

A Review on Role of miRNA in Kidney Diseases

Abhishek Negi, Shahrukh Husain, Priyadarshini*

Department of Biotechnology Jaypee Institute of Information Technology, Noida, Sector 62, UP, India.

Abstract

micro RNA or miRNA are short non-coding RNAs species that inhibit target gene expression by blocking protein translation or by mRNA degradation. Several studies have shown that miRNAs regulate thousands of gene expressions throughout the genomic machinery and play important roles in the physiological and pathological events. In addition, miRNA play important role in pathogenesis of various renal diseases, including diabetic nephropathy, renal carcinoma, polycystic kidney diseases, allograft rejection etc. miRNA as a biomarker has been an area of intensive researches, as it has been discovered to be potential therapeutic tools to manage several diseases. Therapeutic potential of miRNA-based treatment though promising but various challenges such as target specificity is still to be explored. Identification of miRNA and their target can provide a novel therapeutic candidate for curing various kidney diseases. In this review, we have stated the role of various miRNAs in the development of various renal diseases.

Keywords: *miRNA, Polycystic kidney disease (PKD), Chronic kidney disease (CKD), Nephropathy, Urolithiasis.*

Introduction

microRNAs or miRNA are short, endogenously produced, non-coding RNA molecules (21-25 nucleotides) which are located throughout the genome including introns and other non-coding transcription units [1]. Recent studies have shown that they are essential post-transcriptional regulators of gene expression in animals and plants. Functional studies indicate that miRNAs contribute to the regulation of almost every cellular process, and are intrinsically associated with much human pathology [2, 3].

miRNA get loaded onto a RNA-induced silencing complex (RISC), composed of Dicer, TAR RNA-binding protein (TRBP) and Argonaute 2 (Ago2) [16], whose binding to the 3' UTR region of the target mRNA, causes degradation of these mRNA strand.

This binding to target strand is atypically imperfect base pairing, because of which each miRNA is associated with degradation of a number of different mRNA [3, 4]. It was reported by Calin et al that a correlation exists between miRNA abundance and

human disease by indicating an association between the loss of miR-15 and miR-16 and the occurrence of B-cell leukemia [5].

Computational analysis predicts that around 60% of human genes are potential targets of the miRNAs [5]. They modulate the physiological and pathological processes by inhibiting target gene expression.

Manipulation of miRNA function with its sense or antisense oligonucleotide allows coordinated regulation of the entire downstream gene network. They also regulate renal development and contribute in the process of physiology and pathology [6].

miRNAs identification and characterization in various renal diseases give boost to the development of innovative diagnostic tools and therapeutic interventions. In this review article, we first briefly introduce the fundamental aspects of miRNA biogenesis and regulation and then summarize the role of miRNAs in various renal diseases.

Biogenesis

Biogenesis of miRNA occurs in two different pathways, namely canonical and non-canonical pathways. Canonical pathway is Drosha (RNA 3 like protein)/DGCR8 and dicer-dependent while the latter is independent of Drosha/DGCR8 or dicer. In both separate pathways, miRNA undergo processing and editing to get converted into a mature miRNA of a 21-25 nucleotide in size[5], which then get loaded onto a multiprotein structure called RISC that ultimately results in mRNA degradation or translation repression as shown in figure1.

The first step in the biosynthesis of miRNA involved transcription of the miRNA which itself required RNA polymerase II. It results in primary miRNA or pri-miRNA which is of several kilo bases in length and has a well-defined hairpin-like structure that gets

cleaved by the microprocessor complex present in the nucleus.

These microprocessor complexes consist of two major proteins, Drosha, and its cofactor DGCR8. DGCR8 recognizes the hairpin structure of the pri-miRNA, recruit Drosha and cleaves at the stem loop structure of pri-miRNA to give a precursor miRNA or pre-miRNA that is about 70 nucleotides in length. This pre- miRNA is then exported to the cytoplasm by exportin 5 which recognize the stem loop structure of the pre- miRNA. Pre-miRNA is further cleaved to give mature miRNA of 21-25 nucleotides in length by dicer which is an RNase III-type enzyme [6,7].

