Published in final edited form as:

Curr Opin Pediatr. 2015 April ; 27(2): 219–226. doi:10.1097/MOP.0000000000000168.

MicroRNAs in the pathogenesis of cystic kidney disease

Yu Leng Phua^{1,2} and Jacqueline Ho^{1,2,*}

¹Rangos Research Center, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania, United States of America

²University of Pittsburgh, School of Medicine, Department of Pediatrics, Division of Nephrology, Pittsburgh, Pennsylvania, United States of America

Abstract

Purpose of review—Cystic kidney diseases are common renal disorders characterized by the formation of fluid-filled epithelial cysts in the kidneys. The progressive growth and expansion of the renal cyst replaces existing renal tissue within the renal parenchyma, leading to reduced renal function. While several genes have been identified in association with inherited causes of cystic kidney disease, the molecular mechanisms that regulate these genes in the context of post-transcriptional regulation are still poorly understood. There is increasing evidence that miRNA dysregulation is associated with the pathogenesis of cystic kidney disease.

Recent findings—In this review, recent studies that implicate dysregulation of miRNA expression in cystogenesis will be discussed. The relationship of specific specific miRNAs, such as the *miR-17~92* cluster and cystic kidney disease, *miR-92a* and von Hippel-Lindau (VHL) syndrome, and alterations in *LIN28-LET7* expression in Wilms tumor will be explored.

Summary—At present, there are no specific treatments available for patients with cystic kidney disease. Understanding and identifying specific miRNAs involved in the pathogenesis of these disorders may have the potential to lead to the development of novel therapies and biomarkers.

Keywords

cystic kidney disease; miRNA; post-transcriptional regulation; polycystic kidney disease

Introduction

Cystic kidney diseases represent a heterogeneous group of renal disorders that are defined by the presence of distinctive fluid-filled epithelial sacs in the kidney. The etiology of cystic renal diseases can be broadly classified into several categories: inherited, congenital, acquired or in association with renal malignancies or syndromes (Figure 1). Of the inherited cystic kidney diseases, autosomal dominant polycystic kidney disease (ADPKD) is the most

*Corresponding author: Dr. Jacqueline Ho, Rangos Research Center, Children's Hospital of Pittsburgh of UPMC, 4401 Penn Ave, Pittsburgh, Pennsylvania 15224, USA, Phone: 412-692-5303, jacqueline.ho2@chp.edu.

Conflicts of Interest

All sources of funding are noted above. Dr. Yu Leng Phua and Dr. Jacqueline Ho have no conflicts of interest to declare.

common, affecting approximately 500,000 individuals (1:400-1,000 individuals), and is the fourth leading cause of end stage failure in the United States (1)

Although the mechanisms underlying cyst formation from existing nephron tubules are complex and remain poorly understood, cyst formation is thought to stem from perturbations in one or more cellular processes such as abnormal cellular proliferation, apoptosis, altered planar cell polarity-oriented cell division or dysfunctional tubular fluid transport (2–6). Importantly, disruptions to cilia outgrowth and its downstream signaling cascade have also been implicated in a wide variety of ciliopathies including cystic kidney disease (7–12). Irrespective of the underlying etiology, the progressive growth and expansion of renal cysts eventually leads to onset of tubulointerstitial fibrosis and diminishing renal function (13,14). Ultimately, patients develop renal failure, requiring either dialysis or renal transplant, resulting in a significant impact on quality of life.

While previous analyses have successfully identified the causative genes responsible for the onset and inheritance of some cystic kidney diseases such as *PKD1* (ADPKD), *PKD2* (ADPKD), *PKHD1* (autosomal recessive polycystic kidney disease), *HNF-1 β* (renal cysts and diabetes (RCAD) syndrome) and *NPH1-8* (nephronophthisis) (11,12,15–23), there is now a growing body of evidence to suggest that dysregulation of post-transcriptional regulation is also associated with the renal cystic phenotype (24,25). Understanding the mechanisms whereby microRNA- (miRNA) mediated post-transcriptional regulation is involved in renal development and disease is important as it lays the foundation for future medical interventions, and potential diagnostic and prognostic biomarkers that may translate into improved healthcare for patients.

The role and function of microRNAs in kidney development

miRNAs are endogenously produced, small non-coding RNAs (~22-nucleotides long) that play an essential role in a wide variety of cellular processes, primarily by fine-tuning gene expression via messenger RNA (mRNA) repression (26). First transcribed as a primary miRNA (pri-miRNA) by RNA polymerase II, pri-miRNAs are processed sequentially by Drosha into precursor miRNAs (pre-miRNA), and then by Dicer into mature miRNAs during the final biogenesis step (27–29) (Figure 2). The ability for miRNAs to target and bind to the 3' untranslated region (3' UTR) of its target mRNAs is dependent largely on a unique 8 bp-seed sequence present within the miRNA (31,32). Moreover, it is notable that each miRNA is capable of having synergistic effects by targeting multiple transcripts within a single signaling pathway (32). miRNA:mRNA duplex strand binding and formation results in the recruitment of the RNA Induced Silencing Complex (RISC), which then catalyzes the process of decreased mRNA stability and/or translation (33).

