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.

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,
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. 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. 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. 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);
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. 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. For the creation of AKI animal models, ischemia reperfusion (IR) models (pre-renal factor), folic acid or adenine administration models (renal factor), or unilateral ureter obstruction (UUO) models (post-renal factor) have been mostly 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, 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. 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, which explains the inhibitory action of miR-21 against interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). Xu et al. 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. 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. 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. Other reports also showed increased levels of specific miRNAs related to AKI, such as miR-125b, which regulates the AHR in cisplatin-treated mice and IR rats; miR-150, which modulates the expression of IGFR1 in myocardial infarction-induced AKI mice; miR-489, which binds the PARP-1 mRNA in IR mice; miR-494, which downregulates ATF3 in IR mice; and miR-687, which targets PTEN in IR mice. In cisplatin-induced AKI mice, miR-34a was found to be induced via P53 and to control the expression of SIRT1. Another study showed that, in IR mice, miR-34a regulates the autophagic activity in tubular epithelial cells by targeting ATG4B. 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. Saikumar et al. 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. 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. Thirteen million Japanese and 30 million American adults have CKD, and millions of others are at increased risk (National Kidney Foundation). 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. In addition, experimental rodents showing chronic stages due to UUO or diabetes develop CKD associated with renal fibrosis. Several reports helped to clarify the relation between CKD and miRNA (Table 1). Similar to what was observed...
for miR-21 in AKI, an elevated level of miR-21 was reported in the kidneys of mice suffering either from glomerulosclerosis or from UUO and in humans affected either by IgA nephropathy or diabetic nephropathy. In particular, glomerular cells, interstitial fibrotic areas, and atrophic tubules showed elevated miR-21 expression. miR-21 targets several molecules including P53, PDCD4, SMAD7, TGFBR2, TIMP3, CDC25A, CDK6, IGFR1, and fibronectin as downstream factors. An elevated level of miR-192 was reported in kidneys showing CKD due to UUO or diabetes, and in the mouse or rat model of nephrectomy. miR-192 seemed to be targeting SIP1 and ZEB1. As for diabetic nephropathy, miR-216a in mouse mesangial cells appears to be important for the development of sclerosis via PTEN and YBX1. 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. Gebsheuer et al. showed clearly that FSGSS can be induced by miR-193a and that WT1 is an miR-193a target and enforces transgenic expression of miR-193a in mice.

### 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              | miR-21    | Up         | Ischemia/kidney         | MKK3, PDCD4                              | 7–11       |
| Mouse, rat, human| miR-24    | Up         | Ischemia, allograft/kidney| H2AFX, HO1                             | 13         |
| Rat              | miR-36a, c, e | Up    | Contrast-induced nephropathy/kidney | SIRT1, ATG4B                                | 19–21      |
| Mouse            | miR-34a   | Up         | Ischemia, toxic injury/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         |
| Rat              | miR-150   | Up         | Ischemia, myocardial infarction-induced AKI/kidney | -                                          | 9          |
| Mouse, human     | miR-155   | Up         | Ischemia, toxic injury/kidney | -                                         | 9          |
| Mouse            | miR-149   | Up         | Ischemia/kidney         | PARP1                                     | 16         |
| Mouse            | miR-687   | Up         | Ischemia/kidney         | ATF3                                      | 17         |
| 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, IgA, renal fibrosis, remnant kidney model/tubular epithelial cells, glomeruli, tubulointerstitial cells, fibroblasts | HDAC, DKK1, collagens I, II, IV, Tropomyosin | 66–71      |
| Mouse            | miR-34c   | Up         | UUO                     | CTGF, aSMA, collagen type 1, collagen type 3, Fibronectin HIPK2 | 42         |
| Mouse            | miR-141   | Down       | DN, adenine-induced renal fibrosis | NOTCH1, ERBB4, TRAF6, IRAKI              | 58, 59     |
| Mouse, rat, human| miR-146a  | Up/down    | LN, DN, IgAN/tubulointerstitial cells | HIPK2                                      | 52–56      |
| Human            | miR-150   | Up         | LN/proximal tubular cells | SOCS1                                     | 57         |
| Mouse            | miR-192   | Up         | Renal fibrosis/mesangial cells | SIPI, ZEB1                                 | 43–47      |
| Mouse, human     | miR-193a  | Up         | FSFGS/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         |
| Rat              | 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.
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\(^8\), and miR-141 was also shown to regulate the epithelial-mesenchymal transition of the tubular epithelium by targeting HIPK2\(^9\). Furthermore, miR-23b also showed decreased expression in humans, in the mouse model of lupus nephritis\(^6\), and in the mouse model diabetic nephropathy\(^6\). miR-23b was demonstrated to be targeting TAB2, TAB3, and IKK-a\(^6\) in one report and be targeting G3BP2 in another\(^6\). 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\(^6\). Other studies also showed decreased miR-26a expression in the kidneys of mice\(^6\) and rats\(^6\) 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\(^6\). 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\(^6\). The expression of miR-29a, b, and c was also found to be decreased

