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Research Progress of miRNA in Diabetic Nephropathy

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Abstract: Diabetic nephropathy (DN) accounts for approximately 20–40% of diabetic patients. It is one of the most common microvascular complications of diabetes and an important cause of end-stage renal disease (ESRD). Renal biopsy histopathology is an important means for early clinical diagnosis of DN, but because it is an invasive examination, it is not easily accepted by patients due to risks such as bleeding, infection, and thrombosis. Therefore, finding new biomarkers for diagnosing DN is of great significance for early treatment and improving patient prognosis. MicroRNA (miRNA) is a type of highly conserved endogenous non-coding RNA. More and more studies have shown that miRNA is involved in the pathological process of DN and renal fibrosis. This article summarizes the relevant research on miRNA in DN.

Keywords: Diabetic nephropathy; miRNA; circRNA; Fibrosis; Apoptosis; Inflammation

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1. Introduction

China has had the largest number of diabetic patients in the world for the past ten 10 years, and the incidence has been increasing [1]. Diabetic nephropathy (DN) is a chronic kidney disease (CKD) caused by diabetes. About 20–40% of diabetics can progress to DN [2]. CKD is characterized by persistent albuminuria and/or an estimation of the glomerular filtration rate (eGFR) of lower than 60 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ [3]. DN is the leading cause of CKD end-stage renal disease (ESRD) worldwide [4]. In developing countries such as China, glomerulonephritis has always been the main cause of CKD. However, recent studies have shown that since 2011, the incidence of CKD caused by diabetes has increased rapidly, surpassing hypertension and glomerulonephritis. The incidence of related CKD has become the main cause of CKD [5]. Therefore, early diagnosis and intervention of diabetic kidney disease (DKD) are particularly important. At present, renal biopsy histopathology is still the gold standard for diagnosing DKD, but it is invasive and has limited clinical applications. Urinary microalbumin (UmAlb) is a widely used clinical indicator for diagnosing DN, but in type 2 diabetic patients (T2DM) with reduced GFR, about 30–45% showed no increase in UmAlb (> 30mg/g) [6]. In addition, common comorbidities of T2DM such as hypertension or obesity may also damage the glomerular filtration barrier, leading to an increase in UmAlb. This indicates that UmAlb alone is not sensitive or specific enough to diagnose DN.

Therefore, finding new biomarkers for diagnosing DN is of great significance for early treatment and improving patient prognosis. MicroRNA (miRNA) is a type of highly conserved endogenous non-coding RNA. More and more studies have shown that miRNA is involved in the pathological process of DN and renal fibrosis. circRNA can be used as competing endogenous RN (ceRN), that is, as a molecular "sponge" of miRNA to regulate gene expression. This article summarizes the research progress on miRNA in relation to DN.

2. Introduction of miRNA and its physiological functions

miRNAs are a type of endogenous non-coding RNA with highly conserved sequences that are ubiquitous in mammals. They are distributed in various tissues and organs throughout the body and mainly function as post-transcriptional regulators. The miRNA response elements (MREs) in the 3' UTR region of miRNA can bind to mRNA and further inhibit the function of the target gene [7]. In recent years, it has been confirmed that more than 90% of the mammalian genome can be actively transcribed, and most of them belong to the category of non-coding RNAs (ncRNAs) [8,9]. ncRNAs mainly consist of microRNA (miRNA), long non-coding RNA (lncRNA), circular RNA (circRNA), pRNA, and tRNA, which regulate a wide range of biological processes and epigenetic levels post-transcriptionally [10]. circRNA can function as ceRNA (competing endogenous RNA, competing endogenous RNA), that is, as a molecular "sponge" for miRNA. It can also bind to MREs in the 3' UTR region of miRNA to further inhibit the function of miRNA [11], thereby achieving the regulation of gene expression. The biological functions of miRNAs are determined by the mRNAs they control. Effective gene regulation is a combination of miRNA localization, target mRNA levels, affinity of the miRNA-mRNA interaction, and the presence of multiple factors. However, the activity of miRNA on the mRNA target is difficult to determine. Regardless of the mechanism, the ultimate goal is to produce a functional, mature miRNA that can regulate protein expression [12]

