Original Paper

MiR-33a Overexpression Exacerbates Diabetic Nephropathy Through Sirt6-dependent Notch Signaling

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Introduction. Diabetic nephropathy (DN) belongs to the major cause of end-stage kidney disease. We probed the functions of a microRNA miR-33a in inducing podocytes injury during childhood DN (CDN).
Methods. Kidney samples were collected from 20 children with DN. Matrix deposition and glomerular basement membranes thickness were examined by periodic acid-Schiff staining. Immunofluorescence staining was performed to assess kidney function-related proteins. MicroRNA (MiR)-33a mimic together with miR-33a inhibitor was transfected into podocytes for determining the roles of miR-33a. Glomerular podocyte apoptosis was determined by terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) staining along with flow cytometry.
Results. Down-regulation of Nephrin and Podocin and increased podocyte apoptosis rate were observed in the glomerulus of CDN as well as podocytes treated with high glucose. MiR-33a was up-regulated in the glomeruli and glucose-treated podocytes. Injury in podocytes was aggravated with miR-33a elevation but alleviated with miR-33a inhibition. Moreover, the expression of Sirtuin 6 (Sirt6) was decreased while the levels of notch receptor 1 (Notch1) and notch receptor 4 (Notch4) were elevated in the glomerulus and glucose-treated podocytes. Decreased level of Sirt6 upon glucose treatment was abrogated by miR-33a inhibition, and the podocytes injury induced by glucose exposure was relieved by Sirt6 via Notch signaling.
Conclusion. These findings indicated that miR-33a promoted podocyte injury via targeting Sirt6-dependent Notch signaling in CDN, which might provide a novel sight for CDN treatment.

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INTRODUCTION

Diabetic nephropathy (DN) is the most serious and prevalent renal complication in patients with diabetes mellitus, with high morbidity, mortality, and risk deteriorating into end-stage kidney disease.[1] The global rise in childhood obesity is closely related to the rise in the prevalence of DN.[2] The main clinical presentations of DN include proteinuria as well as reduced glomerular filtration rate.[3] The typical features of DN are glomerulosclerosis, extracellular matrix deposition and tubulointerstitial fibrosis.[3,4] Hence, it is

indispensable to explore the molecular mechanisms underlying DN, and explore early diagnostic biomarkers as well as specific therapeutic targets.

Podocyte injury has a crucial function in the pathogenesis of DN.[4] Podocytes are a unique group of terminally differentiated glomerular epithelial cells that are essential for the normal function of the glomerular filtration barrier. Podocytes have very limited proliferative capacity and are not sufficient to replace cells lost in glomerulonephropathy.[5] When podocytes are damaged, the glomerular filtration capacity is compromised, leading to kidney dysfunction.[6] Although childhood diabetes mellitus with proteinuria and glomerular inflammation has been widely reported, its pathogenesis, especially podocyte damage, is still unclear.[7]

Sirtuin 6 (Sirt6) belongs to a key member of the histone deacetylases family that is essential for kidney development and function, and its pleiotropic properties are involved in several catalytic functions such as deacetylation and ribosylation.[8] Deacetylation of H3K9 or H3K56 mediated by Sirt6 has been observed in many diseases,[9] indicating the important function of Sirt6.[10] Recent studies have shown that Sirt6 deficiency induces podocyte damage in angiotensin II-mediated cholesterol accumulation in mouse glomeruli and in diabetic patients,[11] suggesting that Sirt6 is a key player in kidney injury. However, the potential of Sirt6 in podocyte damage in children with diabetes remains elusive.

The Notch pathway in mature podocytes has a key function in the development of glomerular disease and can be stimulated by high-glucose or other stimuli.[12] The Notch pathway consists of 4 Notch receptors (Notch1–4) as well as 5 Notch ligands (Jagged1, Jagged2, Delta-like (Dll) 1, Dll3, and Dll4).[13] Many studies have proved that the Notch pathway is regulated by Sirt6, and may be a possible target of Sirt6.[14,15] More importantly, it has been proved that Sirt6 depletion exacerbates podocyte injury by activating the Notch signaling.[16]

MicroRNAs (miRNAs) belong to small noncoding RNAs that modulate mRNA expression via combining with specific sequences of the 3′untranslated region (3′UTR) of the target mRNA sequence.[17] Recent studies have indicated that miRNAs are implicated in the progression of DN. Guo *et al.* have pointed that miRNA-29c modulates the expression of inflammatory cytokines in DN by targeting tristetraprolin.[18] Zhao *et al.* have discovered that miRNA-337 promotes podocyte injury in DN mice.[19] Jiang *et al.* have proposed that miRNA-342 represses renal interstitial fibrosis in diabetic nephropathy via targeting SOX6.[20] Notably, miR-33a has been proved to take part in the progression of various diseases. For example, miR-33a represses cell growth in renal cancer by regulating MDM4 expression.[21] Low levels of miR-33a are also detected in the serum, urine, along with kidney tissues of patients with IgA nephropathy.[22] It has been documented that miR-33a targeting Sirt6 mediates the pathological processes in multiple tissues.[23,24] Since Sirt6 has been reported to participate in glomerular podocyte damage,[11] it is reasonable to hypothesize that aberrant miR-33a expression may act on Sirt6 to mediate podocyte injury during childhood DN (CDN). In the current research, the potential of miR-33a in CDN and the interaction between miR-33a and Sirt6 in CDN were explored.