| miRNA Target Molecules | Description | Other Name |
|------------------------|-------------|------------|
| AHRR                   | aryl-hydrocarbon receptor repressor | |
| ATF3                   | activating transcription factor 3 | |
| ATG4B                  | autophagy related 4B cysteine 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 | |
| HOI                    | heme oxygenase 1 | |
| IGFI1                  | Insulin-like growth factor 1 receptor | |
| IKK-α                  | conserved helix-loop-helix ubiquitous kinase | CHUK, IKBK |
| 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 | PARD3 |
| PAR3                   | par-3 family cell polarity regulator | |
| 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 | P53 |
| TP53                   | tumor protein 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 | SIP1 |
| ZEB2                   | zinc finger E-box binding homeobox 2 | |
| α-SMA                  | actin, alpha 2, smooth muscle, aorta | ACTA2 |

### 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 4B cysteine 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 | |
| HOI                    | heme oxygenase 1 | |
| IGFI1                  | Insulin-like growth factor 1 receptor | |
| IKK-α                  | conserved helix-loop-helix ubiquitous kinase | CHUK, IKBK |
| 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 | PARD3 |
| PAR3                   | par-3 family cell polarity regulator | |
| 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 | P53 |
| TP53                   | tumor protein 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 | SIP1 |
| ZEB2                   | zinc finger E-box binding homeobox 2 | |
| α-SMA                  | actin, alpha 2, smooth muscle, aorta | ACTA2 |
miRNAs and Kidney Diseases

in the mouse kidneys by UUO\textsuperscript{67} or diabetic condition\textsuperscript{68}. Importantly, miR-29a, b, and c were found to be expressed in glomeruli and renal tubules\textsuperscript{68}. In later studies, miR-29a expression was confirmed in the mouse glomerulus and renal tubules\textsuperscript{69,70} and, in particular, in podocytes\textsuperscript{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\textsuperscript{71}. The miR-29 family has among its targets collagens I and IV\textsuperscript{68}, HDAC\textsuperscript{69} and DKK1\textsuperscript{70}, which are downregulated by miR-29a, and collagen II and tropomyosin 1α, which are under the control of miR-29c\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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\textsuperscript{75}. Valadi et al.\textsuperscript{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\textsuperscript{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\textsuperscript{78}, and the levels of miR-21, miR-200c, and miR-423 were also found to be elevated
in individuals with drug-induced AKI\(^9\). 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-1681, miR-30c, and miR-192\(^2\) 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\(^2\). 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-606\(^87\) 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

| 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, miR-21, miR-200c, miR-423, miR-4640 | Up | Drug-induced AKI | Supernatant | 79 |
| 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 | AKI | Urine | 84 |
| Rat | miR-489 | Up | Drug-induced AKI | Supernatant | 83 |
| Human | 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 | Urinary sediments | 89 |
| 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-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.
the renal expression of miR-193a51, urinary exosomes containing miR-193a also increased in FSGS compared with in a minimal change group59. Furthermore, miR-1825 and miR-1281 were increased in the urine of patients with CKD92, and the levels of miR-451 and miR-16 in urinary exosomes were also increased in rats with diabetic nephropathy93.

The levels of urinary miR-3201 and miR-1273e94 and of miR-29c in urinary exosomes95 were found to be reduced in patients with lupus nephritis. Altered renal expression of miR-29c has also been reported in mice, humans, and rats67, 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 nephropathy90, and of miR-200a, miR-200b, and miR-200c were decreased in the urinary exosomes of CKD patients96, miR-1915 and miR-663 in the urine supernatant from FSGS patients97 and miR-2861, miR-1915, and miR-453294 in urinary exosomes from diabetic nephropathy patients were also found to be decreased. Furthermore, let-7i95 and miR-18198 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 dogs99-101, pigs102, 103, monkeys104, cats99, and cattle105 (Table 4). In addition, urinary levels of miRNAs were also investigated in dogs106 and monkeys107. As for dog CKD, altered urinary exosome levels of miR-26a and miR-21a were detected106.

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 targets34, 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.

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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   | Urinary exosome, healthy kidneys | 106 |
| Dog, Cat| NGS   | Healthy kidney | 99 |
| Monkey  | NGS, PCR | Healthy kidney | 104 |
| Pig     | NGS   | Healthy kidney | 107 |
| Pig     | PCR   | Healthy kidney | 102 |
| Pig     | NGS   | Healthy kidney | 103 |

NGS: next generation sequencing.

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| Dog, Cat| NGS   | Healthy kidney | 99 |
| Monkey  | NGS, PCR | Healthy kidney | 104 |
| Pig     | NGS   | Healthy kidney | 107 |
| Pig     | PCR   | Healthy kidney | 102 |
| Pig     | NGS   | Healthy kidney | 103 |

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