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PCSK9/LOX-1 is associated with T2DM and regulates high glucose-induced lipid metabolism dysfunction in human microvascular endothelial cells

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Abstract

Introduction: The proprotein convertase subtilisin/kexin type 9/lectin-like oxidized low-density lipoprotein receptor-1 (PCSK9/LOX-1) axis plays a crucial role in regulating vascular endothelial cell function, but its specific involvement in type 2 diabetes mellitus (T2DM) remains unclear. This study aims to explore the potential mechanism of the PCSK9/LOX-1 axis in high-glucose (HG)-induced vascular endothelial cell dysfunction.

Material and methods: Peripheral blood samples were collected from T2DM patients to analyse the correlation between PCSK9 and blood lipid levels. Human microvascular endothelial cells (HMEC-1) exposed to high glucose concentration were used as a model of diabetic angiopathy (DA). Levels of PCSK9, reactive oxygen species (ROS), malonodialdehyde (MDA), interleukins (IL): IL-6, IL-1β, superoxide dismutase (SOD), and tumour necrosis factor alpha (TNF-α) were determined by enzyme-linked immunosorbent assay (ELISA) and biochemical methods. Additionally, intracellular total cholesterol (TC) and cholesterol ester (CE) levels were detected using enzyme chemistry. Expression of PCSK9 and LOX-1 was assessed through real-time quantitative polymerase chain reaction (RT-qPCR) and western blotting.

Results: Compared to the normal group, PCSK9 levels were significantly elevated in T2DM patients. Furthermore, PCSK9 levels were found to be positively correlated with body mass index (BMI), waistline, triglyceride (TG), cholesterol, low-density lipoprotein cholesterol (LDL-C), glycated hemoglobin (HbA_{1c}), and intracardiac fat pad levels in T2DM patients. HG exposure led to reduced activity of HMEC-1 cells, along with increased levels of apoptosis, oxidative stress, and inflammation, all of which were counteracted by si-PCSK9. The inhibitory effects of si-PCSK9 on LOX-1 expression, as well as TC and CE contents in HMEC-1 cells induced by HG, were observed. Moreover, intervention with oe-LOX-1 reversed the effects of si-PCSK9 in HG-induced HMEC-1 cells.

Conclusion: Silencing of PCSK9 inhibited HG-induced inflammation, oxidative stress, and lipid metabolic dysfunction in HMEC-1 cells via LOX-1. **(Endokrynol Pol 2025; 76 (1): 116–123)**

Key words: *T2DM; diabetic angiopathy; PCSK9; lipid metabolism; HMEC-1 cells*

Introduction

Diabetes is recognised as a significant global health challenge and a key modifiable risk factor for cardiovascular diseases (CVD) [1]. Type 2 diabetes mellitus (T2DM) is characterised by persistent high blood sugar levels and various molecular, biochemical, and cellular imbalances [2, 3]. The development of T2DM involves multiple factors, affecting the insulin system, α/β cells, specific regions of the kidney and brain, adipose tissue, and liver [4, 5]. Various diabetes-related factors, including metabolic disturbances and oxidative stress, contribute to the buildup of lipids and inflammatory cytokines, leading to impaired endothelial function and vascular dysfunction [6]. Therefore, investigating the pathogenesis of diabetes-related vascular diseases may pave the way for the discovery of innovative treatment approaches.

Chronic vascular complications are the primary causes of disability and mortality in diabetic patients with endothelial dysfunction [7]. Disturbances in lipid metabolism can lead to diabetic angiopathy (DA) [6]. Glucose can increase the expression of hemagglutinin-like oxidised low-density lipoprotein (ox-LDL) receptor 1 (LOX-1) in endothelial cells [8]. LOX-1, a key receptor for ox-LDL, plays a critical role in vascular endothelial dysfunction, foam cell formation, and the stability of atherosclerotic plaques [9]. LOX-1 is considered a major endothelial receptor for ox-LDL and is believed to have pro-atherogenic effects [10, 11]. Therefore, understanding the role of LOX-1 in lipid metabolism dysfunction in vascular endothelial cells is essential.

Elevated levels of proprotein convertase subtilisin/kexin type 9 (PCSK9) have been associated with an increased risk of developing new T2DM and are linked to blood glucose parameters that can potentially

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predict adverse cardiovascular events in individuals with diabetes and coronary artery disease [12]. PCSK9 plays a pivotal role in cholesterol metabolism by acting on LDL receptors in the liver, subsequently leading to a notable increase in circulating LDL cholesterol levels [13]. Notably, loss of LOX-1 has been shown to mitigate endothelial cell apoptosis induced by oxidised LDL through the downregulation of HRD1 [14]. Additionally, PCSK9 is known to promote LOX-1 expression and activate LOX-1 [15]. Heightened apoptosis of vascular endothelial cells can trigger the release of pro-inflammatory cytokines, fuel inflammatory responses, and exacerbate vascular damage [16]. Building on these findings, we hypothesised that the PCSK9/LOX-1 axis may be implicated in diabetes-related vascular complications. Thus, our study delves into the impact of PCSK9/LOX-1 on HG-induced lipid metabolism dysfunction in human microvascular endothelial cells, aiming to establish a novel theoretical framework for the management of the disease.

