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Inhibition of lncRNA-PAX8-AS1-N directly associated with VEGF/TGF-β1/8-OhdG enhances podocyte apoptosis in diabetic nephropathy

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Abstract. – OBJECTIVE: Diabetic nephropathy (DN), the microvascular complications of diabetes, is one of the world's public health hazard. But the detailed mechanism of the occurrence and development remains unclear. Oxidative stress caused by multiple factors is recognized as the main cause of disease, and it is also a research focus. Recently, long non-coding RNAs (lncRNAs) have been declared to involve in a large of important bioactivities in many different diseases. In our study, we aimed to verify whether lncRNA PAX8-AS1-N involved in protecting podocyte apoptosis and directly associated with VEGF/TGF-β1/8-OhdG levels in DN, and further investigated the detailed mechanism that PAX8-AS1-N regulated the pathological process.

MATERIALS AND METHODS: We used blood and urine samples of DN patients to detect the expression of lncRNA-PAX8-AS1-N and VEGF/TGF-β1/8-OhdG by ELISA and quantitative reverse transcription polymerase chain reaction (qRT-PCR). Albuminuria level, relative PAX8-AS1-N and VEGF/TGF-β1/8-OhdG levels, and VEGF/TGF-β1/8-OhdG and cleaved-caspase-3 protein levels were detected by ELISA, qRT-PCR, and Western blot, respectively. CCK8 assay was used to measure the proliferation ability of conditionally immortalized mouse podocytes (MPC5). And we used the TUNEL assay to detect MPC5 apoptosis. Luciferase reporter assay was used to confirm the direct target of PAX8-AS-N and miR-17-5p in MPC5.

RESULTS: We found that the lncRNA PAX8-AS1-N was lowly expressed and high expression of VEGF/TGF-β1/8-OhdG and high level of albuminuria in DN patients and high-glucose-treated MPC5. Besides, we proved that LV-PAX8-AS1-N decreased MPC5 apoptosis and suppressed the expression of VEGF/TGF-β1/8-OhdG *in vitro* experiment. At last, the overexpression of miR-17-5p markedly induced cell apoptosis in MPC5 with high glucose (HG) model. STAT3 reverses the effects of miR-17-5p overexpression in MPC5 with HG model.

CONCLUSIONS: Above that, we found that lncRNA PAX8-AS1-N/miR-17-5p/STAT3 axis was closely related the progression of DN, which could be a potential target for treating DN patients.

Key Words:
Diabetic nephropathy, Diabetes, LncRNA-PAX8-AS1-N, VEGF/TGF-β1/8-OhdG.

Introduction

Diabetic nephropathy (DN) is one of the microvascular complications' disease in diabetes mellitus[1,2]. According to the statistics of the International Diabetes Federation, there are about 285 million people with diabetes worldwide, with an increase of 7 million new patients each year. The prevalence of diabetic nephropathy in type II diabetes is about 20% to 25%, and 10% to 30% of diabetic patients die of diabetic nephropathy[3]. Although there have been significant improvements in the prevention and treatment of DN patients, their prognosis remains poor. Therefore, finding new intervention targets for DN is an urgent problem to be solved.

Long non-coding RNAs (lncRNAs) were first described in the large-scale sequencing of full-length eDNA library in mice in 2002. LncRNA is a group of transcription materials with >200 nucleotides, which lacks a specific complete open reading frame and has no protein-coding function[4,5]. Expression disorders of lncRNA are found in many types of tumors and neurological and cardiovascular diseases[6-8]. Increasing evidence has demonstrated the significant roles of lncRNAs in the pathophysiology of DN, and recently the crosstalk between lncRNA and DN was widely reported. Meng et al[9] declared that

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lncRNA-MALAT1 is dysregulated in diabetic nephropathy and involved in high glucose-induced podocyte injury via its interplay with β-catenin. Moreover, Jiao et al[10] found that lncRNA-PRINS may be involved in diabetic nephropathy by enhancing Smad7 expression and renal apoptosis.

Paired-box gene 8 (PAX8) encodes a transcription factor required for cell growth and differentiation during embryonic development. LncRNA PAX8 antisense RNA 1 (PAX8-AS1) is mapped to chromosome 2q13 in the upstream region of PAX8[11]. Yu et al[12] identified that AK126431 named as lncRNA PAX8-AS1-N, as a novel different isoform of the lncRNA PAX8-AS1, was activated by baicalein to inhibit breast cancer growth. Therefore, we focused on that lncRNA-PAX8-AS1-N might be involved in the development of DN.

Podocyte apoptosis played a key role in the pathogenesis of glomerulosclerosis and albuminuria in DN[13]. Moreover, oxidative stress is the basis of high glucose damage. VEGF, TGF-β1 and 8-hydroxy-guani (8-OhdG) play important roles in this process of oxidative stress[14,15]. Whether regulated lncRNA could influence the expression of these genes in DN is further verified. In our study, we aimed to understand the mechanism of lncRNA PAX8-AS1-N involving in the development of DN in patients via mediating miR-17-5p/STAT3 signaling pathway in MPC5.

