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MicroRNA: a novel biomarker and therapeutic target to combat autophagy in diabetic nephropathy

J. MA, Y. WANG, H.-T. XU, N. REN, N. ZHAO, B.-M. WANG, L.-K. DU

Department of Endocrinology, The First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China

Jian Ma and Yang Wang contributed equally to this work

Abstract. – Diabetic kidney disease (DKD) is a leading cause of end-stage renal disease worldwide and is associated with increased morbidity and mortality in patients with both type 1 and type 2 diabetes. Early treatment of DKD can prevent or slow its progression. Some studies suggest that traditional risk factors such as albuminuria do not effectively predict DKD progression, and other predictors have yet to be characterized and validated. Therefore, there is an urgent need to identify sensitive and easily detectable biomarkers to monitor the decline in renal function. MicroRNAs (miRNAs) have recently emerged as important regulators that are ubiquitous in human tissues and bodily fluids, numerous diseases, including early DKD. Recent developments have revealed that miRNAs-mediated post-transcriptional regulation of gene expression represents an integral part of the autophagy regulatory network. In this review, we explored the utility of miRNAs as biomarkers for the early detection and progression of DKD. We also examined some of the molecular mechanisms by which miRNAs manipulate the autophagic machinery to maintain cellular homeostasis during DKD. A better understanding of the interaction between miRNAs and autophagy may ultimately benefit future DKD diagnosis and therapy.

Key Words:
Diabetic nephropathy, MicroRNA, Autophagy, Diabetes mellitus, Biomarkers.

Introduction

Diabetic nephropathy (DKD) is one of the most serious complications of diabetes mellitus. It is characterized by elevated urinary albumin excretion, impaired glomerular filtration rate (GFR), and a progressive decline in kidney function that ultimately leads to end-stage kidney failure. Identifying patients in the early stage of DKD is an important step for effective management and treatment. Some studies[1] suggest that microalbuminuria, a widely used biomarker for DKD, is a less precise predictor of nephropathy risk than originally thought. Thus, there is an increasing quest to find novel biomarkers to identify and treat individuals at high risk and also to help identify new players in the pathogenesis of glomerular injury in diabetes.

Recently, a novel class of non-coding RNA, microRNA (miRNA), has emerged as potential biomarkers. Mature miRNAs can be detected in a variety of human body fluids, including blood, saliva, and urine, and play important roles in tissue homeostasis and disease progression, thus affecting almost every key cellular function[2]. Normally, miRNAs are protected from endogenous RNase activity, allowing them to remain remarkably stable. Therefore, circulating miRNAs could be potentially useful biomarkers to monitor pathophysiological changes and disease prognosis. A study[3] has confirmed the hypothesis that microRNAs are useful in the early identification of patients who will go on to develop microalbuminuria. A paper[4] reported differences in urinary exosomal microRNAs between normoalbuminuric and microalbuminuric type 1 diabetes patients with incipient nephropathy. It is now appreciated that miRNAs serve as novel and potent regulators of the autophagy pathway.

Autophagy, initially described as a lysosome-dependent degradation of cytoplasmic contents on starvation, has been implicated in almost every facet of human health and can be linked to a myriad of human diseases including DKD. MiRNAs have been identified to have crucial

Corresponding Author: Likun Du, Ph.D; e-mail: 362940257@qq.com

roles in various stages of autophagy, including induction, vesicle nucleation, elongation and completion, docking and fusion, and degradation and recycling. The involvement of different miRNAs in various steps of autophagy suggests the importance of autophagy in cellular homeostasis and disease conditions. However, it is still a matter of debate whether miRNA-regulated autophagy reflects ultimately a protective response or a detrimental process during disease progression. It seems clear that the autophagy pathway does not exist in isolation, but is integrated with other cell signaling networks cross-regulated by miRNAs. It is now well recognized that miRNA-autophagy crosstalk sheds light on new targets for DKD therapies. The aim of this review is to summarize our knowledge regarding DKD-related miRNAs, and whether it might be beneficial to investigate the potential targets of miRNAs and autophagy for possible diagnostic strategies and therapeutic intervention.

