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# LncRNA TCF7 contributes to high glucose-induced damage in human podocytes by up-regulating SEMA3A *via* sponging miR-16-5p

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**Keywords**
Diabetic nephropathy, Podocyte injury, TCF7

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## ABSTRACT

**Aims/Introduction:** Long non-coding RNAs (lncRNAs) exert essential functions in the pathogenesis of diabetic nephropathy (DN). LncRNA T-cell factor 7 (TCF7) and semaphorin-3A (SEMA3A) have been found to be involved in the progression of diabetic nephropathy. However, whether the effect of TCF7 on the pathogenesis of diabetic nephropathy is mediated by SEMA3A remains unclear.

**Materials and Methods:** TCF7, miR-16-5p, and SEMA3A were quantified by a qRT-PCR or immunoblotting method. A CCK-8 assay gauged the cell viability. Measurement of cell apoptosis was done using flow cytometry. RNA immunoprecipitation (RIP), dual-luciferase reporter, and RNA pull-down assays were utilized to assay the targeted interactions among the variables.

**Results:** The TCF7 and SEMA3A levels were elevated in serum from patients with diabetic nephropathy. TCF7 silencing or SEMA3A depletion ameliorated high glucose (HG)-induced podocyte injury. Moreover, TCF7 silencing protected against HG-induced podocyte injury by down-regulating SEMA3A. TCF7 targeted miR-16-5p, and miR-16-5p targeted SEMA3A. Furthermore, TCF7 affected the expression of SEMA3A by competing specifically for shared miR-16-5p.

**Conclusions:** These findings suggested that TCF7 silencing attenuated high glucose-induced podocyte damage partially through the miR-16-5p/SEMA3A regulation cascade.

## INTRODUCTION

Diabetic nephropathy (DN) is a severe complication of diabetes worldwide[1]. Podocytes are members of the filtration barrier in the kidney glomerulus and have a complex cellular organization[2]. The loss of podocytes is an early hallmark of diabetic nephropathy, which contributes to the initiation and progression of diabetic nephropathy[3]. Hyperglycemia, a key factor of podocyte impairment, can drive DN the development of diabetic nephropathy[4]. Nevertheless, the precise molecular mechanisms of podocyte impairment are still largely undefined.

Long non-coding RNAs (lncRNAs) can involve the modulation of gene expression through a wide variety of actions, including sponging microRNAs (miRNAs)[5]. There is some evidence that lncRNAs participate in the pathogenesis of diabetic nephropathy[6,7]. For instance, lncRNA TUG1 affected the mitochondrial bioenergetics in the progression of diabetic nephropathy[8]. LncRNA LINC01619 regulated podocyte damage mediated by endoplasmic reticulum stress *via* miR-27a sequestration[9]. Moreover, Hu and colleagues identified that lncRNA MALAT1 enhanced high glucose (HG)-triggered cytotoxicity in human podocytes by regulating β-catenin[10]. As for lncRNA T-cell factor 7 (TCF7), an up-regulated lncRNA in diabetic nephropathy, it was reported to induce endoplasmic reticulum stress in diabetic nephropathy by acting as a miR-200c sponge[11]. In this project, we sought to define the action of TCF7 in high glucose-induced podocyte damage.

Semaphorin-3A (SEMA3A), a guidance protein, is involved in fundamental development processes by functioning as a chemorepellent or a chemoattractant for axons[12–14]. Previous reports showed that SEMA3A, secreted by podocytes, disrupted the homeostasis of the glomerular filtration barrier and contributed to the development of glomerular disease[15,16]. The

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findings by Aggarwal *et al.*[17] demonstrated that SEMA3A was up-regulated in renal biopsies from patients with diabetic nephropathy and that SEMA3A overexpression enhanced the progression of diabetic nephropathy. Nevertheless, it is still unclear whether the effect of TCF7 on DN the pathogenesis of diabetic nephropathy is mediated by SEMA3A. In our preliminary experiment, we observed a potential interplay between miR-16-5p and TCF7 or SEMA3A using computational methods. We thus hypothesized a putative TCF7/miR-16-5p/SEMA3A regulation cascade in podocyte cytotoxicity induced by high glucose.

Here, we showed that TCF7 and SEMA3A were enhanced in serum from patients with diabetic nephropathy and podocytes treated with high glucose. Consequently, we further explored the precise parts of TCF7 on cell viability, apoptosis, oxidative stress, and inflammation under high glucose stimulation.

## METHODS

### Clinical specimens

The project set consisted of 30 patients with diabetic nephropathy, 30 non-diabetic patients with CKD, and 30 healthy volunteers, who were recruited from Shanghai Jiao Tong University Affiliated Sixth People's Hospital between October 2017 and December 2018. These patients with diabetic nephropathy were defined by having persistent albuminuria (urinary albumin excretion rate >300 mg/24 h) and diabetic retinopathy. The clinical characteristics of the participators are presented in Table 1. Serum specimens were obtained by centrifugation of peripheral blood samples from these subjects and stored in liquid nitrogen. Informed consent was given by each participator, and the study was authorized by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. This work conformed to the provisions of the Declaration of Helsinki in 1995.