Material and methods

Clinical subjects

A total of 17 healthy subjects (Normal) and 39 patients diagnosed with T2DM were hospitalised in the Department of Metabolic Endocrinology. All patients were clinically diagnosed according to the World Health Organization (WHO) (1999) Diabetes Diagnostic Standards [17]. The patients' medical records from the past and current hospitalisation were reviewed to gather information on age, medical history, and previous test results. Patients with acute complications of diabetes, malignant tumours, abnormal liver and kidney function, thyroid dysfunction, and other diseases were excluded from the study. Baseline data including age, sex, body mass index (BMI), waistline, triglycerides (TG), cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), and intracardiac fat pad measurements were recorded. Peripheral blood serum samples were collected to measure PCSK9 levels. All subjects provided informed consent before participating in the study.

Construction of DA cell models and grouping

Human microvascular endothelial cells (HMEC-1, AW-CNH224, Abiowell, China) were provided by Abiowell and cultured by the medium (AW-MC013, Abiowell, China). Cells treated with normal glucose (NG; 5.5 mM, 48 h) as NG. Cells treated with high glucose (HG; 25 mM, 48 h) as the DA models *in vitro* [15]. HMEC-1 cells were randomly divided into NG, HG, HG+si-NC, HG+si-PCSK9, HG+si-PCSK9+oe-NC, and HG+si-PCSK9+oe-LOX-1 groups. NC means negative control, si means oligonucleotides were used for silencing, and oe means oligonucleotides were used for overexpression. To further investigate the biological function of LOX-1, we performed oe-LOX-1 intervention in the NG group, with cells divided into NG, NG+oe-NC, and NG+oe-LOX-1 groups. Based on the HG group, HMEC-1 cells were transfected with si-NC, si-PCSK9 (NM_174936.4, HG-HS174936, HonorGene, China), or oe-NC, oe-LOX-1 (NM_002543.4, HG-HO002543, HonorGene, China) via LIP2000 (11668019, Invitrogen, USA) for 6 h, respectively. The gene sequence for transfection is shown in Table 1.

Intracellular cholesterol measurement

The cells were collected and ultrasonically disrupted to release their contents. Intracellular total cholesterol (TC) and free cholesterol (FC) levels were determined using enzymatic assays as described in reference [18]. The concentrations of TC and FC were calculated based on standard curves generated with 312.5, 625, 1250, and 2500 μmol/L standard cholesterol, and then expressed as μmol/g protein according to reference [19]. Cholesterol ester (CE) levels were calculated by subtracting the FC content from TC.

Table 1. *Gene sequence for transfection*

Gene ID	Sequence	Trademark
Si-NC	5′ - 3′ : CAG UAC UUU UGU GUA GUA CAA	Honorgene
Si-PCSK9	5′ -3′ : UCAUUGAUGACAUCUUUGGCA 5′ -3′ : CCAAAGAUGUCAUCAAUGAGG	Honorgene
Oe-NC	No-live load	Honorgene
Oe-LOX-1	ATGACTTTTGATGAC CTAAAGATCC AGACTGTGAA GGACCAGCCT GATGAGAAGT CAAATGGAAA AAAAGCTAAA GGTCTTCAGT TTCTTTACTC TCCATGGTGG TGCCTGGCTG CTGCGACTCT AGGGGTCCTT TGCCTGGGAT TAGTAGTGAC CATTATGGTG CTGGGCATGC AATTATCCCA GGTGTCTGAC CTCCTAACAC AAGAGCAAGC AAACCTAACT CACCAGAAAA AGAAACTGGA GGGACAGATC TCAGCCCGGC AACAAGCAGA AGAAGCTTCA CAGGAGTCAG AAAACGAACT CAAGGAAATG ATAGAAACCC TTGCTCGGAA GCTGAATGAG AAATCCAAAG AGCAAATGGA ACTTCACCAC CAGAATCTGA ATCTCCAAGA AACACTGAAG AGAGTAGCAA ATTGTTCAGC TCCTTGTCCG CAAGACTGGA TCTGGCATGG AGAAAACTGT TACCTATTTT CCTCGGGCTC ATTTAACTGG GAAAAGAGCC AAGAGAAGTG CTTGTCTTTG GATGCCAAGT TGCTGAAAAT TAATAGCACA GCTGATCTGG ACTTCATCCA GCAAGCAATT TCCTATTCCA GTTTTCCATT CTGGATGGGG CTGTCTCGGA GGAACCCCAG CTACCCATGG CTCTGGGAGG ACGGTTCTCC TTTGATGCCC CACTTATTTA GAGTCCGAGG CGCTGTCTCC CAGACATACC CTTCAGGTAC CTGTGCATAT ATACAACGAG GAGCTGTTTA TGCGGAAAAC TGCATTTTAG CTGCCTTCAG TATATGTCAG AAGAAGGCAA ACCTAAGAGC ACAGTGA	Honorgene

The measurement of cell lipid peroxide levels was performed using a cell lipid peroxide assay kit purchased from Genmed Scientifics, Inc. (Shanghai, China).

