



Can urinary exosomal micro-RNA detection become a diagnostic and prognostic gold standard for patients with lupus nephritis and diabetic nephropathy?

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In 1993, Lee *et al.* (1) observed that *lin-4* gene could control the embryonic development of *C. elegans*, but failed to encode a protein molecule. Besides, the gene transcribes a pair of microRNAs (miRNAs). The short RNA is approximately 22 nucleotides (nt) in length whereas the long one is 61 nt that was predicted to fold a stem loop with anti-sense complementary to 3'-untranslated region (UTR) of *lin-14* messenger RNA (mRNA) (2). In mammalian genomes, these miRNA genes exist as either independent transcription units or are located in the introns of other genes (3). Nowadays, it is conceivable that miRNA is one of the most diverse spectrum of non-coding (nc) RNAs consisting of more than 2,000 different species (4). The crucial function of these miRNAs involves the regulation of mRNA expression through interference with the 3'-UTR. It is estimated that more than 60% of mammalian genes are regulated by these intracellular miRNAs (5). Furthermore, a pool of cell-free miRNAs is found in the biological fluids including plasma, milk, tears, saliva, urine, amniotic fluid, cerebrospinal fluid, semen and ascites (6). This may imply that these extracellular miRNAs act potentially as signaling molecules for cell-cell communication (7). More recent investigations have revealed that these extracellular miRNAs exist in three forms, i.e., exosomes (40–100 nm), microvesicles (100–1,000 nm) and apoptotic bodies (1–4 µm) as shown in *Figure 1*. Exosomes are exocytotic membrane-

derived vesicles that contain a variety of proteins, nucleic acids and lipids for mediating cell-cell communication via proteins and miRNAs delivery (8). Since the amount and composition of exosomal miRNAs are found different among diseases and healthy individuals, they can potentially become novel biomarkers for clinical applications (9).

Pathophysiology of chronic kidney diseases (CKDs) and its correlation to biochemical/immunological biomarkers currently available

CKDs are caused basically by immunological and non-immunological mechanisms. Lupus nephritis (LN) is a typical autoimmune-mediated CKD caused by multiple immunological responses. The immunopathological findings of renal tissues in LN include immune complex deposition with complement activation, glomerular crescents, podocyte injury, inflammatory cell infiltration, vascular injury, and tubulointerstitial lesions (10). In contrast, diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes mellitus (DM) and a representative non-immunologic-mediated CKD. The characteristic pathologic features of DN include thickened glomerular basement membrane, increased extracellular matrix formation, podocyte loss, epithelial-mesenchymal transition (EMT) and renal cell apoptosis (11).

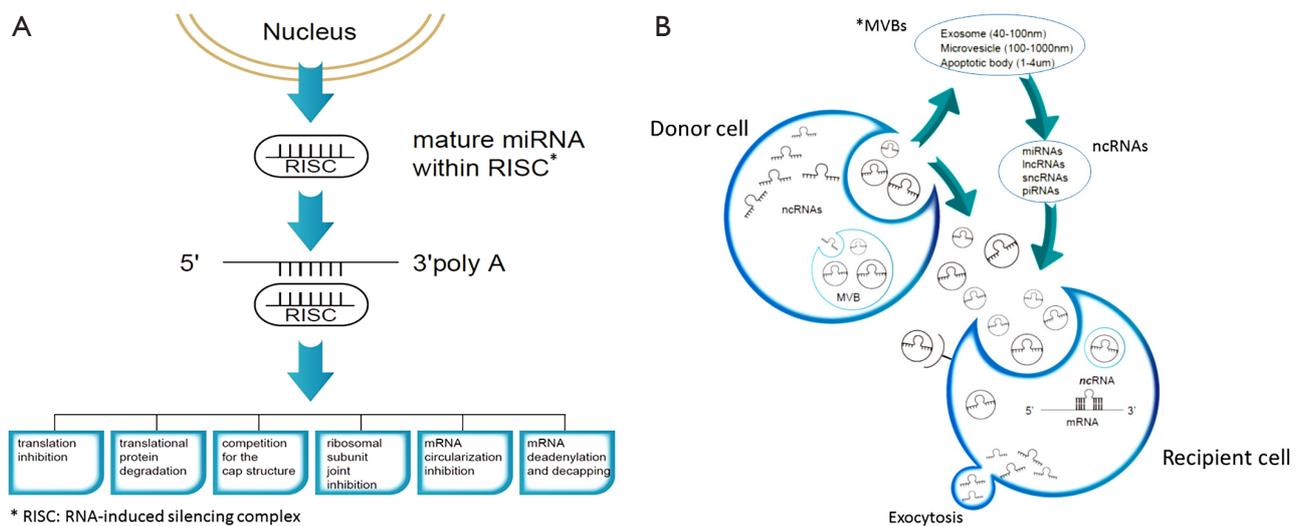


Figure 1 Schematic illustration of the role of non-coding RNAs (ncRNA) on intracellular and extracellular modulation of cell functions. (A) Epigenetic modulation of miRNA on mRNA expression intracellularly; (B) cell-cell communication by non-coding RNAs (ncRNAs) containing multiple vesicle bodies (*MVBs) from donor cells to recipient cells. miRNAs, microRNAs; lncRNAs, long non-coding RNAs; sncRNAs, small non-coding RNAs; piRNAs, piwi-interacting RNAs.

