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Review

MicroRNAs associated with the development of kidney diseases in humans and animals

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Abstract: Mature microRNAs (miRNAs) are single-stranded RNAs with approximately 18–25 bases, and their sequences are highly conserved among animals. miRNAs act as posttranscriptional regulators by binding mRNAs, and their main function involves the degradation of their target mRNAs. Recent studies revealed altered expression of miRNAs in the kidneys during the progression of acute kidney injury (AKI) and chronic kidney disease (CKD) in humans and experimental rodent models by using high-throughput screening techniques including microarray and small RNA sequencing. Particularly, miR-21 seems to be strongly associated with renal pathogenesis both in the glomerulus and tubulointerstitium. Furthermore, abundant evidence has been gathered showing the involvement of miRNAs in renal fibrosis. Because of the complex morphofunctional organization of the mammalian kidneys, it is crucial both to determine the exact localization of the kidney cells that express the miRNAs, which has been addressed mainly using *in situ* hybridization methods, and to identify precisely which mRNAs are bound and degraded by these miRNAs, which has been studied mostly through *in vitro* analysis. To discover novel biomarker candidates, miRNA levels in urine supernatant, sediment, and exosomal fraction were comprehensively investigated in different types of kidney disease, including drug-induced AKI, ischemia-induced AKI, diabetic nephropathy, lupus nephritis, and IgA nephropathy. Recent studies also demonstrated the therapeutic effect of miRNA and/or anti-miRNA administrations. The intent of this review is to illustrate the state-of-the-art research in the field of miRNAs associated with renal pathogenesis, especially focusing on AKI and CKD in humans and animal models. (DOI: 10.1293/tox.2017-0051; J Toxicol Pathol 2018; 31: 23–34)

Key words: microRNA, kidney disease, acute kidney injury, chronic kidney disease, biomarker, exosome

Introduction

Mature microRNAs (miRNAs) act as posttranscriptional regulators by binding mRNAs, and their main function involves the degradation of their target mRNAs. Recent studies revealed altered expression of miRNAs in the kidneys during the progression of acute kidney injury (AKI) and chronic kidney disease (CKD) in humans and experimental animals by using high-throughput screening techniques. Because of the complex morphofunctional organization of the mammalian kidneys, it is crucial both to determine the exact localization of the kidney cells that express the miRNAs, which has been addressed mainly using *in situ* hybridization methods, and to identify precisely which mRNAs are bound and degraded by these miRNAs, which has been studied mostly through *in vitro* analysis. To discover novel biomarker candidates, miRNA levels in urine supernatant, sediment, and exosomal fraction were comprehensively investigated in different types of kidney disease. The intent of this review is to illustrate the state-of-the-art research in the field of miRNAs associated with the pathogenesis of AKI and CKD in humans and animal models.

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The Biosynthesis of microRNAs (miRNAs)

Non-coding RNAs (ncRNAs) are transcribed from the non-coding region of the genome, which is considered to be a "gene desert," and they can regulate the expression of genes on the coding region. miRNAs are one type of ncRNAs; their mature form is a single-stranded RNA molecule between 18 and 25 bases long, and their sequences are highly conserved among animal species. Initially, an miRNA molecule is transcribed by RNA polymerase II as a primary miRNA (pri-miRNA) having a stem-loop structure,

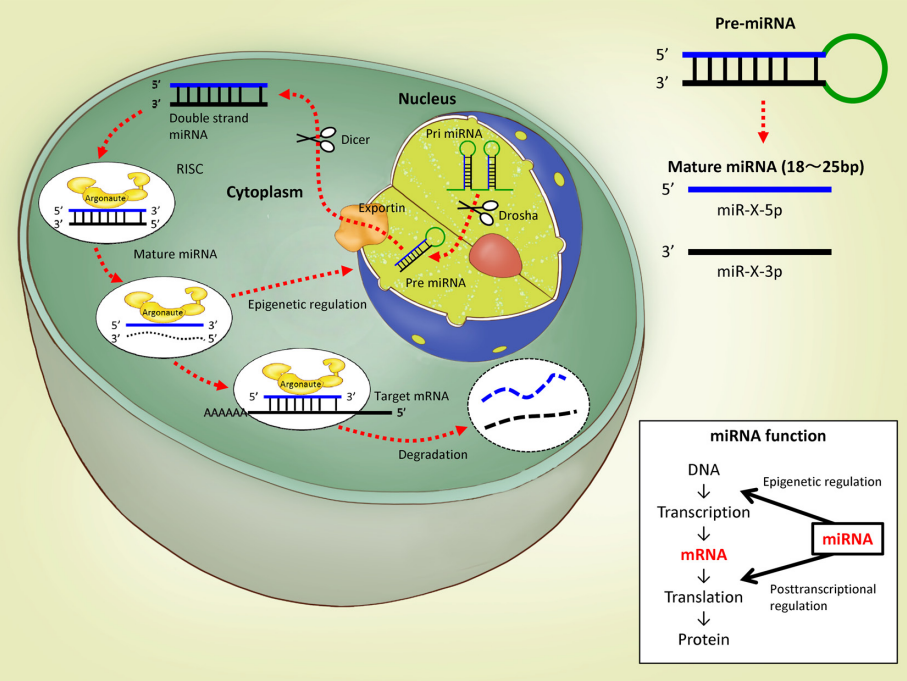

Fig. 1. The biosynthesis and function of miRNAs.

