1^{RC/RC}$ mice (RC, exon 29 of *Pkd1* was replaced with a modified exon in which nucleotide substitutions, AGA to TGC, result in the amino acid substitution of cysteine for arginine at position 3277, p.R3277C) with fenofibrate, a clinically available Pparα agonist, reduced kidney cyst proliferation and inflammatory cell infiltration was exhibited, associated with increased expression of *PPARα* and *FAO/OXPHOS* genes.[71,72] These results suggest that the dysregulation of mitochondrial functions are at least part of the proximate cause of metabolic disruptions in PKD. The specific origin of these metabolic disruptions is being actively researched but they have already become targets for new PKD therapies.

CONCLUSION AND FUTURE DIRECTIONS

Given the accumulating knowledge regarding the dysregulation of miRNA expression in PKDs, along with the pathologic mechanisms of cyst formation, the potential for

miRNAs as biomarkers in these diseases is encouraging and may lead to innovative therapies. We have recently witnessed that the drug candidate RGLS4326, a specific miR-17 inhibitor, can be a feasible therapeutic agent for ADPKD.[40] RG-012, a direct miR-21 inhibitor, has already cleared phase 1 clinical trial and now is being tested in a phase 2 trial involving patients with Alport syndrome. However, whether RG-012 can slow ADPKD progression remains to be seen.[73,74] Despite this early success, unanswered questions lie ahead. An obvious question is whether there will be off-target effects of these drugs. Another concern is whether anti-miR therapy can be safely tolerated over the long term. Considering both technological advances and increasing scientific enthusiasm in miRNA biology, we foresee that these obstacles will soon be overcome and significant progress will be achieved just around the corner.