In recent years, more and more studies have confirmed that miRNAs encoded by the nuclear genome can enter the endoplasmic reticulum, ribosomes, mitochondria, and other organelles to play a gene regulatory role and play an important role in cell metabolism and the occurrence and development of diseases. The regulatory role of miRNA in the occurrence and development of kidney diseases has been highlighted, especially in the topic of diabetic nephropathy, in which the primary focus is on fibrosis, inflammation, and oxidative stress.

3. Characteristics of DN and problems in early diagnosis

DN is one of the major microvascular complications of diabetes and the main cause of ESRD worldwide. The incidence of DN is increasing rapidly in China, and about 20% to 40% of diabetic patients develop DN. DN is also a common cause of CKD. The occurrence and development of DN are related to immune-inflammatory response, oxidative stress, metabolic abnormalities, and epigenetics, among which epigenetics plays an important role in the occurrence and development of DN. In addition to renal biopsy, urinary microalbumin is commonly used clinically as an indicator to diagnose DN. However, increased urinary microalbumin can be caused by many factors. Clinically, obesity combined with other causes of kidney disease can lead to microurinary albumin increases, so this indicator lacks specificity and is insufficient for an early diagnosis.

miRNA is a non-coding RNA with a length of about 20 nucleic acids. It regulates target genes during the transcription process and plays an important role in kidney diseases. Previous studies have shown that miRNA participates in DN by affecting the expression of multiple signaling molecules in multiple signaling pathways. Its pathogenesis is related to renal function damage, interstitial fibrosis, and podocyte apoptosis in DN patients [13,14], which provides new ideas for understanding the pathogenesis of DN.

4. The main mechanism of miRNAs regulating the occurrence and development of diabetic nephropathy

4.1. Research on miRNAs' role in inflammation and oxidative stress in DN

Researchers found 16 types of miRNA in the urine of DN patients, including hsa-miR-514a-5p, hsa-miR-451a, hsa-miR-126-3p, hsa-miR-214, and hsa-miR-503. Eight miRNAs of hsa-miR1-3, hsa-miR-4792, hsa-miR-375, hsa-miR-1268, hsa-miR-501-5p and hsa-miR-582 were down-regulated. Further prediction and pathway analysis of potential target genes by the Kyoto Encyclopedia of Genes and Genomes (KEGG) confirmed that it is related to cellular processes such as apoptosis, inflammation, and tissue transdifferentiation. These effects promote the occurrence of diabetic complications such as DN [15]. However, this study is only a bioinformatics prediction, and further *in vitro* functional studies are needed to confirm the mechanism of their involvement in the pathological process of DN. There has been increasing evidence suggesting that inflammation, oxidative stress, and autophagy disorders contribute to the pathogenesis of DN.

Exosomes (EXO) derived from human umbilical cord mesenchymal stem cells (HUC-MSCS) have been shown to be an effective treatment for DKD, but the underlying mechanisms of this effect remain poorly understood. Wang *et al.* [16] studied the relationship between DN and inflammasome activation, as well as the pathophysiological relevance of EXO-mediated inflammation relief and damage repair in this process. This study co-cultured podocytes and MSC-EXO under high glucose (HG) and injected MSC-EXO into diabetic mice. It was found that HG reduced the viability of podocytes, activated the NLRP3 signaling pathway, increased the number of podocytes, and increased the inflammatory response in diabetic mice. MSCS-EXO can reduce the inflammatory response of podocytes in diabetic mice, inhibit the activation of the NLRP3 signaling pathway, and reduce kidney damage. Further research found that miR-22-3p can reduce the inflammatory response by inhibiting the expression of NLRP3. At the same time, knocking out miR-22-3p can eliminate its anti-inflammatory activity *in vivo* and *in vitro*. This suggests that exons derived from MSC may be a new target for the treatment of DN. Another study [17] found that the combined diagnosis of urinary exosome-derived miRNA-615-3p and ACR is expected to become a more stable and sensitive diagnostic standard for DN. This study confirmed that the expression of miRNA-615-3p in diabetic nephropathy patients is significantly higher than that in normal people and type 2 diabetic patients, and plays a role in mediating inflammation and fibrosis in diabetic nephropathy. (A total of 83 subjects were studied in this study, including 20 normal subjects, 21 T2DM patients, and 42 diabetic nephropathy patients. Exosomes were extracted from their urine for comparison. At the same time, the correlation between inflammation and fibrosis in 42 diabetic nephropathy patients was analyzed.)