and then this pri-miRNA is cleaved into a precursor miRNA (pre-miRNA) of approximately 70 bases by an RNase III enzyme, Drosha (Fig. 1). The pre-miRNA is transported into the cytoplasm through the nuclear export protein Exportin 5 and is cleaved into double-stranded miRNA mainly by Dicer, another RNase III enzyme. The double-stranded miRNA is incorporated into the RNA-induced silencing complex (RISC) containing Argonaute proteins, and one strand of the duplex (called passenger strand, miR-X*, or miR-X-star) is degraded. The other chain of the duplex (called the guide strand or miR-X) is thus free to bind the 3′ untranslated region (UTR) of the target mRNA. In most cases, the guide strand miR-X showed higher abundance compared with the passenger strand miR-X*. On the other hand, to avoid problems if the abundance of each strand changes between tissues, developmental stages, or species, this previously accepted nomenclature has been largely abandoned, and the latest convention is to name mature miRNAs by the arm of the pre-miRNA from which they are derived, regardless of their abundance: those from the 5′ arm are named miR-X-5p, and those from the 3′ arm are named miR-X-3p. If the miRNA sequence corresponds without any mismatches to the target mRNA sequence and if their binding is therefore perfect, RISC will degrade the target mRNA, but if they do not completely match, the mRNA degradation will be delayed, and the miRNA-mRNA duplex will accumulate in the cytoplasmic processing body (P body). For binding to the target mRNA, 2 to 8 bases (called the seed sequence) of the 5′ region of the miRNA are important. Thus, miRNAs regulate the expression of their target mRNAs at the post-transcriptional level. A recent review by Luo *et al.*[1] pointed out the importance of the regulatory circuit existing between epigenetic modulation and miRNAs, with special regard to cancer, as miRNA genes can be epigenetically regulated by DNA methylation and/or histone modification, and in turn, a subclass of miRNAs named “epi-miRNAs” was recognized to directly target epigenetic regulators such as DNA methyltransferases (DNMTs), HDACs, or components of the polycomb repressor complexes.

miRNAs in the Kidneys

At present (August 2017), the miRNA database (miRbase; http://www.mirbase.org/) contains 2,588, 690, 793, 411, 453, 765, and 1,915 miRNAs found in humans, horses, bovines, pigs, dogs, rats, and mice, respectively. In 2004, using miRNA microarray analysis, Sun *et al.*[2] reported that miR-192, miR-194, miR-204, miR-215, and miR-216 are abundantly expressed in the human kidneys. In a later study, Tian *et al.*[3] showed that the expression levels of miR-192 and miR-194 are higher in the cortex than in the medulla of the rat kidneys, while miR-27b was highly expressed in the medulla. These basic comprehensive miRNA expression data are quite important to help clarify the function of miRNAs and identify the miRNAs that could be successfully used as biomarkers in several kidney diseases, because the kidney structures are complexly organized with essential contributions from various cell types, such as glomerulus-composing cells including podocytes, mesangial cells, and endothelial cells; tubulointerstitium-composing cells including the epithelial cells of the proximal tubules (PTs), attenuated tubules, distal tubules (DTs), and collecting ducts (CDs);

capillary endothelial cells; interstitial cells; and immune cells. Renal pathological events can be mainly divided into two types, glomerular and tubulointerstitial lesions. One of the pathological features of renal lesions, i.e., their localization in the organ, differs among kidney diseases. The glomeruli tend to be primarily damaged in focal segmental glomerulosclerosis (FSGS) and in the various types of glomerulonephritis due to genetic mutations or immunological changes and secondarily damaged in systemic diseases such as numerous infectious diseases or autoimmune diseases including systemic lupus erythematosus (SLE; https://www.kidney.org/). On the other hand, the tubulointerstitium tends to be damaged due to the use of antibiotics or anticancer drugs, which usually involve injuries of PTs[4]. Furthermore, the localization of lesions in hereditary kidney diseases depends on the kind of mutated genes. Thus, since renal injury features differ among kidney diseases, we have to carefully examine the miRNA expression profile in each kidney disease in relation to the type of cells expressing the miRNA in question, in particular for application of miRNA molecules as disease markers.

