Circular RNA circ_0000712 regulates high glucose-induced apoptosis, inflammation, oxidative stress, and fibrosis in (DN) by targeting the miR-879-5p/SOX6 axis

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Abstract. Diabetic nephropathy (DN), a frequent diabetes complication, has complex pathogenesis. Circular RNAs (circRNAs) circ_0000712 has been reported to be upregulated in kidney tissues and high glucose (HG)-inducted Mesangial cells (MCs). This study is designed to explore the role and mechanism of circ_0000712 in the HG-inducted MCs injury in DN. Circ_0000712, microRNA-879-5p (miR-879-5p), and SRY-Box Transcription Factor 6 (SOX6) levels were detected by real-time quantitative polymerase chain reaction (RT-qPCR). Cell apoptosis was examined by flow cytometry assay. Protein levels of B-cell lymphoma-2 (Bcl-2), Bcl-2 related X protein (Bax), fibronectin (FN), collagen type I (Col. I), collagen type IV (Col. IV), and SOX6 were assessed by western blot assay. Levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α) were measured by enzyme-linked immunosorbent assay (ELISA). Reactive oxygen species (ROS) generation, Lactate Dehydrogenase (LDH) activity, and Superoxide Dismutase (SOD) activity were detected by the corresponding kits. The binding relationship between miR-879-5p and circ_0000712 or SOX6 was predicted by starBase and Targetscan, and then verified by a dual-luciferase reporter and RNA Immunoprecipitation (RIP) assays. Circ_0000712 and SOX6 were highly expressed, and miR-879-5p was decreased in db/db DN mice and HG-inducted SV40-MES13 cells. Furthermore, circ_0000712 deficiency repressed HG-caused apoptosis, inflammation, oxidative stress, and fibrosis in SV40-MES13 cells. Mechanically, circ_0000712 could regulate SOX6 expression by sponging miR-879-5p. Circ_0000712 knockdown could hinder HG-inducted SV40-MES13 cell injury through targeting the miR-879-5p/SOX6 axis, implying a possible circRNA-targeted therapy for DN.

Key words: Circ_0000712, miR-879-5p, Diabetic nephropathy, High glucose, Mouse mesangial cells

AS A FREQUENT DIABETES COMPLICATION, diabetic nephropathy (DN) has gradually imposed an extremely burdensome on the health and economies of countries worldwide [1, 2]. The complex interaction between hemodynamics and metabolic disorders was considered to the pathogenesis of DN [3]. Mesangial cells (MCs), a class of glomerular proper cells, exert a vital role in the regulation of glomerular filtration by the interconnecting fibrillar networks [4]. Notably, when exposed to high glucose (HG) microenvironments, MCs could cause the ectopic expression of cytokine and fibroin, thus leading to kidney fibrosis [5]. Hence, exploring the molecular mechanisms of DN under HG condition is imperative to develop a more effective diagnosis biomarker and target for clinical treatment.

In recent years, more than 90% of the mammalian genome has been proven to be actively transcribed, most of which are non-coding RNAs [6, 7]. As an emerging non-coding, circular RNAs (circRNAs) have been attracted intensive attention owing to their unique characteristics, with covalently closed loops structure [8]. Emerging evidence indicated that circRNAs were associated with the pathogenesis of certain renal diseases [9], including DN [10]. In fact, it is becoming increasingly apparent that circRNAs acted as essential regulators in DN biology through sequestering their target microRNAs (miRNAs) [11]. For example, Wang et al. reported that the circ_0123996 could aggravate HG-induced mouse mesangial cell injury by regulating Bach1 via sponging mir-149-5p in DN [12]. Similarly, Chen et al. found that circLRP6 as a competing
endogenous RNA (ceRNA) of miR-205 to boost HG-triggered MCs proliferation, oxidative stress, and inflammation in DN [13]. Of note, a prior study indicated that a novel circRNA, circ_0000712 was identified to be increased in kidney tissues and HG-induced MCs [14]. To our knowledge, the precise role and underlying mechanism of circ_0000712 in DN remain largely unknown.