Cell counting kit (CCK-8)

The cells (5×10^3 cells/well) were inoculated in 96-well plates with 100 μL per well. Cell counting kit (CCK8) (10 μL/well, Abiowell, China) solution was added. The cells were incubated (4 h) at 37°C with 5% CO_2. The absorbance values were analysed at 450 nm with an enzyme label (MB-530, HEALES, China) to evaluate cell viability.

Apoptosis

The above treated cells were digested and collected with EDTA-free pancreatic enzyme (AWC0232, Abiowell, China). The cells were washed with phosphate buffered saline (PBS), and centrifuged at 2000 rpm for 5 min, to collect about 3.2×10^5 cells. Binding buffer (500 μL) was added to suspend the cells. The cells were incubated with 5 μL allophycocyanin (APC) and 5 μL propidium iodide (KGA1030, KeyGEN Bio-TECH, China). Subsequently, the apoptosis rate (APC positive ratio + propidium iodide positive ratio) was detected by flow cytometry.

Enzyme-linked immunosorbent assay (ELISA) and biochemical detection

Supernatant from the above treated cells was taken after centrifugation. According to the manufacturer's instructions, PCSK9 (ab209884, abcam, UK), interleukin 1beta (IL-1β) (CBIC-E08053h, CUSABIO, China), interleukin 6 (IL-6) (CBIC-E04638h, CUSABIO, China), tumor necrosis factor alpha (TNF-α) (CBIC-E04740h, CUSABIO, China), malonaldehyde (MDA) (A003-1-2, NJJCBI, China), reactive oxygen species (ROS) (E004-1-1, NJJCBI, China), superoxide dismutase (SOD) (A001-3-2, NJJCBI, China), and TC (A111-1-1, NJJCBI, China) kits were applied to detect inflammation and oxidative stress factors in supernatant or peripheral blood serum on an enzyme-labeled apparatus (MB-530, HEALES, China).

RT-qPCR

Total RNA was extracted from cells by Trizol (15596026, Thermo, USA). The cDNA was synthesised by mRNA (CW2569, CWBIO, China) reverse transcription kit. The primer sequence was as follows: PCSK9: F, 5 '-GGACTCCTCTGTCTTTGCCC-3', R, 5 '-GCCATGACTGTCACTTGC-3'; LOX-1: F, 5 '-AGCAAATTGTTCAGGACTTCATCC-3', R, 5 '-CGCATAAACAGCTCCTCGTTG-3'; β-actin: F, 5 '-ACCCTGAAGTACCCCATCGAG 3', R, 5 '-AGCACAGCCTGGATAGCAAC-3'. The amplification reaction of genes was detected by UltraSYBR Mixture kit (CW2601, CWBIO, China) and fluorescence quantitative PCP instrument (PIKOREAL96, Thermo, USA). The reaction conditions were as follows: 95°C for 10 min, 95°C for 15 s, and 60°C for 30 s with 40 cycles. The expression of target gene was calculated by the $2^{-\Delta\Delta Ct}$ method.

Western blot

After the experiment, cell samples were collected and protein was extracted using radioimmunoprecipitation analysis lysis solution (AWB0136, Abiowell, China). The protein concentration was determined with bicinchoninic acid (BCA) assay. Subsequently, the protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane that was pre-activated with methanol and sealed with 5% buttermilk. The membrane was then incubated with antibodies at 4°C, including anti-PCSK9 (ab31762, 1 μg/mL, Abcam, UK), anti-LOX-1 (ab214427, 1:1000, Abcam, UK), and anti-β-actin (66009-1-Ig, 1:5000, Proteintech, USA). The separate membranes were used for target proteins and beta actin detection. Although the target protein and the internal reference protein were detected on separate membranes, they both originated from the same sample. Subsequently, the membrane was incubated with secondary anti-IgG antibodies (SA00001-1/2, 1:5000/6000, Proteintech, USA) at 37°C for 90 minutes. Finally, the membrane was incubated with ECL chemiluminescence solution (AWB0005, Abiowell, China) for 1 min. The liquid absorbed with filter paper, and the hybrid membrane was wrapped with plastic sealing film. The images were analysed with software (ChemiScope6100, CLiNX, China).

Data analysis

The data in this study were analysed using GraphPad Prism 8.0 statistical software. Measurement data were expressed as mean ± standard deviation. Firstly, normality and homogeneity of variance tests were conducted. Non-paired t-tests were used for comparisons between groups. One-way ANOVA or repeated measures analysis of variance were applied for comparisons among multiple groups. Tukey's post hoc test was conducted. Pearson correlation analysis was used to assess the relationship between PCSK9 and other variables. A p-value of less than 0.05 was considered statistically significant.