Canonical pathway is responsible for major miRNA production but several alternative pathways for miRNA production have been identified in the recent years. Pre-miRNA mimics like mitrons, short hairpin RNA,

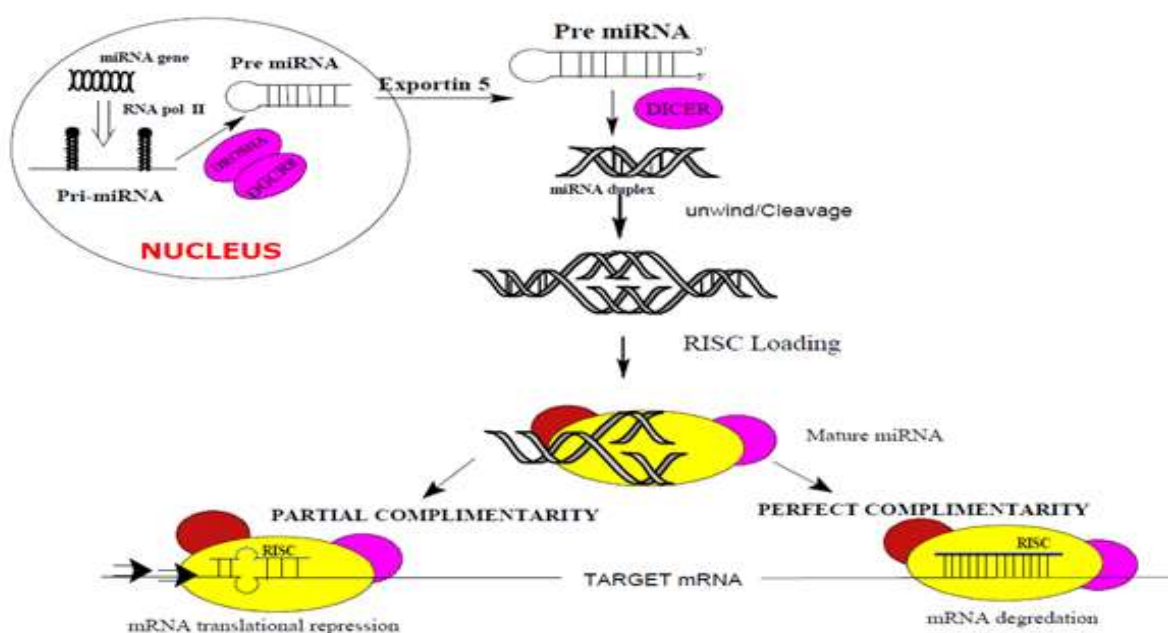


FIGURE1: Schematic diagram representing biogenesis of miRNA.

pseudo gene and other non-coding RNA can enter miRNA synthesis directly after processing with a dicer.

The miRNA effector complex RNA induce silencing complex or RISC contains a catalytic enzyme with endonuclease activity called Ago protein, which is responsible for target mRNA degradation [8]. In human, there are 4 Ago protein, Ago1-4, with an overlapping role in RISC formation [6].

The mature miRNA along with Ago protein get loaded onto the RISC complex, which is

directed to the mRNA based on complementary sequence between miRNA and mRNA. The Ago protein then degrades the mRNA but not always Ago protein is required for RISC formation and mRNA degradation. miRNA can induce target gene degradation or block translation mechanism [9]. In canonical pathway, the RISC complex binds at the 3'UTR region while in the non- canonical pathway the miRNA may bind to the ORF or the 5'UTR region. It's still unclear as to how exactly the binding of RISC complex to the 3'UTR region cause mRNA degradation but

several theories have been postulated for that[10].

Renal Physiology and miRNA

miRNA play critical role in regulating the gene expression that is important for a variety of cellular and physiological activities like cell cycle, growth, apoptosis, metabolism etc. thus maintaining renal homeostasis and whose deregulation can lead to various renal diseases [1].

The genetic ablation of Dicer, one of the enzymes required for the processing of pri-miRNA into mature miRNA, leads to global depletion of miRNA. Several knockout models for dicer have been created in podocytes, proximal tubular cells and juxtaglomerular cells in cultured cells and in mice for kidney [11, 12].