In terms of kidney morphogenesis, the indispensable role of miRNAs in normal renal development and cellular maintenance has been demonstrated in multiple transgenic mouse models (Table 1). Renal lineage specific ablation of Dicer in the Six2⁺ nephron progenitor compartment resulted in renal hypoplasia accompanied by elevated pro-apoptotic protein *Bcl2l11* (*Bim*) mRNA levels and hence premature depletion of the nephron progenitors due to increased cell death (24,34). Loss of miRNAs in the Hoxb7⁺ ureteric bud (UB) lineage

was the first transgenic mouse model to illustrate the functional relevance of miRNAs in cystic kidney disease (35). UB cyst formation was attributed to a primary cilia outgrowth defect, increased cellular proliferation and enhanced apoptosis (Figure 3) (24). Interestingly, loss of miRNAs in proximal tubules had no overt effect on kidney development, but rather is renoprotective against ischemia/reperfusion injury due to reduced tubular apoptosis (40).

The interplay between miRNAs and Polycystic Kidney Disease

miRNA dysregulation has now been associated with a wide spectrum of renal pathophysiology. In the context of cystic kidney diseases, the majority of studies have implicated miRNAs in the pathogenesis of polycystic kidney disease (ADPKD, ARPKD) (25, 42–44). A major challenge continues to be the identification and validation of miRNA:mRNA targets amid a complex network of altered signaling pathways in these diseases. In spite of this caveat, multiple bioinformatics tools including TargetScan, miRanda and microT-CDS have been used with existing mRNA datasets to attempt the prediction of upstream miRNAs based on 3'UTR sequence alignment base pairing (32,45,46). Such an approach was undertaken during the analysis of *Pkd1*^{-/-} mice kidneys, and resulted in the prediction of at least 5000 miRNA:mRNA interactions (47). Collectively, differentially expressed miRNAs in *Pkd1*^{-/-} kidneys such as miRs-10a, 30a, 96, 126-5p, 182, 200a, 204, 488 and 429 that affect signaling pathways known to be strongly associated with ADPKD including calcium sensing, Wnt and Notch signaling have been successfully identified and validated through quantitative PCR measurements (47). In an alternative approach undertaken by Pandey, Brors (48), simultaneous screening for both mRNA and miRNA changes in inbred Han:SPRD-cy rat kidneys (which mimics ADPKD in humans) led to the identification of 935 and 30 differentially expressed mRNA and miRNAs including miRs-21, 31, 128, 147 and 217.

The merit from such transcriptome-wide studies is that they can effectively curate a large cohort and network of miRNA:mRNA interactions which form a comprehensive atlas for conducting future experiments regarding the signaling pathways perturbed by miRNAs in ADPKD. These studies however lack the ability to define the mechanisms whereby miRNA dysregulation results in a renal cystic phenotype. Elucidation of these regulatory mechanisms remains dependent on the characterization of specific miRNA-overexpressing or knockout *in vitro* or *in vivo* models.

The role of the *miR17~92* cluster in the onset of Polycystic Kidney Disease

The *miR-17~92* cluster (which consists of miRs-17, 18a, 19a, 19b, 20a and 92a) is the first miRNA mutation associated with a human syndrome, Feingold Syndrome, and has been implicated in multiple oncogenic tumors (49,50). Although patients diagnosed with Feingold syndrome due to *miR-17~92* deletion have no reported renal defects to date, Feingold syndrome associated with *MYCN* mutations have an 18% incidence rate of congenital anomalies of the kidney and urinary tract (51, 52). miR-92a has also been shown to target *VHL*, a gene that is associated with renal cell carcinoma and von Hippel-Lindau (VHL) syndrome, which is characterized by the formation of cysts in numerous organ systems including the kidney (53–55).

The importance of *miR-17~92* in renal development was recently illustrated by targeted deletion in nephron progenitors (*Six2Cre-miR-17~92^{fl/fl}*), resulting in renal hypoplasia accompanied by signs of progressive renal disease including albuminuria, podocyte foot effacement and glomerulosclerosis during adulthood (56). Mechanistically, this phenotype was thought to be due, at least in part, to elevated levels of the cyclin dependent kinase inhibitor *p21*, which is an mRNA target of miR-17-5p and miR-20a, resulting in decreased nephron progenitor proliferation (57–60).

In the context of PKD, the *miR-17~92* cluster was found to be consistently up-regulated in 4 transgenic mouse models of PKD including *KspCre-Kif3a^{fl/fl}* (a mouse model of ciliopathies), *Pkhd1Cre-Pkd2^{fl/fl}* (ADPKD model), *Pkhd1^{-/-}* null mice (ARPKD model) and *Pkhd1Cre-Hnf1 β ^{fl/fl}* (RCAD model) (Table 2) (25). Kif3a is known to act as a transcriptional suppressor of *c-Myc* (63). Hence, in the absence of Kif3a, c-Myc is up-regulated which in turn promotes transcription of *miR-17~92*, and onset of the renal cyst phenotype (25,63). Ectopic overexpression of *miR-17~92* in the nephron epithelium (*KspCre-CAG STOP^{fl/fl} miR-17~92*) was sufficient to recapitulate the renal cystic phenotype via repression of *Pkd1*, *Pkd2*, *Pkhd1*, *Hnf-1 β* , as well as enhanced tubular proliferation (25). Conversely, loss of *miR-17~92* in compound mutant mice (*KspCre-Kif3a^{fl/fl}/miR-17~92^{fl/fl}*) partially rescued and ameliorated renal cyst formation in *KspCre-Kif3a^{fl/fl}* mice, thereby attesting to the functional relevance of miR-17~92 dosage in the onset of PKD (25).