Results

Fluctuation of PCSK9 level in patients with T2DM

The clinical baseline information of patients with T2DM is presented in Table 2. Compared to the normal group, the PCSK9 level was significantly elevated in T2DM patients (Fig. 1A). Furthermore, significant differences were observed between the normal and T2DM groups in terms of age and intracardiac fat pad measurements (Tab. 2). Pearson coefficient analysis revealed that there was no correlation between PCSK9 levels and the sex or age of the patients (Fig. 1B). These findings suggest that the PCSK9 levels in patients with T2DM were not influenced by sex or age.

Correlation between PCSK9 and lipid parameters in patients with T2DM

The PCSK9 level showed positive associations with BMI, waistline, TG, CHOL, LDL-C, HBA_{1c}, and intracardiac fat pad, as illustrated in Figure 2. These findings indicate that higher levels of PCSK9 detected in patients were correlated with increased BMI, waistline, TG, CHOL, LDL-C, HBALC, and intracardiac fat pad.

Relationship between PCSK9 and carotid artery disease in T2DM patients

No significant difference in PCSK9 levels was observed between the group without carotid artery disease and the group with carotid artery disease (Fig. 3). This result indicates that PCSK9 levels were independent of carotid artery disease in patients with T2DM.

Silencing PCSK9 inhibited oxidative stress, inflammation, and apoptosis in DA cell models

An *in vitro* DA cell model was established using HMEC-1 cells induced by HG, leading to a clear increase in

Table 2. *Baseline characteristics of participants*

Items	Normal	T2DM	p-value
Participants (n)	17	39	
Age [years]	51.18 ± 10.28	61.59 ± 10.71	0.0013
Female (n)	6	17	/
Male (n)	11	22	/
BMI [kg/m²]	25.61 ± 5.22	25.22 ± 2.91	0.7216
Waistline [cm]	88.59 ± 6.55	89.75 ± 8.25	0.6103
TG [mmol/L]	2.71 ± 1.01	2.35 ± 1.74	0.4306
CHOL [mmol/L]	4.67 ± 1.14	4.78 ± 1.07	0.7300
LDL-C [mmol/L]	3.03 ± 1.03	2.82 ± 0.97	0.4678
Intracardiac fat pad [cm]	6.52 ± 2.71	9.94 ± 3.50	0.0007
Carotid artery disease (n)	10	29	/
Without carotid artery disease (n)	7	10	/
PCSK9 [ng/mL]	132.04 ± 84.15	2436.68 ± 809.47	< 0.0001

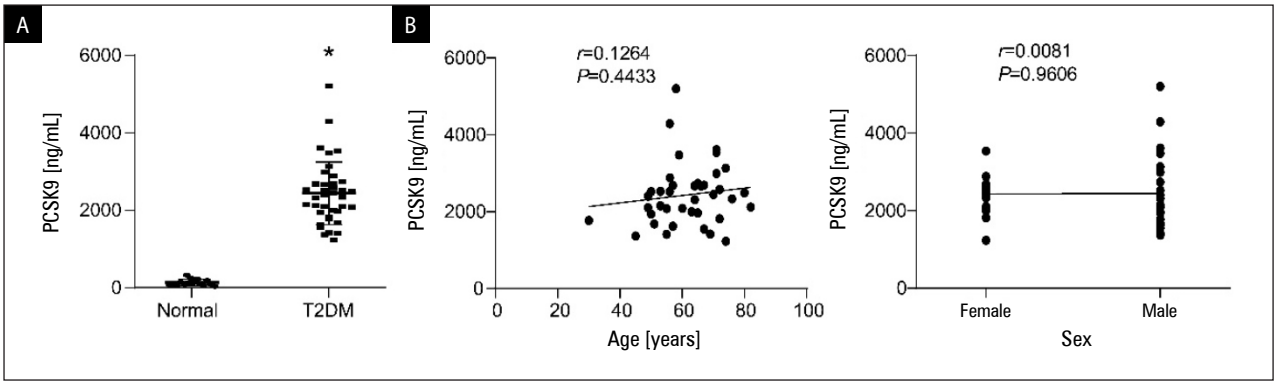

Figure 1. *Proprotein convertase subtilisin/kexin type 9 (PCSK9) level was independent of sex and age in patients with type 2 diabetes mellitus (T2DM). *p < 0.05 vs. normal group. N = 17 in Normal group and N = 39 in T2DM group*

