Diabetes Management

The value of extracellular miRNA in diabetic kidney disease



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Diabetes affects almost 8.3% of the global population and is associated with significant microvascular (renal disease, blindness) and macrovascular (coronary artery disease, stroke, peripheral arterial disease) morbidities and increased mortality. Despite newly developed treatment modalities, 30–40% of patients with diabetes are at risk of developing chronic kidney disease (CKD), where 15–20% of those patients require routine dialysis [1,2]. The annual costs of treating diabetic CKD and providing dialysis for patients are at the order of US$29 and US$49 billion in the USA, respectively [3]. Attempts to mitigate the risk of developing CKD by intensive glycemic [4] or blood pressure [5] control, and treatment with inhibitors of the renin angiotensin aldosterone system [6], had limited success. The dominant paradigm on the progression of kidney ailment is albuminuria; however, 50% of diabetic patients with progressive CKD did not show significant albuminuria [7]. Therefore, a better understanding of the underlying mechanisms for the progression of kidney disease in diabetes is urgently needed to provide a better disease management and identify new therapeutic approaches. To noninvasively assess the pathological progression of kidney disease in diabetic patients is nontrivial. Analyzing urinal molecular changes, such as concentration of specific proteins or metabolites in urine is a direct and logical approach to assess the perturbation of molecular networks in the kidney; however, the results largely just reflect the functional aspect of the kidney. The blood, on the other hand, may provide information on the dysfunction of pancreatic tissue during the progression of diabetes and the effect of deregulated energy metabolism on other organs including the kidney. Recently a number of studies revealed the presence of stable RNA molecules especially miRNAs, a class of short (21–23 nucleotides long) non-coding regulatory RNAs, in various biofluids including urine and blood [8]. These circulating miRNAs are packaged in either lipid vesicles or complexed with proteins or lipoproteins to escape extracellular RNAse activity [9–12]. The functional aspects and mechanisms of how miRNAs are released into the extracellular environment are largely unknown. There are reports that suggest cells actively

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and selectively package some miRNA molecules and release them to the extracellular environment for specific biological reasons. However, there is also contradicting evidence that some of these circulating miRNAs are byproducts of normal cell turnover. Nevertheless, due to the small size of the miRNA repertoire, the readily available isolation methods and measurement tools, the apparent stability of miRNA outside of cells and the important regulatory roles of miRNA on normal cellular activities, the circulating extracellular miRNA has become the center stage of numerous biomarker discovery programs for different pathologies [13]. In the past decade, hundreds of papers have reported concentration changes of specific miRNAs in different types of body fluid that can be linked to various pathological conditions, from cancers to cardiovascular conditions. Even though the origins and biological implications of circulating miRNA are yet to be fully understood, the changes in specific miRNA concentrations in body fluids probably reflect, in part, the alteration of the normal physiology of cells.

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In the normal kidney, miRNAs modulate a wide variety of essential biological processes, for example, the regulation of tonicity, water homeostasis, renin production, sodium and potassium handling, and renal cell development and senescence [14]. Evidence from experimental models and human biopsies indicate the involvement of miRNAs in a variety of renal diseases including polycystic kidney disease, hypertensive nephrosclerosis, lupus nephritis, renal cell cancer and kidney allograft rejection. miRNAs are involved in the progression of diabetic kidney disease, by modulating the biological processes of extracellular matrix production, epithelial to mesenchymal transition, collagen production and fibronectin. Because of such important regulatory roles of miRNA, analyzing changes in the extracellular miRNA spectrum in diabetic patients may provide the key to noninvasively assessing the molecular changes in the development of diabetic kidney disease (DKD).