Podocyte specific dicer knockout model showed various problems like foot process effacement, proteinuria, tubulointerstitial fibrosis and glomerular sclerosis. These finding indicates the importance of dicer for podocyte homeostasis and renal functions [1]. Patel et.al result demonstrated that perturbation of miRNAs in renal epithelial cells contributes to PKD development in the organogenesis. Different animal models of Dicer deletion clearly show that miRNAs play an important role in the physiological functions of kidneys [13].

Mice with Dicer ablation in kidney tubules and collecting ducts showed hydronephrosis, hydro ureter (dilation of the ureter) and cyst formation. Dicer knockout from epithelial tubules showed that mir-200 family(miR-200a, miR-200b, miR-200c, miR-141, and miR-429) involved in targeting polycystin-1 and interfere with tubule formation in IMCD3 (inner medullary collecting duct cell line) cells, regulate polycystin-1 level vital for cystogenesis in PKD.

Another miRNA family, miR-17(miR-20a/b, miR-93, and miR-106a/b), is implicated in PKD (polycystic kidney disease) because it targets polycystin-2, involved in cyst formation. Similarly, dicer knockout in the juxtaglomerular cell leads to reduced expression of renin in the kidney which leads to decreased blood pressure, vascular abnormalities and renal striped fibrosis in the mice model [1, 14].

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Diabetic Nephropathy

Nephropathy is a term used for the diseases that are associated with kidney. Diabetic nephropathy results in kidney glomerular cell inflammation, more precisely mesangial cell inflammation called hypertrophy, which further results in podocyte dysfunction followed by renal fibrosis.

TGF β plays the central critical role in diabetic nephropathy by regulating the miRNA. TGF β pathway activation results in up-regulation of mir-192, which targets the E-box repressor Zeb-1(zinc finger e-box binding homeobox1) and Zeb-2. Since miRNA192 act on repressor of transcription, so all the genes with E-box in their promoter region are up-regulated including mir-217, mir-216a, mir-200b/c and collagen type I $\alpha 2$ (Colla2) gene leading to renal fibrosis.

E-box (Enhancer Box) is a DNA sequence found in some promoter regions in eukaryotes that act as a protein-binding site and has been found to regulate gene expression in neurons, muscles, and other tissues. Its specific DNA sequence, CANNTG (where N can be any nucleotide), with a palindromic canonical sequence of CACGTG is recognized and bound by transcription factors to initiate gene transcription. Once the transcription factors bind to the promoters through the E-box, other enzymes can bind to the promoter and facilitate transcription from DNA to mRNA [15].

mir-217 and mir-216a activate Akt by targeting PTEN and results in mesangial cell hypertrophy. Akt kinase is activated by transforming growth factor- $\beta 1$ (TGF- β) in diabetic kidneys and has important roles in fibrosis, hypertrophy and cell survival in glomerular mesangial cells. However, the mechanisms of Akt activation by TGF- β are not fully understood. TGF- β activates Akt in glomerular mesangial cells by inducing the miRNAs miR-216a and miR-217, both of which target PTEN (phosphatase and tensin homologue) an inhibitor of Akt activation.

These miRNAs are located within the second intron of a non-coding RNA (RP23-298H6.1-001). The RP23 promoter was activated by TGF- β and miR-192 through E-box-regulated mechanisms. Akt activation by these miRs led to glomerular mesangial cell survival and hypertrophy, which were similar to the effects of activation by TGF- β . These studies reveal a mechanism of Akt activation through PTEN downregulation by two miRs, which are regulated by upstream miR-192 and TGF- β . Due to the diversity of PTEN function, this miR-amplifying circuit may have key roles, not only in kidney disorders but also in various other diseases [16].

Renal Cell Carcinoma

miRNA expression level were studied for renal cell carcinoma (RCC) and it was found that decreased level of mir-199a expression causes higher expression of nuclear GSK-3 β (Glycogen synthase kinase 3 beta), which in turn leads to tumor. So repression of this miRNA can help treat RCC. A tumor suppressor von Hippel-Lindau (VHL) also known as pVHL protein encoded by VHL gene is inactivated in RCC that leads to up-regulation of mir210, mir-155, and mir-21 in RCC tumor tissue. In recent studies mir-92, mir-17-5p and mir-224 were found to repress VHL and HIF-1 alpha by directly targeting their expression or their down streaming signaling pathways.