Genetic intersection of miRNAs and Bicc1 in Polycystic Kidney Disease

Apart from the role of the *miR-17~92* cluster in PKD, miR-17, miR-125a and miR-27a have also been implicated in renal cyst formation as modulators of Bicaudal C (Bicc1) function (Table 2) (42,43). Bicc1 is a post-transcriptional regulatory protein containing five K homology domains (KH) and a sterile alpha motif (SAM) that regulates mRNA stability and translation (42,64,65). *Bicc1*-null kidneys have a PKD-like phenotype, and *Pkd2* is significantly down-regulated (42). Interestingly, *Pkd2* contains a conserved miRNA binding site for miR-17 (42). Based on morpholino knockdown studies in the *Xenopus* model, genetic crosstalk between miR-17, *Pkd2* and Bicc1 was uncovered whereby Bicc1 was shown to antagonize the repressive effects of miR-17 on *Pkd2* (42). Hence, in the absence of Bicc1, there is elevated repression of *Pkd2* via miR-17, and onset of renal cyst formation in the *Bicc1* null mice (42).

Elevated miR-17 repression of *Pkd2* is unlikely to be the sole mediator of renal cyst formation in *Bicc1* null mice. Over-expression of a single miRNA from the *miR-17~92* cluster, miR-17, did not result in overt renal cyst formation, suggesting that the renal cystic phenotype in *Bicc1^{-/-}* mice is likely to be the result of a combination of dysregulated pathways (66). Bicc1 has also been shown to bind to the mRNA of the cAMP associated enzyme AC6 via its KH domains, leading to recruitment of miR-125a, which blocks translation of AC6 protein (43). Similarly, Bicc1 inhibits PKI α protein translation via miR-27a activity (43). These findings collectively suggest that several miRNAs play an important role in mediating Bicc1 suppressive activity on renal cyst formation by simultaneously repressing multiple mRNA targets.

miRNAs and Autosomal Recessive Polycystic Kidney Disease

To date, most of the studies linking miRNAs to the pathogenesis of inherited cystic renal diseases have focused on ADPKD. In an experimental PCK rat model of ARPKD, which was used to address the underlying mechanisms of hepatic cystogenesis, miR-15a was found to be reduced in cystic cholangiocytes (61). A potential mRNA target of miR-15a is *Cdc25A*, a cell cycle regulatory protein which modulates cellular transition from G1 to S phase (67). The miR15a:Cdc25A target interaction was validated through 3D cholangiocyte cell culture, whereby knockdown of miR-15a resulted in elevated levels of Cdc25A protein, and hence enhanced cyst formation in culture (61). Whether this mechanism is generalizable to renal cyst formation in ARPKD remains unknown.

Cysts associated with Wilms tumors- the potential contribution of LIN28- *LET7* transcriptional regulation?

Cystic nephroma (CN) and cystic partially differentiated nephroblastoma (CPDN) are rare cystic variants of pediatric Wilms tumor, for which the underlying genetic etiologies are not well understood (68). Recent observations from clinical Wilms tumor samples have implicated the RNA binding protein LIN28 and the conserved miRNA family LET-7 in the pathogenesis of Wilms tumors. LIN28 (which consists of LIN28A and LIN28B) regulates mRNA transcripts either by inhibiting processing of the *LET-7* family or direct mRNA target binding (69–71). *LIN28B* was first identified to be elevated in a subset of clinical Wilms tumor samples (72,73). Recent exome sequencing of Wilms tumor samples has also uncovered the presence of mutations in *DROSHA* and *DICER1*, which result in the impaired processing of tumor suppressing miRNAs, such as LET-7, and subsequent increased expression of LIN28 (74). To address whether elevated levels of *LIN28* is associated with the onset of Wilms tumor, generation of *Lin28* overexpressing transgenic mice [*VasaCre-Ef1a STOP^{fl/fl} Lin28a* (maternal Cre transmission), inducible *Wt1Cre-TetOn LIN28B* (doxycycline induction)] showed that overexpression of mouse *Lin28a* ubiquitously or human *LIN28B* in the renal intermediate mesoderm lineage led to the formation of renal tumors that were pathologically reminiscent to Wilms tumor (73). The enforced expression of *LIN28B* suppresses maturation of *Let-7* isoforms, thereby causing upregulation of *Let-7* target genes that are thought to prolong nephrogenesis and aberrant persistence of undifferentiated nephron progenitors in Wilms tumor (73). Conversely, compound mutants over-expressing *LIN28B* in conjunction with *Let-7g*, a *Let-7* isoform unaffected by Lin28 processing, ameliorated the *LIN28B* over-expression effect, thereby indicating that LIN28B acts in part via *Let-7* post-transcriptional regulatory mechanisms (73).