In this study, bioinformatics analysis first revealed that circ_0000712 possessed some complementary sites with miR-879-5p. Furthermore, miR-879-5p has been pointed out to be downregulated in HG-treated podocytes, which was a kind of highly differentiated glomerular epithelial cell [15]. Therefore, we aimed to demonstrate the role of circ_0000712 in HG-inducted MCs, and to explore whether the regulatory role of circ_0000712 on HG-triggered cell injury in DN in a miRNA-mRNA dependent manner.

**Materials and Methods**

**DN animal models**

Ten-week-old male db/db mice (n = 6) and matched normal littersmates db/m mice (n = 6) (Vital River Laboratory, Beijing, China) were introduced into this study, which was approved by the Animal Ethics Committee of the Affiliated Dongfeng Hospital, Hubei University of Medicine. All mice (weight 18–22 g) were housed in a specific pathogen-free animal facility with a programmed 12 h light/12 h dark cycle for circadian control, and food water was freely available during the exposure. After culture for 12 weeks, all mice were sacrificed to collect the mouse renal cortical tissue for further analysis.

**Cell culture**

Under a humidified atmosphere of 5% CO₂ at 37°C, the mouse mesangial cells (SV40-MES13, American Type Culture Collection, Manassas, VA, USA) were grown in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (PAN Biotech, Aidenbach, Germany). For DN cell models, SV40-MES13 cells were respectively inducted with high glucose (HG, 30 mM Glu) and normal glucose (NG, 5.5 mM Glu).

**Real-time quantitative polymerase chain reaction (RT-qPCR)**

According to the operation manual of Trizol reagent (Gibco), total RNA was isolated from tissues and cells, followed by reverse transcription with PrimeScript RT Master Mix (TaKaRa, Dalian, China) and High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Subsequently, the synthesized cDNA was subjected to PCR using SYBR Green PCR kit (TaKaRa) on an ABI 7900 System (Applied Biosystems), and GAPDH (for circ_0000712, SRY-Box Transcription Factor 6 (SOX6)) and U6 (for miR-879-5p) were used as the reference. Finally, the fold changes were analyzed according to the 2^(-ΔΔCt) method [16]. The sequences of primers in this assay were as follows:

- circ_0000712: 5'-GTCTGAGCTGTCGAGGAGGACC-3' (Forward primer), 5'-TTTACAACCTCACGGCGCCA G-3' (Reverse primer);
- miR-879-5p: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGGCTTAGA-3' (Loop primer), 5'-GTCGAGACGTTAGTCGAGT-3' (Reverse primer);
- SOX6: 5'-GGACAGCGTTCTGTCATCTC-3' (Forward primer), 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGCTTAGA-3' (Loop primer), 5'-GGACAGCGTTCTGTCATCTC-3' (Reverse primer);
- U6: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGCTTAGA-3' (Loop primer), 5'-GGACAGCGTTCTGTCATCTC-3' (Reverse primer);
- GAPDH: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGCTTAGA-3' (Loop primer), 5'-GGACAGCGTTCTGTCATCTC-3' (Reverse primer).

**RNase R treatment**

In short, RNase R (3 U/μg, Epicentre Technologies, WI, USA) were applied for the treatment with total RNA, followed by incubation for 15 min at 37°C. After purification, the samples were subjected to RT-qPCR analysis of circ_0000712 and GAPDH.