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REFERENCES

1. Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. *Cell*. 2014;157:77-94.
2. Matsui M, Corey DR. Non-coding RNAs as drug targets. *Nat Rev Drug Discov*. 2017;16:167-179.
3. Eddy SR. Non-coding RNA genes and the modern RNA world. *Nat Rev Genet*. 2001;2:919-929.
4. Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. *Nat Rev Cancer*. 2018;18:5-18.
5. Van der Hauwaert C, Glowacki F, Pottier N, Cauffiez C. Non-coding RNAs as new therapeutic targets in the context of renal fibrosis. *Int J Mol Sci*. 2019;20:1977.
6. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. *Nat Cell Biol*. 2019;21:542-551.
7. Bergmann C, Guay-Woodford LM, Harris PC, Horie S, Peters DJM, Torres VE. Polycystic kidney disease. *Nat Rev Dis Primers*. 2018;4:50.
8. Ferre S, Igarashi P. New insights into the role of HNF-1beta in kidney (patho)physiology. *Pediatr Nephrol*. 2019;34:1325-1335.
9. Harris PC. Molecular basis of polycystic kidney disease: PKD1, PKD2 and PKHD1. *Curr Opin Nephrol Hypertens*. 2002;11:309-314.
10. Salmanidis M, Pillman K, Goodall G, Bracken C. Direct transcriptional regulation by nuclear microRNAs. *Int J Biochem Cell Biol*. 2014;54:304-311.
11. Liu B, Shyr Y, Cai J, Liu Q. Interplay between miRNAs and host genes and their role in cancer. *Brief Funct Genomics*. 2018;18:255-266.
12. Yheskel M, Patel V. Therapeutic microRNAs in polycystic kidney disease. *Curr Opin Nephrol Hypertens*. 2017;26:282-289.
13. Bartel DP. Metazoan microRNAs. *Cell*. 2018;173:20-51.
14. Patel V, Williams D, Hajarnis S, et al. miR-17∼92 miRNA cluster promotes kidney cyst growth in polycystic kidney disease. *Proc Natl Acad Sci U S A*. 2013;110:10765-10770.
15. Hajarnis S, Lakhia R, Yheskel M, et al. microRNA-17 family promotes polycystic kidney disease progression through modulation of mitochondrial metabolism. *Nat Commun*. 2017;8:14395.
16. Sun L, Zhu J, Wu M, et al. Inhibition of miR-199a-5p reduced cell proliferation in autosomal dominant polycystic kidney disease through targeting CDKN1C. *Med Sci Monit*. 2015;21:195-200.
17. Patel V, Hajarnis S, Williams D, Hunter R, Huynh D, Igarashi P. MicroRNAs regulate renal tubule maturation through modulation of Pkd1. *J Am Soc Nephrol*. 2012;23:1941-1948.
18. Tee JB, Dnyanmote AV, Lorenzo MK, et al. Pkd1-targeted mutation reveals a role for the Wolffian duct in autosomal dominant polycystic kidney disease. *J Dev Orig Health Dis*. 2020;11:78-85.
19. Lakhia R, Hajarnis S, Williams D, et al. MicroRNA-21 aggravates cyst growth in a model of polycystic kidney disease. *J Am Soc Nephrol*. 2016;27:2319-2330.
20. Lee SO, Masyuk T, Splinter P, et al. MicroRNA15a modulates expression of the cell-cycle regulator Cdc25A and affects hepatic cystogenesis in a rat model of polycystic kidney disease. *J Clin Invest*. 2008;118:3714-3724.
21. Ilatovskaya DV, Levchenko V, Pavlov TS, et al. Salt-deficient diet exacerbates cystogenesis in ARPKD via epithelial sodium channel (ENaC). *EBioMedicine*. 2019;40:663-674.
22. Dweep H, Sticht C, Kharkar A, Pandey P, Gretz N. Parallel analysis of mRNA and microRNA microarray profiles to explore functional regulatory patterns in polycystic kidney disease: using PKD/Mhm rat model. *PLoS One*. 2013;8:e53780.
23. Pandey P, Qin S, Ho J, Zhou J, Kreidberg JA. Systems biology approach to identify transcriptome reprogramming and candidate microRNA targets during the progression of polycystic kidney disease. *BMC Syst Biol*. 2011;5:56.
24. Liu G, Kang X, Guo P, et al. miR-25-3p promotes proliferation and inhibits autophagy of renal cells in polycystic kidney mice by regulating ATG14-Beclin 1. *Ren Fail*. 2020;42:333-342.
25. Lakhia R, Yheskel M, Flaten A, et al. Interstitial microRNA miR-214 attenuates inflammation and polycystic kidney disease progression. *JCI Insight*. 2020;5:e133785.
26. Chau BN, Xin C, Hartner J, et al. MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. *Sci Transl Med*. 2012;4:121ra118.
27. Kim DY, Woo YM, Lee S, et al. Impact of miR-192 and miR-194 on cyst enlargement through EMT in autosomal dominant polycystic kidney disease. *FASEB J*. 2019;33:2870-2884.
28. de Stephanis L, Mangolini A, Servello M, et al. MicroRNA501-5p induces p53 proteasome degradation through the activation of the mTOR/MDM2 pathway in ADPKD cells. *J Cell Physiol*. 2018;233:6911-6924.