Interestingly, cellular specific overexpression of *Lin28* in specific renal cell lineages, or when induced postnatally, did not result in tumor formation (73). Instead, overexpression of *Lin28a* or *LIN28B* in the nephron progenitor lineage and its epithelial derivatives (*Six2Cre*) or in postnatal day 10 kidneys upon doxycycline induction (*Wt1Cre-TetOn LIN28B*) led to renal cyst formation (Table 2) (73). Such findings are suggestive for a novel paradigm whereby Lin28/*let-7* transcriptional regulation is necessary to prevent ectopic renal cyst formation. It is plausible to speculate that ectopic overexpression of *LIN28* in specific

nephron epithelium could result in a cystic Wilms tumor phenotype, and be associated with CN or CPDN in patients, although this remains to be determined.

Translation from bench to bedside: Clinical aspects of miRNAs in cystic kidney diseases

The potential use of miRNAs as diagnostic and prognostic markers in human cystic kidney diseases is promising given their functional relevance to cyst formation. Additionally, the relative stability of miRNAs in biological fluids such as urine makes them attractive as non-invasive biomarkers (75). Large-scale clinical studies looking at miRNA expression in patients with cystic kidney disease remain limited. Nevertheless, reappraisal of a publicly available microarray data on Gene Expression Omnibus (GSE7869) (76), which was initially used to look for mRNA changes in human ADPKD cyst samples, showed up-regulation of MIR143HG and MIR100HG. Enrichment of MIR143 was also independently validated through a separate high throughput RNA-sequencing of ADPKD patient urine samples (77), thereby highlighting the concordance of miRNA dysregulation in ADPKD.

At present, there are no specific therapies available for the heterogeneous group of disorders that cystic kidney diseases encompass, although there is emerging evidence that novel therapies that target cyst formation may be beneficial. In this review, recent studies were highlighted that demonstrate the involvement of miRNAs in the pathogenesis of cystic kidney diseases. Targeting appropriate candidate miRNAs may serve as one avenue of exploration for innovative therapies, since anti-miR oligonucleotides has been successfully utilized in mouse model systems to reduce renal injury induced fibrosis (78).

Conclusion

miRNAs are increasingly relevant to cystic renal diseases in patients given the growing body of knowledge regarding the dysregulation of miRNA expression in these diseases, along with the association of specific miRNAs with the pathologic mechanisms of cyst formation. The potential for miRNAs to be used as biomarkers in these diseases is encouraging, but remains in the preliminary stages of investigation. As more insights are gained regarding the mechanisms by which miRNAs drive cyst formation, this may lead to innovative therapies for patients with renal cystic diseases.

Acknowledgments

The work in Dr. Ho's laboratory is supported by funding by an NIDDK R00DK087922, a March of Dimes Basil O'Connor Starter Scholar Award, and a Satellite Healthcare Norman S. Coplon Extramural grant.

References and Recommended Reading

Papers of particular interest, published within the annual period of review, (18 months/2013–2014) have been highlighted as:

- of special interest
- of outstanding interest

1. Masoumi A, Reed-Gitomer B, Kelleher C, et al. Developments in the Management of Autosomal Dominant Polycystic Kidney Disease. *Ther Clin Risk Manag*. 2008; 4(2):393–407. [PubMed: 18728845]
2. Woo D. Apoptosis and Loss of Renal Tissue in Polycystic Kidney Diseases. *N Engl J Med*. 1995; 333(1):18–25. [PubMed: 7776989]
3. Fischer E, Legue E, Doyen A, et al. Defective Planar Cell Polarity in Polycystic Kidney Disease. *Nat Genet*. 2006; 38(1):21–23. [PubMed: 16341222]
4. Avner ED, Sweeney WE Jr, Nelson WJ. Abnormal Sodium Pump Distribution During Renal Tubulogenesis in Congenital Murine Polycystic Kidney Disease. *Proc Natl Acad Sci U S A*. 1992; 89(16):7447–7451. [PubMed: 1323837]
5. Saburi S, Hester I, Fischer E, et al. Loss of Fat4 Disrupts PCP Signaling and Oriented Cell Division and Leads to Cystic Kidney Disease. *Nat Genet*. 2008; 40(8):1010–1015. [PubMed: 18604206]
6. Hanaoka K, Guggino WB. Camp Regulates Cell Proliferation and Cyst Formation in Autosomal Polycystic Kidney Disease Cells. *J Am Soc Nephrol*. 2000; 11(7):1179–1187. [PubMed: 10864573]
7. Pazour GJ, Dickert BL, Vucica Y, et al. Chlamydomonas Ift88 and Its Mouse Homologue, Polycystic Kidney Disease Gene Tg737, Are Required for Assembly of Cilia and Flagella. *J Cell Biol*. 2000; 151(3):709–718. [PubMed: 11062270]
8. Yoder BK, Hou X, Guay-Woodford LM. The Polycystic Kidney Disease Proteins, Polycystin-1, Polycystin-2, Polaris, and Cystin, Are Co-Localized in Renal Cilia. *J Am Soc Nephrol*. 2002; 13(10):2508–2516. [PubMed: 12239239]
9. Hou X, Mrug M, Yoder BK, et al. Cystin, a Novel Cilia-Associated Protein, Is Disrupted in the Cpk Mouse Model of Polycystic Kidney Disease. *J Clin Invest*. 2002; 109(4):533–540. [PubMed: 11854326]
10. Low SH, Vasanth S, Larson CH, et al. Polycystin-1, Stat6, and P100 Function in a Pathway That Transduces Ciliary Mechanosensation and Is Activated in Polycystic Kidney Disease. *Dev Cell*. 2006; 10(1):57–69. [PubMed: 16399078]
11. Nauli SM, Alenghat FJ, Luo Y, et al. Polycystins 1 and 2 Mediate Mechanosensation in the Primary Cilium of Kidney Cells. *Nat Genet*. 2003; 33(2):129–137. [PubMed: 12514735]
12. Koulen P, Cai Y, Geng L, et al. Polycystin-2 Is an Intracellular Calcium Release Channel. *Nat Cell Biol*. 2002; 4(3):191–197. [PubMed: 11854751]
13. Phua YL, Martel N, Pennisi DJ, et al. Distinct Sites of Renal Fibrosis in Crim1 Mutant Mice Arise from Multiple Cellular Origins. *J Pathol*. 2013; 229(5):685–696. [PubMed: 23224993]
14. Okada H, Ban S, Nagao S, et al. Progressive Renal Fibrosis in Murine Polycystic Kidney Disease: An Immunohistochemical Observation. *Kidney Int*. 2000; 58(2):587–597. [PubMed: 10916082]
15. Burn TC, Connors TD, Dackowski WR, et al. Analysis of the Genomic Sequence for the Autosomal Dominant Polycystic Kidney Disease (PKD1) Gene Predicts the Presence of a Leucine-Rich Repeat. The American Pkd1 Consortium (APKD1 Consortium). *Hum Mol Genet*. 1995; 4(4):575–582. [PubMed: 7633406]
16. Hughes J, Ward CJ, Peral B, et al. The Polycystic Kidney Disease 1 (PKD1) Gene Encodes a Novel Protein with Multiple Cell Recognition Domains. *Nat Genet*. 1995; 10(2):151–160. [PubMed: 7663510]
17. Mochizuki T, Wu GQ, Hayashi T, et al. PKD2, a Gene for Polycystic Kidney Disease That Encodes an Integral Membrane Protein. *Science*. 1996; 272(5266):1339–1342. [PubMed: 8650545]
18. Brasier JL, Henske EP. Loss of the Polycystic Kidney Disease (PKD1) Region of Chromosome 16p13 in Renal Cyst Cells Supports a Loss-of-Function Model for Cyst Pathogenesis. *J Clin Invest*. 1997; 99(2):194–199. [PubMed: 9005987]
19. González-Perrett S, Kim K, Ibarra C, et al. Polycystin-2, the Protein Mutated in Autosomal Dominant Polycystic Kidney Disease (ADPKD), Is a Ca²⁺-Permeable Nonselective Cation Channel. *Proc Natl Acad Sci U S A*. 2001; 98(3):1182–1187. [PubMed: 11252306]
20. Boucher C, Sandford R. Autosomal Dominant Polycystic Kidney Disease (ADPKD, MIM 173900, PKD1 and PKD2 Genes, Protein Products Known as Polycystin-1 and Polycystin-2). *Eur J Hum Genet*. 2004; 12(5):347–354. [PubMed: 14872199]