Biogenesis and Functional Mechanism of MicroRNA-Mediated Translational Repression

MiRNAs (microRNAs) are endogenously produced short non-coding RNAs with a length of 22-25 nucleotides and are present in all mammals, plants, and viruses. They have been shown to play a key role in mammalian post-transcriptional gene expression by repressing translation or inducing target degradation, ultimately resulting in gene silencing[5,6]. It is currently estimated that miRNAs regulate the expression of at least 60% of all protein coding genes, and alterations in miRNA expression profiles have been observed in numerous pathological processes. Consequently, there is much interest in miRNAs both as novel biomarkers and as potential targets for therapeutic intervention.

The biogenesis of miRNAs is a multistep process that begins with nuclear transcription. Primary miRNA transcripts (pri-miRNA) are transcribed by RNA polymerase II, the same polymerase that transcribes protein-coding genes. Pri-miRNAs vary in length, in some cases spanning kilobases, and have a distinctive stem-loop structure that encodes the functional miRNA sequences in the stem[7]. This stem loop structure is cleaved within the nucleus into precursor miRNAs (pre-miRNAs) by a multiprotein complex, formed by Dicer (an RNase III type enzyme) and its cofactor DiGeorge syndrome critical region gene 8 (DGRC8). The pre-miRNAs are then exported out of the nucleus to the cytoplasm by Exportin 5, where they undergo further processing.

After strand separation of the duplexes, a strand of the duplex is selected to be loaded onto the RNA-induced silencing complex (RISC), whereas the complementary strands are likely degraded. The main constituents of the RISC are members of the Argonaute (AGO) family that have robust endonuclease activity to degrade the target mRNAs or to block protein translation. The RISC-bound miRNA is able to recognize and bind the 3′-UTR region of the target mRNA via a sequence complementarity. If there is a perfect complementarity between miRNA and target mRNA 3′-UTR, the target will be cleaved by AGO2. However, imperfect complementarity results in the inhibition of the target's translation or degradation in processing bodies (P-bodies), ultimately leading to the gene silencing[8].

miRNAs in Diabetic Nephropathy

DKD is a progressive kidney disease and a major debilitating complication of both type 1 and type 2 diabetes that can lead to end-stage renal disease (ESRD). The major pathological features of DKD are characterized by expansion of the glomerular mesangium (hypertrophy), tubulointerstitial fibrosis and glomerular basement membrane thickening due to the accumulation of extracellular matrix (ECM) proteins such as collagen, and podocyte dysfunction along with proteinuria. Several key pathways have been found to be induced by hyperglycemia to promote renal dysfunction including an abnormally active renin-angiotensin system (RAS), overproduction of reactive oxygen species (ROS), increased advanced glycation end products (AGEs), TGF-β1protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), and enhanced pro-inflammatory cytokine signaling[9]. Current therapies for DKD are aimed at controlling blood glucose levels and blood pressure, and in particular, inhibition of the RAS to reduce or abrogate the development of albuminuria and progression of DKD. However, these methods of treatment can slow but usually not prevent progressive injury in diabetic nephropathy. Therefore, therapeutic interventions directed are important at preventing the progression of DKD.

Over the past decade, a vast number of studies exploring the significant contribution of miRNAs to human health and disease have underscored the critical relevance of miRNAs to basic and translational biology. MiRNAs are critically involved