Materials and Methods

Patients

Blood samples were collected from 30 patients with DN and 32 healthy control patients included as a control group who were diagnosed and treated at the First People's Hospital of Yunnan Province from January 2016 to January 2018. All patients were diagnosed according to the standard established by the Chinese Medical Association (2014) [13]. All patients were diagnosed and treated for the first time. Patients with other more severe diseases were excluded. No significant differences in age and sex were found between the groups. The experiment protocol was approved by the Institutional Review Board of the First People's Hospital of Yunnan Province.

Blood Sample Collection, RNA Extraction and Reverse Transcription-Quantitative Polymerase Chain Reaction

We collected the blood samples (25 ml) from each participant 24 h after admission. Total RNA was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Total RNA concentration was measured using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and RNA samples with an A260/A280 ratio of 1.8-2.0 were reverse transcribed into cDNA using SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA) through the following thermocycling conditions: 25°C for 5 min; 50°C for 20 min and 80°C for 20 min. qPCR reaction systems were prepared using SYBR® Green master mix (Bio-Rad Laboratories, Hercules, CA, USA). The following primer pairs were used for the qPCR: for PAX8-AS1-N, 5′-GGATGTGTTGTTTTGACAG-3′ (sense) and 5′-AGAGTCGCTGAAGTTCTG-3′ (antisense); for VEGF, 5′-AACCATGAACTTTCTGCTGTCTTG-3′ (sense) and 5′-TTCACCACTTCGTGATGATTCTG-3′(antisense); for TGF-β1,5'-CCCAGCATCTGCAAAGCTC-3' (sense) and 5'-GTCAATGTACAGCTGCCGCA-3' (antisense); for β-actin, 5'-GACCTCTATGCCAACACAGT-3' (sense) and 5'-AGTACTTGCGCTCAGGAGGA-3' (antisense). All experiments were repeated three times at least.

Cell Culture and Transfection

The conditionally immortalized mouse podocytes (MPC5) were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) and antibiotics (120 U/mL penicillin and 120 μg/mL streptomycin) (Sigma-Aldrich Co., St. Louis, MO, USA) at 37°C with 5% CO_2 atmosphere for 24 hours. Then, we divided the cells into three groups: normal glucose (NG) group, high glucose (HG) group, and HG+NGR1 group. In the NG group, RPMI 1640 medium was used to incubate cells supplemented with 5 mM glucose. In the HG group, cells were incubated in RPMI 1640 medium supplemented with 30 mM glucose. In the HG+NGR1 group, cells were treated with 25 μmol/L of NGR1 for 24 hours. si-control and si-PAX8-AS1-N were transfected into MPC5 cells in the HG+NGR1 group using lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

ELISA

8-OhdG and albuminuria concentration in blood serum and urine was detected by 8-OhdG ELISA kit (Shanghai Westang Biotech, Shanghai,

China) and albuminuria ELISA kit (Nanjing Jinyibo Biotechnology, Jiangsu, China) according to the manufacturer's instructions.

CCK-8 Assay

MPC5 cells transected with LV- PAX8-AS1-N seeded in 96-well plates ($3x10^3$ per well) were incubated for 24 h, 48 h, 72 h, and 96 h in HG, respectively. MPC5 cells transected with NC-FAM-LV-RNA were used as negative control in HG. Consequently, the cell ability of proliferation was evaluated by the CCK-8 assay.

TUNEL Assay

MPC5 cell apoptosis was detected by Dead End™ Fluorometric TUNEL System (Promega, Madison, WI, USA) in accordance with the manufacturer's protocol. Cells were fixed with 1% polytetrafluoroethylene in PBS for 10 minutes at room temperature. Cooled ethanol was used to permeabilize fixation cells. Then, the cells were immersed in equilibration buffer. The working strength TdT enzyme was added and incubated at 37°C for 1 hour. The stop buffer was added to stop the reaction. Cells were washed with PBS and incubated with anti-digoxigenin conjugate at room temperature for 30 minutes in the dark. The results were acquired using Zeiss photomicroscope (Carl Zeiss, Oberkochen, Germany) and quantified using Image-Pro plus 6.0 software via counting at least five random fields.

Luciferase Activity

The wild-type PAX8-AS1-N, STAT3 and a mutant PAX8-AS1-N, STAT3 sequences were amplified and cloned into the downstream of the stop codon of the firefly luciferase in basic vector (Promega, Madison, WI, USA). MPC5 were cultured overnight after being added into a 24-well plate, co-transfected with the WT-PAX8-AS1-N/ MUT-PAX8-AS1-N reporter gene plasmid and miR-17-5p mimics or miR-17-5p inhibitor. Renilla expression vector was transfected into each group to serve as a normalized control. After 48 hours transfected, firefly and Renilla luciferase activities were detected by Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA). Data were normalized against the activity of the Renilla luciferase gene.