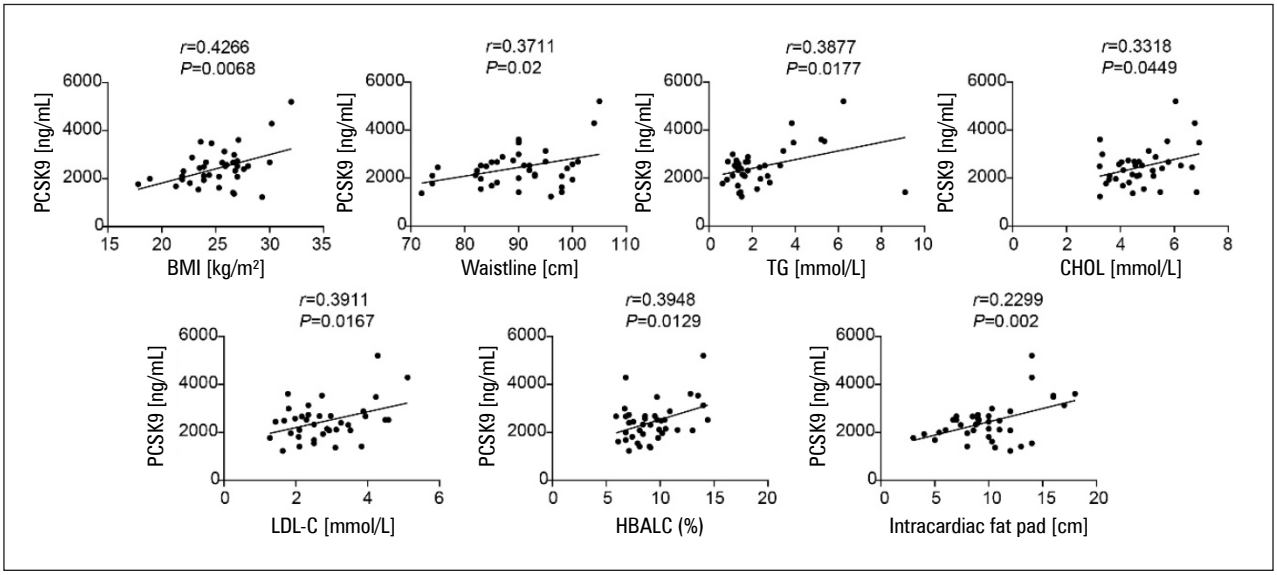

Figure 2. *Association of proprotein convertase subtilisin/kexin type 9 (PCSK9) levels with body mass index (BMI), waistline, triglycerides (TG), cholesterol (CHOL), low-density lipoprotein (LDL-C), HBA_{1c}, and intracardiac fat pad. N = 17 in Normal group and N = 39 in T2DM group*

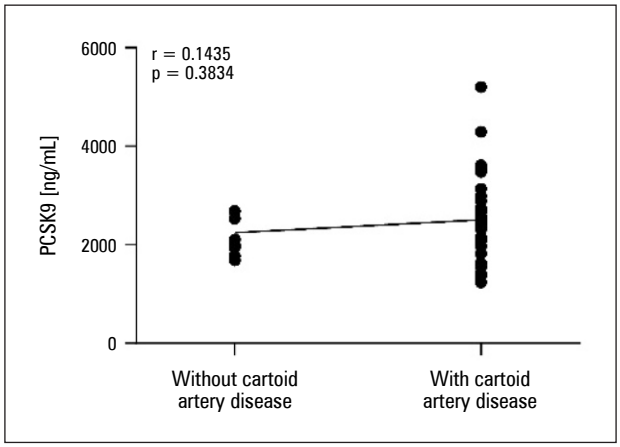

Figure 3. *Comparison of proprotein convertase subtilisin/kexin type 9 (PCSK9) levels in type 2 diabetes mellitus (T2DM) patients with or without carotid artery disease. N = 17 in Normal group and N = 39 in T2DM group*

the level of PCSK9 (Fig. 4AB). Furthermore, transfection with si-PCSK9 resulted in the inhibition of PCSK9 expression in the HG group (Fig. 4AB). HG exposure induced a decrease in HMEC-1 cell activity and an increase in apoptosis levels, which were both attenuated by si-PCSK9 (Fig. 4CD). Additionally, HG triggered an elevation in oxidative stress and inflammation in HMEC-1 cells, as shown by increased levels of ROS, MDA, IL-6, IL-1β, and TNF-α, along with a decrease in SOD levels (Fig. 4EF). Importantly, si-PCSK9 transfection was found to down-regulate oxidative stress and inflammation in HMEC-1 cells induced by HG (Fig. 4EF). Taken together, these results indicate that silencing PCSK9 effectively suppressed inflammation, oxidative stress, and apoptosis in the DA cell model.

PCSK9 regulated oxidative stress, inflammation, apoptosis, and lipid metabolism disorders in DA cell models through LOX-1

Further analysis of lipid metabolism revealed that HG induced an increase in LOX-1 expression in HMEC-1 cells, which was inhibited by si-PCSK9 (Fig. 5A). Moreover, HG led to elevated levels of TC and CE in HCMEC-1 cells (Fig. 5B). Silencing PCSK9 resulted in decreased TC and CE contents in HMEC-1

