IncRNA KCNQ1OT1 regulated high glucose-induced proliferation, oxidative stress, extracellular matrix accumulation, and inflammation by miR-147a/SOX6 in diabetic nephropathy (DN)

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Abstract. Long non-coding RNAs (lncRNAs) have been proved to play critical roles in diabetic nephropathy (DN). This study aimed to investigate the functions and underlying mechanism of potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1 (KCNQ1OT1) in DN. Blood samples were obtained from 33 DN patients and 30 healthy volunteers. Kidney biopsies tissues of DN patients (n = 10) and patients with normal kidney morphology (n = 10) were collected. We found that KCNQ1OT1 was markedly overexpressed in the blood and kidney biopsies tissues of DN patients, as well as in high glucose (HG)-cultured human glomerular mesangial (HGMC) cells. Knockdown of KCNQ1OT1 suppressed proliferation, extracellular matrix (ECM) accumulation, inflammation, and oxidative stress in HG-treated HGMC cells in vitro. KCNQ1OT1 functioned as a sponge for microRNA-147a (miR-147a), and SRY-Box Transcription Factor 6 (SOX6) was directly targeted by miR-147a. Downregulation of miR-147a or upregulation of SOX6 partly overturned the prohibitive effects of KCNQ1OT1 knockdown or miR-147a overexpression on proliferation, ECM accumulation, inflammation, and oxidative stress in HG-treated HGMC cells. Altogether, KCNQ1OT1 mediated the proliferation, ECM accumulation, inflammation, and oxidative stress in HG-treated HGMC cells via miR-147a/SOX6 axis, which might be a novel target for DN therapy.

Key words: Long non-coding RNAs (lncRNAs), KCNQ1OT1, miR-147a, SOX6, Diabetic nephropathy

DIABETIC NEPHROPATHY (DN) is a progressive and universal complication of diabetes and also the major reason for renal dysfunction, which is characterized by glomeruli capillaries injury, depressed renal glomerular filtration rate, and dysfunction of kidney organ [1, 2]. In the past decade, the incidence of DN has been growing rapidly worldwide, which is the major cause of deaths due to diabetes [3, 4]. It is necessary to investigate the mechanism underlying DN progression to develop and identify effective therapeutic strategy for DN.

Long non-coding RNAs (lncRNAs) are functional RNAs (>200 nucleotides in length) and exert their regulatory functions through competing endogenous RNAs (ceRNAs) mechanism [5, 6]. Extensive lncRNA profiling revealed that lncRNAs were abnormally expressed in DN and were closely linked to the pathogenesis of DN, which might be promising targets for the treatment and prevention of DN [7]. Potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1 (KCNQ1OT1) was considered to be an important regulator in tumorigenesis [8-10], which was also suggested to be involved in multiple diabetic complications, including cardiomyopathy [11], diabetic corneal endothelial keratopathy [12], and nephropathy [13]. Nevertheless, the regulatory mechanism of KCNQ1OT1 in DN progression still needs further investigation.

MicroRNAs (miRNAs), a small non-coding RNAs that could bind to complementary binding sequences (miRNA response elements) in the 3’ untranslated region (UTR) of target genes, thereby leading to transcription inhibition or degradation of mRNA [14]. Surely, miRNAs were dysregulated and played significant roles in multiple human malignancies through acting as modulators of gene expression [15]. A previous research uncovered that...
miR-147a level was dysregulated in DN [16], however, whether miR-147a is implicated in DN progression remains unclear.

SRY-Box Transcription Factor 6 (SOX6) is a main representative of SRY-related high mobility group box family and plays a significant role in numerous cellular processes [17]. A previous report revealed that dysregulation SOX6 is linked to different disease conditions, including diabetes [18]. Importantly, the in vivo study also displayed that SOX6 is overexpressed in DN mice [19]. However, the biologic function of SOX6 has not yet been fully elucidated.

In present research, the expression of IncRNA KCNQ1OT1 was investigated in the blood and kidney biopsies tissues of DN, and HG-treated human glomerular mesangial (HGMC) cells. Besides, we also explored the interaction between KCNQ1OT1 and miR-147a or SOX6 and their regulatory mechanism in DN. This study aimed to search a potential biomarker for DN diagnosis and treatment.