**Cell transfection**

In this assay, circ_0000712 small interference RNA (si-circ_0000712), si-SOX6, miR-879-5p mimic (miR-879-5p), miR-879-5p inhibitor (in-miR-879-5p), and their negative controls (si-NC, miR-NC, in-miR-NC) was acquired from Ribobio (Guangzhou, China). Meanwhile, the overexpression vector of SOX6 (SOX6) was generated by inserting the SOX6 sequence (Accession: NM_001277327.1) into pcDNA vector (Invitrogen, Carlsbad, CA, USA), and the empty vector acted as a negative control (pcDNA). Whereafter, the oligonucleotides and vector were transfected into SV40-MES13 cells (2 × 10⁵ cells/well), as per the supplier’s direction of Lipofectamine 3000 (Invitrogen). After transfection for 48 h, the harvested cells were used for further study.

**Cell apoptosis assay**

In short, treated SV40-MES13 cells were collected and washed with PBS (Invitrogen), followed by re-suspended in 100 μL binding buffer. Following stain
with 5 μL Annexin (V-fluorescein isothiocyanate) V-FITC/Propidium Iodide (PI) (Beyotime, Nantong, China) for 15 min, the detection of apoptosis rate was carried out using FACS San flow cytometry (BD Bioscience, San Jose, CA, USA) and Cell Quest software (BD Biosciences).

**Western blot assay**

Generally, total proteins from tissues and cells were lysed referring to the user’s guidebook of RIPA lysis buffer (Beyotime), followed by separation with 10% SDS-PAGE and transferring with a nitrocellulose membrane (Millipore). And then, the membrane was subjected to standard blocking with 5% non-fat milk, hybridization with primary antibodies at 4°C overnight, and incubation with secondary antibody at room temperature for 1 h. At last, the bands were detected according to the instruction of ECL detection kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The primary antibodies were presented as below: B-cell lymphoma-2 (Bcl-2, 1:1,000; ab194583, Abcam, Cambridge, UK), Bcl-2 related X protein (Bax, 1:1,000; ab53154, Abcam), fibronectin (FN, 1:1,000; ab45688, Abcam), collagen type I (Col. I, 1:1,000; ab34710, Abcam), collagen type IV (Col. IV, 1:1,000; ab227616, Abcam), SOX6 (1:1,000; ab64946, Abcam), and β-actin (1:5,000; ab8227, Abcam). And the horse-radish peroxidase (HRP)-conjugated secondary antibody (ab205178, 1:10,000, Abcam).

**Enzyme-linked immunosorbent assay (ELISA)**

In this assay, the levels of inflammatory cytokines containing interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α) were detected by ELISA kits (R&D Systems, Minneapolis, MN, USA). After HG or normal glucose (NG) treatment for 24 h, the culture medium of SV40-MES13 cells was harvested and assessed severally using the corresponding commercial kits (R&D Systems) based on the supplier’s direction. And then, a Spectra Max Plus plate reader (Molecular Devices, Sunnyvale, CA, USA) was applied to read the results at 450 nm.

**Measurement of ROS generation, LDH activity, and SOD activity**

For the measurement of reactive oxygen species (ROS) generation, SV40-MES13 cells were collected after treatment with HG or NG treatment for 24 h, followed by incubation with 10 nM Dichlorofluoresceindiacetate (DCFH-DA, Beyotime, Shanghai, China) for 30 min at 37°C. And then, the samples were assessed under a fluorescence microscope (485nm excitation, 530 nm emission), followed by quantification with a FACS-Calibur (BD Bioscience). For the detection of Lactate Dehydrogenase (LDH) activity and Superoxide Dismutase (SOD) activity, treated SV40-MES13 cells were harvested and lysed, followed by an examination in the cell extract using LDH kit (Beyotime) and a SOD kit-WST (Dojindo, Kumamoto, Japan), in line with the manufacturer’s instructions.