However, some immunological components such as increased innate immune receptors of Toll-like receptor 2 and 4 (12) and activation of the complement system (13) are also implicated in DN. In clinical practice, kidney biopsy remains the gold standard for differential diagnosis and treatment decision for various CKDs. However, the biopsy procedure is rather invasive with complication risks. Instead, the non-invasive biomarkers obtained from serum or urine specimens have been extensively investigated by authors for predicting, diagnosing and prognostic purposes. Hsieh *et al.* (14) reviewed the literature and concluded that currently reported serum or urine biomarkers for LN are exclusively inflammatory or immune-related molecules that are unable to reflect the ongoing scenario of pathological changes in LN. The similar impasse is also encountered in DN where urine-specific miRs have been found positively correlated to the albuminuria and TGF- β 1 levels, but not comparable with the gold-standard histological findings (15).

Urinary exosomal miRNAs are potential biomarkers for LN and DN

The physiologic functions of extracellular vesicles in normal renal glomeruli include immune modulation, cell proliferation, tissue regeneration, matrix synthesis, and cell-

cell communication (16). Jia *et al.* (15) and Eissa *et al.* (17) have found that increased urinary exosomal miR-192, miR-156, miR-34a and miR-636 are correlated to albuminuria/proteinuria in patients with DN. In addition, diverse urinary exosomal proteins, lipids, mRNAs, mitochondrial DNAs and miRNAs have been reported in different acute kidney diseases, CKDs and DN by Bhatt *et al.* (18) and Street *et al.* (19). Recently, Cardenas-Gonzalez *et al.* (20) profiled 2401 urinary exosomal miRNAs by identifying, confirming and replicating them in patients with LN and DN in two-run cohort study. They conclusively clarified some urinary exosomal miRNAs as potential sensitive and specific urine biomarkers after correlating them with renal functions and histopathology of the biopsy-proven CKDs. They demonstrated down-regulation of miR-3201 and miR-1273e in correlation with endocapillary glomerular inflammation in LN. In addition, down-regulation of miR-2861, miR-1915-3P and miR-4532 were correlated to decreased serum estimated glomerular filtration rate (eGFR), renal interstitial fibrosis and tubular atrophy in DN. The authors proposed that these novel urinary exosomal miRNAs could be regarded as liquid biopsy in patients with LN and DN. *Table 1* summarizes the correlation of urinary exosomal miRNAs with histological changes or functional impairments in LN and DN in the literature.

Table 1 Correlation of urinary exosomal miRNA expression and pathological changes/functional impairment in patients with lupus nephritis and diabetic nephropathy

Exosomal miRNA expression	Correlation with
Lupus nephritis	
Increased expression	Glomerulonephritis (14)
miR-125a	
miR-150	
miR-155	
miR-146	
Decreased expression	
miR-141	
miR-192	
miR-200a	
miR-200c	
miR-221	
miR-222	
miR-429	
Decreased expression	Endocapillary glomerular inflammation (20)
miR-3201	
miR-1273e	
Diabetic nephropathy	
Increased expression	Proteinuria albuminuria (16)
miR-130a	
miR-145	
miR-15b	
miR-34a	
miR-192	
Decreased expression	
miR-155	
miR-424	
Decreased expression	Decrease in eGFR interstitial fibrosis tubular atrophy (20)
miR-2861	
miR-1915-3p	
miR-4532	

miR, microRNA; eGFR, estimated glomerular filtration rate.

A likelihood of miRNAs in urine to be biomarkers for LN and DN

A clinically useful disease biomarker should fulfill the criteria of high sensitivity, high specificity, general availability and reasonable cost for predicting, monitoring and/or foreseeing the outcome of the disease. More than 2,000 miRNomes are now available for the detection of

global urinary miRNAs. It is believed that CKD jeopardizes patients to the occurrence of cardiovascular diseases, end-stage renal diseases and mortality (21). To directly reflect the renal histopathologic changes, the detection of excreted molecules by diseased kidney in the urine seems feasible and practical. Exosomes are small extracellular vesicles carrying proteins and nucleic acids that can be transferred in active form to nearby or distant tissues for cell-cell communication. In patients with DM, blood glucose homeostasis requires a constant communication between insulin-secreting and insulin-sensitive cells. In addition to endocrine modulation, exosomes released from pancreatic beta cells, skeletal muscle, adipose tissue and liver can serve as new players in metabolic organ cross-talk (22). In case of deranged exosomal miRNA cross-talk among these metabolic organs, in-time blood glucose regulation cannot be achieved, leading to DM. The high blood glucose level subsequently elicits glomerular podocyte dysfunction and depletion through an EMT together with growth hormone-induced podocyte apoptosis in DN (23). Similarly, the autoimmune-induced inflammation in LN may also cause podocyte injury as reported by Yu *et al.* (10). It remains unclear why only some particular exosomal miRNA biogenesis decreases in LN and DN as reported by Cardenas-Gonzalez *et al.* (20). The sophisticated interactions among these aberrant miRNAs in LN and DN are still open questions. Undoubtedly, these perplexities need more investigations to clarify. In addition to exosomal miRNAs, it is quite interesting that long nc RNAs (lncRNAs) and short nc RNAs (sncRNAs) with molecular weight of approximately 200-nt in length are also participants in transcriptomic regulation of immune cells and autoimmune diseases (24). These larger ncRNAs were found more specific in regulating gene expression than the multi-targeting miRNAs (24). It is of equal interesting to know whether or not these lncRNAs and sncRNAs appear in urinary exosomes for cell-cell communications in CKDs.

Conclusions

Transcriptomics and bioinformatics have discovered the expression of thousands of ncRNAs which are functional RNAs for regulating mRNAs expression. These ncRNAs may serve as potential biomarkers for diagnosis, monitoring, or prognosis of patients with DN or LN via so-called “liquid biopsy” from urine or other body fluids instead of invasive tissue biopsy. A simple assay kits for detecting urinary exosomal ncRNAs are expected to be developed for the

“precision medicine” in the management of DN and LN instead of the potentially hazardous renal biopsy.

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Footnote

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