Knockdown of plasmacytoma variant translocation 1 (PVT1) inhibits high glucose-induced proliferation and renal fibrosis in HRMCs by regulating miR-23b-3p/early growth response factor 1 (EGR1)

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Abstract. Long noncoding RNAs (lncRNAs) have been reported to play critical role in the development of diabetic nephropathy (DN). However, the effects and mechanism of plasmacytoma variant translocation 1 (PVT1) remain poorly understood. The expression of PVT1, miR-23b-3p, early growth response factor 1 (EGR1), Fibronectin (FN), Collagen IV (Col IV), alpha smooth muscle actin (α-SMA), E-cadherin, and vimentin, transforming growth factor (TGF)-β1 was examined by quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation was assessed by Cell Counting-8 (CCK-8) assay. Western blot assay was conducted to measure the protein levels of FN, Col IV, E-cadherin, α-SMA, vimentin, TGF-β1, and EGR1. The interaction between miR-23b-3p and PVT1 or EGR1 was predicted by starBase or TargetScan and confirmed by the dual luciferase reporter assay. The oxidative stress factors were analyzed by corresponding kits. We found that the expression of PVT1 and EGR1 was increased and miR-23b-3p was decreased in serum samples of DN patients and HG-induced HRMCs. Knockdown of PVT1 significantly inhibited HG-induced proliferation, extracellular matrix (ECM) accumulation, epithelial-mesenchymal transition (EMT), and oxidative stress in HRMCs, while these effects were abated by inhibiting miR-23b-3p. In addition, EGR1 was confirmed as downstream target of miR-23b-3p and miR-23b-3p could specially bind to PVT1. Besides, downregulation of PVT1 inhibited the progression of DN partially via upregulating miR-23b-3p and downregulating EGR1. In conclusion, our results suggested that PVT1 knockdown suppressed DN progression though functioning as ceRNA of miR-23b-3p to regulate EGR1 expression in vitro, providing potential value for the treatment of DN.

Key words: Diabetic nephropathy, Plasmacytoma variant translocation 1 (PVT1), MiR-23b-3p, Early growth response factor 1 (EGR1), Renal fibrosis

DIABETIC NEPHROPATHY (DN) is one of most frequent complications of diabetes mellitus (DM) and has become the primary cause of end-stage renal disease (ESRD) in the world [1]. DN is characterized by thickening of the basement membrane, renal tubal epithelial–mesenchymal transition (EMT), extracellular matrix (ECM) accumulation, glomerulosclerosis, and finally leading to kidney failure [2-5]. The DN pathogenesis remains poorly understood, and there is no particularly effective and specific strategy for treatment of DN. Hence, it is important to further explore its exact molecular mechanisms to develop potential effective therapies for DN.

Long noncoding RNAs (lncRNAs) have been shown to play critical regulatory roles in various diseases, including DN [6]. For example, lncRNA CYP4B1-PS1-001 repressed growth and fibrosis of mesangial cells and might be a potential therapeutic target for DN [7]. LncRNA taurine-upregulated gene 1 (TUG1) alleviated...
ECM accumulation by mediating miR-377 targeting of peroxisome proliferator-activated receptor γ (PPARγ) in DN [8]. Human placental amnion variant translocation 1 (PVT1), which maps to chromosome 8q24, is closely associated with various diseases [9, 10]. PVT1 has been shown to participate in DN progression and its expression was upregulated by glucose treatment in human mesangial cells [11]. However, the specific mechanisms of PVT1 in development and progression of DN need to be further clarified.

It is generally accepted that lncRNAs may function as competing endogenous RNAs (ceRNAs) via same microRNA (miRNA) response elements (MREs) for miRNA targets, thus blocking a one miRNA or some miRNAs from binding to their targets [12]. MiR-23b, located on chromosome number 9 in a cluster with miR-27b/24-1, has been suggested to be downregulated in DN [13]. Moreover, a previous study showed that PVT1/miR-23b-3p axis played a key roles in DN [14]. Therefore, we aimed to explore the downstream genes of PVT1/miR-23b-3p axis. Recently, it has been shown that early growth response factor 1 (EGR1) contributes to DN through elevating ECM production [15]. Interestingly, online bioinformatics database showed that PVT1 and EGR1 had complementary binding sequence for miR-23b-3p, which prompted us to assume the ceRNA network of PVT1/miR-23b-3p/EGR1 axis.