Acute Kidney Injury and miRNAs

AKI is defined as a sudden renal dysfunction or kidney damage that occurs within 48 hours or 7 days, according to international criteria such as RIFLE, AKIN, and KDIGO criteria[5]. It includes renal dysfunction as indicated by a decrease in glomerular filtration and increase in serum creatinine and include a wide range of diseases including early renal tubular injuries. It is commonly observed in hospitalized patients, in particular elderly patients. AKI can be induced by decreased blood flow due to shock, bleeding, severe diarrhea, organ failure, and drugs/chemicals[5]. For the creation of AKI animal models, ischemia reperfusion (IR) models (prerenal factor), folic acid or adenine administration models (renal factor), or unilateral ureter obstruction (UUO) models (post-renal factor) have been globally adopted to examine the pathogenesis of these various lesions mainly using experimental rodents including mice and rats. Based on several experimental data and clinical evidence, hepatitis A virus cellular receptor 1 (Havcr1, also known as kidney injury molecule-1; KIM-1/TIM-1), lipocalin 2 (LCN2, also known as neutrophil gelatinase-associated lipocalin; NGAL), and liver-type fatty acid binding protein (L-FABP) have been found to be excellent markers to predict tubulointerstitial lesions in AKI[6], as they are localized in the PTs.

miRNAs associated with the development of AKI have mainly been reported in mouse, rat, and humans showing ischemic status after surgical operations (Table 1). To avoid confusion, we have provided a list of miRNA target molecules with their descriptions (Table 2). The strongest evidence was obtained for miR-21[7–9]. In fact, its expression was found to be increased in the mouse IR kidneys and showed a pathological significance by targeting several factors including MKK3[10], which explains the inhibitory action of miR-21 against interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). Xu *et al.*[11] reported PDCD4 as a target of miR-21, clarifying the observed protective effect of miR-21 against apoptosis. Similarly, after IR, miR-146a-deficient mice exhibited more severe tubulointerstitial lesions than wild-type mice, and overexpression of miR-146a reduced both IRAK1 and CXCL8/CXCL1 expression in injured tubular cells[12]. *In vivo*, more severe renal ischemia-reperfusion injury (IRI) was also found to be associated with increased miR-146a expression in both allografts and urine of human kidney transplant recipients[12]. miR-24 was upregulated in mouse IR kidneys and in human ischemic kidneys, especially in renal endothelial and tubular epithelial cells, where it was shown to exert a deleterious pro-apoptotic effect by targeting the H2A.X and HO1[13]. Other reports also showed increased levels of specific miRNAs related to AKI, such as miR-125b, which regulates the AHRR in cisplatin-treated mice[14] and IR rats[8]; miR-150, which modulates the expression of IGF1R in myocardial infarction-induced AKI mice[15]; miR-489, which binds the PARP-1 mRNA in IR mice[16]; miR-494, which downregulates ATF3 in IR mice[17]; and miR-687, which targets PTEN in IR mice[18]. In cisplatin-induced AKI mice, miR-34a was found to be induced via P53[19] and to control the expression of SIRT1[20]. Another study showed that, in IR mice, miR-34a regulates the autophagic activity in tubular epithelial cells by targeting ATG4B[21]. In the IR rat kidneys, increased miR-127 expression appeared to be positively associated with cell-matrix and cell-cell adhesion maintenance through downregulation of KIF3B[22]. Saikumar *et al.*[9] reported that miR-21 as well as miR-155 showed increased levels in a rat model of AKI induced by IR or gentamicin administration. Contrast media also induces AKI and is associated with an increased risk of cardiovascular events. Gutiérrez-Escolan *et al.*[23] revealed increased levels of miR-30 family members (miR-30a, c, e) in the rat kidney showing contrast-induced nephropathy. Thus, several reports showed increased expression for various miRNAs in kidney tissues affected by AKI in the species examined, while similar reports showing decreased miRNA expression levels were relatively scarce.

Chronic Kidney Disease and miRNA

CKD is defined as abnormalities of kidney structure or function continuing for more than 3 months[24]. Thirteen million Japanese[25] and 30 million American adults and have CKD, and millions of others are at increased risk (National Kidney Foundation, https://www.kidney.org/). Complex factors can lead to CKD, such as diabetes, hypertension, autoimmune diseases, systemic infections, urinary tract infections, urinary stones, neoplasia, hereditary mutations, AKI, or drugs/chemicals. Spontaneous animal models for CKD are scarce, but several mouse strains showing autoimmune diseases have been adopted as CKD models[26]. In addition, experimental rodents showing chronic stages due to UUO or diabetes develop CKD associated with renal fibrosis[27–30].