MATERIALS AND METHODS

Tissue Specimens

Sample size was determined using Gpower 3.1.3.[25] Kidney biopsy samples from 20 CDN or 20 healthy children were acquired from the Nephrology Division of Nanyang Second General Hospital. CDN patients had not received any treatment before enrolling in this study. The CDN patients included 10 boys and 10 girls, aged 6-17 years with an average age of 11.90 ± 3.05 years. The healthy children included 8 boys and 12 girls, aged 5-17 years with an average age of 11.87 ± 3.16 years. No difference was discovered in general data between CDN patients and healthy children ($P > .05$). All human experiments were performed in line with the Declaration of Helsinki. This study was approved by the Ethics Committee of Children's Hospital of Nanjing Medical University, and the Ethics number was NYLL-001A, which was issued by Nanyang Second General Hospital in November 2022. Written informed consent was signed by all participants and their guardians.

Histology Analysis

Kidney tissues were fixed in formalin, embedded in paraffin, followed by slicing into sections (3 μm in thickness). Matrix deposition and basement membranes thickness of the glomeruli were

examined by periodic acid-Schiff (PAS) staining. Renal tissues were treated with 0.1% periodic acid and incubated in Schiff's reagent. After washing, the tissues were counterstained with Mayer's hematoxylin and dehydrated. Next, the samples were cleared with xylene and mounted with Entellan.

Immunofluorescence

Immunofluorescence staining was performed on renal tissue samples or podocytes as previously described.[26] Renal tissue samples or podocytes were fixed using cold methanol for 40 min, followed by cultivation for a night at 4 °C with primary antibodies containing anti-Nephrin, anti-Podocin, anti-Synaptopodin and anti-Sirt6. Next, secondary antibodies conjugated with Alexa Fluor® 488 or 594 (1/1000, ab150077, Abcam) were incubated with kidney tissue samples or podocytes for 1 h. At last, sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and visualized with the help of a Zeiss LSM 880 confocal microscope. The details of primary antibodies were listed in Table 1.

Podocytes Culture

A human podocyte cell line was commercially bought from Beijing Fubo Biotechnology Co., Ltd. (Beijing, China), which was provided by Austrian Evercyte Company (Austrian). The cells were cultivated in RPMI 1640 medium including 11.0 mmol/L glucose, as previously documented.[26] High-glucose (HG, a final dose of 5 or 30 mmol/L in culture media) stimuli were conducted to induce injury in this study.

Western Blotting Analysis

Protein samples from human glomerulus or cultured podocytes were extracted by using a lysis buffer (pH = 7.4), which contained tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl, 10 mM), ethylenediamine tetraacetic acid (EDTA, 1 mM), and sucrose (250 mM), aprotinin (15 µg/mL), leupeptin (5 g/mL), polymethoxyselenoflavone (PMSF, 0.1 mM), ^{18}F-sodium fluoride (NaF, 1 mM), and Na_3VO_4 (1 mM). Specific protein levels were measured by using antibodies by western blotting. Briefly, protein samples were shifted onto a polyvinylidene fluoride membrane (Millipore, USA), followed by separation in sodium dodecyl sulfatepolyacrylamide gel electrophoresis. The membrane was hatched with corresponding primary antibodies against Nephrin, Podocin, Synaptopodin, Sirt6, notch receptor 1 (Notch1), notch receptor 4 (Notch4) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) after blocking with 5% skim milk overnight at 4 °C. The horseradish peroxidase (HRP)-related secondary antibody (1:10,000 dilution; Abcam, ab205718) and a commercial enhanced chemiluminescence (ECL) detection kit (Millipore) was implemented for detecting the immunoreactive bands. The details of primary antibodies are listed in Table 1.

Apoptosis Assay

Glomerular podocyte apoptosis was determined by terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) staining using a TUNEL detection kit (purchased from Roche, Basel, Switzerland). Images of sections were evaluated with a fluorescence microscope (Olympus BX60, Center Valley, PA, USA).

Flow cytometry was applied to detect podocyte apoptosis in this study. Cells were digested by using

Table 1. Antibodies

Antibody	Manufacturer (Cat. #)	Titration for IF	Titration for WB
Anti-Nephrin	Abcam (ab227806)	1:1000	1:1000
Anti-Podocin	Abcam (ab181143)	1:500	1:1000
Anti-Synaptopodin	Abcam (ab259976)	1:100	1:1000
Anti-Sirt6	Abcam (ab191385)	1:1000	1:1000
Anti-Notch1	Abcam (ab52627)	1:1000	1:1000
Anti-Notch 4	Abcam (ab184742)	1:1000	1:1000
Anti-BAX	Abcam (ab32503)	1:100	1:1000
Anti-BCL2	Abcam (ab32124)	1:500	1:1000
Anti-Caspase3	Abcam (ab32042)	1:100	1:1000
Anti-GAPDH	Abcam (ab8245)	1:1000	1:10000

Abbreviations: IF; immunofluorescence, WB; western blotting.

trypsin (no EDTA), washed, and then resuspended with 100 μL of fluorescence-activated cell sorting (FACS) buffer. Next, the samples were incubated with annexin (V-fluorescein isothiocyanate) and propidium iodide (PI) (Thermo Fisher Scientific, USA). Apoptotic cells were detected by FACSCalibur flow cytometry (BD Bioscience, USA).