Western Blot Assays

RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) was used to lyse renal tissues or MPC5 cells on ice. Protein samples were isolated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membrane (Thermo Fisher Scientific, Waltham, MA, USA). The membrane was blocked with 5% skim milk for 2 hours and incubated with primary antibody against VEGF/8-OhdG (Thermo Fisher Scientific, Waltham, MA, USA), cleaved-caspase 3 (Thermo Fisher Scientific, Waltham, MA, USA), and GAPDH (Abcam, Cambridge, MA, USA) overnight. The secondary horseradish peroxidase (HRP) conjugated antibody (Abcam, Cambridge, MA, USA) was added and incubated for 2 hours. β-actin was used as an internal control. The blots were visualized by ChemiDoc MP imaging system (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Statistical Analysis

All statistical analyses were performed with Statistical Product and Service Solutions (SPSS) 18.0 software (SPSS Inc., Chicago, IL, USA) and presented as mean ± standard error. GraphPad Prism 7.0 (GraphPad, La Jolla, CA, USA) software was used to draw graphs. Each assay was applied at least three independent experiments or replicates. Student's *t*-test, one-way analysis of variance (ANOVA) and multiple comparison between the groups were performed by SNK method. p value<0.05 was regarded as statistically significant.

Results

LncRNA PAX8-AS1-N Was Lowly Expressed and VEGF/TGF-β1/8-OhdG were Highly Expressed in Patients of DN and HG-Induced MPC5 Cell

In order to estimate the metabolism of kidney in DN patients, we detected the albuminuria concentration in urine. The experiment revealed that DN patients had higher albuminuria concentration compared with control group (Figure 1A). Then, the expression of PAX8-AS1-N was detected by RT-qPCR in blood samples from patients with DN group and control group. We found that the expression of PAX8-AS1-N was significantly decreased in all patient groups compared with controls group (Figure 1B). At the same time, we measure the VEGF/ TGF-β1/8-OhdG level in serum. We found that the level of VEGF/TGF-β1/8-OhdG was also highly expressed compared with control group (Figure 1C-E). Moreover, we examined the expression of PAX8-AS1-N and VEGF/ TGF-β1/8-OhdG in MPC5 cells. Compared with the NG group, HG

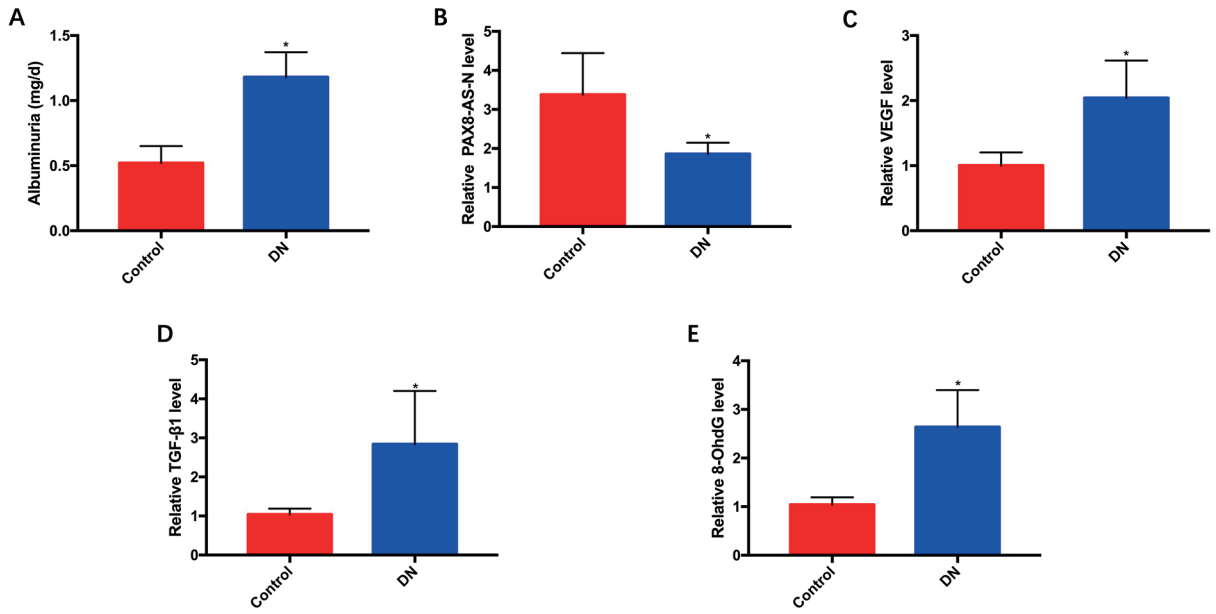

Figure 1. PAX8-AS1-N was lowly expressed and VEGF/TGF-β1/8-OhdG were highly expressed in patients of DN. **(A)** Elisa was performed to measure albuminuria concentration. * $p<0.05$. **(B-D)** The expressions of PAX8-AS1-N/VEGF/TGF-β1 were detected by qRT-PCR assay. * $p<0.05$. **(E)** Elisa was performed to measure the level of 8-OhdG. * $p<0.05$.

significantly decreased the PAX8-AS1-N level and the expression of VEGF/ TGF-β1/8-OhdG was remarkably increased in MPC5 (Figure 2A-2E). From these results, we indicated that the low expression of PAX8-AS1-N and high expression of VEGF/TGF-β1/8-OhdG were closely related to renal damage in DN patients.