Several reports helped to clarify the relation between CKD and miRNA (Table 1). Similar to what was observed

Table 1. miRNAs Showing Altered Expression in the Kidneys in AKI Or CKD

	Species	miRNA	Expression	Status/organs or cells	Putative target or downstream molecules	References
AKI	Mouse, rat, human	miR-21	Up	Ischemia/kidney	MKK3, PDCD4	7–11
	Mouse, human	miR-24	Up	Ischemia, allograft/kidney	H2AFX, HO1	13
	Rat	miR-30a, c, e	Up	Contrast-induced nephropathy/kidney	-	23
	Mouse	miR-34a	Up	Ischemia, toxic injury/kidney	SIRT1, ATG4B	19–21
	Mouse, rat	miR-125b	Up	Ischemia/kidney	AHRR	8, 14
	Rat	miR-127	Up	Ischemia/proximal tubular cell	KIF3B	22
	Mouse, human	miR-146a	Up	Ischemia, allograft/proximal tubular cell	IRAK, CXCL8/CXCL1	12
	Mouse	miR-150	Up	Ischemia, myocardial infarction-induced AKI/kidney	IGF1R	15
	Rat	miR-155	Up	Ischemia, toxic injury/kidney	-	9
	Mouse	miR-489	Up	Ischemia/kidney	PARP1	16
	Mouse	miR-494	Up	Ischemia/kidney	ATF3	17
	Mouse	miR-687	Up	Ischemia/kidney	PTEN	18
CKD	Mouse, human	miR-21	Up	UUO, glomerulosclerosis, IgAN, DN/interstitial fibrotic areas, atrophic tubules	TP53, PDCD4, SMAD7, TGFBR2, TIMP3, CDC25A, CDK6, MAPK1, PTEN, PPARA, MPV17L, DDAH1, RECK	31–38
	Mouse, human	miR-23b	Down	LN, DN	TAB2, TAB3, IKK-a, G3BP2	60.61
	Mouse, rat, human	miR-26a	Down	LN, DN/podocytes	CTGF	62–64
	Mouse, rat, human	miR-29a–c	Down/up	UUO, IgAN, renal fibrosis, remnant kidney model/tubular epithelial cells, glomeruli, tubulointerstitial cells, fibroblasts	HDAC, DKK1, collagens I, II, IV, Tropomyosin 1α	66–71
	Mouse	miR-34c	Up	UUO	CTGF, aSMA, collagen type 1, collagen type 3, Fibronectin	42
	Mouse	miR-141	Down	DN, adenine-induced renal fibrosis	HIPK2	58, 59
	Mouse, rat, human	miR-146a	Up/down	LN, DN, IgAN/tubulointerstitial cells	NOTCH1, ERBB4, TRAF6, IRAK1	52–56
	Human	miR-150	Up	LN/proximal tubular cells	SOCS1	57
	Mouse	miR-192	Up	Renal fibrosis/mesangial cells	SIP1, ZEB1	43–47
	Mouse, human	miR-193a	Up	FSGS/podocytes	WT1	51
	Mouse	miR-200a–c	Down/up	UUO, proximal tubules	ZEB1, ZEB2	65, 66
	Mouse	miR-216a	Up	Mesangial cells	PTEN, YBX1	48, 49
	Mouse	miR-377	Up	DN/tubular epithelial cells, podocytes, mesangial cells	Fibronectin, PAK2, SOD	50
	Mouse	miR-382	Up	UUO/outer and inner medulla, fibrotic area	Kallikrein 5	40
	Mouse	miR-433	Up	UUO/tubulointerstitial area	Azin1	39
	Rat	miR-491	Up	UUO/glomeruli, tubular epithelial cells	PAR3	41

UUO: unilateral ureteral obstruction. IgAN: IgA nephropathy. DN: diabetic nephropathy. LN: lupus nephritis. FSGS: focal segmental glomerulosclerosis.