Real-time Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from human glomeruli or podocytes with TRIzol reagent (Invitrogen), after which M-MLV reverse transcriptase (Promega, USA) was applied for reverse transcription of the isolated RNA (miR-33a, Notch1, and Notch4) into complementary DNA (cDNA). SYBRs PremixExTaq^{TM} reagent (TaKaRa) on ABI Prisms 7500 Sequence Detection System (Applied Biosystems, USA) was implemented to amplify the cDNA, and the expression of miR-33a as well as Notch1 and Notch4 were normalized with miR-16 or GAPDH, respectively. Primer sequences used in our study are listed in Table 2.

Lentivirus Construction and Cell Transfection

MiR-33a mimic or inhibitor was acquired from Biomics (China). The sequences were as below: miR-33a mimic sense 5′-UAACACUGCCUGGUAACGAUGU-3′ and anti-sense 5′-ACAUCGUUACCAUUCAGUGUUA-3′, miR-33a inhibitor sequences were 5′-ACAUCGUUACCAGACAGUGUUA-3′. Sirt6 over-expression achieved by Sirt6-lentiviral transfection (LV5-Sirt6) along with the negative control was purchased from GenePharma (Shanghai, China). Podocytes were planted in 6 well plates in RPMI 1640 with 10% FBS at 37 °C for 7 days to induce differentiation. After 48 h incubation, cells transfection was implemented by Lipofectamine 2000 (Invitrogen, USA). The nucleotides of miR-33a Mimic and miR-33a Inhibitor were adopted at a final concentration of 50 nM in antibiotic-free Opti-MEM medium (Gibco, USA). RT-qPCR together with western blotting was employed to verify the transfection efficiency.

Table 2. Primer Sequences in this Study

Gene	Sequences (5’ to 3’)
miR-33a	Forward: GTGCATTGTAGTTGCATTG
	Reverse: GAACATGTCTGCGTATCTC
miR-16	Forward: AGCAGCACGTAAATATTGG
	Reverse: GAACATGTCTGCGTATCTC
NOTCH1	Forward: GGTGAACTGCTCTGAGGAGATC
	Reverse: GGATTGCAGTCGTCCACGTTGA
NOTCH4	Forward: TTCCACTGTCCTCCTGCCAGAA
	Reverse: TGGCACAGGCTGCCTTGGAATC
GADPH	Forward: GTCTCCTCTGACTTCAACAGCG
	Reverse: ACCACCCTGTTGCTGTAGCCAA

Statistical Analysis

Data were exhibited as means ± SEM. Statistical analyses were conducted by using Graphpad Prism (Windows Edition 6.0, Graphpad, San Diego, CA). Statistical comparisons were made by one-way ANOVA tests and Dunnett’s post hoc tests. Student’s *t-test* was implemented to determine the significant difference between the independent groups. A P value of less than .05 was considered significant.

RESULTS

Podocyte Damage and High Expression of miR-33a Are Observed in Glomeruli of Diabetic Children and Glucose-exposed Differentiated Podocytes

To investigate miR-33a expression as well as the presence of podocyte damage during CDN, kidney tissues were collected for RT-qPCR and histopathological analyses with PAS and immunofluorescent staining. MiR-33a over-expression as well as glycogen deposition and glomerular basement membrane thickening were observed in nephridial tissues of patients with CDN or glucose-exposed differentiated podocytes (Figure 1A-C). Moreover, the expression of podocyte marker proteins Nephrin and Podocin down-regulated, while podocyte apoptosis rate up-regulated in glomeruli of CDN, but not in glomeruli of healthy children, as observed by immunofluorescence, western blotting and TUNEL analysis (Figure 1D-F and J). Additionally, differentiated podocytes were treated with 5- or 30-mM glucose. It was shown that podocyte apoptosis could be enhanced (96 h), but Nephrin and Podocin protein levels were down-regulated (72 h) (Figure 1G-I and K). Moreover, the results of western blotting illustrated that Bcl-2 protein level was down-regulated in glomeruli of CDN and podocytes with 5-or 30-mM glucose, while BAX together with cleaved caspase 3 levels presented up-regulation (Figure 1L-M). These findings indicated that podocyte apoptosis accompanied by miR-33a over-expression were confirmed in glomerulus of CDN.