**Dual-luciferase reporter assay**

According to the analysis of starBase (http://starbase.sysu.edu.cn) and TargetsScan (www.targetscan.org) software, the possible binding relationship between miR-879-5p and circ_0000712 or SOX6 was proved by a dual-luciferase reporter assay, in brief, the sequences of circ_0000712 or SOX6 3’ un-translated region (3’UTR) containing the wild-type (WT) or mutant (MUT) sequence (miR-879-5p binding site mutation) were constructed and cloned into psiCHECK-2 vector (Promega, Madison, WI, USA), termed as circ_0000712 WT/MUT or SOX6 3’UTR WT/MUT. And then, the SV40-MES13 cells were co-transfected with these reporter vectors along with miR-879-5p or miR-NC, following the producer’s instructions of the Lipofectamine 3000 (Invitrogen). The luciferase activities in cell lysates were determined using a dual-luciferase reporter assay kit (Promega) after transfection for 48 h.

**RNA Immunoprecipitation (RIP)**

In this assay, RIP assay was performed to verify the relationship between miR-879-5p and circ_0000712, in accordance with the instructions of Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA). Briefly, SV40-MES13 cells were lysed in the complete RIP lysis buffer, followed by incubation with magnetic beads loaded with Argonaute 2 antibody (Ago2; Millipore) and a negative control IgG (Millipore) for 24 h. And the Input was as a positive control. After treatment with proteinase K, the samples were purified and examined by RT-qPCR assay.

**Statistical analysis**

The data in this study were operated using GraphPad Prism7 (GraphPad Software, La Jolla, CA, USA) and exhibited as the mean ± standard deviation (SD). Student’s t-test or one-way analysis of variance (ANOVA) with Tukey’s tests was utilized to compare the difference between two groups or multiple groups, and a p-value <0.05 was considered significant.

**Results**

**Circ_0000712 expression was increased in db/db DN mice and MCs treated with high glucose**

Firstly, to investigate the functional role of circ_0000712 in DN progression, its expression profile
was assessed by using RT-qPCR assay. As exhibited in Fig. 1A, compared with the normal db/m mice (n = 6), the upregulation of circ_0000712 was noticed in the DN db/db DN mice (n = 6). Subsequently, SV40-MES13 cells were induced with HG (30 mM Glu) and NG, (5.5 mM Glu) in vitro. Data suggested that circ_0000712 level was apparently upregulated in the 30 mM group at various treatment times, presenting a time-dependence characteristic (Fig. 1B). These results implied that the high expression of circ_0000712 might participate in the DN progression. Moreover, to further identify the circular nature of circ_0000712, SV40-MES13 cells were treated with RNase R+ or RNase R-. Results displayed that the treatment of RNase R+ reduced the mRNA level of GAPDH, while had little effect on circ_0000712 expression (Fig. 1C), verifying that circ_0000712 is indeed a circRNA resistant to treatment with RNase R digestion.

Silencing of Circ_0000712 suppressed HG-caused apoptosis, inflammation, oxidative stress, and fibrosis in MCs

In view of the high expression of circ_0000712 in HG-treated SV40-MES13 cells, we knocked down circ_0000712 in treated-SV40-MES13 cells. The transfection efficiency of si-circ_0000712 was assessed and shown in Fig. 2A. Whereafter, we used the knocking down system to further explore the function of circ_0000712 on MCs progression. Flow cytometry results exhibited that enhanced cell apoptosis caused by the treatment of HG was abolished by circ_0000712 downregulation in SV40-MES13 cells (Fig. 2B). Simultaneously, the transfection efficiency of miR-879-5p mimic or miR-879-5p inhibitor was detected and presented in SV40-MES13 cells treated with HG (Fig. 3B). To verify the interaction of mir-879-5p with circ_0000712 was mediated by the putative binding sites, the dual-luciferase reporter assay was conducted. Data analysis suggested that the luciferase activity in SV40-MES13 cells transfected with circ_0000712 WT and miR-879-5p mimic was distinctly declined relative to