The purpose of this study was to determine whether and how they (PVT1, miR-23b-3p and EGR1) regulated renal fibrosis in DN. Moreover, we also explored the ceRNA regulatory network of PVT1/miR-23b-3p/EGR1 to better understand the molecular mechanism of DN.

Materials and Methods

Serum collection and RNA isolation

The blood samples were obtained from The First People’s Hospital of Lanzhou New District. All participants did not receive chemotherapy or radiotherapy. Human blood samples were obtained from ninety patients with were diagnosed with DN (stage I, III, IV and V) based on generally accepted clinical criteria. Control subjects were from ninety age- and sex-matched healthy volunteers. This research was approved by the ethics committee of The First People’s Hospital of Lanzhou New District, and written informed consent was acquired from all participants.

Blood samples were collected using a special tube containing separation gel and clot activator and then centrifuged at 3,000 rpm for 10 min at room temperature. The supernatant was transferred to a new tube, followed by centrifugation at 1,500 rpm for another 30 min at 4°C to remove cell debris. Next, the final supernatant was transferred to labeled EP tubes and stored at –80°C until RNA extraction. Serum total RNA including miRNAs was isolated using mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) and the serum total mRNA was extracted by using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA).

Cell culture and transfection

Human renal mesangial cells (HRMCs) were purchased from ScienCell (San Diego, CA, USA). HRMCs were grown in Dulbecco’s modified Eagle’s medium (DMEM; Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) in a moist atmosphere with 5% CO2 at 37°C.

Small interfering RNA (siRNA) against PVT1 (si-PVT1), si-EGR1, siRNA negative control (si-NC), miR-23b-3p mimics (miR-23b-3p), negative control (NC), PVT1 overexpression vector (PVT1), empty vector, miR-23b-3p (anti-23b-3p), and anti-NC were purchased from GenePharma (Jiangsu, China). HRMCs were transfected with oligonucleotides or vectors using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Following transfection, HRMCs were then exposed to high glucose (HG, 30 mM) or with normal glucose (NG, 5.5 mM).

Quantitative real-time polymerase chain reaction (qRT-PCR)

HRMCs were collected and the total RNA was extracted using the Trizol reagent (Invitrogen). The reverse transcription (RT) reaction of lncRNAs and mRNA was carried out using the PrimeScript RT Reagent Kit (TaKaRa, Osaka, Japan), and the RT reaction of miRNA was performed with the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA). HRMCs were collected on an ABI7700 Real Time PCR system (Applied Biosystems, Foster City, CA, USA). All primers were purchased from Sangon Biotech (Shanghai, China): PVT1 (forward, 5’-GTCTCCCTATGGAATGTAAG-3’; reverse, 5’-AGTGTCTTGCGATGAAAAGG-3’); Fibronectin (FN) (forward, 5’-CGGTGGTCTGTCATGCGTTC-3’); vimentin (forward, 5’-GAGAACTGCAATGCGTTC-3’); Collagen IV (Col IV) (forward, 5’-GCCATGTTCTATCGGGTACTTC-3’); transforming growth factor (TGF)-β1 (forward, 5’-CATCCATGACATGAAAACAG-3’; reverse, 5’-GCCATGTCTTATCCGATC-3’); alpha smooth muscle actin (α-SMA) (forward, 5’-AAAAAGACAGCTACGTGGGTGA-3’; reverse, 5’-ACAGAAGTTGGGCATGGTAGCCCTT-3’); E-cadherin (forward, 5’-TGCCCCAGAAATGGAAAAAGG-3’; reverse, 5’-GTTATGTTGCACCAGCTTAC-3’).
TTGCGGTGGAAAGC-3'; reverse, 5'-GCTTCCCTGTAGG TGCAATCC-3'; miR-23b-3p (forward, 5'-CGGGCATC ACATTGCAAGG-3'; reverse, 5'-CAGCCACAAAGA GCACAAT-3'); EGFR (forward, 5'-CTACGACCTTTCC AGTCTGTC-3'; reverse, 5'-TGCTGTCAATGTCATAAG ACCC-3'); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward, 5'-ACCC-3'; reverse, 5'-TGTTGCAGCAAAATCTTGTT-3'); U6 (forward, 5'-GCTTCCGAGCACATATACTAA AT-3'; reverse, 5'-CGCTTCAGAATTTGCGTGTC AT-3'). Relative expression of genes was evaluated with 2-ΔΔCt method and normalized to GAPDH (control for lncRNA and mRNA) or U6 small nuclear RNA (control for miRNA), respectively.