Overexpressed PAX8-AS1-N Would Suppress the Level of VEGF/TGF-β1/8-OhdG

To further research the role of lncRNA PAX8-AS1-N in DN, we upregulated the expression of PAX8-AS1-N in HG-induced MPC5. The results showed that VEGF/TGF-β1/8-OhdG level was suppressed in HG-treated MPC5 cells after PAX8-AS1-N overexpressed (Figure 3A-3B, 3D). Meanwhile, the protein level of VEGF/TGF-β1 was detected by Western blot, and we got the same data (Figure 3C). The results indicated oxidative stress damaged the kidney via regulating PAX8-AS1-N.

PAX8-AS1-N Regulated Podocyte Apoptosis and Promoted Cells Growth in HG

The degree of destruction in the podocyte determines the level of renal function. It's important to regulate podocyte apoptosis for the prevention and treatment of DN. In order to study whether PAX8-AS1-N modulated the function of kidney, we detected the level of apoptosis and the proliferation. The results showed that podocyte apoptosis was significantly upregulated in the HG group compared with the NG group. After PAX8-AS1-N overexpressed, podocyte apoptosis was remarkably decreased (Figure 4A). Meanwhile, the apoptosis gene level of Bad, Bax and cleaved-caspase-3 was significantly reduced when PAX8-AS1-N upregulated in the HG group (Figure 4B). Furthermore, compared with NG, the proliferation of MPC5 was decreased in HG. Then, we upregulated the PAX8-AS1-N level, and we found that LV-PAX8-AS1-N reversed this effect (Figure 4C). In conclusion, PAX8-AS1-N could regulate podocyte apoptosis and promote cell proliferation in DN.

PAX8-AS-N Affects the Podocyte Apoptosis via Directly Binding with miR-17-5p in HG

As we have found that PAX8-AS1-N could promote the renal function via regulating podocyte apoptosis, the molecular mechanism remained unclear. PAX8-AS1-N was mainly situated in the cytoplasm. Many cytoplasmic lncRNAs have been reported to act as competing endogenous RNAs (ceRNAs) via competitively binding common miRNAs[12]. We hypothesized that

PAX8-AS1-N regulated cell proliferation through interaction with miR-17-5p. To further verify the relationship of PAX8-AS1-N and miR-17-5p, a WT-PAX8-AS1-N luciferase reporter vector (WT-PAX8-AS1-N), a MUT-PAX8-AS1-N 3'UTR luciferase reporter vector (MUT-PAX8-AS1-N) with mutations on predicted miR-17-5p binding site in PAX8-AS1-N was constructed (Figure 5A). After transfected miR-17-5p mimics and miR-17-5p NC into MPC5 for 24 h, we detected the relative luciferase activity. The results revealed that co-transfection with miR-17-5p mimic and wt-PAX8-AS1-N significantly decreased the luciferase activity of MPC5, compared with pmiR-GLO vector. Meanwhile, the luciferase activity in pmiR-PAX8-AS1-N-MT was enriched, compared with transfected with pmiR-PAX8-AS1-N-WT (Figure 5B). To investigate the potential relationship between PAX8-AS1-N and miR-17-5p, we examined the expression of miR-17-5p in the 25 serum samples of DN patients. The data demonstrated the expression of miR-17-5p was negatively correlated with the expression of PAX8-AS1-N (Figure 5C). To verify the influence of PAX8-AS-N on miR-17-5p expression, MPC5 was transfected with LV-PAX8-AS-N. The cells transfected with LV-PAX8-AS-N presented significantly decreased miR-17-5p expression (Figure 5D). To further investigate whether the effects of PAX8-AS-N were mediated by miR-17-5p in DN, the miR-17-5p mimics were introduced into LV-PAX8-AS-N transfected MPC5. The results showed that LV-PAX8-AS-N-induced decrease in apoptosis was remarkably reversed by upregulating the expression of miR-17-5p (Figure 5E). The protein levels of Bad, Bax and cleaved-caspase-3 are consistent with the trend of apoptosis, but the level of Bcl-2 was opposite (Figure 5F). These data demonstrated that PAX8-AS-N impacted MPC5 apoptosis by suppressing miR-17-5p expression.

MiR-17-5p Inhibited STAT3 Expression by Direct Interaction

We predicted the complementary sequences between miR-17-5p and STAT3-3'UTR with StarBase and TargetScan software (Figure 6A). The Dual-Luciferase reporter assay showed that miR-17-5p reduced the luciferase activity of STAT3-WT reporter rather than miR-NC (Figure 6B). What's more, transfected with miR-17-5p inhibitor or overexpressed PAX8-AS-N would result in the promotion of STAT3 protein expression in MPC5 (Figure 6C). To further explore whether the effects of miR-17-5p were regulated by STAT3 in DN, miR-17-5p mimics and pcDNA-STAT3 were co-transfected into MPC5 in NG model. The results showed that miR-17-5p upregulating led to