**Materials and Methods**

**Patient specimens**

Blood specimens were collected from DN patients (n = 33), while control blood samples were acquired from 30 healthy volunteers at Huangshi central Hospital. Besides, human kidney biopsies tissues (n = 10) were obtained from of diagnostic renal biopsies performed at Huangshi central Hospital. Normal kidney tissues (n = 10) were obtained from healthy transplant donor kidney biopsies and used as controls. All patients and volunteers provided the written the informed consents. This research was permitted by the Ethics Committee of Huangshi central Hospital.

**Cell lines and cell culture**

HGMC cells were purchased from the Nanjing Key Gen Biotech (Nanjing, China) and cultured Dulbecco’s modified Eagle medium (GIBCO BRL, Grand Island, NY, USA) containing 10% (v/v) fetal bovine serum (FBS; GIBCO BRL) at 37°C with 5% CO2. The medium would be replaced every 2 or 3 days. HGMC cells were treated with 30 mM of glucose to mimic the DN cellular model in vitro, HGMC cells that incubated 5.5 mM of glucose were used as normal glucose (NG) group, and HGMC cells with 5.5 mM glucose and 24.5 mM mannitol treatment were used as osmolar control.

**Quantitative real-time polymerase chain reaction (RT-qPCR)**

TRIzol Kit (Omega, Norcross, GA, USA) was used to isolate total RNA. SuperScript Reverse Transcriptase Kit (Vazyme, Nanjing, China) was applied to reverse transcribe RNA into cDNA. The RT-qPCR assay was conducted with 2 μL of cDNA, primers, 5 μL of 2X Power SYBR Green master mix (Vazyme), and nuclease-free water in StepOne Plus Real-time PCR System (Roche Applied Science, Mannheim, Germany). The expression level of RNA was measured by the comparative threshold cycle (Ct) method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 as references. The primers were: KCNQ1OT1, upstream 5'-CCTCCCCAC TGAGCTTT GG-3' and R-5'-GTGCGACCCTATACCG GAAG-3'; miR-147a, upstream 5'-GCCGAGGTGTGTTGG AAAAT-3' and downstream 5'-CAGTGCAAGGTCCGAG GTAT-3'; SOX6, upstream 5'-AGGGAGTCTTGCCGAT GCAGTGTG-3' and downstream 5'-CAGGCTCTCACGGT GTACCTTA-3'; U6, upstream 5'-ATCCTTACGCACCCA GTCCA-3' and downstream 5'-GAACGCTTCACGAAT TTGC-3'; GAPDH, upstream 5'-GAACGCTTCACGAAT TTGC-3' and downstream 5'-TCTTACTCCTGGAGG GCACA-3'.

**Transfection assay**

Short interfering RNA (siRNA) targeting KCNQ1OT1 (si-KCNQ1OT1), and control (si-NC), SOX6-overexpression vector (SOX6), and negative control was synthesized by Genscript Company (Guangzhou, China). The mimic of miR-147a and negative control (miR-147a mimic and mimic NC), inhibitors of miR-147a and negative control (miR-103a-3p inhibitor and inhibitor NC) were bought from Ribobio Company (Shanghai, China). HGMC were transfected with vector (1 μg), si-circRNA and miRNA inhibitor (40 nM), or miRNA mimic (80 nM) by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) referring to the user’s guideline, and then were used for further experiments.

**Cell proliferation assay**

Cell Counting Kit-8 (CCK-8) was bought from Dojindo Laboratories, (Kumamoto, Japan) for measuring cell viability. A total of 1 × 10^4 HGMC cells were cultured in 96-well plates. HGMC cells were incubated with 20 μL of CCK-8 solution after culture 48 h. The absorbance was read under microplate reader (Bio-Rad, Hercules, CA, USA) at 450 nm wavelength. All detections were performed in triplicate.