While in another study it was found that VHL-dependent mir-204 up-regulation could suppress tumor progression by preventing macro autophagy through targeting LC3B [6]. It was found that mir-205 level was reduced in renal cancer cell lines and RCC samples, which was involved in suppression of genes targets encoding Src, Lyn, Yes and Lck that cause's cell migration, invasion, and cell proliferation. Local administration of this miRNA might reduce the tumor growth. Expression of mir-584 too was downregulated in RCC sample, which was correlated with higher expression of ROCK-1 protein that control cell motility.

Downregulation of mir-1285, which target oncogenic genes that inhibit cancer cell proliferation, invasion, and migration, thus contributing to RCC development. Repression of this miRNA could help treat RCC.

Polycystic Kidney Disease

Pathogenic mutations in miRNA are rare and most polymorphism lies outside the mature sequence of miRNA. Polymorphism in 3'UTR modifying the miRNA targeting site is usually associated with different diseases. Many genes are important for the formation and functioning of kidney, mutation in those gene causes hypodysplasia (condition in which an organ, most often the kidneys, is abnormally small and malformed)[2].

Autosomal dominant polycystic kidney disease (ADPKD), one of the most common inherited renal disease is caused by mutation in PKD-1 and PKD-2 genes. Differential expression of polycystic-2 causes abnormal proliferation of renal tubular and biliary epithelial cells, which lead to cystogenesis.

The regulating role of various miRNAs controlling the expression of PKD genes has been explored recently. mi-RNAs such as miR-17 directly targets the 3'UTR of PKD2 and post-transcriptionally represses the expression of PKD2 on the other hand over-expression of miR-17 enhances cell proliferation through post-transcriptional repression of PKD2 in HEK293T cells [20].

While in another study on rat model for autosomal recessive polycystic kidney disease (PKD) shows that mir-15a played a critical role in PKD. This miRNA was associated with repression of cell cycle regulator Cdc25a mRNA. Overexpression of Cdc25a leads to proliferation and cytogenesis, which ultimately promotes cyst formation.

CKD (Chronic Kidney Disease)

The two main causes of chronic kidney disease are diabetes and high blood pressure, which are responsible for up to two-thirds of the CKD cases. Diabetes mellitus is a leading cause of CKD, and hypertension is a major contributor to the progression, as many kidney diseases result in increased blood pressure, which in turn promotes the progression of kidney disease [21].

In CKD patient, there is a correlation between the decreases in circulating miRNAs with the severity of renal injury. In CKD mouse kidneys; there is an elevated expression of mir-146a, which apparently correlates with the pathological phenotype development [6]. Also the fact that, miR-146a

expression in the kidneys and its urinary excretion was specifically associated with the development of interstitial lesions and correlated with inflammatory cell infiltration.

In microarray analysis of kidneys, the expression of miR-146a was elevated in B6.MRLc1 CKD mice that spontaneously develop renal inflammation with age.

Table 1: Mirna in Different Physiological and pathological processes.

S.No.	miRNA	Target	Function	References
1	mir-92, mir-17-5p and mir-224	Repress VHL and HIF-1 alpha	RCC (renal cell carcinoma)	6
2	mir-205	genes targets encoding Src, Lyn, Yes and Lck	cause's cell migration, invasion and cell proliferation	6
3	mir-200	polycystin-1	regulate polycystin-1 level vital for cystogenesis in PKD	14
4	mir-192	E-box repressor Zeb-1 and Zeb-2	Up-regulation of all genes with E-box in there promoter region leading to renal fibrosis	16
5	mir-217 & 216a	PTEN (phosphatase & tensin homologue)	activate Akt kinase and results in mesangial cell hypertrophy	16
6	miR-17	3'UTR of PKD2	transcriptionally represses the expression of PKD2	14,20
7	miR-31	GNA13 mRNA	Control cell invasion	26
8	miR-155	transcription factor c/ebp β	regulating cytokine production	27,28
9	mir-21	TCF21 and PTEN	Affect KISS1 associated renal cell carcinoma cell invasion pathway	34
10	mir-10b	Not yet demonstrated	Cell cycle arrest	35
11	mir-34a	CD44 transcriptional complex	Suppresses cell proliferation and metastasis	36
12	miR-141, 200c and miR-429	Not yet known	Regulates epithelial-mesenchymal transition (EMT)	19,37