**Western blot assay**

After transfection or treatment, total protein was extracted from HRMCs using radio-immunoprecipitation assay (RIPA) lysis buffer (Invitrogen) containing phenyl-methanesulfonyl fluoride (PMSF; Beyotime, Shanghai, China). Protein concentration was evaluated using a bicinchoninic acid (BCA) protein assay kit (Tanon, Shanghai, China). Then, protein was electrophoresed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked by 5% nonfat milk, and then probed with antibodies against FN (1:500, ab6382, Abcam, Cambridge, UK), Col IV (1:1,000, ab6586, Abcam), TGF-β1 (1:500, ab9485, Abcam), E-cadherin (1:500, ab15148, Abcam), vimentin (1:500, ab137321, Abcam), α-SMA (1:500, ab5694, Abcam), EGR1 (1:500, ab216964, Abcam), and GAPDH (1:2,500, ab9485, Abcam) overnight at 4°C, followed by incubation with secondary antibodies. Finally, all protein bands were visualized using an enhanced chemiluminescence detection reagent (ECL; Tanon). Relative protein expression was calculated with ImageJ software and normalized to the GAPDH.

**Cell proliferation assay**

Cell Counting Kit-8 assay (CCK-8; Beyotime, Shanghai, China) was used to detect cell proliferation ability. Briefly, HRMCs were seeded into a 96-well plate. After transfection/treatment for 1 day, 2 days, 3 days, 4 days, and 5 days, respectively, CCK-8 solution (10 μL) was added to per well using a pipette tip. Absorbance of per well was measured using a microtiter plate reader (Thermo Fisher Scientific, Bonn, German) at 450 nm.

**Dual-luciferase reporter assay**

PVT1 or 3'UTR of EGR1 containing with putative or mutated binding site of miR-23b-3p was synthesized and then inserted into a pLG3 promoter vector (Promega, Madison WI, USA), named as PVT1 wt, PVT1 mut, EGR1 wt, or EGR1 mut. The reporter vectors with miRNA or miR-23b-3p were co-transfected into HRMCs using Lipofectamine 3000. Luciferase activity was measured after transfection for 48 h using a dual luciferase reporter assay system (Promega).

**Statistical analysis**

All data were obtained from at least three independent experiments and expressed as mean ± standard deviation (SD). The statistical analyses and graphing were performed using GraphPad Prism version 6.0 software (GraphPad Software, San Diego California, USA). Spearman’s correlation tests were applied to analyze the association among PVT1, miR-23b-3p and EGR1. Statistical comparisons were analyzed using the student’s t-test (for two groups) and a one-way analysis of variance (ANOVA; for more than two groups) followed by Turkey’s post-hoc test. P < 0.05 was considered statistically significant.