**Protein extraction and western blot assay**

The protein was collected by ice-cold RIPA lysis buffer (Beyotime, Shanghai, China). After quantifying by the Bradford assay kit (Bio-Rad), protein (100 μg) was isolated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride membranes (PVDF, Bio-Rad).
Afterwards, the membranes were soaked in 5% skim milk solution and then immunoblotted by appropriate primary antibodies overnight at 4°C, followed by washing with Tris-buffered saline with Tween 20 incubation. Then membranes were interacted with HRP-conjugated secondary goat anti-rabbit antibodies (ab1500771; 1:1,800 dilution; Abcam, Cambridge, MA, USA) for 1.5 h. Finally, western band was visualized by enhanced chemiluminescence detection kit (Pierce Biotechnology, Rockford, IL, USA). Antibodies used in this study were purchased from Abcam, such as SOX6 (ab245215; 1:1,200 dilution), Proliferating Cell Nuclear Antigen (PCNA; ab92552; 1:1,200 dilution), Collagen I (ab34710; 1:1,200 dilution), Collagen IV (ab6586; 1:1,200 dilution), Fibronectin (ab2413; 1:1,200 dilution), and GAPDH (ab181602; 1:1,800 dilution).

Enzyme linked immunosorbent assay (ELISA)

HGMC cell supernatant was lower centrifuged (2,500 × g) for 15 min at 4°C, and then 50 μL of cell supernatant was collected. Tumor necrosis factor-α (TNF-α) level of samples was determined by using TNF alpha Human ELISA Kit (Invitrogen). The samples (50 μL/well) were added to a 96-well plate treated with goat anti-mouse IgM, followed by incubation for 1 h. After washing, each well was added with TMB solution (100 μL/well) for incubation 10 min. The absorbance read at 450 nm under microplate reader (Bio-Rad). Similarly, interleukin-6 level (IL-6) was analyzed by IL-6 Human ELISA Kit (Invitrogen).

Oxidative stress assay

Malondialdehyde (MDA) level and superoxide dismutase (SOD) activity were assessed by Malondialdehyde Assay Kit (Nanjing Jiancheng Bioengineering, Nanjing, China) and Superoxide Dismutase Colorimetric Activity Kit (Nanjing Jiancheng Bioengineering), correspondingly.

Dual-luciferase reporter experiment

The prediction of the binding sites of KCNQ1OT1 and miR-147a were presented by Starbase (http://starbase.sysu.edu.cn/). The target gene of miR-147a was predicted by TargetScan (http://www.targetscan.org/mamm_31/). The KCNQ1OT1 segments were synthesized with either mutant or wild-type containing miR-147a binding sites and then cloned into the downstream of the firefly luciferase gene in pGL3-basic vectors (Realgene, Nanjing, China). Similarly, a wild-type or mutant of 3'UTR fragment of SOX6 mRNA was cloned into pGL3-basic vectors to perform dual-luciferase reporter assay. HGMC cells (1 × 10^5 cells/well) were co-transfected with wild-type or mutated type vectors and miR-147a mimic or control using Lipofectamine 2000 (Invitrogen). The dual-luciferase reporter assay kit (Promega, Madison, WI, USA) was used to assess firefly luciferase activity, with Renilla luciferase activity as control.

RNA immunoprecipitation (RIP) assay

Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) was applied to perform RIP assay. In brief, HGMC cells were lysed in the RIP lysis buffer containing 0.5 M EDTA and 0.1% RNase inhibitor and then interacted with magnetic beads covered with anti-Argonaute2 (AGO2) at 4°C for 4 h, with IgG as control. Proteinase K was used for digestion of the protein. Relative expression levels of immunoprecipitated RNAs were quantified by RT-qPCR.

Statistical analysis

The data were assessed by SPSS 21.0 software (IBM, Somers, NY, USA) and displayed as mean ± standard deviation. Statistical analysis was determined by Student’s t-test or analysis of variance with multiple comparisons with post hoc Bonferroni test. Pearson’s correlation coefficient analysis was used to analyze linear correlation. P-value less than 0.05 was deemed as a significant difference.