Another study found that AIF-1 regulates inflammation, oxidative stress, and autophagy levels in human renal glomerular endothelial cells through the miR34a/*ATG4B* pathway, thereby participating in regulating the pathogenesis of diabetic nephropathy. *In vivo* experiments found that the levels of *AIF-1*, miR-34a, oxidative stress, and inflammatory factors were significantly increased in the blood and urine samples of DN patients and mouse models, these inflammatory factors were also correlated with urinary protein levels. *In vitro* experiments observed that the expression of AF-1/miR-34a/ROS and inflammatory factors in human glomerular endothelial cells increased when induced by HG, while the expression of *ATG4B* and other autophagy-related proteins decreased. It was further confirmed that miR-34a had a targeting effect on *ATG4B*. When the *AIF-1* gene was overexpressed, the levels of miR-34a, reactive oxygen species (ROS), and inflammatory factors were significantly increased, and the expression of autophagy-related proteins such as *ATG4B* was down-regulated, while the *AIF-1* gene was upregulated. In addition, miR-34a inhibited the expression of *ATG4B* and autophagy-related proteins and increased ROS and inflammation levels. When the *ATG4B* gene was overexpressed, the level of autophagy was increased and the inflammatory factors were downregulated. In contrast, when

the *ATG4B* gene was inhibited, autophagy levels were downregulated and the inflammatory factors were upregulated. Then, autophagy inducers suppressed the inflammation and ROS levels [18]. Bai *et al.* found that mi-20a may regulate high glucose-induced apoptosis, inflammatory response, and cell proliferation through the miR-20a/CXCL8 axis by regulating the expression of *CXCL8* and the MEK/ERK pathway [19].

4.2. Research progress of miRNAs in renal fibrosis in DN

DN is characterized by the progressive accumulation of glomerular mesangial extracellular matrix (ECM) and basement membrane thickening, leading to glomerulosclerosis, that is, renal interstitial fibrosis caused by increased ECM protein deposition [20]. Hyperglycemia is the main driving force for the progression of DN, while micro-inflammation and increased ECM are the main reasons for progression. Some studies have emphasized the important role of chronic low-grade inflammation in the progression of DN. Animal experiments and clinical experiments have also verified the activation of inflammatory signaling pathways and increased expression of cell adhesion molecules, chemokines, and pro-inflammatory factors in DN kidney tissue. These all point to inflammation as the main mechanism for the occurrence and development of DN [21]. Many studies have shown that miRNAs (miRNA-17-5p, miRNA-29, and miRNA-223) can regulate the occurrence and development of cellular inflammation [22-24]. Many scholars have experimentally proven that miRNAs (miRNA-186, miRNA-140, and miRNA-578) can affect the deposition of ECM [25-26]. The latest research [27] shows that CircLARP1B is a molecular sponge of miR-578, targeting TLR4 to play a role in apoptosis, inflammation, and fibrosis. The study confirmed that CircLARP1B and TLR4 are highly expressed in cells induced by high glucose, miR- 578 has low expression, and knocking out circLARP1B can inhibit high glucose-induced apoptosis and inflammation. *MMP2* is recognized as a biomarker of DKD fibrosis. Yin *et al.* [28] found that miR-106b-5p and miR-93-5p can act as upstream signal regulators to regulate the expression of *MMP2* and participate in the process of DKD fibrosis. Circ_0114428 and Circ_0123996 promote the progression of DN by regulating the miR-203a-3p/SOX6 and miR-185-5p/SMAD3 axes to promote high glucose-induced glomerular mesangial cell proliferation, fibrosis, and EMT processes [29,30].