**Results**

**The level of PVT1 was increased in serum samples of DN patients**

To explore the potential role of PVT1 in DN, the level of PVT1 was determined by qRT-PCR in serum samples from patients with DN. Results showed that the level of PVT1 was increased in serum samples of DN patients (n = 90) compared with control group (n = 90) (Fig. 1A). Moreover, the level of PVT1 was higher in patient at IV/V clinical stages than those at I/III clinical stages (Fig. 1B). These results indicated that PVT1 might play crucial role in development of DN.

**Knockdown of PVT1 inhibited HG-induced proliferation, ECM accumulation, and EMT in HRMCs**

To study the effect of PVT1 on the HG-induced proliferation, ECM accumulation, and EMT, HRMCs were transfected with either si-PVT1 or si-NC and then treated with HG. QRT-PCR was used to confirm the success of transduction. As presented in Fig. 2A, the expression of PVT1 was increased after treatment with HG in HRMCs, while transfection of si-PVT1 reversed the effect. And the expression of PVT1 was also decreased in HRMCs transfected with si-PVT1 under NG conditions while transfection of PVT1 showed an opposite effect (Supplementary Fig. 1A and 1B), suggesting that the transfection of si-PVT1 or PVT1 was successful. CCK-8 assay demonstrated that the cell proliferation was increased at different time points in HG-induced HRMCs compared to...
those HRMCs treated with NG; however, the effect was abated by transfection of si-PVT1 (Fig. 2B). To test the effect of PVT1 on ECM accumulation and EMT progression in DN, the mRNA and protein levels of ECM (Col-IV, FN and TGF-β1) and EMT (E-cadherin, α-SMA and vimentin) markers were determined using qRT-PCR and western blotting assays, respectively. Results showed that the mRNA and protein expression of Col-IV, FN, TGF-β1, α-SMA and vimentin (mesenchymal markers) were elevated but E-cadherin (an epithelial marker) was reduced in HG-induced HRMCs compared with HRMCs cultured in NG group, which could be abolished by inhibiting PVT1 (Fig. 2C–2F). These findings suggested that inhibition of PVT1 suppressed HG-induced proliferation, ECM accumulation, and EMT in HRMCs.

**PVT1 directly targeted miR-23b-3p**

Recently, many lncRNA transcripts have been confirmed to function as competing ceRNA through competitively binding common microRNAs. We performed a bioinformatics analysis using starBase and found that PVT1 contained binding sites for miR-23b-3p (Fig. 3A). To further confirm whether miR-23b-3p was a target of PVT1, the PVT1 containing a wild-type or mutant binding sites of miR-23b-3p was cloned into luciferase reporter vector. The results indicated that overexpression of miR-23b-3p decreased the luciferase activity in HRMCs transfected with PVT1-wt, but did not suppressed the luciferase activity in PVT1-mut group (Fig. 3B). The expression of miR-23b-3p was measured in HRMCs transfected with si-NC, si-PVT1, PVT1, or vector. Results

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**Fig. 1**  Relative expression of PVT1 in serum samples with DN patients. (A and B) The expression of PVT1 was measured in serum samples with DN patients, healthy subjects, and clinical stages by qRT-PCR. **p < 0.01.

**Fig. 2**  Effects of PVT1 on cell proliferation, ECM accumulation, and EMT in HG-induced HRMCs. HRMCs were transfected with si-NC or si-PVT1 and then simulated with HG, and NG-treated HRMCs served as a control group. (A) PVT1 level was determined by qRT-PCR. (B) CCK-8 assay was conducted to assess proliferation. (C–F) The mRNA and protein levels of FN, Col IV, TGF-β1, E-cadherin, α-SMA and Collagen IV were detected by qRT-PCR and western blot, respectively. **p < 0.01.
showed that knockdown of PVT1 promoted the expression of miR-23b-3p, while its overexpression inhibited the expression of miR-23b-3p in HRMCs (Fig. 3C). In addition, qRT-PCR analysis indicated that the expression of miR-23b-3p was lower in serum samples of DN patients than control group (Fig. 3D). Moreover, we also found an obvious negative correlation between PVT1 and miR-23b-3p expression \((p < 0.001, r = -0.6295)\) (Fig. 3E). Next, we detected the level of miR-23b-3p was decreased in HG-stimulated HRMCs. The results suggested that the expression of miR-23b-3p was decreased in HG-stimulated HRMCs compared with control cells (Fig. 3F). Thus, our data demonstrated that miR-23b-3p was a target of PVT1 and was negatively regulated by PVT1.