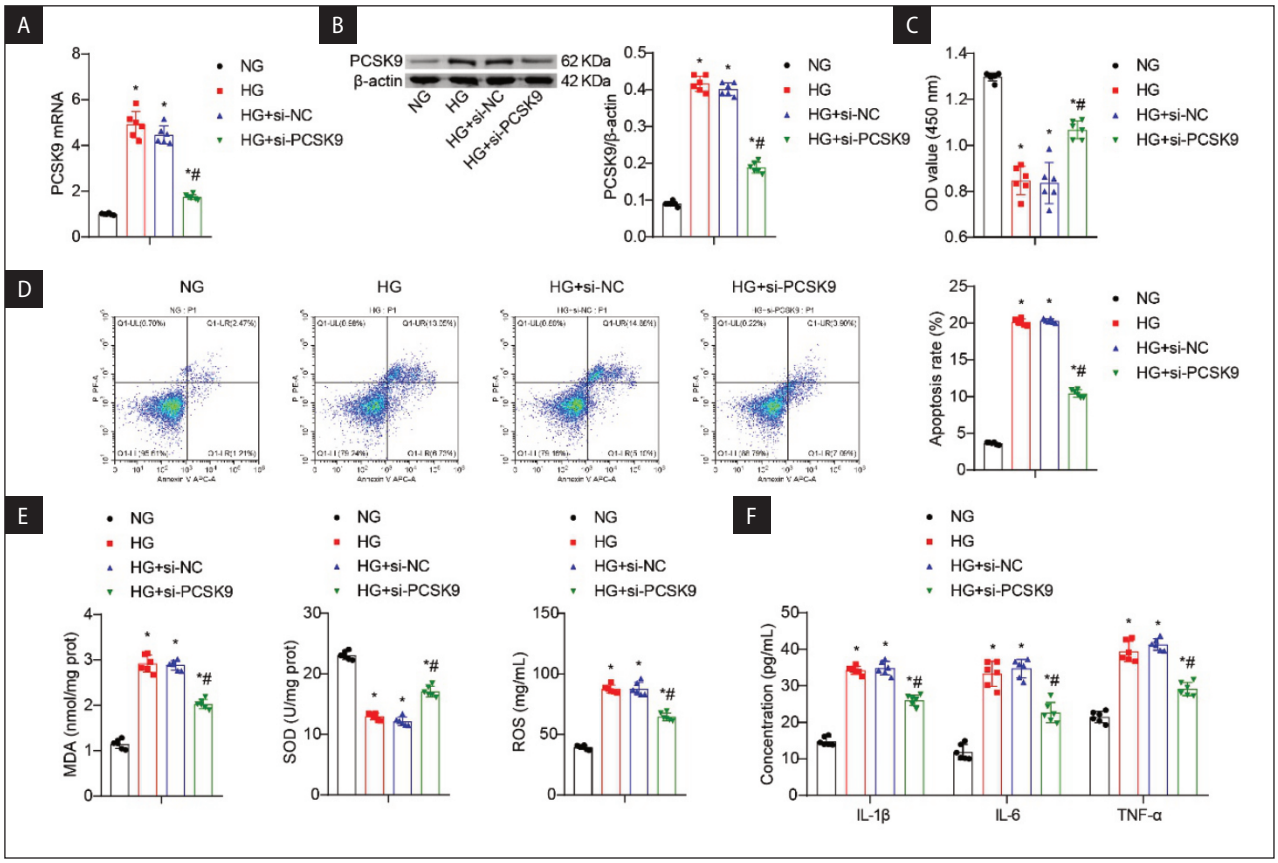

Figure 4. *Silencing proprotein convertase subtilisin/kexin type 9 (PCSK9) affected oxidative stress, inflammation, and apoptosis of diabetic angiopathy (DA) model cells.* **AB.** *PCSK9 levels were detected by RT-qPCR or western blot.* **C.** *Cell counting kit 8 (CCK-8) was applied to measure cell viability;* **D.** *Apoptosis.* **EF.** *Reactive oxygen species (ROS), malonaldehyde (MDA), superoxide dismutase (SOD), interleukin 6 (IL-6), interleukin 1beta (IL-1β), and tumor necrosis factor alpha (TNF-α) levels were determined by biochemical and enzyme-linked immunosorbent assay (ELISA) methods. *p < 0.05 vs. NG, #p < 0.05 vs. HG + si-NC. N = 6 repetitions*