in many biological processes, and accumulating evidence also points to an important role of miRNAs in the pathogenesis of both diabetes and diabetes-related complications[10]. In mice, conditional knockout of Dicer in specific renal cell types leads to developmental phenotypes, supporting a role of miRNAs in kidney development. In podocytes, selective inactivation of Dicer results in foot process effacement, proteinuria, tubulointerstitial fibrosis, and glomerulosclerosis at 2-4 weeks after birth, and finally animals die likely due to severe kidney failure[11,12]. There are 2214 and 848 known miRNAs in humans and mice. Precise control of miRNA levels is crucial to maintain normal cellular functions, and the dysregulation of miRNA is often associated with human diseases, such as DKD. Under diabetic conditions, several miRNAs are upregulated in diabetic kidney. These miRNAs bind to the 3′-UTR of renoprotective genes which leads to their decreased expression. As a result, these upregulated miRNAs contribute to the pathogenesis of DKD. There are also downregulated miRNAs. It is reasonable that these downregulated miRNAs are DKD-inhibiting miRNAs which lead to the decrease of these DKD-inducing factors.

Different miRNAs are involved in targeting the features of DKD disease progression. HG/TGF-β is mainly involved in causing DKD regulation by different pathways. Analyses of miRNA expression profiles have identified a set of miRNAs expressed mainly in the adult human kidney (including miR-215, miR-146a, and miR-886); other miRNAs, such as miR-192, miR-194, miR-21, miR-200a, miR-204, and let-7a-g, are enriched in the kidney as well as in other organs[13]. TGF-β regulates the miRNA transcriptional profile in the kidney in a cell-dependent and context-dependent manner through Smad signaling. Zhong et al[14] found that TGF-β upregulates the expression of the profibrotic miR-21 in cultured proximal tubular epithelial cells (PTECs) via Smad3 signaling, both at the transcriptional and post-transcriptional levels. The overexpression of miR-21 acts as a central moderator of signal transduction pathways in PTECs by downregulation of PTEN and activation of the P13k/Akt pathway–a pathogenic pathway in DKD[15]. In several studies, Kato et al[16-20] identified a miRNA circuit in TGF-β dependent renal mesangial cell injury in which miR-192 is the central miRNA up-regulated by TGF-β activation. They observed that miR-192 was upregulated along with an increased mRNA level of collagen 1 alpha 2 (COL1α2) compared with nondiabetic control in glomeruli isolated from streptozotocin (STZ)-induced type 1 diabetic mice and db/db type 2 diabetic mice. Conversely, studies[21] from another laboratory found a decreased miR-192 expression in the advanced-stage human DKD renal biopsy samples accompanied, by a low estimated GFR and tubulointerstitial fibrosis. Further studies also found that TGF-β treatment decreased the expression of miR-192 in rat proximal tubular cells (NRK-52E), primary rat mesangial cells, human podocytes, and the kidneys of apolipoprotein E diabetic mice. The discrepancies (anti- and pro-fibrotic effects) might be due to differences in cell types and animal species. Further studies are needed to explain the differences between these results.

Autophagy

Autophagy is a lysosomal protein degradation pathway that delivers intracellular constituents to lysosomes for degradation to maintain homeostasis and cell integrity. It has two major roles in cells: to recycle intracellular energy resources in response to nutrient-depleted conditions and to remove cytotoxic proteins and organelles under stressful conditions. Three major types of autophagy have been recognized in cells, namely macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy, hereafter referred to as autophagy, has been the most intensively investigated. The process of autophagy is initiated by the formation of a double-membrane bound vesicle known as the autophagosome with sequestered materials inside. The autophagosome subsequently fuses with the lysosome or endosomes to form single-membrane autolysosomes, and the enclosed contents are degraded and recycled.

Autophagy is a well-coordinated multi-step process regulated by *ATG* (autophagy-related) gene products originally identified in yeast. Four major steps are involved in the formation of autophagosomes: initiation, nucleation, elongation, and closure. Autophagy induction is controlled by the gatekeeper, the mammalian target of rapamycin complex 1 (mTOC1), which can sense and integrate stress signals from various sources, including growth factors, amino acids, hypoxia, and energy levels. Under normal conditions, mTORC1 is active and represses autophagy by precluding the assembly of Unc-51-like kinase 1/2 (Ulk1/2) complex (mammalian ortholog of the yeast Atg1), including Ulk1/2, Atg13, FIP200 (mammalian homolog of the yeast Atg 17)[2-24], and