There are a number of reports examining the changes in miRNA in serum or plasma in both Type I and Type II diabetes patients [15]. However, very few studies have conducted systematic profiling of extracellular miRNAs in body fluids with the goal of understanding the perturbed molecular processes, and identifying and validating specific miRNAs as diagnostic or prognostic markers for DKD. In addition, most of the studies are cross-sectional with pooled samples and did not include patients with confirmed DKD. To demonstrate the possibility of using extracellular miRNA to reflect the perturbation of molecular processes in the kidney, we examined the changes of urinal miRNA spectrum from a set of longitudinal samples collected from Type I diabetes patients who developed diabetic CKD [16]. The study uncovered changes in several miRNAs associated with specific disease states (e.g., patients with overt disease or intermittent microalbuminuria) rather than a quantitative trend of change in specific miRNA concentration in urine paralleling the severity of albuminuria (indicator for CKD). These altered miRNAs, although different in different disease stages, target common pathways or biological processes that are known to be involved in the development of kidney disease and fibrosis (e.g., the transforming growth factor pathway). Our findings suggested the possibility of assessing the molecular changes in the kidney through analyzing the changes of the miRNA spectrum in urine. Future studies should thus focus on validating miRNA biomarkers for early diagnosis and monitoring disease progression and/or prognosis, utilizing prospective measurements against the rate of disease progression in both Type 1 and Type 2 diabetes patients.

To fully realize the potential of extracellular miRNAs as diagnostic tools for diabetes-associated kidney diseases, it is imperative that such studies acknowledge the limitations as well as the significance of albuminuria-based assessment for CKD. In particular, a study design based on both the rate of renal filtration loss and albuminuria is needed for a number of reasons. Firstly, studies in both Type 1 and Type 2 diabetes patients show that the rate of renal function decline may be a more important predictor for the development of renal failure (stage 5 CKD) than proteinuria. Secondly, the level of renal function has been associated with a decline in the concentration of a number of circulating miRNAs [17]. Lastly, emerging evidence suggests that some of the miRNA changes may be reflective of the level of proteinuria rather than a specific renal pathology (e.g., diabetic vs non-diabetic CKD) [18]. To enhance interpretability of the results and improve diagnostic specificity or prognostic accuracy, systems approaches that consider the change of specific miRNA concentration as well as their affected biological processes should be explored as clinical outcomes predictors.

One cautionary note on extracellular miRNA biomarker is the significant inconsistency of different studies. This is not unique in diabetic studies,

it is a common issue associated with miRNA measurement, especially for circulating miRNA. Some of these inconsistencies are simply caused by the quickly evolving nomenclature guideline, for example, the use of -3p and -5p instead of the asterisk sign for miRNA name, which creates some confusion concerning the identity of the specific miRNAs that are reported. In addition, the nature of the miRNA molecule – short sequence length and highly conserved family members, makes the design of specific probes or primers to measure particular miRNAs a challenging task. The lack of a specific assay causes significant measurement- and platform-associated difference. The type of sample included in the study, sample storage, handling conditions and even RNA isolation method all add additional variables on the accuracy of miRNA measurement. Therefore, it is important to understand the differences in sample type and measurement platform before combining or comparing different studies [19]. In the coming years, with increasing interest in circulating miRNA, it is very likely that a few cell-free miRNAs in either urine or circulation will be identified as diagnostically or prognostically relevant.

Though clinically important, the biological significance of these findings on extracellular miRNA can only be understood by using systems approaches with *in vitro* and *in vivo* models to identify and establish the mechanistic relevance of miRNAs as biomarkers to reflect the perturbed network during the progression of DKD. Such insights on the etiology and progression of DKD will pave the way to develop novel miRNA-based surrogate end points for specific clinical outcomes. Although still at an early stage, RNA-based therapeutics may hold great promise treating DKD [20]. A number of *in vivo* studies have reported the accumulation of therapeutic synthetic RNA in the kidney which, although undesirable for other targeted organs, provides an opportunity for modulating certain diseases in the kidney using RNA. Once we understand how miRNA are involved in disease-associated networks, it may be reasonable to test the possibility of using synthetic miRNAs or antimiRs (antisense of miRNA) to revert the perturbed pathways. Insofar as the combination of clinical observation, systems biology/bioinformatics and basic molecular research facilitate the discovery of relevant miRNAs, one may reasonably anticipate the successful translation into effective RNA-based therapies for DKD.

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