Results

KCNQ1OT1 was overexpressed while miR-147a was downregulated in DN

Herein, the expression of KCNQ1OT1 was remarkably overexpressed in the blood of DN patients (n = 33) compared with the healthy controls (n = 30) (Fig. 1A). Besides, we also detected the expression of KCNQ1OT1 in kidney biopsies tissues of DN patients (n = 10) and patients with normal kidney morphology (n = 10), and found that KCNQ1OT1 expression in kidney biopsies tissues of DN patients was significantly higher than that in normal group (Supplementary Fig. 1). Besides, the level of miR-147a was downregulated in the blood of patients with DN as compared to controls (Fig. 1B). In order to investigate the potential association between KCNQ1OT1 and miR-147a, Pearson’s correlation analysis was performed. As presented in Fig. 1C, a negative correlation between KCNQ1OT1 and miR-147a in the blood of DN patients was confirmed. To detect KCNQ1OT1 expression upon D-glucose stimulation, HGMC cells were exposed to different concentrations of D-glucose for 48 h, and mannitol (MA, 30 mmol/L) was used as the negative control. As presented in Supplementary Fig. 2A, KCNQ1T1 expression was elevated in D-glucose-induced HGMC cells in a dose dependent manner, while its expression was not affected by MA.
treatment. Besides, we found that KCNQ1T1 expression was significantly increased in HGMC cells with with the increasing time for D-glucose treatment (Supplementary Fig. 2B). Hence, the effect of glucose on KCNQ1T1 expression has dose and time dependency, and HGMC cells incubated with 30 mmol/L glucose for 48 h were selected to simulate the condition of DN. As shown in Fig. 1D, relative expression level of KCNQ1OT1 was increased about 2.5-fold in HG-treated HGMC cells. Furthermore, to exclude osmotic pressure side effects, we detected the expression level of KCNQ1OT1 in osmolar control group, NG group and HG group. And the results uncovered that KCNQ1OT1 level was significantly elevated in HG-cultured group, while its expression had almost no difference in NG group and osmolar control group (Supplementary Fig. 3). Hence, the changes of osmotic pressures exhibited no effect on KCNQ1OT1 expression. On the contrary, HG treatment inhibited miR-147a expression in HGMC cells (Fig. 1E). Therefore, these results suggested that KCNQ1OT1 and miR-147a might play significant roles in DN progression.

**Depletion of KCNQ1OT1 inhibited proliferation, extracellular matrix (ECM) accumulation, inflammation, and oxidative stress in HG-treated HGMC cells**

In order to investigate the potential roles of KCNQ1OT1 in DN, siRNA targeting KCNQ1OT1 was used to knock down the expression of KCNQ1OT1. As displayed in Fig. 2A, KCNQ1OT1 was significantly downregulated in si-KCNQ1OT1-transfected cells compared with that in si-NC group. HG treatment dramatically increased cell viability, and this increased tendency was weakened by si-KCNQ1OT1 transfection in HGMC cells (Fig. 2B). Besides, PCNA was upregulated in HG-treated HGMC cells, and this effect was reversed by KCNQ1OT1 knockdown (Fig. 2C). The protein levels of Collagen I, Collagen IV, and Fibronectin were increased in HG-induced HGMC cells, which were abolished by depletion of KCNQ1OT1 (Fig. 2D). Moreover, HG treatment enhanced the inflammation of HGMC cells by promoting the release of TNF-α and IL-6, while these effects were partly reversed by KCNQ1OT1 inhibition (Fig. 2E–2F). Additionally, we also found that oxidative stress was enhanced in HG-treated HGMC cells, while si-KCNQ1OT1 transfection notably decreased MDA level, but elevated SOD activity (Fig. 2G–2H). In summary, these results indicated an important function of KCNQ1OT1 in regulating proliferation, ECM accumulation, inflammation, and oxidative stress in HG-treated HGMC cells.