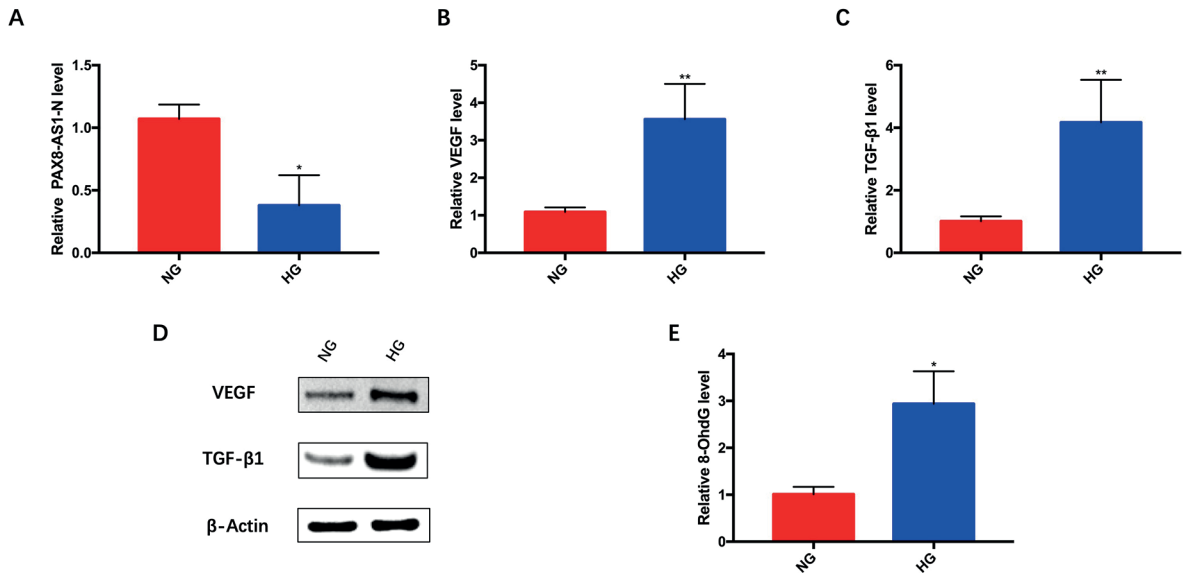

Figure 2. LncRNA PAX8-AS1-N was lowly expressed and VEGF/TGF-β1/8-OhdG were highly expressed in HG-induced MPC5 cell. **(A-C)** The expressions of PAX8-AS1-N/VEGF/TGF-β1 were detected by qRT-PCR assay. * $p<0.05$, ** $p<0.01$. **(D)** The protein level of VEGF/TGF-β1 were detected by Western blot. * $p<0.05$. **(E)** The level of 8-OhdG was measured by Elisa. * $p<0.05$.