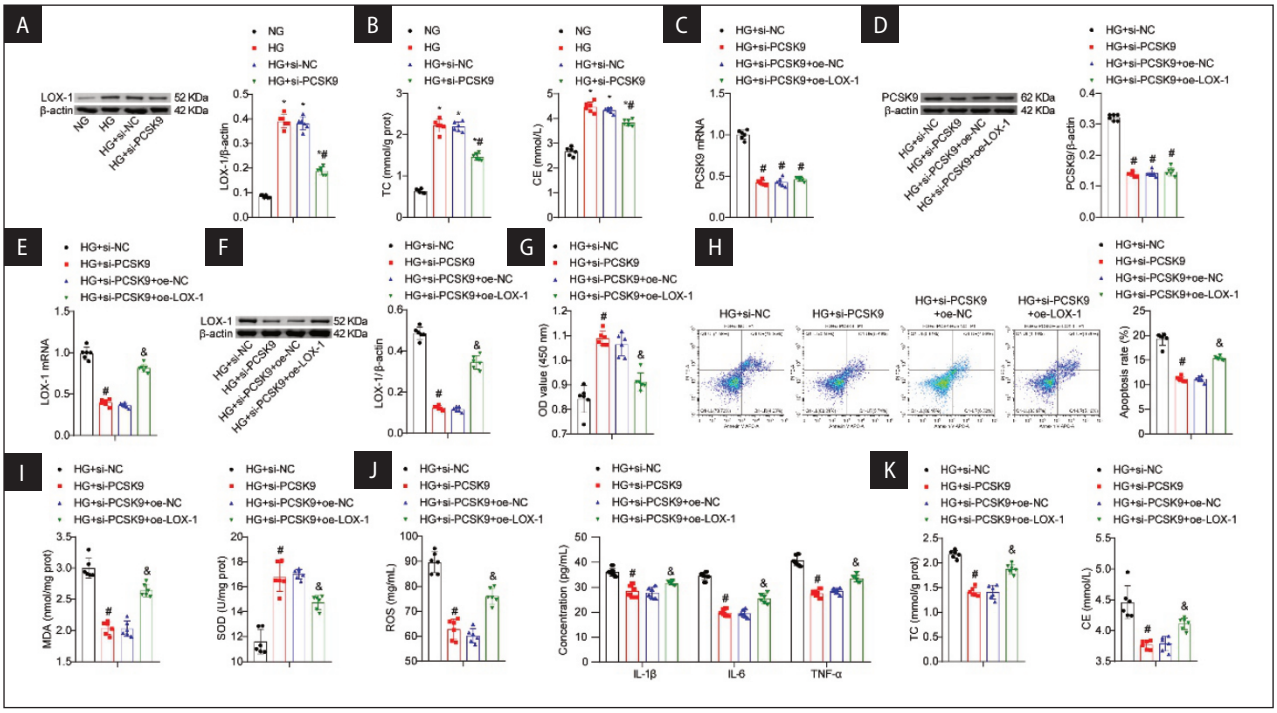

Figure 5. *Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulated oxidative stress, inflammation, apoptosis, and lipid metabolism disorders in diabetic angiopathy (DA) cell models through lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1).* **A.** *LOX-1 level was detected by western blot;* **B.** *The contents of total cholesterol (TC) and cholesteryl ester (CE) were detected by enzyme chemistry;* **C–F.** *The PCSK9 and LOX-1 levels were detected by real-time quantitative polymerase chain reaction (RT-qPCR) or western blot;* **G.** *Cell counting kit 8 (CCK-8) was applied to measure cell viability;* **H.** *Apoptosis was detected by flow cytometry;* **IJ.** *Reactive oxygen species (ROS), malonaldehyde (MDA), superoxide dismutase (SOD), interleukin 6 (IL-6), interleukin 1beta (IL-1β), and tumor necrosis factor (TNF-α) levels were determined by biochemical and enzyme-linked immunosorbent assay (ELISA) methods;* **K.** *The contents of TC and CE were determined by enzyme chemistry. *p < 0.05 vs. HG + si-NC, #p < 0.05 vs. HG + si-PCSK9 + oe-NC. N = 6 repetitions*

cells exposed to HG (Fig. 5B). To further investigate the biological function of LOX-1, we performed oe-LOX-1 intervention in the NG group. Compared with the NG+oe-NC group, the expression of LOX-1 mRNA and protein was significantly elevated in the NG+oe-LOX-1 group (Fig. S1AB). Overexpression of LOX-1 suppressed the viability of NG group cells and promoted cell apoptosis (Fig. S1C-D). In addition, overexpression of LOX-1 led to increased levels of MDA and ROS in NG group cells while decreasing SOD levels (Fig. S1E). Overexpression of LOX-1 also elevated the IL-6, IL-1β, and TNF-α levels in NG group cells (Fig. S1F). Furthermore, overexpression of LOX-1 increased the levels of TC and CE in NG group cells (Fig. S1G). There were no significant changes of PCSK9 level between the HG+si-PCSK9+oe-NC group and the HG+si-PCSK9+oe-LOX-1 group (Fig. 5C-D). Compared to the HG+si-PCSK9+oe-NC group, both LOX-1 gene and protein expression were significantly elevated in the HG+si-PCSK9+oe-LOX-1 group (Fig. 5EF). Additionally, overexpression of LOX-1 in the HG+si-PCSK9 group inhibited the proliferation of HMEC-1 cells and promoted apoptosis (Fig. 5GH). Compared to the HG+si-PCSK9+oe-NC group, oe-LOX-1 increased levels of ROS, MDA, IL-6, IL-1β, and TNF-α, along with a decrease in SOD levels (Fig. 5IJ). Upregulation of LOX-1 also increased the levels of TC and CE in HMEC-1 cells treated with HG+si-PCSK9 (Fig. 5K). Collectively, these findings indicate that the interplay between LOX-1 and PCSK9 influenced lipid metabolism in the DA cell models.