**MiR-147a was a direct target of KCNQ1OT1**

The putative targets of KCNQ1OT1 were predicted by Starbase software. Several miRNAs were predicted to contain the binding sites in KCNQ1OT1, in which eight miRNAs (miR-27a-3p [20], miR-383-5p [21], miR-205-5p [22], miR-144-3p [23], miR-147a [16], miR-654-3p [24], miR-326 [25] and miR-9-5p [26]) that had been suggested to play a vital role in diabetes and its complications were selected. As displayed in Supplementary Fig. 4A, miR-147a was the most significant upregulated miRNA in HGMC cells with KCNQ1OT1 knockdown, indicating the strongest correlation between KCNQ1OT1 and miR-147a. Then, dual-luciferase reporter assay and RIP assay were performed to further confirm
this correlation. The complementary binding sequences between KCNQ1OT1 and miR-147a were presented in Fig. 3A. Besides, miR-147a expression level was elevated by 12-fold in cells with miR-147a mimic transfection (Fig. 3B). Furthermore, overexpression of miR-147a decreased luciferase activity of WT-KCNQ1OT1 group, while it had no significant effect on luciferase activity of MUT-KCNQ1OT1 group (Fig. 3C). In addition, miR-147a and KCNQ1OT1 could be enriched by AGO2 but not IgG, suggesting the correlation between KCNQ1OT1 and miR-147a (Fig. 3D). MiR-147a expression was lower in HGMC cells transfected with miR-147a inhibitor, as compared to control (Fig. 3E). Importantly, KCNQ1OT1 knockdown increased the expression of miR-147a, which was abolished by transfection with miR-147a inhibitor (Fig. 3F). Hence, KCNQ1OT1 acted as a sponge for miR-147a.

**Knockdown of miR-147a reversed KCNQ1OT1 inhibition-mediated effects on HG-treated HGMC cells**

Considering that miR-147a was a direct target of KCNQ1OT1, we further investigated the effects of KCNQ1OT1/miR-147a in HG-treated HGMC cells. KCNQ1OT1 inhibition impeded cell proliferation in HG-treated HGMC cells, which was abated by miR-147a inhibitor transfection (Fig. 4A). Besides, the protein levels of PCNA, Collagen I, Collagen IV, and Fibronectin were decreased by silencing of KCNQ1OT1 in HG-treated HGMC cells, whereas these effects were counteracted by miR-147a knockdown (Fig. 4B–4C). Furthermore, downregulation of KCNQ1OT1 decreased the release of TNF-α and IL-6 in HG-treated HGMC cells, whereas these anti-inflammatory effects were overturned by miR-147a downregulation (Fig. 4D–4E). In addition, knockdown of miR-147a partly reversed the...
suppressive effects of si-KCNQ1OT1 on oxidative stress in HG-treated HGMC cells by MDA level and decreasing SOD activity (Fig. 4F–4G). These data confirmed that KCNQ1OT1 mediated cell proliferation, ECM accumulation, inflammation, and oxidative stress in HG-treated HGMC cells by sponging miR-147a.

**SOX6 was targeted by miR-147a in HGMC cells.**

To figure out the mechanism of miR-147a-mediated effects on HG-treated HGMC cells, the target genes of miR-147a was predicted. There were eight genes were predicted to contain the complementary binding sites of miR-147a, and SOX6 expression was the most significant decreased by miR-147a mimic transfection (Supplementary Fig. 4B). Hence, SOX6 was chosen for subsequent investigation. As shown in Fig. 5A, SOX6 contained the complementary binding sites of miR-147a. The luciferase activity was reduced in HGMC cells by co-transfecting with WT-SOX6 3’UTR and miR-147a mimic (Fig. 5B). RIP assay also indicated that SOX6 and miR-147a were significantly enriched in the anti-AGO2 group but not anti-IgG group (Fig. 5C). In addition, the mRNA and protein levels of SOX6 were increased in the serum of DN patients (Fig. 5D–5E), and HG-treated HGMC cells (Fig. 5F). Pearson’s correlation analysis confirmed that there was a negative correlation between SOX6 and miR-147a in the serum of DN patients (Fig. 5G). The transfection efficiency of SOX6 overexpression vector was confirmed by western blot assay (Fig. 5H). And upregulation of SOX6 partly abolished the suppressive effect of miR-147a mimic on SOX6 protein level in HGMC cells (Fig. 5I). Altogether, SOX6 could be a direct target of miR-147a in HGMC cells.