29. Woo YM, Kim DY, Koo NJ, et al. Profiling of miRNAs and target genes related to cystogenesis in ADPKD mouse models. *Sci Rep*. 2017;7:14151.
30. Duplomb L, Droin N, Bouchot O, et al. A constitutive BCL2 down-regulation aggravates the phenotype of PKD1-mutant-induced polycystic kidney disease. *Hum Mol Genet*. 2017;26:4680-4688.
31. Streets AJ, Magayr TA, Huang L, et al. Parallel microarray profiling identifies ErbB4 as a determinant of cyst growth in ADPKD and a prognostic biomarker for disease progression. *Am J Physiol Renal Physiol*. 2017;312:F577-F588.
32. Bartram MP, Hohne M, Dafinger C, et al. Conditional loss of kidney microRNAs results in congenital anomalies of the kidney and urinary tract (CAKUT). *J Mol Med (Berl)*. 2013;91:739-748.
33. Piazzon N, Maisonneuve C, Guilleret I, Rotman S, Constam DB. Bicc1 links the regulation of cAMP signaling in polycystic kidneys to microRNA-induced gene silencing. *J Mol Cell Biol*. 2012;4:398-408.
34. Duan J, Huang H, Lv X, et al. PKHD1 post-transcriptionally modulated by miR-365-1 inhibits cell-cell adhesion. *Cell Biochem Funct*. 2012;30:382-389.
35. Sun H, Li QW, Lv XY, et al. MicroRNA-17 post-transcriptionally regulates polycystic kidney disease-2 gene and promotes cell proliferation. *Mol Biol Rep*. 2010;37:2951-2958.
36. Masyuk T, Masyuk A, LaRusso N. Cholangiociliopathies: genetics, molecular mechanisms and potential therapies. *Curr Opin Gastroenterol*. 2009;25:265-271.
37. Hopp K, Ward CJ, Hommerding CJ, et al. Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *J Clin Invest*. 2012;122:4257-4273.
38. Pei Y, Paterson AD, Wang KR, et al. Bilineal disease and transheterozygotes in autosomal dominant polycystic kidney disease. *Am J Hum Genet*. 2001;68:355-363.
39. Yheskel M, Lakhia R, Cobo-Stark P, Flaten A, Patel V. Anti-microRNA screen uncovers miR-17 family within miR-17∼92 cluster as the primary driver of kidney cyst growth. *Sci Rep*. 2019;9:1920.
40. Lee EC, Valencia T, Allerson C, et al. Discovery and preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 for the treatment of polycystic kidney disease. *Nat Commun*. 2019;10:4148.
41. Shin Y, Kim DY, Ko JY, Woo YM, Park JH. Regulation of KLF12 by microRNA-20b and microRNA-106a in cystogenesis. *FASEB J*. 2018;32:3574-3582.
42. Tetreault MP, Yang Y, Katz JP. Kruppel-like factors in cancer. *Nat Rev Cancer*. 2013;13:701-713.
43. Gu S, Chan WY. Flexible and versatile as a chameleon-sophisticated functions of microRNA-199a. *Int J Mol Sci*. 2012;13:8449-8466.
44. Riccio A, Cubellis MV. Gain of function in CDKN1C. *Nat Genet*. 2012;44:737-738.
45. Venugopal J, McDermott J, Sanchez G, et al. Ouabain promotes partial epithelial to mesenchymal transition (EMT) changes in human autosomal dominant polycystic kidney disease (ADPKD) cells. *Exp Cell Res*. 2017;355:142-152.
46. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol*. 2008;10:593-601.
47. Sonoda H, Lee BR, Park KH, et al. miRNA profiling of urinary exosomes to assess the progression of acute kidney injury. *Sci Rep*. 2019;9:4692.
48. Chi SW, Zang JB, Mele A, Darnell RB. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature*. 2009;460:479-486.
49. Fierro-Fernandez M, Busnadiego O, Sandoval P, et al. miR-9-5p suppresses pro-fibrogenic transformation of fibroblasts and prevents organ fibrosis by targeting NOX4 and TGFBR2. *EMBO Rep*. 2015;16:1358-1377.
50. Miguel V, Busnadiego O, Fierro-Fernandez M, Lamas S. Protective role for miR-9-5p in the fibrogenic transformation of human dermal fibroblasts. *Fibrogenesis Tissue Repair*. 2016;9:7.
51. Fierro-Fernandez M, Miguel V, Marquez-Exposito L, et al. MiR-9-5p protects from kidney fibrosis by metabolic reprogramming. *FASEB J*. 2020;34:410-431.
52. Matsuzaka T, Shimano H. Elovl6: a new player in fatty acid metabolism and insulin sensitivity. *J Mol Med (Berl)*. 2009;87:379-384.
53. Norman J. Fibrosis and progression of autosomal dominant polycystic kidney disease (ADPKD). *Biochim Biophys Acta*. 2011;1812:1327-1336.
54. Drummond IA. Polycystins, focal adhesions and extracellular matrix interactions. *Biochim Biophys Acta*. 2011;1812:1322-1326.
55. Lee K, Battini L, Gusella GL. Cilium, centrosome and cell cycle regulation in polycystic kidney disease. *Biochim Biophys Acta*. 2011;1812:1263-1271.
56. Fischer E, Legue E, Doyen A, et al. Defective planar cell polarity in polycystic kidney disease. *Nat Genet*. 2006;38:21-23.
57. Wallace DP. Cyclic AMP-mediated cyst expansion. *Biochim Biophys Acta*. 2011;1812:1291-1300.
58. Delghandi MP, Johannessen M, Moens U. The cAMP signalling pathway activates CREB through PKA, p38 and MSK1 in NIH 3T3 cells. *Cell Signal*. 2005;17:1343-1351.
59. Casey SC, Baylot V, Felsher DW. The MYC oncogene is a global regulator of the immune response. *Blood*. 2018;131:2007-2015.
60. Fuziwara CS, Kimura ET. Insights into regulation of the miR-17-92 cluster of miRNAs in cancer. *Front Med (Lausanne)*. 2015;2:64.
61. Ji M, Rao E, Ramachandrareddy H, et al. The miR-17-92 microRNA cluster is regulated by multiple mechanisms in B-cell malignancies. *Am J Pathol*. 2011;179:1645-1656.
62. Lin F, Hiesberger T, Cordes K, et al. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc Natl Acad Sci U S A*. 2003;100:5286-5291.
63. Zhang Y, Huang B, Wang HY, Chang A, Zheng XFS. Emerging role of microRNAs in mTOR signaling. *Cell Mol Life Sci*. 2017;74:2613-2625.
64. Torres VE, Boletta A, Chapman A, et al. Prospects for mTOR inhibitor use in patients with polycystic kidney disease and hamartomatous diseases. *Clin J Am Soc Nephrol*. 2010;5:1312-1329.
65. Pulito C, Mori F, Sacconi A, et al. Metformin-induced ablation of microRNA 21-5p releases Sestrin-1 and CAB39L antitumoral activities. *Cell Discov*. 2017;3:17022.
66. Jiang LP, He CY, Zhu ZT. Role of microRNA-21 in radiosensitivity in non-small cell lung cancer cells by targeting PDCD4 gene. *Oncotarget*. 2017;8:23675-23689.
67. Yu X, Li R, Shi W, et al. Silencing of microRNA-21 confers the sensitivity to tamoxifen and fulvestrant by enhancing autophagic cell death through inhibition of the PI3K-AKT-mTOR pathway in breast cancer cells. *Biomed Pharmacother*. 2016;77:37-44.