Discussion and Conclusion

PCSK9 loss-of-function gene variants are linked to lower LDL cholesterol levels, but are also associated with higher blood sugar levels and an increased risk of T2DM [20]. PCSK9 inhibitors have been shown to decrease the risk of major cardiovascular events and may serve as a therapeutic option for dyslipidemia [21]. Elevated circulating PCSK9 levels have been positively correlated with age, diabetes, obesity, and hypertension [22]. Serum PCSK9 levels have the potential to serve as a novel biomarker for predicting cardiovascular events [23]. Our research confirmed an increase in PCSK9 levels in individuals with T2DM, which positively correlated with blood lipid levels, glycosylated haemoglobin, and intracardiac fat deposition. Targeting PCSK9 could

be a promising approach to improve dyslipidaemia associated with T2DM.

In aortic sinus tissue of ApoE–/– mice fed a high-fat diet, the expression levels of PCSK9, LOX-1, and aging markers were found to be increased [24]. Previous research has suggested that nuclear factor kappa B (NF- **B**), LOX-1, and syndecan-4 serve as potential targets of PCSK9 and participate in mediating pro-inflammatory responses in macrophages [25]. Specifically, PCSK9 has been shown to enhance the expression of the inflammatory mediator LOX-1 and to interact with it, thereby promoting the inflammatory response [26]. Furthermore, HG treatment has been shown to inhibit cell proliferation and induce apoptosis, ROS production, inflammation, and oxidative stress in HMEC-1 cells [16]. Our study revealed that silencing PCSK9 using siRNA prevented the HG-induced decrease of activity, increase in apoptosis, oxidative stress, inflammation, and LOX-1 expression in HMEC-1 cells. These findings imply that targeting PCSK9 via gene silencing may represent an effective strategy for alleviating HG-induced endothelial cell dysfunction.

The development of monoclonal antibodies targeting PCSK9 heralds a new era of drug therapy for dyslipidaemia [27]. In randomised trials, the administration of monoclonal antibodies against PCSK9 has been shown to reduce mortality and cardiovascular events in patients [28]. Furthermore, inhibition of PCSK9 through liposomal immunogenic fusion with the PCSK9 tetanus vaccine has been found to improve glucose and insulin tolerance impairment as well as lipid status in diabetic patients [29]. It has been observed that LOX-1 and PCSK9 positively influence the inflammatory response [30]. PCSK9 also interacts with LOX-1 in a mutually reinforcing manner [31]. Cholesterol accumulates as CE in cytoplasmic vesicles [32]. Our study demonstrated that silencing PCSK9 inhibited the expression of LOX-1 and reduced TC and CE levels in HMEC-1 cells induced by HG. These findings support the potential of PCSK9 as a target for treating HG-induced vascular endothelial cell dysfunction in DA.

Combined inhibition of cholesterol ester transfer protein inhibitors (CETP) and PCSK9 has an additive effect on lipid characteristics and disease risk, including a reduced risk of coronary artery disease [33]. PCSK9 and LOX-1 positively amplify pro-inflammatory activity and gene expression in endothelial cells, vascular smooth muscle cells, and macrophages [34]. *Gynostemma pentaphyllum* decreased the expression of PCSK9 and LOX-1, as well as the secretion of adhesion factors in ox-LDL stimulated human umbilical vein endothelial cells (HUVECs) [35]. Aerobic exercise training reduced PCSK9, LOX-1, and VCAM-1 expression in the common carotid artery of rats on a high-fat diet compared to the control group [36]. Additionally, downregulation of LOX-1 exerted an anti-atherosclerotic effect by inhibiting excessive cholesterol deposition in the aorta [37]. Our study confirmed that oe-LOX-1 intervention blocked the effect of si-PCSK9 in HG-induced HMEC-1 cells. All these prove that silencing PCSK9/LOX-1 could help improve the vascular endothelial dysfunction of DA as an effective treatment.

Therefore, the PCSK9 levels in peripheral blood were found to be positively correlated with BMI, waistline, TG, CHOL, LDL-C, HBALC, and intracardiac fat pad levels in T2DM patients. Moreover, PCSK9 was found to regulate HG-induced inflammation, oxidative stress, and lipid metabolic dysfunction in HMEC-1 cells via LOX-1, revealing new possibilities for the treatment of T2DM.

Acknowledgments
Not applicable.

Authors' contributions
Y.Y. performed the experiments, collected and analysed the data, prepared Figures 1–5, and wrote the manuscript. H.L. provided critical input in the study design, data interpretation, and manuscript revision. All authors have reviewed and approved the final version of the manuscript.

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Data availability statement
All data found and analysed in this study are in the manuscript or can be obtained from the corresponding author.

Ethical approval
This study followed the principles of the Declaration of Helsinki. Approval was granted by the Zhuzhou Central Hospital. Written informed consent was obtained from all participating patients and healthy persons.

Conflict of interest
There is no conflict of interest.

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