**Overexpression of SOX6 reversed the effects of miR-147a on proliferation, ECM accumulation, inflammation, and oxidative stress in HG-treated HGMC cells**

The biological association between miR-147a and SOX6 was analyzed in HG-treated HGMC cells. Overexpression of miR-147a abolished the increase of cell viability in HG-induced HGMC cells, while this effect was overturned by SOX6 overexpression (Fig. 6A). Transfection of miR-147a mimic decreased the protein levels of PCNA, Collagen I, Collagen IV, and Fibronectin in HG-treated HGMC cells, while SOX6 overexpression partly counteracted these effects (Fig. 6B–6C). Besides, upregulation of miR-147a impeded the release of TNF-α and IL-6 elevated by HG treatment, and these effects could be abrogated by SOX6 upregulation (Fig. 6D–6E). In addition, increased SOD activity and decreased MDA level were found in HG-treated HGMC cells with miR-147a overexpression, whereas these effects were abrogated by co-transfection of SOX6 vector (Fig. 6F–6G). Altogether, miR-147a regulated cell proliferation, ECM accumulation, inflammation, and...
oxidative stress in HG-treated HGMC cells by interacting with SOX6.

**KCNQ1OT1 regulated miR-147a/SOX6 axis to activate Wnt/β-Catenin pathway in HG-treated HGMC cells**

The association among KCNQ1OT1, miR-147a, and SOX6 was further explored in HG-treated HGMC cells. We found that silencing of KCNQ1OT1 weakened the elevated mRNA and protein levels of SOX6 in HG-induced HGMC cells, while these effects were abolished by miR-147a inhibition (Fig. 7A–7B). In addition, KCNQ1OT1 silence inhibited the protein levels of β-catenin, c-myc, and cyclinD1 that were elevated by HG treatment in HG-treated HGMC cells, whereas the function of KCNQ1OT1 was eliminated by transfection of miR-147a inhibitor (Fig. 7C). Hence, KCNQ1OT1 regulated miR-147a/SOX6 axis to activate Wnt/β-Catenin pathway and regulate DN progression *in vitro*.

**Discussion**

To date, IncRNAs have been suggested to play important roles in regulating the pathological processes of
human disease. In current research, we found that lncRNA KCNQ1OT1 is upregulated in the blood and kidney biopsies tissues of DN patients, as well as in HG-induced HGMC cells. More importantly, we found that KCNQ1OT1 knockdown blocked the progression of DN by regulating cell proliferation, oxidative stress, ECM accumulation, and inflammatory in HG-induced HGMC cells. Mechanically, KCNQ1OT1 acted as a sponge for miR-147a to regulate the expression of SOX6, thereby activating Wnt/β-catenin pathway and promoting DN progression.

The pathogenesis of DN was multifactorial and complicated. It has been reported that dysfunction of glomerular mesangial cells was involved in the development of DN [27]. Furthermore, glucose metabolic dysfunction was a feature in DN, leading to consistent micro-inflammation [28]. ECM protein deposition, including Collagen and Fibronectin, was related to the development of renal glomerular fibrosis in DN [29]. Besides, HG treatment induced ECM deposition, inflammation, and oxidative stress in HGMC cells, which were key pathological features of multiple renal diseases and widely reported in previous studies [30, 31]. To investigate the biological role of KCNQ1OT1 in DN, HGMC cells treated with 30 mM glucose were used as the cellular model for DN.

Indeed, recent evidence has indicated that KCNQ1OT1 was upregulated in response to HG stimulation and promoted DN progression. For example, Jie et al. reported that KCNQ1OT1 knockdown blocked the progression of DN by regulating cell proliferation, oxidative stress, ECM accumulation, and inflammatory in HG-induced HGMC cells. Mechanically, KCNQ1OT1 acted as a sponge for miR-147a to regulate the expression of SOX6, thereby activating Wnt/β-catenin pathway and promoting DN progression.

Fig. 5 MiR-147a targeted SOX6 in HGMC cells. (A) The binding sites between miR-147a and SOX6 were presented. (B–C) Dual-luciferase reporter and RIP assays were carried to measure the association between miR-147a and SOX6. (D–F) The relative level of SOX6 was estimated by RT-qPCR in peripheral blood tissues from diabetic nephropathy patients and controls, as well as in HGMC cells treated with high glucose (HG) or normal glucose (NG). (G) The correlation between SOX6 and miR-147a was analyzed by Pearson’s correlation coefficient analysis. (H–I) The protein level of SOX6 was evaluated by western blot assay in HGMC cells transfected with vector or SOX6, as well as in HGMC cells transfected with mimic NC, miR-147a mimic, miR-147a mimic + vector, or miR-147a mimic + SOX6. * p < 0.05.