21. Bergmann C, Senderek J, Küpper F, et al. PKHD1 Mutations in Autosomal Recessive Polycystic Kidney Disease (ARPKD). *Human Mutat.* 2004; 23(5):453–463.
22. Bellanne-Chantelot C, Chauveau D, Gautier JF, et al. Clinical Spectrum Associated with Hepatocyte Nuclear Factor-1beta Mutations. *Ann Intern Med.* 2004; 140(7):510–517. [PubMed: 15068978]
23. Otto EA, Trapp ML, Schultheiss UT, et al. Nek8 Mutations Affect Ciliary and Centrosomal Localization and May Cause Nephronophthisis. *J Am Soc Nephrol.* 2008; 19(3):587–592. [PubMed: 18199800]
24. Nagalakshmi VK, Ren Q, Pugh MM, et al. Dicer Regulates the Development of Nephrogenic and Ureteric Compartments in the Mammalian Kidney. *Kidney Int.* 2011; 79(3):317–330. [PubMed: 20944551]
- 25**. 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(26):10765–10770. This article investigates the role of *miR-17~92* in Polycystic Kidney Disease. [PubMed: 23759744]
26. Bartel DP. Micronas: Genomics, Biogenesis, Mechanism, and Function. *Cell.* 2004; 116(2):281–297. [PubMed: 14744438]
27. Lee Y, Ahn C, Han J, et al. The Nuclear RNase III Drosha Initiates microRNA Processing. *Nature.* 2003; 425(6956):415–419. [PubMed: 14508493]
28. Hutvagner G, McLachlan J, Pasquinelli AE, et al. A Cellular Function for the RNA-Interference Enzyme Dicer in the Maturation of the Let-7 Small Temporal RNA. *Science.* 2001; 293(5531):834–838. [PubMed: 11452083]
29. Ketting RF, Fischer SE, Bernstein E, et al. Dicer Functions in RNA Interference and in Synthesis of Small RNA Involved in Developmental Timing in *C. Elegans*. *Genes Dev.* 2001; 15(20):2654–2659. [PubMed: 11641272]
30. Ho J, Kreidberg JA. The Long and Short of microRNAs in the Kidney. *J Am Soc Nephrol.* 2012; 23(3):400–404. [PubMed: 22302196]
31. Grimson A, Farh KK, Johnston WK, et al. MicroRNA Targeting Specificity in Mammals: Determinants Beyond Seed Pairing. *Mol Cell.* 2007; 27(1):91–105. [PubMed: 17612493]
32. Lewis BP, Burge CB, Bartel DP. Conserved Seed Pairing, Often Flanked by Adenosines, Indicates That Thousands of Human Genes Are MicroRNA Targets. *Cell.* 2005; 120(1):15–20. [PubMed: 15652477]
33. Hammond SM, Bernstein E, Beach D, et al. An RNA-Directed Nuclease Mediates Post-Transcriptional Gene Silencing in *Drosophila* Cells. *Nature.* 2000; 404(6775):293–296. [PubMed: 10749213]
34. Ho J, Pandey P, Schatton T, et al. The Pro-Apoptotic Protein Bim Is a MicroRNA Target in Kidney Progenitors. *J Am Soc Nephrol.* 2011; 22(6):1053–1063. [PubMed: 21546576]
35. Pastorelli LM, Wells S, Fray M, et al. Genetic Analyses Reveal a Requirement for Dicer1 in the Mouse Urogenital Tract. *Mamm Genome.* 2009; 20(3):140–151. [PubMed: 19169742]
36. Shi S, Yu L, Chiu C, et al. Podocyte-Selective Deletion of Dicer Induces Proteinuria and Glomerulosclerosis. *J Am Soc Nephrol.* 2008; 19(11):2159–2169. [PubMed: 18776119]
37. Harvey SJ, Jarad G, Cunningham J, et al. Podocyte-Specific Deletion of Dicer Alters Cytoskeletal Dynamics and Causes Glomerular Disease. *J Am Soc Nephrol.* 2008; 19(11):2150–2158. [PubMed: 18776121]
38. Ho J, Ng KH, Rosen S, et al. Podocyte-Specific Loss of Functional microRNAs Leads to Rapid Glomerular and Tubular Injury. *J Am Soc Nephrol.* 2008; 19(11):2069–2075. [PubMed: 18832437]
39. Zhdanova O, Srivastava S, Di L, et al. The Inducible Deletion of Drosha and microRNAs in Mature Podocytes Results in a Collapsing Glomerulopathy. *Kidney Int.* 2011; 80(7):719–730. [PubMed: 21544061]
40. Wei Q, Bhatt K, He HZ, et al. Targeted Deletion of Dicer from Proximal Tubules Protects against Renal Ischemia-Reperfusion Injury. *J Am Soc Nephrol.* 2010; 21(5):756–761. [PubMed: 20360310]