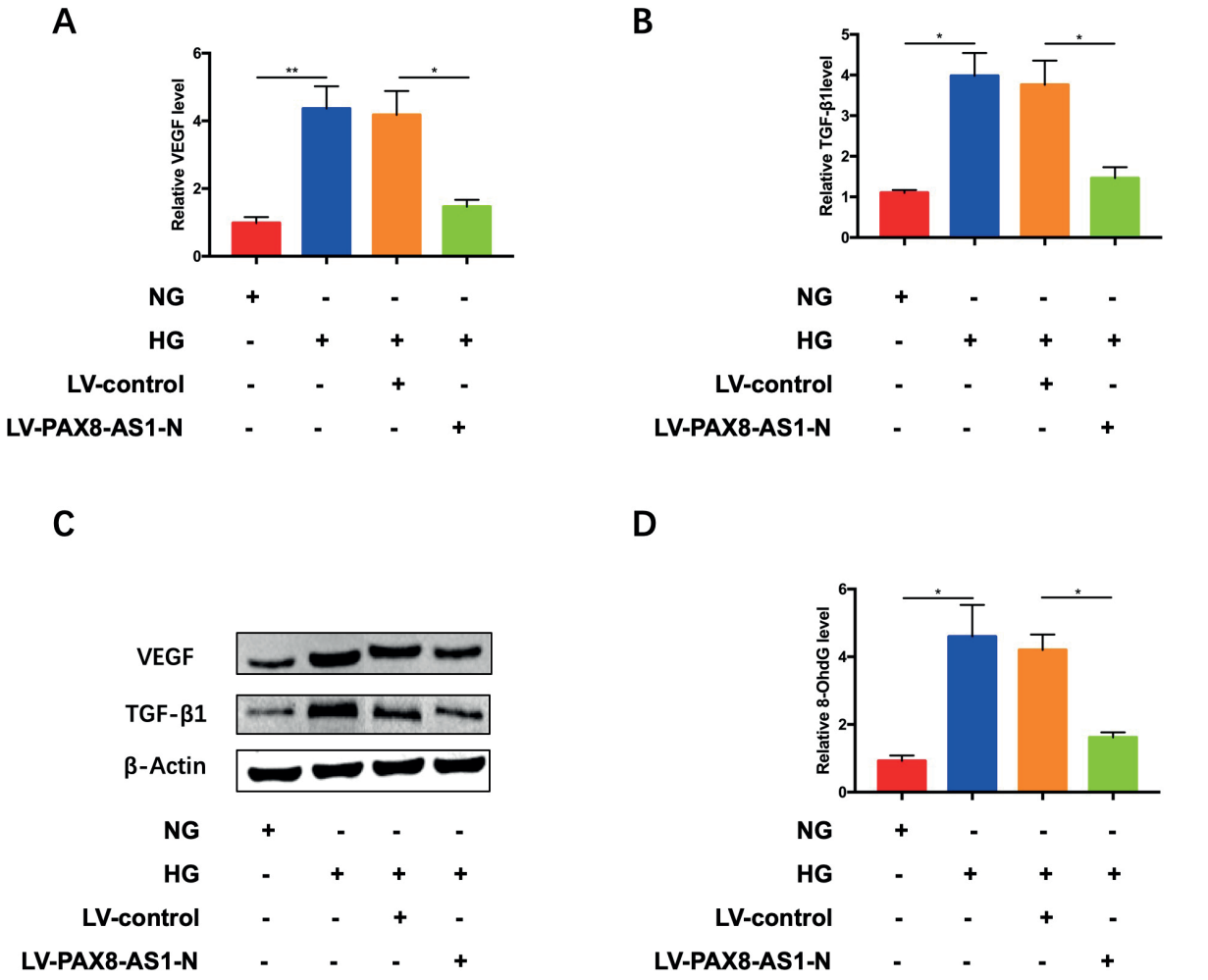

Figure 3. Overexpressed PAX8-AS1-N would suppress the level of VEGF/TGF-β1/8-OhdG. **(A-C)** After transfected with LV-PAX8-AS1-N, the mRNA and protein level of VEGF/TGF-β1 was detected by qRT-PCR and Western blot in HG-treated MPC5 cells. ** $p<0.01$, * $p<0.05$. **(D)** After transfected with LV-PAX8-AS1-N, the 8-OhdG level was detected by Elisa in HG-treated MPC5 cells. * $p<0.05$.

a significant promotion in apoptosis of MPC5, while these effects were substantially reversed following improved STAT3 expression (Figure 6D). As previously mentioned, the protein level of Bad, Bax and cleaved-caspase-3 are consistent with the trend of apoptosis, but the level of Bcl-2 was opposite (Figure 6E). Above that, PAX8-AS-N regulates the apoptosis via the miR17-5p/STAT3 signaling pathway.

Discussion

DN is one of the complications of microvascular disease in diabetes. In recent years, the clinical and experimental studies have shown that glucose metabolism and the change of renal hemodynamic play important roles in the pathogenesis of DN [16]. At the same time, it was also considered that a variety of cytokines abnormal expression, genetic susceptibility, oxidative stress and other factors play important roles. At present, it is mainly to control hyperglycemia and hypertension, reduce proteinuria, correct lipid metabolism disorders, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists to inhibit renin-angiotensin system (RAS) activation for delaying the corresponding treatment of diabetic nephropathy [17]. However, it still could not effectively limit the progress of the disease, or there are quite diabetic patients with kidney disease progress to end-stage renal disease (ESRD).

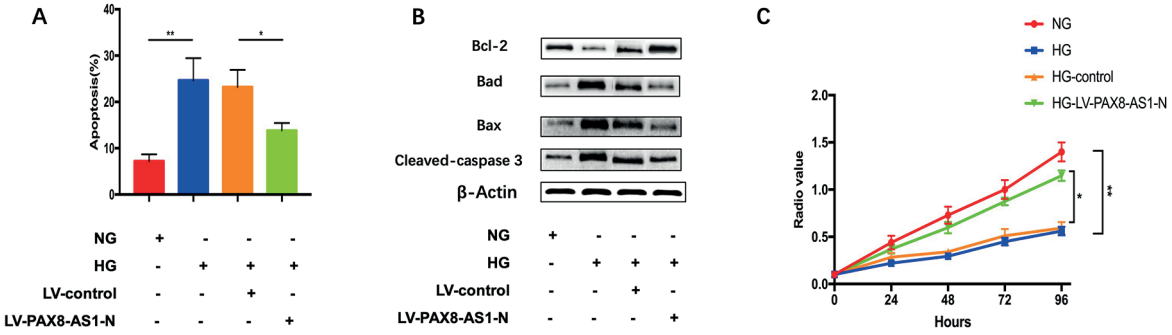

Figure 4. PAX8-AS1-N regulated podocyte apoptosis and promoted cells growth in HG. **(A)** After transfected with LV-PAX8-AS1-N, podocyte apoptosis was detected by TUNEL assay in the HG-treated MPC5 cells. ** $p<0.01$, * $p<0.05$. **(B)** After transfected with LV-PAX8-AS1-N, the protein levels of cleaved-caspase-3, Bax, Bad and Bcl-2 were detected by Western blot in the HG-treated MPC5 cells. **(C)** After transfected with LV-PAX8-AS1-N, the MPC5 proliferation was measured by CCK8 in NG and HG. * $p<0.05$, ** $p<0.01$.

Therefore, further searching for novel targets for diabetic nephropathy is a focus that needs to be solved urgently.