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proliferation, oxidative stress, ECM accumulation, and inflammatory in HG-induced HGMC cells. In addition, KCNQ1OT1 was suggested to contain the complementary binding sites of miR-147a.

MiR-147a has been reported to take part in the pathogenesis of DN [16]. Here, we found that miR-147a is lower expressed in DN blood and tissues. And KCNQ1OT1 could regulate the activity of miR-147a by sponging miR-147a. Besides, miR-147a downregulation partly overturned the suppression effects of miR-147a on DN progression. Hence, miR-147a as a key regulator in DN progression. Moreover, we found that miR-147a directly targeted SOX6. Notably, Jiang et al. demonstrated that the upregulation of miR-342-3p impeded HG-induced renal interstitial fibrosis by decreasing SOX6 expression [19]. Circ_WBSCR17 targeted miR-185-5p/SOX6 regulatory axis to enhance inflammatory responses and fibrosis [34]. Herein, SOX6 expression was significantly suppressed in the blood of DN patients, and was negatively regulated by miR-185-5p. Moreover, we found that SOX6 overexpression partly overturned the suppression effects of miR-147a in HG-induced HGMC cells.

It was noteworthy that the activation of Wnt/β-catenin signaling pathway played a vital role in DN, which might be a possible therapeutic target for DN [35, 36]. Hence, we also investigated whether KCNQ1OT1/miR-147a/ SOX6 axis regulated DN progression by modulating Wnt/β-catenin signaling. Interestingly, SOX6 could inhibit the development of clear cell renal cell carcinoma by negatively regulating the Wnt/β-catenin signaling, confirming the association between SOX6 and Wnt/β-catenin.

Fig. 6 MiR-147a regulated proliferation, ECM accumulation, inflammation, and oxidative stress in high glucose-treated HGMC cells by targeting SOX6. (A–H) HGMC cells were divided into six groups: NG, HG, HG + mimic NC, HG + miR-147a mimic, HG + miR-147a mimic + vector, and HG + miR-147a mimic + SOX6. (A) Cell viability was analyzed by CCK-8. (B–C) Western blot assay was employed to detect protein levels of PCNA, Collagen I, Collagen IV, and Fibronectin in HGMC cells. (D–E) After collecting supernatant, expression levels of TNF-α and IL-6 were evaluated by ELISA. (F–G) The oxidative stress in HGMC cells was assessed by MDA level and SOD activity. * p < 0.05.
signaling [37]. We confirmed that KCNQ1OT1 contributed to DN progression and activated Wnt/β-catenin signaling by targeting miR-147a/SOX6 axis.

Together, these findings identified the KCNQ1OT1/miR-147a/SOX6 regulatory axis in DN progression. Besides, KCNQ1OT1 knockdown inhibited proliferation, ECM accumulation, inflammation, oxidative stress and activated Wnt/β-catenin pathway in HG-treated HGMC cells through miR-147a/SOX6 axis, indicating the clinically relevant implications of KCNQ1OT1 in DN biology.

**Conclusion**

Taken together, KCNQ1OT1 promoted proliferation, ECM accumulation, inflammation, and oxidative stress in HG-treated HGMC cells via acting as miR-147a sponge to increase SOX6 expression, which enhanced our understanding of DN mechanisms and highlighted the role of KCNQ1OT1 in DN.

**Competing Interests**

The authors declare that they have no conflict of interest.

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**References**

1. Wada J, Makino H (2013) Inflammation and the pathogenesis of diabetic nephropathy. *Clin Sci (Lond)* 124: 139–152.
2. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, et al. (2010) Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol* 21: 556–563.
3. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, et al. (2019) Global and regional diabetes prevalence
estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9 th edition. Diabetes Res Clin Pract 157: 107843.

4. Valenti G, Tamma G (2016) History of diabetes insipidus. G Ital Nefrol 33 Suppl 66: 33.S66.1.

5. Qian X, Zhao J, Yeung PY, Zhang QC, Kwok CK (2019) Revealing IncRNA structures and interactions by sequencing-based approaches. Trends Biochem Sci 44: 33–52.

6. Qi X, Zhang DH, Wu N, Xiao JH, Wang X, et al. (2015) ceRNA in cancer: possible functions and clinical implications. J Med Genet 52: 710–718.