requires the activity of the class III phosphatidylinositol 3-kinase (PI3K), Vps34[25]. The Vps34 activity is enhanced by its interaction with Beclin 1, and the Vps34-Atg14L complex facilitates vesicle nucleation and phagophore formation. The phosphorylation of Atg13 and FIP200 by Ulk1 is essential for triggering autophagy. The class I PI3K complex and a small GTPase (Ras) could activate the PI3K-PKD1-AKT pathway and the Ras-Raf-1-MEK1/2-ERK1/2 pathways, respectively. This, in turn, activates mTORC1, causing the inhibition of autophagy

During autophagosome elongation/closure, two novel conjugation systems are involved: LC3 (microtubule-associated protein 1 light chain 3) and Atg12[26]. LC3 is an ubiquitin-like protein that is part of one of the systems, and it undergoes post-translational modifications involving the conversion of a soluble form (LC3-I) to a membrane-associated form (LC3-II). The conversion of LC3-I conjugated to phosphatidylethanolamine through two consecutive ubiquitination-like reactions that are catalyzed by E1-like enzyme Atg7 and the E2-like enzyme Atg3 to form LC3-II. Thus, LC3-II formation is recognized as a marker of autophagosome formation in cell or animal experiments[27,28].

Autophagy in Kidney Disease and Diabetic Nephropathy

Autophagy is basically a cell-protection mechanism because it maintains the cell's energy level under nutrient-depleted conditions, regulates the turnover of aged or abnormal proteins, and eliminates damaged organelles. However, it may also promote cell death through excessive degradation of cellular constituents, depending on the cellular and environmental context[29]. Previously, the study of autophagy has been undertaken in lower species. In mammalian systems, autophagy is advancing rapidly and has revealed that mammalian autophagy is involved in the pathogenesis of various metabolic or age-related diseases.

Recently, more attention has been paid to the effect of autophagy in kidney disease and DKD. A growing body of evidence indicates that autophagy is important in many biological processes, and deficiency in autophagy contributes to the pathogenesis of kidney diseases[30]. The regulation and function of autophagy in the kidney are likely cell type and context specific. Hartleben et al[31] showed that podocytes have a high level of basal autophagy, which may serve as a mechanism for their maintenance of cellular homeostasis. Furthermore, podocyte-specific deletion of the Atg5 gene led to the development of glomerulopathy in aging mice, with oxidized and ubiquitinated protein accumulation and ER stress that ultimately led to more severe albuminuria, loss of podocytes and glomerulosclerosis, compared with control mice. Similar changes have been found in mesangial cells[32], glomerular endothelial cells[33], and proximal tubular epithelial cells[34]. All these findings suggest that autophagy serves as an essential mechanism to maintain homeostasis of glomeruli and tubules, and plays an important role in the pathogenesis of DKD in both type 1 diabetes (T1D) and type 2 diabetes (T2D).

MicroRNAs as Emerging Players in Kidney Disease Targeting Autophagy

Mounting evidence indicates that microRNAs (miRNAs)-mediated post-transcriptional regulation of gene expression represents an integral part of the autophagy regulatory network and may have a substantial effect on autophagy-related physiological and pathological conditions. Moreover, miRNAs modulate autophagy at different stages, such as autophagic induction, vesicle nucleation, vesicle elongation and completion, by targeting autophagy complexes via different miRNAs. Although a growing body of evidence indicates that miRNAs modulate autophagy, their target genes and precise roles in the autophagy pathways have not been fully defined yet.