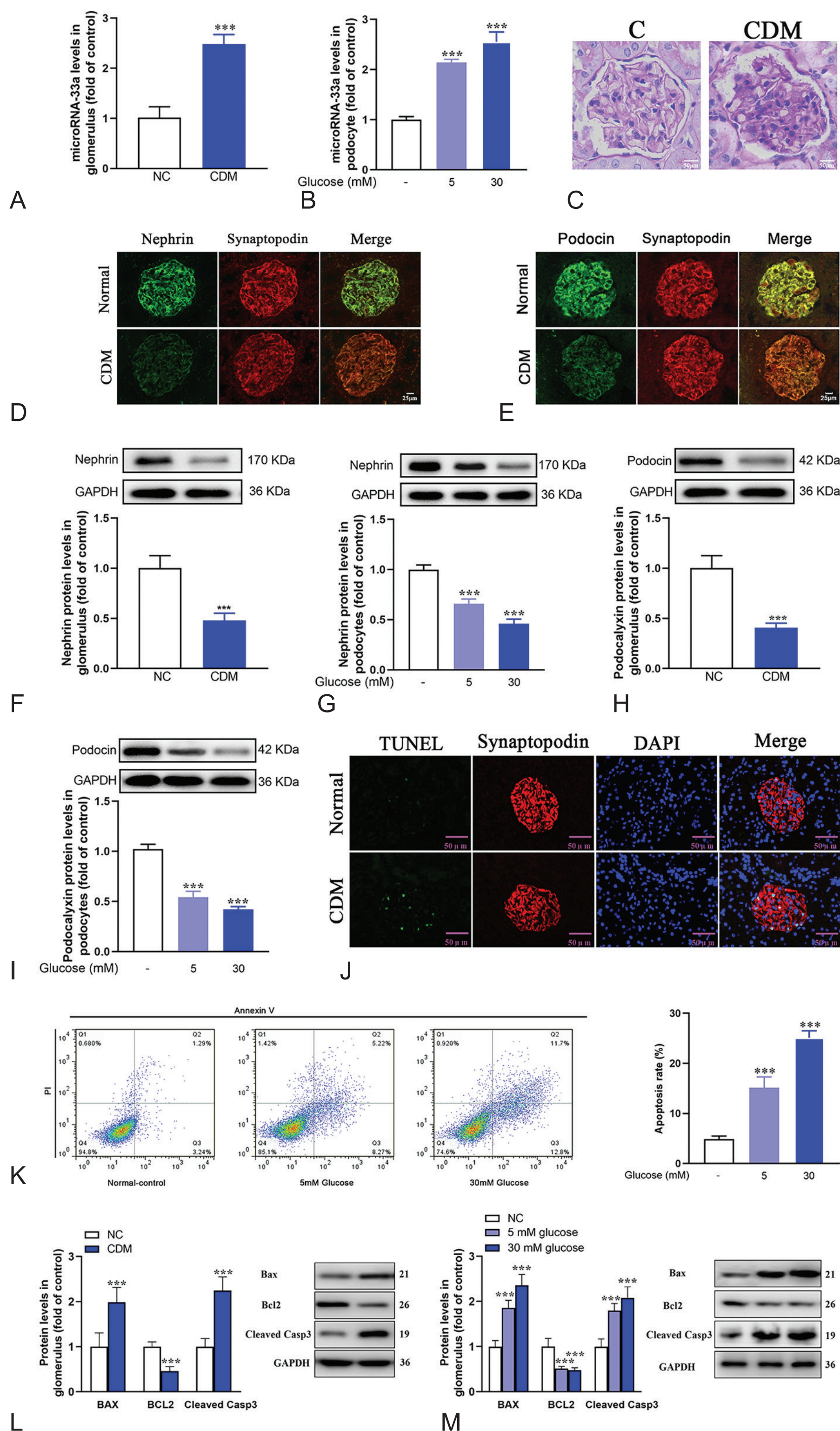

Figure 1. High expression of miR-33a in glomeruli of diabetic children and glucose-exposed differentiated podocytes. (A-B) RT-qPCR analysis of miR-33a levels in glomeruli or podocytes. (C) PAS analysis of glomerular tissue. (D-E) Representative immunofluorescence images of kidney glomeruli stained with nephrin and podocin (green), and synaptopodin (red; scale bar, 25 μm). (F-I) Relative nephrin and podocin protein levels in glomeruli or podocytes were detected by Western blot analysis. (J) Podocyte apoptosis analysis in glomeruli by TUNEL (scale bar, 50 μm). (K) Flow cytometry analysis in differentiated podocytes (96 h). (L-M) Relative BAX, BCL2 and Cleaved casp3 protein levels in glomeruli or differentiated podocytes were detected by Western blot analysis (****P* < .001).