Primary-microRNA analysis found that elevated miR-146a [sol] b expression in the kidneys of B6.MRLc1 mice were mainly derived from miR-146a rather than miR-146b, and this expression increased with the development of CKD [22].

Problems with CKD screening are related to the need for both more efficient screening strategies and the development of better screening tests for pre-clinical disease detection, facilitating earlier stage treatment and leading to a better outcome.

In Cancer

In a comparative study on tumor tissue and normal tissue, it was found that most of the miRNA genes are located at the fragile sites of human genome that are subjected to various chromosomal abbreviation that leads to improper expression of these miRNA genes in tumor tissue, leading to a potentially faulty regulation of their target mRNAs.

Urolithiasis

The term refers to the formation of renal calculi of calcium oxalate monohydrate (COM) in the various parts of the urinary system. These renal calculi are formed when the urine is supersaturated with salt and minerals such as calcium oxalate, struvite

(ammonium magnesium phosphate), uric acid and cystine. 60-80% of stones contain calcium when the urine is persistently acidic.

Depending upon the location of formation of the calcium oxalate calculi in the urinary system there are majorly three forms of urolithiasis i.e. inside the urinary bladder (cystolithiasis), in the ureter (ureterolithiasis), inside the kidney (nephrolithiasis). The major reason behind the formation of these calculi is urinary stasis which due to the failure of emptying the bladder completely on urination. The classical feature of these renal calculi is sudden severe pain in the kidney or in the region of the ureter, resulting in dilatation, stretching and spasm of the ureter.

Urolithiasis affects approximately 10% of individuals in Western societies by the seventh decade of life. The most common form, idiopathic calcium oxalate urolithiasis, results from the interaction of multiple genes and their interplay with dietary and environmental factors. To date, considerable progress has been made in identifying the metabolic risk factors that predispose to this complex trait, among which hypercalciuria predominates. The specific genetic and epigenetic factors involved in urolithiasis have remained less clear, partly owing to the

candidate gene and linkage methods that have been available until now, being inherently low in their power of resolution and in assessing modest effects in complex traits. However, together with investigations of rare, Mendelian forms of urolithiasis associated with various metabolic risk factors, these methods have afforded insights into biological pathways that seem to underlie the development of stones in the urinary tract.

Monogenic diseases account for a greater proportion of stone formers in children and adolescents than in adults. Early diagnosis of monogenic forms of urolithiasis is of importance owing to associated renal injury and other potentially treatable disease manifestations, but diagnosis is often delayed because of a lack of familiarity with these rare disorders[32].

Urolithiasis affects around 10% of the US population with an increasing rate of prevalence, recurrence, and penetrance. The causes for the formation of most urinary calculi remain poorly understood, but obtaining the chemical composition of these stones might help identify key aspects of this process and new targets for treatment. The majority of urinary stones are composed of calcium that is complexed in a crystalline matrix with organic and inorganic components. Surprisingly, mitigation of urolithiasis risk by altering calcium homeostasis has not been very effective.

Thus, studies to identify other therapeutic stone-specific targets, using proteomics, metabolomics, and microscopy techniques, have been conducted, revealing a high level of complexity. The data suggest that numerous metals other than calcium and many non-metals are present within calculi at measurable levels and several have distinct distribution patterns. Manipulation of the levels of some of these elemental components of calcium-based stones has resulted in clinically beneficial changes in stone chemistry and rate of stone formation.

The elementome-the full spectrum of elemental content-of calcium-based urinary calculi is emerging as a new concept in stone research that continues to provide important insights for improved understanding and prevention of urinary stone disease [33].