MiRNAs are involved in the induction of autophagy and regulate the major autophagy cascades. One of the initial events upon autophagy induction is the activation of the ULK1 complex, which is directly controlled by AMPK-mTORC1. Several miRNAs have been reported to interfere with upstream autophagy signaling by targeting AMPK-mTORC1. Lu et al[35] observed that glucose-induced up-regulation of mesangial cell miRNA-21 expression up-regulates phosphatase and tensin homolog (PTEN) expression, inhibits the activation of the PI3K/Akt/mTOR signaling pathway, and enhances autophagy to reduce the accumulation of the extracellular matrix and ameliorate cell hypertrophy and proliferation. Mir-21 can also target PTEN and PRAS40, which in turn may activate Akt and TORC1 respectively, causing renal fibrosis and hypertrophy[15]. PTEN as a negative regulator of the PI3K signaling pathway was also demonstrated as a hot target of a number of miRNAs, including miR-214, miR-216a, miR-217, miR-26a, and miR-18a, that are involved in the regulation of several cell types[36-38]. MiR-

NAs are also involved in the regulation of two conjugation systems. By targeting the LC3 beta isoform (LC3B), miR-204 impeded autophagy and suppressed the growth of renal clear cell carcinoma[39]. Moreover, miRNA was shown to silence the expression of the ATG family member. MiR-375 can suppress autophagy by targeting Atg7, independent of its regulation of oncogenic AKT/mTORC1 signaling[40]. The miRNA-autophagy crosstalk reprograms the biological functions of autophagy during kidney disease development and also provides new ideas for therapies.

Conclusions

Over a period of decades, a large number of studies have attempted to reveal the molecular mechanisms underlying DKD and to develop new therapeutic strategies. However, the incidence of end-stage kidney disease due to DKD continues to increase worldwide. Albuminuria is widely used as a biomarker for DKD. However, its clinical relevance as a surrogate outcome in chronic kidney disease is controversial, and recent studies suggest that microalbuminuria is a less precise predictor of nephropathy risk than originally thought. Thus, there is an increasing quest to identify novel biomarkers reflecting early effects during disease development. In addition, early biomarker discovery potentially allows monitoring predictive and/or prognostic treatment effects in the pathogenesis of the glomerular injury in diabetes. There is consistent evidence supporting the hypothesis that miRNA analyses show promise for these transcripts as both biomarkers and therapies in kidney pathologies[41]. *In vitro* and *in vivo* animal models have shown a critical role of miRNAs in the development of DKD and in the progression of kidney fibrosis[42]. The study of miRNAs in the regulation of kidney development, physiology and pathology has emerged as an important and potentially fruitful area of research, especially in the last few years. It has been recognized that miRNAs can rapidly rise and then decrease under various cellular conditions. However, the research field of miRNAs in the development of kidney diseases is still in its early stage, but is expanding rapidly.

Autophagy is an intracellular catabolic process in which proteins and organelles are degraded via lysosomes to maintain intracellular homeostasis. Autophagy has garnered widespread interest as an important pathway in many biological functions. It plays key roles in normal and disease states, including immunity, inflammation, development and aging, metabolic and neurodegenerative disorders, and cancer[43]. Thus, autophagy represents an essential cytoprotective pathway. However, altered autophagy is often associated with pathologies within metabolic processes. It is now appreciated that miRNAs serve as novel and potent regulators of the autophagy pathway. The study of autophagy and miRNAs has only recently begun; therefore, evidence showing their involvement in the pathogenesis of DKD is still emerging. A great number of issues remain to be resolved.

Clearly, research will advance our understanding of the molecular mechanisms of miRNA and autophagy in DKD. The clinical observational discussion of this study has some limitations. It is an open question as to what role – and under what conditions-miRNA response to autophagic dying cells may play in various physiological environments and settings. The identification and characterization of the miRNAs in various kidney diseases may lead to breakthroughs in the development of novel diagnostic tools and therapeutic interventions. Nonetheless, further research is needed to define whether autophagy is beneficial or pathological in more specific disease contexts. Future studies should be designed specifically to investigate the role of autophagy in T2D using miRNA as the main outcome. A better understanding of the interaction between miRNAs and cellular autophagy may ultimately benefit future DKD diagnosis and therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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