Recent studies have shown that lncRNA regulates gene expression on a variety of levels, mainly including epigenetic transcription and posttranscriptional modification, and the regulation modes include chromosomal modification, transcriptional interference, or transcriptional activation. Accumulating evidence has indicated the significant roles of lncRNAs in the pathophysiology of DN, and the crosstalk between lncRNA and DN was

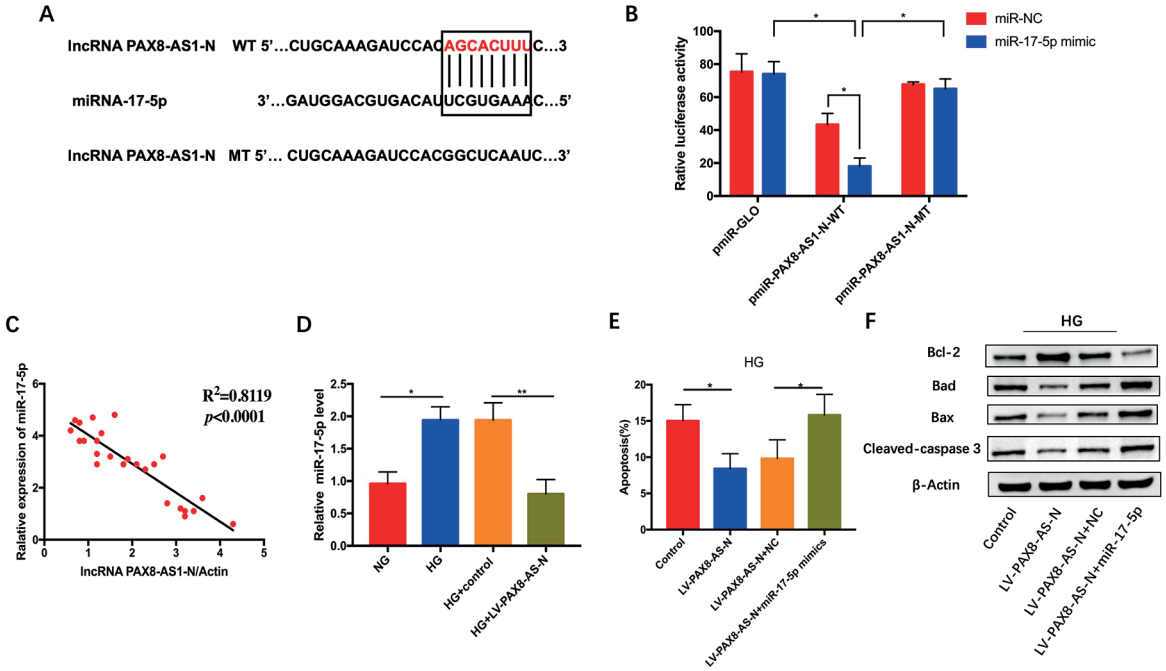

Figure 5. PAX8-AS-N affects the podocyte apoptosis via directly binding with miR-17-5p in DN. **(A)** Bioinformatics analysis predicted binding sites between PAX8-AS1-N and miRNA-17-5p. **(B)** The luciferase reporter assay. Co-transfection with miR-17-5p and PAX8-AS1-N WT significantly decreased the luciferase activity of MPC5 compared with others. * $p<0.05$. **(C)** The LncRNA PAX8-AS1-N expression level was negatively correlated with miRNA-17-5p expression in MPC5. $R^2=0.8119$, **** $p<0.0001$. **(D)** The expression levels of miR-17-5p were determined by qPCR in MPC5 after transfection with LV-PAX8-AS-N. ** $p<0.01$, * $p<0.05$. **(E)** After transfected with LV-PAX8-AS1-N and miR-17-5p mimics, podocyte apoptosis was detected by TUNEL assay in the HG-treated MPC5 cells. * $p<0.05$. **(F)** After transfected with LV-PAX8-AS1-N and miR-17-5p mimics, the protein levels of cleaved-caspase-3, Bax, Bad and Bcl-2 were assayed by Western blot in the HG-treated MPC5 cells.