**Inhibition of miR-23b-3p partially blocked the effects of PVT1 downregulation on HG-mediated proliferation, ECM accumulation, and EMT in HRMCs**

To explore the effects of miR-23b-3p on HG-induced proliferation, ECM accumulation and EMT, HRMCs were transfected with either si-NC or si-PVT1 and then treated with HG. We found that miR-23b-3p overexpression could weaken HG-proliferation, ECM accumulation, and EMT in HRMCs (Supplementary Fig. 2A–2E). To demonstrate whether PVT1 directly targeted miR-23b-3p to repress HG-induced proliferation, ECM accumulation, and EMT in HRMCs, we transfected with si-NC, si-PVT1, anti-miR-NC, or si-PVT1 + anti-miR-23b-3p into HRMCs and stimulated with HG. Results revealed that inhibition of miR-23b-3p reversed the si-PVT1-mediated promotion of miR-23b-3p expression in HG-induced HRMCs (Fig. 4A). Besides, we found the transfection of anti-miR-23b-3p decreased the expression of miR-23b-3p and transfection of miR-23b-3p mimic presented an opposite effect (Supplementary Fig. 1C and 1D), implying the successful introduction of anti-miR-23b-3p or miR-23b-3p mimic into HRMCs under NG conditions. Moreover, deficiency of miR-23b-3p could neutralize the inhibitory effect of PVT1 knockdown on proliferation in HG-induced HRMCs (Fig. 4B). Furthermore, inhibition of miR-23b-3p abolished the effect of PVT1 interference on downregulation of mRNA and protein levels of Col-IV, FN, TGF-β1, α-SMA and vimentin and upregulation of E-cadherin expression in HG-stimulated HRMCs (Fig. 4C–4F). Altogether, these data indicated that the effects of PVT1 on HG-induced proliferation, ECM accumulation, and EMT were regulated by miR-23b-3p.

**EGR1 was a direct target of miR-23b-3p**

To further explore the underlying mechanism by which miR-23b-3p exerted its functional effect, Target Scan was used to search the functional target of miR-23b-3p. As shown in Fig. 5A, EGR1 might be a potential target of miR-23b-3p. To verify this hypothesis,
ypothesis, EGR1 wt or EGR1 mut and miR-23b-3p or miR-NC were co-transfected into HRMCs. Results showed that overexpression of miR-23b-3p repressed the luciferase activity of wild type 3’UTR of EGR1 rather than the mutant group (Fig. 5B). Next, the effect of miR-23b-3p on EGR1 expression was investigated in HRMCs. Upregulation of miR-23b-3p reduced EGR1 mRNA and protein expression and its knockdown presented an opposite effect (Fig. 5C and 5D). The mRNA expression of EGR1 was expressed at a high level in serum samples of DN patients (Fig. 5E). Moreover, miR-23b-3p level was inversely correlated with the level of EGR1 in serum samples with DN (p < 0.001, r = –0.6711) (Fig. 5F). Additionally, the mRNA level and protein expression of EGR1 were increased in HRMCs treated with HG compared with NG (Fig. 5G and 5H). Collectively, these data demonstrated that EGR1 was a downstream target of miR-23b-3p and negatively correlated to miR-23b-3p.