Circ_0000712 directly interacted with miR-879-5p

Then, to further explore the molecular mechanism of circ_0000712, we investigated putative circ_0000712-interacting miRNAs using the online software starBase. As a result, miR-879-5p was found to have some complementary bases pairing with circ_0000712 (Fig. 3A). Simultaneously, the transfection efficiency of mir-879-5p mimic or miR-879-5p inhibitor was detected and presented in SV40-MES13 cells treated with HG (Fig. 3B). To verify the interaction of mir-879-5p with circ_0000712 was mediated by the putative binding sites, the dual-luciferase reporter assay was conducted. Data analysis suggested that the luciferase activity in SV40-MES13 cells transfected with circ_0000712 WT and miR-879-5p mimic was distinctly declined relative to

Fig. 1 Circ_0000712 was elevated in db/db DN mice and MCs treated with high glucose. (A) RT-qPCR assay was applied to measure the expression level of circ_0000712 in db/db DN mice (n = 6) and db/m non-DN mice (n = 6). (B) Circ_0000712 level was detected in mouse mesangial cells (SV40-MES13) treated with normal glucose (NG, 5.5 mM Glu) and high glucose (HG, 30 mM Glu). (C) Expression levels of circ_0000712 and linear GAPDH were examined in SV40-MES13 cells treated with RNase R+ or RNase R-. * p < 0.05.
that in cells transfected with circ_0000712 WT and miR-NC, while there was no remarkable effect in the cells with circ_0000712 MUT (Fig. 3C). Also, to further prove the mutual effect of circ_0000712 and miR-879-5p at endogenous levels, we carried out RIP assay using antibody AGO2, a core component of the RNA-induced
silencing complex (RISC) [19]. Results indicated that circ_0000712 and miR-879-5p were greatly enriched in the Anti-AGO2 group compared with both the Anti-IgG group and the Input group in SV40-MES13 cells (Fig. 3D). Interestingly, we viewed that miR-879-5p was expressed at a low level in the DN db/db DN mice in comparison with the normal db/m mice (Fig. 3E). Consistently, miR-879-5p level was markedly decreased in vitro mouse MCs treated with HG (30 mM Glu) versus those treated with NG (5.5 mM Glu) (Fig. 3F). Besides, we further assessed the impact of circ_0000712 on miR-879-5p expression in HG-treated SV40-MES13 cells. As presented in Fig. 3G, the silencing of circ_0000712 improved the expression level of miR-879-5p, whereas the introduction of in-miR-879-5p could effectively counteract these effects. Overall, these data discovered that circ_0000712 regulated the abundance of miR-879-5p.

Circ_0000712 deficiency mitigated HG-induced MCs damage through targeting miR-879-5p in vitro

Considering the regulatory role of circ_0000712 on miR-879-5p in HG-triggered MCs, we further explored whether the influence of circ_0000712 on HG-caused SV40-MES13 cells injury was related to miR-879-5p. As displayed in Fig. 4A and 4B, the reduced expression of miR-879-5p abated the suppression action of circ_0000712 knockdown on apoptosis rate in treated SV40-MES13 cells, while the transfection of in-miR-879-5p notably mitigated these effects in HG-stimulated SV40-MES13 cells (Fig. 4C–4E). Apart from that, circ_0000712 knockdown-mediated decrease in oxidative stress was obviously abrogated by the downregulation of miR-879-5p in HG-induced SV40-MES13 cells, as evidenced by increased ROS generation and LDH activity, and declined SOD activity (Fig. 4F–4H). In addition, the inhibition action of FN, Col. I, and Col IV because of circ_0000712 knockdown was reversed by miR-879-5p inhibitor in HG-triggered SV40-MES13 cells, suggesting that the downregulation of miR-879-5p could abolish the repression of si-circ_0000712 on fibrosis (Fig. 4I). Together, these results indicated that circ_0000712 knockdown could hinder HG-caused MCs injury through interacting with miR-879-5p in vitro.
SOX6 was a direct target of miR-879-5p