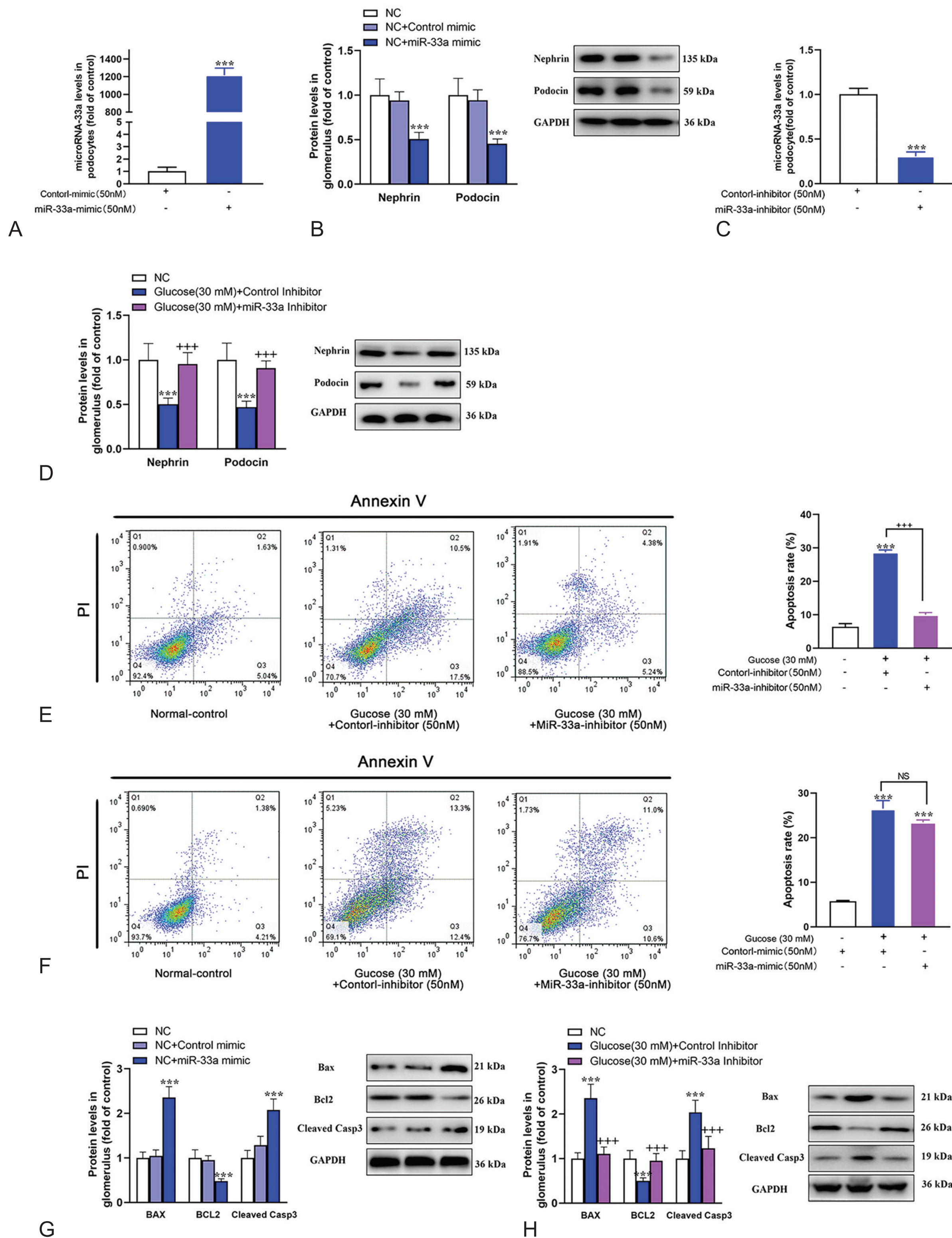

Figure 2. miR-33a high expression induces glomerular podocyte apoptosis in diabetic children. (A) The transfection efficiency of miR-33a mimic in podocytes was evaluated by RT-qPCR analysis. (B) Western blot analysis of nephrin and podocin levels in podocytes after miR-33a elevation. (C) The transfection efficiency of miR-33a inhibitor in podocytes was evaluated by RT-qPCR analysis. (D) Western blot analysis of nephrin and podocin levels. (E-F) Flow cytometry analysis of HG-exposed differentiated podocytes after miR-33a inhibition and up-regulation. (G) Western blot analysis of BAX, BCL2 and Cleaved casp3 levels. (H) Western blot analysis of BAX, BCL2 and Cleaved casp3 levels (***P < .001, ^{+++}P < .001; NS means no significance).

MiR-33a High Expression Induces Glomerular Podocyte Apoptosis in Diabetic Children

To further verify whether miR-33a participates in podocyte apoptosis, differentiated podocytes were transiently transfected with miR-33a mimic or miR-33a inhibitor to specifically enhance or decline miR-33a expression, respectively. RT-qPCR analysis verified the successful transfection in differentiated podocytes (Figure 2A and C). Over-expressed miR-33a decreased Nephrin and Podocin levels in differentiated podocytes (72 h), while resulted in no effect on glucose-stimulated podocyte apoptosis (Figure 2B, 2F and 2G). In contrast, miR-33a reduction up-regulated Nephrin and Podocin protein levels (72 h), and repressed podocyte apoptosis (96 h) in glucose-induced differentiated podocytes (Figure 2D, 2E and 2H). These results indicated that miR-33a overexpression aggravated podocytes apoptosis during CDN.

Sirt6 Downregulated by miR-33a Overexpression Mediates Podocyte Apoptosis in CDN

As reported, Sirt6 belongs to a target mRNA of miR-33a,[23,24] we further verified the functionality of their binding sites. The sequence of wild-type Sirt6 3′UTR or a mutated Sirt6 3′UTR in the proposed binding site were cloned to pMIR-report vectors to perform reporter luciferase assays (Figure 3A-B). It was unveiled that miR-33a over-expression reduced the relative luciferase activity of Sirt6 3′UTR wild-type, whereas it had no influence on that of Sirt6 3′UTR mutant-type in differentiated podocytes (Figure 3B). We then confirmed Sirt6 under-expression in glomerulus of CDN and glucose-exposed differentiated podocytes by immunofluorescence and western blotting analysis (Figure 3C-F). MiR-33a inhibition promoted Sirt6 protein levels (72 h), while miR-33a mimic transfection had no effect on Sirt6 expression in glucose-exposed differentiated podocytes (Figure 3G-H). All above outcomes indicated that glucose could elevate miR-33a expression to decrease Sirt6, which further induced podocyte apoptosis.