for miR-21 in AKI, an elevated level of miR-21 was reported in the kidneys of mice suffering either from glomerulosclerosis[31] or from UUO[32] and in humans affected either by IgA nephropathy[33] or diabetic nephropathy[34]. In particular, glomerular cells[34, 35], interstitial fibrotic areas[36, 37], and atrophic tubules[35] showed elevated miR-21 expression. miR-21 targets several molecules including P53, PDCD4, SMAD7, TGFBR2, TIMP3[31], CDC25A, CDK6[34], ERK/MAPK, PTEN, PPARA, MPV17L, DDAH1, and RECK[35, 38]. In UUO-treated mice or rats, elevated expression levels were reported for miR-433[39], miR-382[40], and miR-491[41] in the tubulointerstitial area, inner and outer medulla, and glomerulus or tubular epithelial cells, respectively. miR-34c showed increased levels in mouse UUO kidneys, and it was reported to have CTGF, α-SMA, collagen type 1, collagen type 3, and fibronectin as downstream factors[42]. An elevated level of miR-192 was reported in kidneys showing CKD due to UUO[43] or diabetes[44, 45] and in the mouse or rat model of nephrectomy[46]. miR-192 seemed to be targeting SIP1[44] and ZEB1[47]. As for diabetic nephropathy, miR-216a in mouse mesangial cells appears to be important for the development of sclerosis via PTEN[48] or YBX1[49]. On the other hand, Wang *et al*. showed that miR-377 is upregulated in tubular epithelial cells, podocytes, and mesangial cells and can lead to increased fibronectin production in diabetic mice[50]. Gebeshuber *et al.*[51] showed clearly that FSGS can be induced by miR-193a and that WT1 is an miR-193a target and enforces transgenic expression of miR-193a in mice. Besides, elevated levels of miR-146a in the kidneys are reported in individuals developing lupus nephritis[52] or IgA nephropathy[53] and in the mouse model for lupus nephritis[54] as well as in the rat model for diabetes[55]. However, the miR-146a level was decreased in diabetic mice[55]. miR-146a appears to be targeting TRAF6 and IRAK1[55] or NOTCH1 and ERBB4[56]. In addition, regarding human lupus nephritis, Zhou *et al.*[57] showed increased miR-150 in the kidneys and found SOCS1

Table 2. miRNA Targets Referred to in this Review

miRNA target molecules	Description	Other name
AHRR	aryl-hydrocarbon receptor repressor	
ATF3	activating transcription factor 3	
ATG4B	autophagy related 4Bcysteine peptidase	
CDC25A	cell division cycle 25A	
CDK6	cyclin dependent kinase 6	
CTGF	connective tissue growth factor	
CXCL1	C-X-C motif chemokine ligand 1	
CXCL8	C-X-C motif chemokine ligand 8	
DDAH1	dimethylarginine dimethylaminohydrolase 1	
DKK1	dickkopf WNT signaling pathway inhibitor 1	
ERBB4	erb-b2 receptor tyrosine kinase 4	
G3BP2	G3BP stress granule assembly factor 2	
H2AFX	H2A histone family member X	H2A.X
HDAC	histone deacetylase 9	HDAC9
HIPK2	homeodomain interacting protein kinase 2	
HO1	heme oxygenase 1	
IGF1R	Insulin-like growth factor 1 receptor	
IKK-α	conserved helix-loop-helix ubiquitous kinase	CHUK, IKBKA
IRAK1	interleukin 1 receptor associated kinase 1	
KIF3B	kinesin family member 3B	
MAPK1	mitogen-activated protein kinase 1	ERK, MAPK
MKK3	mitogen-activated protein kinase kinase 3	
MPV17L	MPV17 mitochondrial inner membrane protein like	
NOTCH1	notch 1	
PAK2	p21 (RAC1) activated kinase 2	
PAR3	par-3 family cell polarity regulator	PARD3
PARP1	poly(ADP-ribose) polymerase 1	
PDCD4	programmed cell death 4	
PPARA	peroxisome proliferator activated receptor alpha	PPARalpha
PTEN	phosphatase and tensin homolog	
RECK	reversion inducing cysteine rich protein with kazal motifs	
SIRT1	sirtuin 1	
SMAD7	SMAD family member 7	
SOCS1	suppressor of cytokine signaling 1	
SOD	superoxide dismutase	
TAB2	TGF-beta activated kinase 1/MAP3K7 binding protein 2	
TGFBR2	transforming growth factor beta receptor 2	
TIMP3	TIMP metallopeptidase inhibitor 3	
TP53	tumor protein p53	P53
TRAF6	TNF receptor associated factor 6	
WT1	Wilms tumor 1	
YBX1	Y-box binding protein 1	
ZEB1	zinc finger E-box binding homeobox 1	
ZEB2	zinc finger E-box binding homeobox 2	SIP1
α-SMA	actin, alpha 2, smooth muscle, aorta	ACTA2

as its target.

It should, however be noted that decreased levels of miR-141 were reported in the kidneys of mice developing diabetic nephropathy and adenine-induced renal fibrosis[58], and miR-141 was also shown to regulate the epithelial-mesenchymal transition of the tubular epithelium by targeting HIPK2[59]. Furthermore, miR-23b also showed decreased expression in humans, in the mouse model of lupus nephritis[60], and in the mouse model diabetic nephropathy[61]. miR-23b was demonstrated to be targeting TAB2, TAB3, and IKK-a[60] in one report and be targeting G3BP2 in another[61]. In our previous study, we clarified the role of decreased miR-26a levels in the glomerulus of mice and individuals developing lupus nephritis and in the glomerulus of individuals developing IgA nephropathy, and we suggested this decrease was related to podocyte injury[62]. Other studies also showed decreased miR-26a expression in the kidneys of mice[63] and rats[64] developing diabetic nephropathy, and the authors of those studies explained the correlation between low miR-26a and nephropathy with findings showing that miR-26a negatively regulates CTGF. Decreased expression of miR-200a was detected in the proximal tubules of UUO-treated rodents, and miR-200a seemed to suppress the expression of ZEB1 and ZEB2[65]. However, another report showed that expression of the miR-200 family, particularly of miR-200b, was increased in a time-dependent manner in the kidneys of UUO mice, that expression of ZEB1 and ZEB2 was also increased after UUO, and that administration of the miR-200b precursor suppressed these increases[66]. The expression of miR-29a, b, and c was also found to be decreased