Next, bioinformatics software Targetscan was utilized to predict the latent downstream target genes of miR-879-5p. As presented in Fig. 5A, there were some complementary sites between miR-879-5p and SOX6 3’UTR, as validated by a dual-luciferase reporter assay. Data suggested that the forced expression of miR-879-5p reduced distinctly the luciferase activity of SOX6 3’UTR WT report vector but not that of SOX6 3’UTR MUT reporter vector in SV40-MES13 cells (Fig. 5B). Of note, RT-qPCR and western blot results indicated that both mRNA level and protein level of SOX6 were significantly upregulated in the db/db DN mice when compared to their respective controls (Fig. 5C and 5D). Also, the expression level of SOX6 in HG-induced SV40-MES13 cells was higher than that of cells treated with NG (Fig. 5E). Besides, we further determined that the impact of miR-879-5p on SOX6 expression in HG-triggered SV40-MES13 cells. As exhibited in Fig. 5F, the overexpression of miR-879-5p blocked the SOX6 protein level, which was strikingly undermined after the co-transfection of pcDNA-SOX6 in HG-stimulated SV40-MES13 cells. Notably, the results from western blot assay showed that the silencing of circ_0000712 curbed SOX6 protein level, while the reduced expression of miR-879-5p could overturn the suppression effect of si-circ_0000712 on SOX6 expression in HG-treated SV40-MES13 cells (Fig. 5G), implying the regulatory role of the circ_0000712/miR-879-5p/SOX6 axis in MCs. In summary, these data suggested that SOX6 served as a target of miR-879-5p.

Overexpression of SOX6 attenuated the inhibitory effect of miR-879-5p on apoptosis, inflammation, oxidative stress, and fibrosis in HG-induced MCs in vitro

As mentioned above, miR-879-5p presented the key role in HG-MCs damage in vitro, and SOX6 acted as an underlying target of miR-879-5p. Therefore, we further explored whether miR-879-5p could exert its biological activity by interacting with SOX6. Functionally, reduced cell apoptosis due to the upregulation of miR-879-5p in HG-treated SV40-MES13 cells was abolished by the
re-introduction of SOX6, as depicted by decreased Bcl-2 and elevated Bax (Fig. 6A and 6B). Synchronously, the overexpression of SOX6 partly weakened the negative action of miR-879-5p on pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in HG-induced SV40-MES13 cells (Fig. 6C–6E), suggesting that the suppression of inflammatory response caused by miR-879-5p was overturned by pcDNA-SOX6 in HG-treated MCs. In addition, the upregulation of SOX6 also could impair the inhibitory effect of miR-879-5p on oxidative stress, as evidenced by elevated ROS generation and LDH activity, and declined SOD activity in HG-triggered SV40-MES13 cells (Fig. 6F–6H). Consistently, the renal fibrosis-related proteins, including FN, Col. I, and Col IV were similarly blocked on account of the overexpression of miR-879-5p, which was evidently reversed by SOX6 upregulation in HG-induced SV40-MES13 cells (Fig. 6I). In addition, we also proved that the downregulation of SOX6 could hinder HG-caused MCs damage (Fig. S1). All of these results concluded that the miR-879-5p could repress the HG-caused MCs injury by negatively regulating SOX6 in vitro.

Discussion

At present, the function and molecular mechanism of circRNAs have been increasingly identified due to the development of RNA sequencing technologies [20]. It has been widely accepted that circRNAs, unlike the linear RNA, could serve as appealing biomarkers on account of the stability and resistance to RNase activity [21]. Furthermore, the abnormality of circRNAs expression has an inextricable relationship with the progression of renal diseases [22], containing DN. Since the abnormal development of MCs was correlated with the pathological changes of DN, MCs have been widely utilized for DN research in vitro [23, 24]. In current work, the upregulation of circ_0000712 in the db/db DN mice and HG-triggered MCs was verified, in agreement with the previous study [14]. Also, our data showed the stability of circ_0000712, suggesting the underlying diagnostic and prognostic biomarker for DN. More importantly, functional analysis discovered that the knockdown of circ_0000712 could dampen HG-caused apoptosis, inflammation, oxidative stress, and fibrosis of MCs, implying that the circ_0000712 downregulation might exert a repression role in HG-induced MCs injury.