Sirt6 Deficiency Induces Podocyte Apoptosis Through Targeting Notch Signaling

It has been reported that Sirt6 deficiency exacerbates podocyte damage via Notch signaling in two different mouse models-DN together with Adriamycin-stimulated nephropathy.[16] We then investigated whether Sirt6 down-regulation promoted podocytes injury by activating Notch pathway during CDN. We first confirmed the high expression of Notch1 and Notch4 in glomerulus of CDN and glucose-exposed differentiated podocytes (Figure 4A-B). Then, LV5-Sirt6 was transiently transfected into differentiated podocytes to increase Sirt6 expression (Figure 4C). As shown in Figure 4D, Sirt6 protein levels were significantly increased compared with the LV5-GFP-transfected differentiated podocytes (48 hours). More importantly, over-expression of Sirt6 down-regulated the mRNA and protein levels of Notch1 together with Notch4 in glucose-exposed differentiated podocytes (Figure 4E-F). Over-expression of Sirt6 also reversed glucose-induced upregulation of Nephrin and Podocin (72 hours), and blocked glucose-stimulated apoptosis at 96 h in differentiated podocytes (Figure 4G-I), implying Sirt6 down-regulation may activate Notch signaling and thereby inducing podocyte apoptosis. All these results indicated that Sirt6 deficiency induced podocyte apoptosis in CDN through targeting Notch signaling.

DISCUSSION

In this study, we discovered that miR-33a presented up-regulation in the glomeruli of CDN, which was further confirmed in glucose-treated differentiated podocytes. Many studies have displayed that miR-33a has a role in the pathological process of diseases, such as hepatocellular carcinoma, atherosclerosis and obesity,[27-29] and it can act as a noninvasive biomarker for cancer therapy.[30] Besides, recent studies have also emphasized the influence of aberrant miR-33a expression on the modulation of renal function,[21,22] thus this research was intended to further explore the potential of miR-33a on the podocyte damage of CDN. To the best of our knowledge, podocyte apoptosis is considered to be the initial event leading to proteinuria, chronic kidney disease and even kidney failure.[31-33] Using renal biopsies in CDN and a glucose-treated podocyte model in vitro, our study found that miR-33a over-expression aggravated podocytes apoptosis during CDN, implying that miR-33a may participate in the progression of CDN.