Knockdown of PVT1 inhibited progression of DN by regulating miR-23b-3p and EGR1

It has been demonstrated that miR-23b-3p was negatively regulated by PVT1, we investigated whether PVT1 could act as a ceRNA of miR-23b-3p to affect EGR1 expression and explored their relationships in HG-induced HRMCs. The protein expression of EGR1 was reduced in HRMCs transfected with si-EGR1 compared to si-NC group under NG conditions (Supplementary Fig. 1E), indicating that transfection of si-EGR1 was successful. QRT-PCR and western blot analysis revealed that knockdown of miR-23b-3p elevated EGR1 mRNA and protein expression, whereas it was ablated by inhibition of EGR1 in HRMCs transfected with si-PVT1 and treat with HG (Fig. 6A and 6B). In addition, downregulation of EGR1 alleviated the promoting effect of miR-23b-3p knockdown on proliferation in HRMCs transfected with si-PVT1 and stimulated with HG (Fig. 6C). Moreover, silencing EGR1 reversed the promotive effects of miR-23b-3p knockdown on ECM accumulation and EMT (Fig. 6D-6F). Therefore, our results suggested that knockdown of PVT1 suppressed HG-induced proliferation, ECM accumulation, and EMT through upregulating miR-23b-3p and downregulating EGR1.

Knockdown of PVT1 inhibited HG-induced oxidative stress by upregulating miR-23b-3p in HRMCs

Oxidative stress is one of features of DN. Next, we explored the effect of PVT1 on oxidative stress in HG-induced HRMCs. We found that HG treatment increased oxidative stress by increasing reactive oxygen species (ROS) and malondialdehyde (MDA) levels, and...
decreasing superoxide dismutase (SOD) activity (Supplementary Fig. 3A–3C), which were partially abated by downregulating PVT1 (Supplementary Fig. 3D–3F). In addition, interference of miR-23b-3p reversed the suppressive impact of PVT1 downregulation on oxidative stress (Supplementary Fig. 3D–3F). These results indicated that PVT1 played a critical role in oxidative stress in HG-induced HRMCs.

Discussion

DN is a common diabetic complication and chronic progressive kidney disease [16]. Kidney fibrosis is considered to be the main pathologic change in DN, and there is no specific treatment to reverse fibrosis in DN. EMT increases ECM synthesis, and ECM are the main characteristic of kidney fibrosis [17, 18]. HG, oxidative stress and TGF-β1 are common mediators of diabetic kidney damage and fibrosis [19, 20]. Recent studies have shown that lncRNAs are closely related to various chronic diseases, including diabetic complications [6, 21]. Therefore, we used the HG-stimulated HRMCs to mimic DN and explore the potential role of PVT1 in DN.

Increasing amount of evidence suggested that PVT1 was usually dysregulated in many cancers [22, 23]. In addition, PVT1 has been confirmed to be tightly associated with ESRD attributed to type 1/2 diabetes [24, 25]. Besides, previous study showed that human mesangial cells treated with glucose could increase PVT1 abundance and might regulate the development and progression of DN via involving ECM accumulation [11]. Our study also demonstrated that level of PVT1 was increased in serum samples of DN patients and HG-induced HRMCs. FN and Col-IV are crucial components of ECM and considered to be key factors in myofibroblast differentiation. Besides, TGF-β is a crucial mediator of ECM accumulation and EMT [26, 27]. EMT is characterized by decreased epithelial surface markers (such as E-cadherin) and induced mesenchymal markers (such as vimentin and α-SMA), resulting in the
formation of more motile fibroblast-like cells [28]. In this study, we also proved that PVT1 downregulation weakened HG-induced proliferation, ECM accumulation (Col-IV, FN and TGF-β1 expression upregulated), EMT (α-SMA and vimentin expression elevated, E-cadherin level reduced), and oxidative stress in HRMCs, suggesting that it is a critical regulatory event in ECM accumulation and EMT changes.