68. Rowe I, Chiaravalli M, Mannella V, et al. Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy. *Nat Med*. 2013;19:488-493.
69. Chiaravalli M, Rowe I, Mannella V, et al. 2-Deoxy-d-glucose ameliorates PKD progression. *J Am Soc Nephrol*. 2016;27:1958-1969.
70. Menezes LF, Lin CC, Zhou F, Germino GG. Fatty acid oxidation is impaired in an orthologous mouse model of autosomal dominant polycystic kidney disease. *EBioMedicine*. 2016;5:183-192.
71. Padovano V, Podrini C, Boletta A, Caplan MJ. Metabolism and mitochondria in polycystic kidney disease research and therapy. *Nat Rev Nephrol*. 2018;14:678-687.
72. Lakhia R, Yheskel M, Flaten A, Quittner-Strom EB, Holland WL, Patel V. PPARalpha agonist fenofibrate enhances fatty acid beta-oxidation and attenuates polycystic kidney and liver disease in mice. *Am J Physiol Renal Physiol*. 2018;314:F122-F131.
73. Guo J, Song W, Boulanger J, et al. Dysregulated expression of microRNA-21 and disease-related genes in human patients and in a mouse model of Alport syndrome. *Hum Gene Ther*. 2019;30:865-881.
74. Gomez IG, MacKenna DA, Johnson BG, et al. Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. *J Clin Invest*. 2015;125:141-156.