The adhesion of COM crystals to the renal tubular cells results in the change of expression profiles of various miRNAs. This

differential expression of the miRNAs is associated with apoptosis, regulation of metabolic process, intracellular signaling cascade, insulin signaling pathway and type 2 diabetes[38].

In a study, a multi-step approach combining microarray miRNA and mRNA expression profile and bioinformatics analysis was adopted to analyze dysregulated miRNAs and genes in genetic hypercalciuric stone-forming (GHS) rat kidneys, using normal Sprague-Dawley (SD) rats as controls. This strategy showed 2418 mRNAs and 19 miRNAs as significantly differentially expressed and also they suggested that rno-miR-674-5p, rno-miR-672-5p, rno-miR-138-5p and rno-miR-21-3p may play important roles in the regulatory mi-RNA gene network through network analysis[39].

miRNA as Drug Targets

There is hardly any disease that doesn't show significant change in miRNA expression level when compared with normal tissue. Their extensive involvement in human diseases has given a new idea for development of therapeutic strategies. The principle for developing miRNA-based therapies remain the same as for any other drug with target identification, validation, and development of effective delivery system to assure satisfactory efficacy, specificity and lack of toxicity. There are two ways of developing the miRNA-based therapeutics: miRNA antagonists and miRNA mimics [40, 41].

miRNA mimics are used to restore a loss of function also known as 'miRNA replacement therapy' in which miRNA are re-introduced into the diseased cells that are normally expressed in healthy cell such that they lead to reactivation of normal cellular pathways. While miRNA antagonists are generated to inhibit endogenous miRNAs that show a gain-of-function in diseased tissues. It's working approach is similar to any other inhibitory therapeutics wherein these miRNA therapeutics would target a specific miRNA strand forming an irreversible duplex miRNA structure which is not processed by RISC and/or degraded[40].

Delivery System of miRNA-Based Drugs

Various diseases discussed above provide mounting evidence that miRNA-based therapies hold great promise in curing them

[29]. But critical hurdles often involved in the delivery of these miRNA-targeting agents like off-target binding and side effects, poor in-vivo stability, disruption of endogenous RNA machinery etc. need to be overcome before being used commercially. Both viral vectors and non viral delivery systems can be developed to avoid these challenges [30].

Adeno associated viruses (AAV) are commonly used for miRNA-based drugs as tissue specific promoters increased their delivery efficiency to target organs. In a study AAV-mediated delivery of mir-26a for a mouse model of liver cancer, progress of cancer was found to be hindered [3]. Liposomes are also used for delivery of conventional drugs and miRNA-based drugs, they have a high drug to lipid ratio and penetrate to a tumor in high concentration [5].

But because they penetrate in all tissue and can have deleterious effect in off-target tissues these therapeutic drugs could be used with promoters that are solely for a particular tumor type for better result.

miRNA as Biomarkers

miRNAs are quite stable, much more stable than mRNAs. As a matter of fact, miRNAs are detectable in various body fluids such as serum, saliva, tears, and urine. Recent studies have proved the possibility of finding miRNA in plasma and serum of humans and animals, opening the possibility of using them as potential biomarkers for various diseases [31].

Their stability and presence in body fluids pave the way for the use of miRNAs as diagnostic and prognostic biomarkers for human diseases miRNA biomarkers have several remarkable features.

Firstly, as stated above, miRNAs are highly stable. Secondly, miRNA can be reliably analyzed and quantified by real-time PCR. The analysis is relatively simple and extremely sensitive. Thirdly, because the analysis is sequence-based, it is very specific.

Finally, specific miRNAs play important pathogenic roles in diseases, making it possible to use these miRNAs to monitor the occurrence and progression of the diseases [6].

Future Challenges

There are several challenges in exploring the role of miRNA in different diseases, their biology and most importantly regulation of the miRNA production mechanism. Although computational analysis has shown that around 60% of human genes could be a potential target for miRNAs, but it has been proved experimentally in only a few cases. Further, their mechanism for degradation of the target mRNA is still unclear. Using them as therapeutic agents for various diseases still faces major challenges regarding development of a reliable and safe target specific delivery system and avoiding off-target toxicity.