Multiple lncRNAs have been indicated to act as miRNA sponges and modulate their biological functions [29]. To explore the regulatory mechanism of PVT1 on the progression of DN, we used bioinformatics analysis and further experimental validation to confirm that PVT1 directly targeted miR-23b-3p. More importantly, a previous also demonstrated that miR-23b-3p was a direct of PVT1, and PVT1 knockdown alleviated HG-induced proliferation and fibrosis in human mesangial cells by upregulating miR-23b-3p/WT1 axis [30]; however, whether PVT1/miR-23b-3p axis has other downstream genes and its role in HG-induced oxidative stress are unclear. Moreover, previous reports have demonstrated that miR-23b-3p was a critical regulator in cellular possess, such as cell growth, EMT, migration, and invasion [30, 31]. Besides, it has been demonstrated that miR-23b-3p expression was reduced in kidney cells exposed to HG and its overexpression attenuated kidney...
fibrosis [32]. In the present research, results displayed that miR-23b-3p expression was downregulated in serum samples of DN patients and HG-induced HRMCs, which was consistent with previous studies. Besides, inhibition of miR-23b-3p partially blocked the effects of PVT1 downregulation on proliferation, ECM accumulation, EMT, and oxidative stress in HRMCs exposed to HG. To study how miR-23b-3p affected progression of DN, potential target was predicted by TargetScan. It represented that there were potential binding sites of EGR1 in miR-23b-3p. Next, we probed that EGR1 was a downstream target of miR-23b-3p.

A number of evidence has proven that EGR1 was involved in progression of DN. For example, EGR1 has been shown to be responsible for activating the heparanase promoter under diabetic conditions [33]. Besides, the expression of EGR1 was increased in HG-induced HK2 cells and miR-192 could target EGR1 to delay tubular interstitial fibrosis [34]. Here, we demonstrated that EGR1 expression was enhanced in serum samples of DN patients and HRMCs treated with HG. Further experiments indicated that EGR1 expression was negatively correlated with the miR-23b-3p level. Moreover, we further proved that inhibition of PVT1 suppressed HG-induced proliferation, ECM accumulation, and EMT by regulating miR-23b-3p and EGR1 expression. In our study, we found that high glucose could promote mesangial cell proliferation and oxidative stress, which was in line with previous study [35]. The relationship between oxidative stress and cell proliferation under high glucose conditions will be the focus of our future research. Collectively, our data disclosed that PVT1 functioned as a sponge of miR-23b-3p to facilitate EGR1 expression, and thereby promoting proliferation and fibrosis in HG-induced HRMCs (Fig. 7).

In summary, results showed that PVT1 and EGR1 were increased and miR-23b-3p was decreased in serum samples of DN patients and HG-induced HRMCs. Moreover, knockdown of PVT1 could reverse the HG-induced proliferation, ECM accumulation, EMT, and oxidative stress in HRMCs. Furthermore, PVT1 could bind to miR-23b-3p to modulate EGR1 expression. Besides, downregulation of PVT1 inhibited progression of DN by upregulating miR-23b-3p and downregulating EGR1. Collectively, PVT1 knockdown blocked progression of DN partially via regulating miR-23b-3p/EGR1 axis, providing a new insight for understanding the functions of PVT1 in development and progression of DN.

**Acknowledgement**

Not applicable

**Highlights**

1: Knockdown of PVT1 inhibited HG-induced proliferation, ECM accumulation, EMT, and oxidative stress in HRMCs
2: Downregulation of miR-23b-3p partially reversed the effects of PVT1 knockdown on proliferation, ECM accumulation, EMT, and oxidative stress in HG-induced HRMCs3.
3: PVT1 knockdown blocked progression of DN through functioning as a ceRNA of miR-23b-3p to regulate EGR1 expression.

**Disclosure of Interest**

The authors declare that they have no financial conflict of interest.
**Authors’ Contribution**

Conceptualization and Methodology: Xiaohong Yang and Yong Zhu; Formal analysis and Data curation: Fenyan Xu, Hong Zhang and Zhiqiang Qiu; Validation and Investigation: Dongmei Yu, Xiaohong Yang and Hong Zhang; Writing-original draft preparation and Writing-review and editing: Dongmei Yu, Xiaohong Yang and Yong Zhu; Approval of final manuscript: all author

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**Data Availability**

All the data used and analyzed during the study are available from the corresponding author on the request.

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