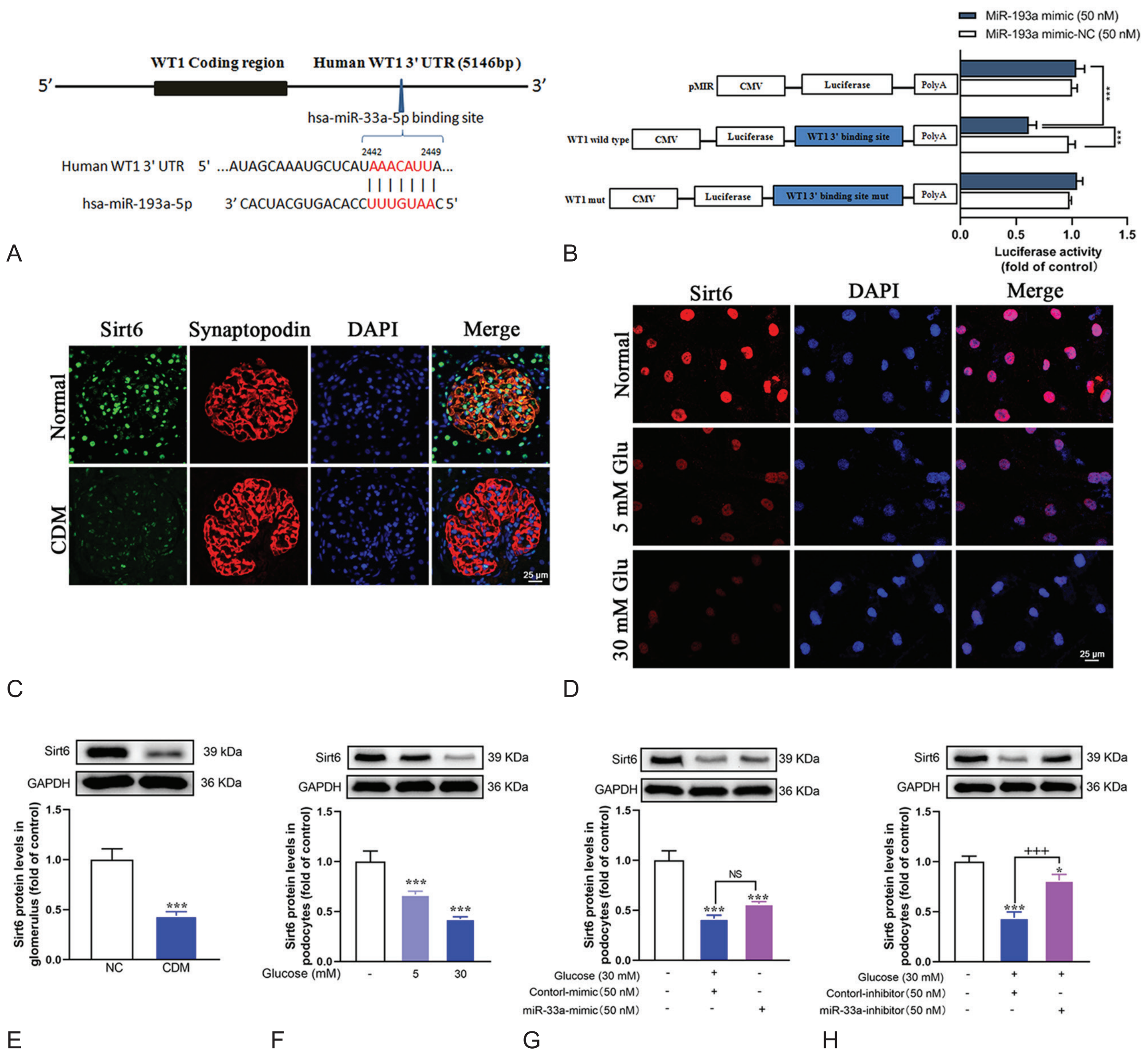

Figure 3. MiR-33a overexpression decreasing Sirt6 mediates podocyte apoptosis in CDN. (A) Binding site of Sirt6 and miR-33a sequence in human. (B) Schematic diagram of the luciferase reporter plasmids. Regulation of miR-33a on binding site of Sirt6 in podocytes by luciferase reporter assay. (C) Immunofluorescence analysis of Sirt6 (green) in glomeruli (scale bar, 25 μm). (D) Immunofluorescence analysis of Sirt6 (green, 72 h) in podocytes. (E-F) Relative Sirt6 protein levels in glomeruli or podocytes were detected by Western blot analysis. (G-H) Western blot analysis of Sirt6 levels after transfection with miR-33a mimic or inhibitor (*$P < .05$, ***$P < .001$, $^{+++}P < .001$; NS means no significance).

Consistently, previous study has also proved that miR-33a presented up-regulation in the plasma of hypercholesterolemic children,[34] and miR-33a can serve as an early biomarker for hypercholesterolemia in childhood.

MiRNAs exert their biological roles post-transcriptionally through binding to 3′UTR of their target mRNAs to suppress mRNAs expression.[35] As reported previously, miR-33a represses the adipogenic differentiation of ovine adipose-derived stromal vascular fraction cells through targeting SIRT6.[23] Consistently, our study also probed the target mRNA of miR-33a and found the binding sequences of miR-33a and 3′UTR region of Sirt6 mRNA, which was confirmed by dual luciferase reporter assay. Likewise, many miRNAs participate in DN progression via targeting their mRNAs,[36] which further supports the regulatory mechanism