References

1. Schena FP, Serino G, Sallustio F. MicroRNAs (2014) in kidney diseases: new promising biomarkers for diagnosis and monitoring. *Nephrol Dial Transplant*(4):755-63.
2. Chua JH, Armugam A, Jeyaseelan K. MicroRNAs: (2009) biogenesis, function and applications. *Curr Opin Mol Ther*; 11(2):189-99.
3. Chandrasekaran K, Karolina DS, Septhamian S, Armugam A, Wintour EM, Bertram J. (2012) al. Role of microRNAs in kidney homeostasis and disease. *Kidney Int*;81(7):617-27.
4. Saal S, Harvey SJ. Micro RNAs (2009) and the kidney: coming of age. *Curr Opin Nephrol Hypertens* ;18(4):317-23.
5. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. (2002) Frequent deletions and down regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA*; 99(24):15524-9.
6. Li JY, Yong TY, Michael MZ, Gleadow JM. Review: (2010) The role of microRNAs in kidney disease. *Nephrology (Carlton)*; 15(6):599-608.
7. Lee RC, Feinbaum RL, Ambros V. The C. (1993) elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*;75:843-54.
8. Khvorovova A, Reynolds A, Jayasena SD. Functional siRNAs miRNAs (2003) exhibit strand bias. *Cell* 2003;115(2):209-16.
9. Park CY, Choi YS, McManus MT. Analysis (2010) of microRNA knockouts in mice. *Hum mol Genet*.19(R2):R169-75.
10. Cheloufi S, Dos Santos CO, Chong MM,