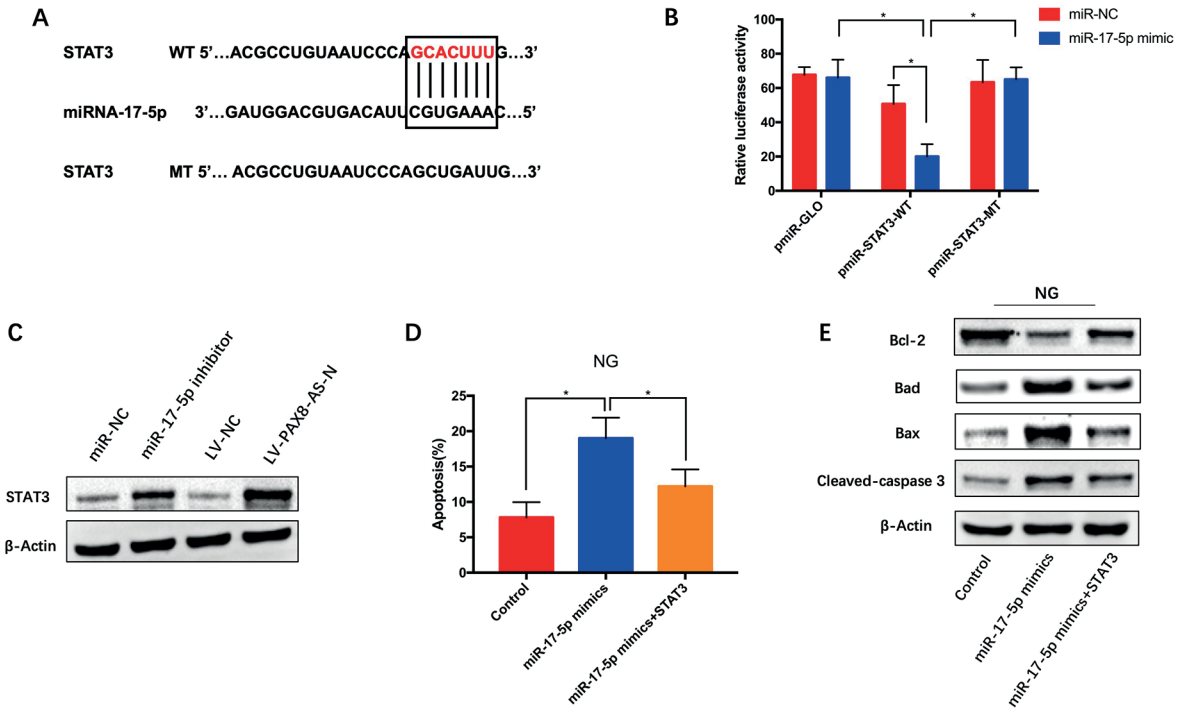

Figure 6. MiR-17-5p inhibited STAT3 expression by direct interaction. **(A)** Bioinformatics analysis predicted binding sites between miRNA-17-5p and STAT3. **(B)** The luciferase reporter assay. Co-transfection with miR-17-5p and STAT3 WT significantly decreased the luciferase activity of MPC5 compared with others. * $p<0.05$. **(C)** Western blot assay of STAT3 protein expression in MPC5 after transfection with miR-217 inhibitor or LV-PAX8-AS-N. **(D)** After transfected with STAT3 and miR-17-5p mimics, podocyte apoptosis was detected by TUNEL assay in the NG-treated MPC5 cells. * $p<0.05$. **(E)** After transfected with STAT3 and miR-17-5p mimics, the protein levels of cleaved-caspase-3, Bax, Bad and Bcl-2 were determined by Western blot in the NG-treated MPC5 cells.

widely reported in recent years. Wang et al[18] found that LncRNA CYP4B1-PS1-001 inhibited proliferation and fibrosis of mesangial cells in diabetic nephropathy by interacting with nucleolin.

Under the condition of hyperglycemia of diabetes, a variety of factors mainly involved in the disorder of glucose metabolism in renal tissue, caused by excessive production of oxidative stress in renal tissue, destroying the normal clearance ability of renal tissue and leading to the destruction of podocytes. VEGF/ TGF-β1/8-OhdG plays key roles in oxidative stress and participate in the progression of various diseases. So that we aimed to verify the role of VEGF/ TGF-β1/8-OhdG in DN.

From our research, it is the first report of PAX8-AS1-N being participated in the progression of DN via regulating the expression of VEGF/ TGF-β1/8-OhdG. In this study, we used ELISA kit to detect the albuminuria concentration and found albuminuria concentration was significantly increased in DN patients. Then, we demonstrated that lncRNA PAX8-AS1-N was down-regulated and VEGF/ TGF-β1/8-OhdG was increased in DN, and LV-PAX8-AS1-N could reverse this effect of promotion. Additionally, we verified that PAX8-AS1-N indirectly adjusted the cell apoptosis and proliferation by regulating miR-150-5p. Collectively, we know that PAX8-AS1-N/miR-17-5p/STAT3 axis regulated cell apoptosis and proliferation, and further modulated the VEGF/ TGF-β1/8-OhdG level for relieving the kidney damage.

In summary, we identified a novel regulation mechanism that PAX8-AS1-N exerts its suppression effects for cell apoptosis via activating miR-17-5p/STAT3 in DN. And we detected albuminuria concentration was increased and demonstrated that relieved PAX8-AS1-N could promote VEGF/ TGF-β1/8-OhdG levels to trigger oxidative stress in DN. Furthermore, PAX8-AS1-N governed the high glucose damage through miR-17-5p/STAT3 signal pathway in DN. Therefore, our results provided a new insight for treating DN, and indicated that enhancing the expression of PAX8-AS1-N would be a potential therapeutic strategy.

Conclusions

Our study firstly found that PAX8-AS1-N was attenuated in DN and played a key role in the occurrence of oxidative stress and the progression of high glucose damage in DN. And we found that PAX8-AS1-N/miR-17-5p/STAT3 axis could regulate podocyte apoptosis, which may be a novel potential therapeutic strategy for treatment and predicting DN.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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