7. Leti F, Morrison E, DiStefano JK (2017) Long noncoding RNAs in the pathogenesis of diabetic kidney disease: implications for novel therapeutic strategies. Per Med 14: 271–278.

8. Li Y, Li C, Li D, Yang L, Jin J, et al. (2019) IncRNA KCNQ1OT1 enhances the chemoresistance of oxaliplatin in colon cancer by targeting the miR-34a/ATG4B pathway. Onco Targets Ther 12: 2649–2660.

9. Kang Y, Jia Y, Wang Q, Zhao Q, Song M, et al. (2019) Long noncoding RNA KCNQ1OT1 promotes the progression of non-small cell lung cancer via regulating miR-204-5p/ATG3 axis. Onco Targets Ther 12: 10787–10797.

10. Wang J, Zhang H, Situ J, Li M, Sun H (2019) KCNQ1OT1 aggravates cell proliferation and migration in bladder cancer through modulating miR-145-5p/PCBP2 axis. Cancer Cell Int 19: 325.

11. Yang F, Qin Y, Wang Y, Li A, Ly J, et al. (2018) LncRNA KCNQ1OT1 mediates pyroptosis in diabetic cardiomyopathy. Cell Physiol Biochem 50: 1230–1244.

12. Zhang Y, Song Z, Li X, Xu S, Zhou S, et al. (2020) Long noncoding RNA KCNQ1OT1 induces pyroptosis in diabetic corneal endothelial keratopathy. Am J Physiol Cell Physiol 318: C346–C359.

13. Jie R, Zhu P, Zhong J, Zhang Y, Wu H (2020) LncRNA KCNQ1OT1 affects cell proliferation, apoptosis and fibrosis through regulating miR-18b-5p/SORBS2 axis and NF-kB pathway in diabetic nephropathy. Diabetol Metab Syndr 12: 77.

14. Liu B, Li J, Cairns MJ (2014) Identifying miRNAs, targets and functions. Brief Bioinform 15: 1–19.

15. Lee YS, Dutta A (2009) MicroRNAs in cancer. Annu Rev Pathol 4: 199–227.

16. Ji TT, Qi YH, Li XY, Tang B, Wang YK, et al. (2020) Loss of IncRNA MIAT ameliorates proliferation and fibrosis of diabetic nephropathy through reducing E2F3 expression. J Cell Mol Med 24: 13314–13323.

17. Saleem M, Hodgkinson CP, Xiao L, Gimenez-Bastida JA, Rasmussen ML, et al. (2020) Sox6 as a new modulator of renin expression in the kidney. Am J Physiol Renal Physiol 318: F285–F297.

18. Saleem M, Barturen-Larrea P, Gomez JA (2020) Emerging roles of Sox6 in the renal and cardiovascular system. Physiol Rep 8: e14604.
pyroptosis of renal tubular epithelial cells. *Diabetes Metab Syndr Obes* 13: 365–375.

33. Li J, Li M, Bai L (2021) KCNQ1OT1/miR-18b/HMG2 axis regulates high glucose-induced proliferation, oxidative stress, and extracellular matrix accumulation in mesangial cells. *Mol Cell Biochem* 476: 321–331.

34. Li G, Qin Y, Qin S, Zhou X, Zhao W, *et al.* (2020) Circ_WBSCR17 aggravates inflammatory responses and fibrosis by targeting miR-185-5p/SOX6 regulatory axis in high glucose-induced human kidney tubular cells. *Life Sci* 259: 118269.

35. Bose M, Almas S, Prabhakar S (2017) Wnt signaling and podocyte dysfunction in diabetic nephropathy. *J Investig Med* 65: 1093–1101.

36. Guo Q, Zhong W, Duan A, Sun G, Cui W, *et al.* (2019) Protective or deleterious role of Wnt/beta-catenin signaling in diabetic nephropathy: an unresolved issue. *Pharmacol Res* 144: 151–157.

37. Chen L, Xie Y, Ma X, Zhang Y, Li X, *et al.* (2020) SOX6 represses tumor growth of clear cell renal cell carcinoma by HMG domain-dependent regulation of Wnt/β-catenin signaling. *Mol Carcinog* 59: 1159–1173.