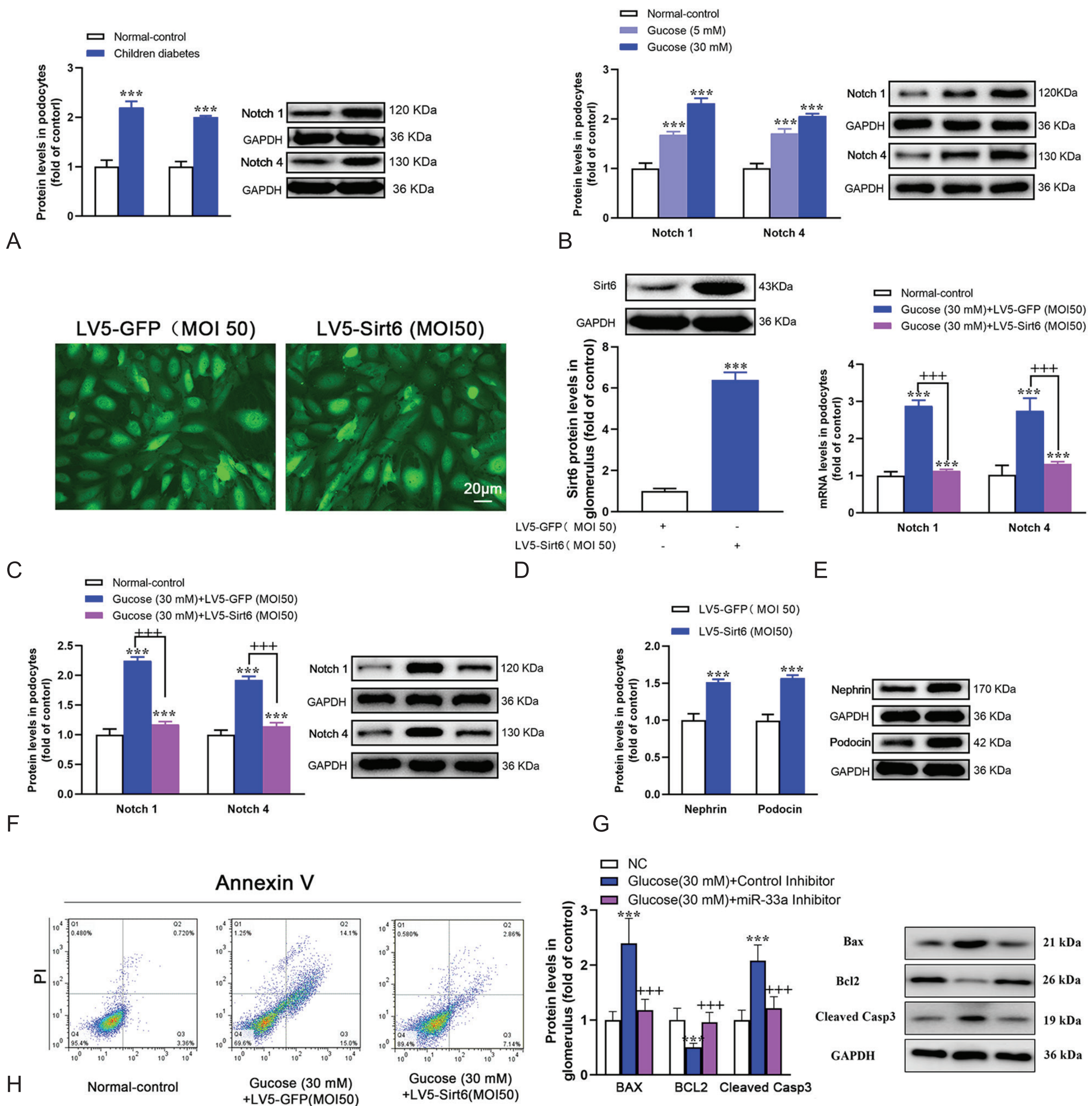

Figure 4. Sirt6 deficiency exacerbates apoptosis through targeting Notch signaling. (A-B) Western blot analysis of Notch1 and Notch4 levels in glomeruli or differentiated podocytes, (C-D) The transfection efficiency of LV5-Sirt6 in differentiated podocytes was detected by epifluorescent microscopy (original magnification, ×200, 24 h) and Western blot analysis (48 h, n = 6), (E-F) The mRNA and protein levels of Notch1 and Notch4 in podocytes, (G) Western blot analysis of nephrin and podocin levels, (H) Flow cytometry analysis of differentiated podocytes. (I) Western blot analysis of BAX, BCL2 and Cleaved casp3 levels (***P < .001, ^{+++}P < .001).

of miR-33a in DN.

Sirt6, abundantly expressed in normal glomeruli, possesses pleiotropic protective actions containing anti-inflammatory, anti-oxidative stress along with anti-apoptosis.[37] Moreover, Sirt6 can insufficiently induce podocyte apoptosis by downregulation of Notch to inhibit autophagy in 2 independent mouse models, DN together with Adriamycin-stimulated nephropathy.[16] Likewise, our study further demonstrated that Sirt6 was reduced in glomeruli of CDN and glucose-exposed differentiated podocytes. In addition, we found that miR-33a reduction could increase Sirt6 protein levels, while miR-33a over-expression had no effect on

Sirt6 expression in glucose-exposed differentiated podocytes, implying glucose may up-regulate miR-33a expression to down-regulate Sirt6, which further induced podocyte apoptosis.

As reported previously, the Notch signaling pathway is closely linked to podocyte apoptosis in DN.[38] Inhibiting the Notch signaling pathway can reduce podocyte apoptosis in DN.[12] In addition, a recent study has shown that Notch1 over-expression is strongly associated with higher Bax/Bcl-2 ratio, consistent with a significant cell apoptosis in chronic lymphocytic leukaemia.[39] Previous published literatures have widely indicated that Sirt6 can regulate the Notch signaling in the progression of diseases.[16] Sirt6 deficiency represses corneal epithelial wound healing via regulating Notch signaling.[40] Sirt6 reduces the proliferation of ovarian cancer cells through reduction of Notch 3 expression.[41] All above findings suggest that SIRT6 may negatively regulate the Notch pathway. In this study, we found that silence of Sirt6 resulted in Notch1/4 upregulation, as well as inhibited Bcl-2 and enhanced Bax and cleaved caspase-3, leading to podocyte apoptosis in the presence of high glucose condition.

LIMITATIONS OF THE STUDY

There are several limitations of our study. First, the sample size of our study is small. Besides, the relation between clinical features of CDN patients and miR-33a expression is not explored. Therefore, further large-scale, and in-depth studies should be carried out in the near future.

CONCLUSION

MiR-33a activates the Notch signaling to promote podocyte apoptosis in CDN via targeting Sirt6. Therefore, targeting miR-33a-Sirt6-Notch signaling may lay a foundation for further assays on the molecular mechanism of podocyte injury and highlight that miR-33a may be a promising target for CDN treatment.

AUTHORS' CONTRIBUTIONS

Yingying Wang, Shasha Dai, and Jing Yang designed the study, drafted the manuscript, and performed assays. Jun Ma, Peng Wang, Xiaowei Zhao, Juan Liu performed assays. Ao Xiao and Yahui Song interpreted data. Lipin Gao analyzed data and revised the manuscript.

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DATA AVAILABILITY STATEMENT

Data sets for the duration of this study and/or the analysis period are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no competing interests.

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