Previous research has documented that circRNAs could act as ceRNAs for miRNAs, which is a vital mechanism in modulating diverse biological processes [25]. In this paper, circ_0000712 was verified to interact with miR-879-5p and block its expression for the first time.

Fig. 6 Overexpression of SOX6 diminished the inhibitory effect of miR-879-5p on HG-triggered MCs damage in vitro. SV40-MES13 cells were transfected with miR-NC, miR-879-5p, miR-879-5p + pcDNA, and miR-879-5p + SOX6, followed by stimulation with HG for 24. (A) Apoptosis rate of treated SV40-MES13 cells was analyzed. (B) Protein levels of Bcl-2 and Bax of treated SV40-MES13 cells were analyzed. (C–E) Levels of IL-1β, IL-6, and TNF-α of treated SV40-MES13 cells were detected. (F–H) ROS generation, LDH activity, and SOD activity of treated SV40-MES13 cells were tested. (I) Protein levels of FN, Col. I, and Col IV of treated SV40-MES13 cells were examined. * p < 0.05.
Apart from that, miR-879-5p has been reported to be related to the HG-triggered podocytes damage in DN [15]. The present work revealed that miR-879-5p expression was decreased in the db/db DN mice and HG-treated MCs. Furthermore, our results proved that the reduced expression of miR-879-5p could mitigate the inhibitory role of circ_0000712 deficiency on HG-caused cell injury. That was to say, circ_0000712 silencing exerts a protective role to ameliorate the damage of MCs through interacting with miR-879-5p.

As widely believed, miRNAs customarily function through directly binding to their target mRNA [26]. In our work, SRY-Box Transcription Factor 6 (SOX6) was identified as a target of miR-879-5p. SOX6, a member of the Sry-related high mobility group box (Sox) family of transcription factors [27], has been confirmed to be involved in the pathogenesis of diabetes mellitus [28, 29]. Consistent with an earlier study, our data verified the upregulation of SOX6 in the db/db DN mice and HG-triggered MCs [30]. Functional analysis indicated that the overexpression of SOX6 partly reversed the miR-879-5p-mediated decrease in HG-induced MCs damage. Besides, the promoting role of SOX6 on HG-triggered apoptosis, inflammatory responses, and fibrosis also proved in a prior document [30]. In addition, our data further discovered that circ_0000712 could regulate SOX6 expression by acting as a sponge of miR-879-5p in HG-treated MCs, supporting the regulatory role of the circ_0000712/miR-879-5p/SOX6 axis in HG-induced cell damage (Fig. 7).

**Fig. 7** Schematic model of the circ_0000712/miR-879-5p/SOX6 on HG-induced SV40-MES13 cells injury in DN. The knockdown of circ_0000712 could repress HG-triggered apoptosis, oxidative stress, inflammatory response, and fibrosis of SV40-MES13 cells through the miR-879-5p/ SOX6 axis.

**Conclusions**

These results suggested that circ_0000712 was increased in the db/db DN mice and HG-treated MCs. Moreover, circ_0000712 could function as the ceRNA of miR-879-5p to increase SOX6 expression, thereby regulating HG-induced MCs injury. Our findings provided a crucial preclinical basis for the treatment of DN.

**Ethics Approval and Consent to Participate**

Not Applicable.

**Consent for Publication**

Not Applicable.

**Availability of Data and Material**

Not Applicable.

**Competing Interests**

The authors declare that they have no conflicts of interest.

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None.

**Authors’ Contributions**

Li Zhao designed and performed the research; Huaqian Chen, Yan Zeng, Kun Yang, Ren Zhang, Zhengdong Li, Tao Yang, Hualing Ruan analyzed the data; Li Zhao wrote the manuscript. All authors read and approved the final manuscript.

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