41. Sequeira-Lopez ML, Weatherford ET, Borges GR, et al. The microRNA-Processing Enzyme Dicer Maintains Juxtaglomerular Cells. *J Am Soc Nephrol*. 2010; 21(3):460–467. [PubMed: 20056748]
42. Tran U, Zakin L, Schweickert A, et al. The RNA-Binding Protein Bicaudal C Regulates Polycystin 2 in the Kidney by Antagonizing mir-17 Activity. *Development*. 2010; 137(7):1107–1116. [PubMed: 20215348]
43. Piazzon N, Maisonneuve C, Guilleret I, et al. Bicc1 Links the Regulation of Camp Signaling in Polycystic Kidneys to microRNA-Induced Gene Silencing. *J Mol Cell Biol*. 2012; 4(6):398–408. [PubMed: 22641646]
44. 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(5):382–389. [PubMed: 22411058]
45. Enright AJ, John B, Gaul U, et al. MicroRNA Targets in *Drosophila*. *Genome Biol*. 2004; 5(1):R1–R1. [PubMed: 14709173]
46. Paraskevopoulou MD, Georgakilas G, Kostoulas N, et al. DIANA-microT Web Server V5.0: Service Integration into miRNA Functional Analysis Workflows. *Nucleic Acids Res*. 2013; 41(Web Server issue):W169–173. [PubMed: 23680784]
47. Pandey P, Qin S, Ho J, et al. Systems Biology Approach to Identify Transcriptome Reprogramming and Candidate microRNA Targets During the Progression of Polycystic Kidney Disease. *BMC Syst Biol*. 2011; 5:56. [PubMed: 21518438]
48. Pandey P, Brors B, Srivastava PK, et al. Microarray-Based Approach Identifies microRNAs and Their Target Functional Patterns in Polycystic Kidney Disease. *BMC Genomics*. 2008; 9:624. [PubMed: 19102782]
49. de Pontual L, Yao E, Callier P, et al. Germline Deletion of the mir-17~92 Cluster Causes Skeletal and Growth Defects in Humans. *Nat Genet*. 2011; 43(10):1026–1030. [PubMed: 21892160]
50. O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-Regulated MicroRNAs Modulate E2f1 Expression. *Nature*. 2005; 435(7043):839–843. [PubMed: 15944709]
51. Celli J, van Bokhoven H, Brunner HG. Feingold Syndrome: Clinical Review and Genetic Mapping. *Am J Med Genet A*. 2003; 122A(4):294–300. [PubMed: 14518066]
52. Marcelis CL, Hol FA, Graham GE, et al. Genotype-Phenotype Correlations in Mycn-Related Feingold Syndrome. *Hum Mutat*. 2008; 29(9):1125–1132. [PubMed: 18470948]
53. Gnarra JR, Tory K, Weng Y, et al. Mutations of the VHL Tumour Suppressor Gene in Renal Carcinoma. *Nat Genet*. 1994; 7(1):85–90. [PubMed: 7915601]
54. Lonser RR, Glenn GM, Walther M, et al. Von Hippel-Lindau Disease. *The Lancet*. 2003; 361(9374):2059–2067.
55. Ghosh AK, Shanafelt TD, Cimmino A, et al. Aberrant Regulation of pVHL Levels by microRNA Promotes the HIF/VEGF Axis in CLL B Cells. *Blood*. 2009; 113(22):5568–5574. [PubMed: 19336759]
- 56*. Marrone AK, Stolz DB, Bastacky SI, et al. MicroRNA-17~92 Is Required for Nephrogenesis and Renal Function. *J Am Soc Nephrol*. 2014; 25(7):1440–1452. This article investigates the role of miR-17~92 in relation to kidney development. [PubMed: 24511118]
57. Cloonan N, Brown MK, Steptoe AL, et al. The miR-17–5p microRNA Is a Key Regulator of the G1/S Phase Cell Cycle Transition. *Genome Biol*. 2008; 9(8):R127. [PubMed: 18700987]
58. Fontana L, Fiori ME, Albin S, et al. Antagomir-17–5p Abolishes the Growth of Therapy-Resistant Neuroblastoma through p21 and Bim. *PLoS One*. 2008; 3(5):e2236. [PubMed: 18493594]
59. Wong P, Iwasaki M, Somervaille TC, et al. The mir-17–92 microRNA Polycistron Regulates MLL Leukemia Stem Cell Potential by Modulating p21 Expression. *Cancer Res*. 2010; 70(9):3833–3842. [PubMed: 20406979]
60. Gibcus JH, Kroesen BJ, Koster R, et al. MiR-17/106b Seed Family Regulates p21 in Hodgkin's Lymphoma. *J Pathol*. 2011; 225(4):609–617. [PubMed: 21953646]
61. 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(11):3714–3724. [PubMed: 18949056]
- 62*. Urbach A, Yermalovich A, Zhang J, et al. Lin28 Sustains Early Renal Progenitors and Induces Wilms Tumor. *Genes Dev*. 2014; 28(9):971–982. This article contains supporting evidence for

the potential contributory role of Lin28-Let7 regulation in cystic kidney disease. [PubMed: 24732380]

63. 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(9):5286–5291. [PubMed: 12672950]
64. Maisonneuve C, Guilleret I, Vick P, et al. Bicaudal C, a Novel Regulator of Dvl Signaling Abutting RNA-Processing Bodies, Controls Cilia Orientation and Leftward Flow. *Development*. 2009; 136(17):3019–3030. [PubMed: 19666828]
65. Mahone M, Saffman EE, Lasko PF. Localized Bicaudal-C RNA Encodes a Protein Containing a KH Domain, the RNA Binding Motif of FMR1. *EMBO J*. 1995; 14(9):2043–2055. [PubMed: 7538070]
66. Shan SW, Lee DY, Deng Z, et al. MicroRNA miR-17 Retards Tissue Growth and Represses Fibronectin Expression. *Nat Cell Biol*. 2009; 11(8):1031–1038. [PubMed: 19633662]
67. Jinno S, Suto K, Nagata A, et al. Cdc25a Is a Novel Phosphatase Functioning Early in the Cell-Cycle. *EMBO J*. 1994; 13(7):1549–1556. [PubMed: 8156993]
68. Joshi VV, Beckwith JB. Multilocular Cyst of the Kidney (Cystic Nephroma) and Cystic, Partially Differentiated Nephroblastoma. Terminology and Criteria for Diagnosis. *Cancer*. 1989; 64(2):466–479. [PubMed: 2544253]
69. Viswanathan SR, Daley GQ, Gregory RI. Selective Blockade of microRNA Processing by Lin28. *Science*. 2008; 320(5872):97–100. [PubMed: 18292307]
70. Newman MA, Thomson JM, Hammond SM. Lin-28 Interaction with the Let-7 Precursor Loop Mediates Regulated microRNA Processing. *RNA*. 2008; 14(8):1539–1549. [PubMed: 18566191]
71. Wilbert ML, Huelga SC, Kapeli K, et al. Lin28 Binds Messenger RNAs at Ggaga Motifs and Regulates Splicing Factor Abundance. *Molecular Cell*. 2012; 48(2):195–206. [PubMed: 22959275]
72. Viswanathan SR, Powers JT, Einhorn W, et al. Lin28 Promotes Transformation and Is Associated with Advanced Human Malignancies. *Nat Genet*. 2009; 41(7):843–848. [PubMed: 19483683]
- 73*. Rakheja D, Chen KS, Liu Y, et al. Somatic Mutations in DROSHA and DICER1 Impair MicroRNA Biogenesis through Distinct Mechanisms in Wilms Tumours. *Nat Comm*. 2014; 2 This article demonstrates evidence for *DROSHA* and *DICER1* mutations in Wilms tumor which affect biogenesis of Lin28 and Let7.
74. Piskounova E, Viswanathan SR, Janas M, et al. Determinants of microRNA Processing Inhibition by the Developmentally Regulated RNA-Binding Protein Lin28. *J Biol Chem*. 2008; 283(31): 21310–21314. [PubMed: 18550544]
75. Cheng L, Sun X, Scicluna BJ, et al. Characterization and Deep Sequencing Analysis of Exosomal and Non-Exosomal miRNA in Human Urine. *Kidney Int*. 2014; 86(2):433–444. [PubMed: 24352158]
76. Song X, Di Giovanni V, He N, et al. Systems Biology of Autosomal Dominant Polycystic Kidney Disease (ADPKD): Computational Identification of Gene Expression Pathways and Integrated Regulatory Networks. *Hum Mol Genet*. 2009; 18(13):2328–2343. [PubMed: 19346236]
77. Ben-Dov IZ, Tan YC, Morozov P, et al. Urine MicroRNA as Potential Biomarkers of Autosomal Dominant Polycystic Kidney Disease Progression: Description of miRNA Profiles at Baseline. *PLoS One*. 2014; 9(1):e86856. [PubMed: 24489795]
78. Chau BN, Xin C, Hartner J, et al. MicroRNA-21 Promotes Fibrosis of the Kidney by Silencing Metabolic Pathways. *Sci Transl Med*. 2012; 4(121):121ra118.