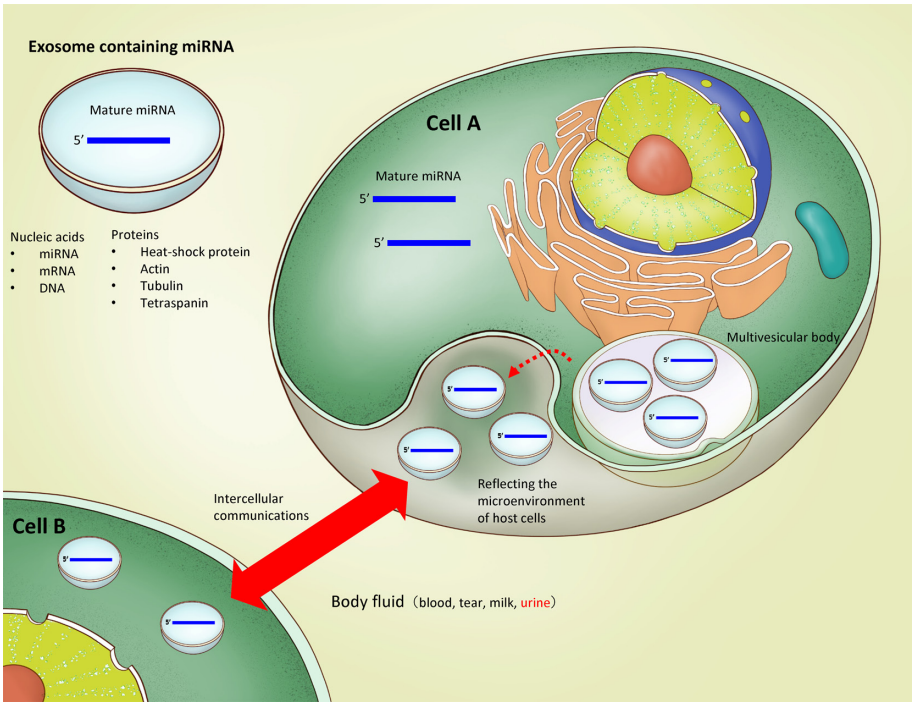

Fig. 2. The biosynthesis and function of exosomes containing miRNAs.

in the mouse kidneys by UUO[67] or diabetic condition[68]. Importantly, miR-29a, b, and c were found to be expressed in glomeruli and renal tubules[68]. In later studies, miR-29a expression was confirmed in the mouse glomerulus and renal tubules[69, 70] and, in particular, in podocytes[69]. Besides, decreased levels of miR-29c were reported in the kidneys of individuals developing IgA nephropathy with renal fibrosis and in the 5⁄6 nephrectomy rat[71]. The miR-29 family has among its targets collagens I and IV[68], HDAC[69] and DKK1[70], which are downregulated by miR-29a, and collagen II and tropomyosin 1α, which are under the control of miR-29c[71].

Urinary miRNA and Kidney Diseases

The kidney is a nonregenerative organ, and confirmed diagnosis of kidney disease relies on highly invasive renal biopsy. Identification of biomarkers in body fluids that can help to identify the renal pathology type will be essential for the development of noninvasive diagnostic methods. Urinary albumin, blood urea nitrogen, serum creatinine level, etc., are useful diagnostic indicators, but further information is still needed to be able to grasp the kidney disease type. For the progress of kidney disease research, it will be of crucial importance to evaluate carefully miRNAs as "disease-specific nucleic acid biomarkers."

miRNAs are present in body fluids. Weber *et al.*[72] have comprehensively analyzed miRNA extracted from cerebrospinal fluid, tears, pleural effusion, trachea wash, milk, colostrum, ascites, urine, semen, amniotic fluid, and plasma from healthy humans. The number of detected species of miRNA was highest in saliva with 458 miRNAs and lowest in urine with 204 miRNAs. Notably, these authors identified miRNAs that appear specifically in body fluids, such as tear fluid miR-637 and cerebrospinal fluid miR-577, but they were unable to identify any urine-specific miRNAs.