- Hannon GJ. A (2010) dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature*;465:584-89.
11. Harvey SJ, Jarad G, Cunningham J, Goldberg S, Schermer B, Harfe BD et al. (2008) Podocyte-specific deletion of dicer alters cytoskeletal dynamics and causes glomerular disease. *J Am Soc Nephrol*; 19: 2150–8.
 12. Ho J, Ng KH, Rosen S, Dostal A, Gregory RI, Kreidberg JA. (2008) Podocyte-specific loss of functional microRNAs leads to rapid glomerular and tubular injury. *J Am Soc Nephrol*; 19(11):2069–75.
 13. Patel V, Hajarnis S, Williams D, Hunter R, Huynh D, Igarashi P. MicroRNAs(2012) regulate renal tubule maturation through modulation of Pkd1. *J Am Soc Nephrol*; 23(12):1941–8.
 14. Hertweck A, O'Connor E, Godwin J et al. *J Exp Med* 2005;201(9):1367-73.
 15. Wang V, Wu W. MicroRNA-based (2009) therapeutics for cancer. *BioDrugs*; 23(1):15–23.
 16. Wei Q, Mi QS, Dong Z. The (2013) regulation and function of microRNAs in kidney diseases. *IUBMB Life* 65(7):602-14.
 17. Lodygin D, Tarasov V, Epanchintsev A, Berking C, Knyazeva T, Körner H et al. (2008) Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. *Cell cycle*;7(16):2591–2600.
 18. Kort EJ, Farber L, Tretiakova M, Petillo D, Furge KA, Yang X et al. (2008) The E2F3-Oncomir-1 axis is activated in Wilms' tumor. *Cancer Res*;68(11):4034-4038.
 19. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G et al. (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol*; 10(5):593–601.
 20. Kato M, Arce L, Natarajan R. MicroRNAs (2009) and their role in progressive kidney diseases. *Clin J Am Soc Nephrol*; 4(7):1255-66.
 21. Beltrami C, Clayton A, Phillips AO, Fraser DJ, Bowen T. Analysis (2012) of urinary microRNAs in chronic kidney disease. *Biochem Soc Trans*;40(4):875-9.
 22. Qingqing Wei, Qing-Sheng Mi, Zheng Dong. (2013) The Regulation and Function of MicroRNAs in Kidney Diseases. *IUBMB Life*; 65(7):602–614.
 23. Godwin JG, Ge X, Stephan K, Jurisch A, Tullius SG, Iacomini J. Identification(2010) of a microRNA signature of renal ischemia reperfusion injury. *Proc. Natl Acad. Sci. USA*;107(32),14339-44.
 24. Scian MJ, Maluf DG, Mas VR. Mi RNAs (2013) in Kidney Transplantation: potential role as new biomarkers. *Expert Rev Mol Diagn*;13(1):93-104.
 25. Sui W, Dai Y, Huang Y, Lan H, Yan Q, Huang H. Microarray(2008) analysis of microRNA expression in acute rejection after renal transplantation. *Transpl. Immunol*; 19(1):81–5.
 26. Yi T, Zhai B, Yu Y, Kiyotsugu Y, Raschle T, Etzkorn M et al. (2014) Quantitative phosphoproteomic analysis reveals system-wide signaling pathways downstream of SDF-1/CXCR4 in breast cancer stem cells. *Proc. Natl. Acad. Sci. USA*; 111(21):E2182- 90.
 27. Tang J, Ahmad A, Sarkar FH. (2012) The role of microRNAs in breast cancer migration, invasion and metastasis. *Int J Mol Sci*;13(10):13414–37.
 28. Van Schooneveld E, Wildiers H, Vergote I, Vermeulen PB, Dirix LY, Van Laere SJ. (2015) Dysregulation of microRNAs in breast cancer and their potential role as prognostic and predictive biomarkers in patient management. *Breast Cancer Res*;17:21.
 29. Takahashi RU, Miyazaki H, Ochiya T. (2015) The Roles of Micro RN as in Breast Cancer. *Cancers (Basel)*;7(2):598-6.
 30. Ma MT, He M, Wang Y, Jiao XY, Zhao L, Bai XF, et al. (2013) MiR-487a resensitizes mitoxantrone (MX)-resistant breast cancer cells (MCF-7/MX) to MX by targeting breast cancer resistance protein (BCRP/ABCG2). *Cancer Lett*; 339(1),107–15.
 31. Takahashi RU, Miyazaki H, Ochiya T (2015) The Roles of Micro RN as in Breast Cancer. *Cancers (Basel)*;7(2):598-6.
 32. Andreas G Bader and Paul Lammers. The Therapeutic Potential of microRNAs, *Innovations in Pharmaceutical Technology*.
 33. Eva van Rooij, Angela L. Purcell, Arthur A. Levin. *Developing MicroRNA Therapeutics*.
 34. Bohan Wang, Bolin Wu, Jun Liu, Weimin Yao, Ding Xia, Lu Li et. al. (2014) Analysis of Altered MicroRNA Expression Profiles in Proximal Renal Tubular Cells in Response to Calcium Oxalate Monohydrate Crystal Adhesion: Implications for Kidney Stone Disease. *Plos One*. 9(7):e101306.
 35. Lu Y, Qin B, Hu H, Zhang J, Wang Y, Wang (2016) Q et al. Integrative microRNA-gene expression network analysis in genetic hypercalciuric stone-forming rat kidney. *Peer*;4:e1884.

36. Zhang H1, Guo Y, Shang C, Song Y, Wu B.(2012)miR-21 downregulated TCF21 to inhibit KISS1 in renal cancer. Urology. Dec; 80(6):1298-302.e1.
37. Cheng He, Xiaokun Zhao, Hongyi Jiang, ZhaohuiZhong, Ran Xu. (2015) Demethylation of miR-10b plays a suppressive role in ccRCC cells.Int J ClinExpPathol;8(9):10595-10604.
38. Zhang Y1, Wang Z, Gemeinhart RA. (2013) Progress in microRNA delivery. J Control Release;172(3):962-74.
39. Chim SS, Shing TK, hung EC, lueng TY, lau TK, Chiu RW, (2008) et al. Detection and characterization of placental microRNA in maternal plasma. Clin Chem.; 54(3):482-90.
40. Yu G, Li H, Wang J, Gumireddy K, Li A, Yao W, et. al. (2014) miRNA-34a suppresses cell proliferation and metastasis by targeting CD44 in human renal carcinoma cells. J Urol. Oct;192(4):1229-37.
41. Kun Tang, Hua Xu (2015) Prognostic value of meta-signature miRNAs in renal cell carcinoma: an integrated miRNA expression profiling analysis. Scientific Reports 5, Article number:10272.