Key Points

1. Dysregulation of miRNAs is associated with the pathogenesis and progression of cystic kidney disease.
2. The *miR-17~92* cluster has been shown to play a functional role in the pathogenesis of Polycystic Kidney Disease in mice.
3. miRNAs may become useful biomarkers and drug targets for renal cystic diseases.

Figure 1. Classification of cystic kidney diseases and their associated genetic mutations
Genes and miRNAs that are known to be involved in the cystic kidney phenotype are indicated.

Figure 2. miRNA biogenesis pathway

Primary miRNAs (pri-miRNAs) are sequentially processed by Drosha into precursor miRNAs (pre-miRNAs), and then Dicer into mature miRNAs. The mature miRNA then represses its target mRNA by first binding to the mRNA 3'UTR region, followed by recruitment of the RISC complex which down-regulates the expression of mRNA transcripts via decreased mRNA stability or decreased translation. Figure reproduced with permission from JASN (30).

Figure 3. Hematoxylin and eosin stained histological section of postnatal day 0 *Hoxb7Cre-Dicer^{fl/fl}* kidney
Deletion of *Dicer*, the endoribonuclease complex required for miRNA synthesis, in the *Hoxb7⁺* ureteric bud lineage results in tubular cyst formation (J. Ho, unpublished observation). Tubular cysts are indicated by asterisks.

Table 1

Renal phenotypes in mouse models which lack miRNA biogenesis in specific cell lineages.

Renal lineage	Renal phenotype	Mechanisms	Reference
Six2⁺ Nephron progenitor	Premature nephron progenitor depletion	Increased <i>Bim</i> levels, leading to elevated cell death	(24,34)
Hoxb7⁺ Ureteric bud	Ureteric bud epithelium cysts. Adult mice develop hydronephrosis and hydroureter	Increased cellular proliferation and apoptosis leading to cyst formation. Perturbations to primary cilia outgrowth	(24,35)
Podocyte	Adult mice develop proteinuria and glomerulosclerosis	Podocyte foot effacement and loss of cellular maintenance	(36–39)
Proximal tubule	No overt renal phenotype. Kidney is however resistant against ischemia/reperfusion injury	Reduced renal damage and tubular apoptosis	(40)
Renin cell	Decreased renin cells accompanied by disrupted blood pressure regulation. Characteristic striped fibrosis.	Depletion of juxtaglomerular apparatus cells	(41)

Table 2

Murine models of cystic kidney disease with dysregulated miRNA activity.

Transgenic rodents	Dysregulated miRNA activity	miRNA target genes	Functional relevance to humans	Reference
<i>KspCre- CAG STOP^{fl/fl} miR-17~92</i>	Overexpression model of <i>miR-17~92</i> in nephron epithelium	<i>Pkd1, Pkd2, Pkhd1, Hnf-1β</i>	ADPKD/ARPKD/RCAD	(25)
<i>Bicc1^{-/-}</i>	miRs-17, 125a, 27a	<i>Pkd2, AC6, PKIa</i>	ADPKD	(42,43)
PCK rat	miR-15a?	<i>Cdc25A?</i>	ARPKD	(61)
<i>Six2Cre-Ef1a STOP^{fl/fl} Lin28a</i>	Overexpression model of <i>Lin28a</i> in nephron progenitors and epithelial derivatives	Undefined	Cystic phenotype is still undefined	(62)
<i>Wt1Cre-TetOn LIN28B</i> (doxycycline induction)	Overexpression model of <i>LIN28B</i> postnatally at day10	Undefined	Cystic phenotype is still undefined	(62)