How do miRNAs exist in these body fluids? Johnstone *et al.*[73] discovered vesicles secreted from sheep reticulocytes and named them exosomes (Fig. 2). Exosomes are a type of extracellular vesicles which include microvesicles, apoptotic bodies, and ectosomes[74]. They are derived from the budding of endosomal membranes, resulting in the formation of multivesicular bodies (MVBs), and are produced by the fusion of MVBs with the plasma membrane. Therefore, exosomal membrane molecules such as CD9, CD63, and CD81 are used as representative exosomal markers[75]. Valadi *et al.*[76] reported that mRNAs and miRNAs are present in the exosomes, and the miRNAs in body fluids are thought to exist in exosome-encapsulated form. Urinary miRNAs are considered stable because they are also included in exosomes[77]. In recent years, research has increasingly been conducted to analyze miRNAs in urine in different renal diseases and to compare healthy and disease groups. It is still unknown whether miRNAs could be a good biomarker for kidney disease, but we believe it is worthwhile anyway to summarize the results so far obtained in this field of research.

Urinary miRNAs and AKI

As summarized in Table 3, miR-21, miR-200c, miR-423, and miR-4640 appeared to be increased in the urine supernatant of individuals with AKI[78], and the levels of miR-21, miR-200c, and miR-423 were also found to be elevated

Table 3. miRNAs Showing Altered Levels in the Urine of AKI or CKD

	Species	miRNA	Expression	Status	Specimen	References
AKI	Human	miR-16	Up	AKI	Urine	81
	Human	miR-21	Up	AKI after cardiac surgery	Supernatant	7
	Human	miR-21	Up	AKI	Urine	9
	Human	miR-21, miR-200c, miR-423.	Up	Drug-induced AKI	Supernatant	79
	Human	miR-21, miR-200c, miR-423, miR-4640	Up	AKI	Supernatant	78
	Rat, human	miR-30c-5p, miR-192-5p	Up	AKI, cardiac surgery	Supernatant	82
	Human	miR-146a	Up	Ischemia-reperfusion injury	Supernatant	12, 80
	Mouse, human	miR-494	Up	Ischemia-reperfusion injury, AKI	Urine	84
	Rat	miR-489	Up	Drug-induced AKI	Supernatant	83
	Rat	miR-21	Down	Drug-induced AKI	Urine	9
	Human, rat	miR-155	Down	AKI, Drug-induced AKI	Urine	9
CKD	Human	miR-15	Up	IgAN	Urinary sediments	89
	Human	miR-15b, miR-34a, miR-636	Up	DN	Urinary exosomes, sediments	86
	Mouse, human	miR-26a	Up	LN	Urinary exosomes	62
	Human	miR-133b, miR-342, miR-30a	Up	DN	Urinary exosomes	85
	Human	miR-146a	Up	LN	Urinary exosomes	88
	Mouse	miR-146a	Up	Glomerulonephritis, CKD	Urinary sediments	54
	Human	miR-193a	Up	FSGS	Urinary exosomes	91
	Human	miR-215, miR-378i	Up	IgAN	Urinary exosomes	90
	Rat	miR-451, miR-16	Up	DN	Urinary exosomes	93
	Human	miR-320c, miR-6068	Up	DN	Urinary exosomes	87
	Human	miR-1825, miR-1281	Up	CKD	Supernatant	92
	Human	let-7a	Down	CKD	Supernatant	92
	Human	miR-15	Down	Diabetic glomerulosclerosis	Urinary sediments	89
	Human	miR-29c	Down	LN	Urinary exosomes	95
	Human	miR-29c, miR-205	Down	IgAN	Urinary exosomes	96
	Human	miR-181	Down	CKD	Urinary exosomes	98
	Human	miR-2861, miR-1915-3p, miR-4532	Down	DN	Urine	94
	Human	miR-29a, miR-29c, miR-200b, miR-200c	Down	CKD	Urinary exosomes	96
	Human	miR-3201, miR-1273e	Down	LN	Urine	94
	Human	mir-1915, miR-663	Down	FSGS	Supernatant	97

IgAN: IgA nephropathy. DN: diabetic nephropathy. LN: lupus nephritis. FSGS: focal segmental glomerulosclerosis.

in individuals with drug-induced AKI[79]. As for miR-21, an increase in its urinary levels was observed in individuals with AKI[7, 9]; however, rats with drug-induced AKI showed decreased miR-21 urinary levels[9]. miR-146a[12, 80], miR-16[81], miR-30c, and miR-192[82] were increased in the urine of patients diagnosed with AKI. In particular, miR-146a and miR-30c were also found to be elevated in the kidneys of patients and mice suffering from AKI[12] and in the kidneys of rats with contrast-induced nephropathy[23], respectively. In addition, increased urine levels of miR-489[83] were reported in rats with gentamicin-induced AKI. Elevated levels of miR-494 were described in mice and individuals developing AKI[17], and an increase in miR-494 levels was also detected in the kidney of mice with AKI[16, 17, 84]. For miR-155, reduced levels in the urine were reported in rats with AKI induced by gentamicin and in patients with AKI[9]. However, to date, evidence of a truly diagnostic value of the AKI-associated miRNAs found in urinary exosomes is still scarce.