4.3. Research on miRNAs and podocyte damage caused by DN

Podocytosis is a glomerular disease characterized by reduced number and density of podocytes, thickening of the basement membranes, and a combination of foot processes under an electron microscope. Podocyte injury is closely related to proteinuria and the decrease in GFR. In DN, podocyte damage is induced by multiple signaling pathways. It has been discovered that the signaling pathway related to podocyte damage in diabetic nephropathy is TGF-B1/SMAD7, which can directly induce apoptosis of podocytes in diabetic nephropathy [31]. TGF-1/MAP kinase/bcl-2/Bax induces podocyte apoptosis, thereby activating the caspase-3 apoptosis pathway [32]. TGF-1 can also induce ROS production leading to increased podocyte apoptosis. The mTORC1 inhibitor, rapamycin, has been shown to inhibit podocyte damage caused by HG in DN. The JAK-STAT signaling pathway has also been reported to play an important role in DN. In glomerular cells, including podocytes, this pathway is even activated in patients with early-stage DN. Treatment with the JAK1/2 inhibitor baricitinib reduces albuminuria in patients with DN [33].

miRNAs, which are critical for regulating transcription following podocyte gene expression, have emerged as biomarkers in certain podocyte-damaging diseases and as potential targets for future treatments. Several studies have reported that the overexpression or knockout of different miRNAs has a significant impact on podocyte damage in diabetic nephropathy in highly stimulated podocytes cultured *in vitro*. High glucose stimulation can inhibit human podocyte miR-16-5p and promote VEGF production. Overexpression of miR-16-5p can reduce podocyte damage in rats with DN. This protective effect is achieved by inhibiting the production

of VEGF. Overexpression of miRNA-21 in cultured mouse podocytes reduces the expression level of nephrin and increases the expression level of a-SMA by activating the B-catenin pathway and the TGF-β1/SMAD pathway. A study found that the expression of miR-26a-5p and nephrin decreased and the expression of TRPC6 increased in podocytes induced by high glucose, and further confirmed that miR-26a-5p targetedly regulates TRPC6 expression to reduce DKD podocyte damage. Another study found that the protein expression of miR-31, TGF-B1, and a-SMA in human glomerular podocytes induced by high glucose was significantly increased, while the expression level of FIH-1 protein was significantly down-regulated, further confirming that miR-31 has a targeting relationship with FIH-1 to regulate high glucose-induced epithelial-mesenchymal transition in human glomerular podocytes. There are many other miRNAs involved in the regulation of podocyte damage. miRNAs play a key role in the development of podocyte damage in DN, suggesting that miRNAs may be potential biomarkers and therapeutic targets, and further clinical studies are still needed to investigate their usefulness in detail.

5. Summary and outlook

In summary, miRNAs are widely distributed in the cells of various organs throughout the body, and participate in cellular metabolic processes such as inflammation, oxidative stress, tissue fibrosis, apoptosis, autophagy, and aging through multiple signaling pathways, thereby affecting the occurrence and development of kidney diseases. Specific miRNAs in blood or urine are expected to be used as early non-invasive diagnostic biomarkers, and are expected to become new targets for disease molecular treatment by regulating the circRNA /miRNA/target gene axis. However, current research on the role of miRNA in the occurrence and development of diabetic nephropathy is still limited to the laboratory level, and safety and specificity issues still need to be resolved in order to translate the laboratory findings into clinical studies. It is expected to be found in animal models of kidney disease that regulating certain miRNAs can specifically improve cell metabolism and overall kidney function.

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Disclosure statement

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