Urinary miRNA and CKD

In order to identify possible candidate miRNAs to be exploited as biomarkers of CKD, a thorough analysis of the exosomal fractions from human urinary samples was performed. miR-133b, miR-342, miR-30a[85], miR-15b, miR-34a, miR-636[86], miR-320c, and miR-6068[87] were found to be increased in urinary exosomes from diabetic patients (Table 3). With regard to lupus nephritis, elevated levels of miR-146a[88] and miR-26a[62] in urinary exosomes were reported in humans; conversely, the miR-26a level was found to be decreased in the kidneys of mice with lupus nephritis[62]. An elevated level of miR-146a in the urinary sediment was also observed in a mouse model of lupus nephritis[54]. Patients with IgA nephropathy showed increased amounts of miR-15 in urinary sediment[89] or increased amounts of miR-215 and miR-378i in urinary exosomes[90]. The detection of miR-15 in urinary sediment also indicates the presence of cells in the urine, which can be of diagnostic significance. However, the miR-15 levels in urinary sediment of diabetic glomerulosclerosis patients were found to be decreased[89]. Similar to

the renal expression of miR-193a[51], urinary exosomes containing miR-193a also increased in FSGS compared with in a minimal change group[91]. Furthermore, miR-1825 and miR-1281 were increased in the urine of patients with CKD[92], and the levels of miR-451 and miR-16 in urinary exosomes were also increased in rats with diabetic nephropathy[93].

The levels of urinary miR-3201 and miR-1273e[94] and of miR-29c in urinary exosomes[95] were found to be reduced in patients with lupus nephritis. Altered renal expression of miR-29c has also been reported in mice, humans, and rats[67, 68, 71]. In agreement with these reports, miR-29c, as well as miR-205, was found to be decreased in the urinary exosomes of individuals developing IgA nephropathy[90], and miR-29a, miR-29c, miR-200b, and miR-200c were decreased in the urinary exosomes of CKD patients[96]. miR-1915 and miR-663 in the urine supernatant from FSGS patients[97] and miR-2861, miR-1915, and miR-4532[94] in urinary exosomes from diabetic nephropathy patients were also found to be decreased. Furthermore, let-7a[92] and miR-181[98] showed decreased levels in urine supernatant and urinary exosomes of CKD patients, respectively.

miRNAs and Animals

Abundant evidence of altered expression in the kidneys and of abnormal levels in the urine of patients with kidney disease was reported for miR-21 and for the miR-29 family. However, further basic data obtained using rodents as well as other animal species are needed; particularly, the availability of further evidence from dogs, pigs, and monkeys, which are major animal species, would be helpful when extrapolation of experimental results from animals to humans is required. Several studies investigated miRNA expression in the kidneys, including those of dogs[99-101], pigs[102, 103], monkeys[104], cats[99], and cattle[105] (Table 4). In addition, urinary levels of miRNAs were also investigated in dogs[106] and monkeys[107]. As for dog CKD, altered urinary exosome levels of miR-26a and miR-21a were detected[106].

Conclusion

One kind of miRNA seems to target multiple mRNAs. Therefore, this class of small ncRNAs would strongly affect molecular biological processes by functioning upstream of the central dogma and have a great influence on renal pathogenesis. For the analysis of miRNAs associated with renal pathogenesis, identification of disease type-specific miRNAs and their expressing cells and elucidation of the molecular network controlled by these miRNAs in the cells would be important because the kidneys are structurally and functionally remarkably complex organs. In particular, evidence of miR-21 being associated with both AKI and CKD is strong. Recently, miRNAs have been adopted not only as disease markers but also as therapeutic targets[34, 45]. We strongly hope that next-generation biomarker diagnosis and/or nucleic acid delivery therapy developed by future miRNA researches will be applied not only for human kidney disease control but also for managing and containing kidney disease in animals.

Table 4. miRNA Studies Reporting the miRNA Expression in the Kidney or Urine of Various Animal Species

Species	miRNA	Organ	References
Cattle	NGS	Healthy kidney	105
Dog	PCR	Healthy kidney	101
Dog	NGS	MDCK cells	100
Dog	NGS, PCR	Urinary exosome, healthy kidneys, diseased kidneys	106
Dog, Cat	NGS	Healthy kidney	99
Monkey	NGS, PCR	Healthy kidney	104
Monkey	NGS	Urine	107
Pig	PCR	Healthy kidney	102
Pig	NGS	Healthy kidney	103

NGS: next generation sequencing.

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