### Cell culture

Human immortalized podocytes (provided by Dr Saleem M) were cultivated in 10% FBS RPMI-1640, 1 mg/mL insulin, 0.5 μg/mL sodium selenite, and 0.55 mg/mL human transferrin (all from Thermo Fisher Scientific, Paisley, UK) with 5% $CO_2$ at 37°C. Podocytes of ~60% confluence were treated with 30 mM D-glucose (Solarbio, Beijing, China) for 48 h and defined as the high glucose (HG) group. Medium containing 5.5 mM D-glucose and 24.5 mM mannitol (Solarbio) was defined as being a normal glucose (NG) group, and 24.5 mM mannitol + 5.5 mM D-glucose was used to mimic the osmotic condition.

### qRT-PCR

The TRIzol reagent (Invitrogen, Paisley, UK) was used for the preparation of total RNA from serum specimens and treated podocytes, as described previously[18]. For the TCF7 and SEMA3A quantification, RNA extracts were reverse-transcribed into cDNA with a Prime-Script RT Kit (TaKaRa, Dalian, China), and qRT-PCR was performed using SYBR Premix Ex Taq (TaKaRa) as recommended by the manufacturers. GAPDH served as a reference gene. Quantification of miR-16-5p was performed using a TaqMan RT Kit and TaqMan MicroRNA Assay Kit (all from Applied Biosystems, Darmstadt, Germany), with U6 snRNA used as a reference gene. All reactions were done in quintuplicate on an ABI 7500 Fast Real-time PCR System (Applied Biosystems). $\Delta\Delta C_t$ was calculated by subtracting the $C_t$ values of the control and the $C_t$ values of GAPDH or U6 from the $C_t$ values of the sample. Relative expression was determined using the $2^{\Delta\Delta C_t}$ method. The primers (BGI, Shenzhen, China) for PCR amplification are listed as follows: TCF7-forward, 5′-CCGTCTACTCCGCCTTCAAT-3′ and TCF7-reverse, 5′-CTGCGGGGTCCACTTACC-3′; SEMA3A mRNA-forward, 5′-ACCCAACTATCAATGGGTGCCTTA-3′ and

**Table 1** | Clinical characteristics of patients with diabetic nephropathy, non-diabetic patients with CKD, and healthy volunteers

| Variable | NC (30) | DN (30) | non-diabetic CKD (30) | NC vs DN | Non-diabetic CKD vs DN |
|---|---|---|---|---|---|
| Age (years ± SD) | 52.4 ± 6.1 | 49.8 ± 4.9 | 50.12 ± 5.3 | 0.065 | 0.072 |
| Sex (M:F) | 15:15 | 13:17 | 15:15 | | |
| BW (kg) | 68.2 ± 5.97 | 59 ± 6.82 | 60.13 ± 3.33 | 0.082 | 0.077 |
| Glu (mmol/L) | 5.43 ± 1.21 | 10.26 ± 0.89 | 7.35 ± 1.06 | 0.031 | 0.046 |
| HbA1c (%) | 5.24 ± 0.49 | 6.61 ± 0.64 | 5.52 ± 0.81 | 0.038 | 0.067 |
| UACR (g/gCr) | 20.1 ± 1.25 | 351.2 ± 41.6 | 320.4 ± 36.31 | 0.0071 | 0.061 |
| Duration of DM | – | 5.21 ± 0.56 | – | | |
| Retinopathy existence | – | Yes | – | | |
| Systolic BP (mmHg) | 110 ± 3.52 | 140 ± 5.81 | 128 ± 7.64 | 0.018 | 0.059 |
| eGFR value | 108.33 ± 1.25 | 70.13 ± 5.68 | 80.24 ± 7.15 | 0.027 | 0.066 |
| ARB/ACEi | No | Yes | Yes | | |

BW, body weight; DN, diabetic nephropathy; Glu, blood glucose; HbA1c-A, glycosylated hemoglobin; NC, healthy volunteers; UCAR, urine albumin creatinine ratio.

SEMA3A mRNA-reverse, 5′-AACACTGGATTGTACATGGCT GGA-3′; miR-16-5p-forward, 5′-CGCGCTAGCAGCACGTAA AT-3′ and miR-16-5p-reverse, 5′-GTGCAGGGTCCGAGGT-3′; GAPDH-forward, 5′-GTCAGCCGCATCTTCTTTTG-3′ and GAPDH-reverse, 5′-GCGCCCAATACGACCAAATC-3′; U6-forward, 5′-GCTTCGGCAGCACATATACTAA-3′ and U6-reverse, 5′-AAAATATGGAACGCTTCACGA-3′.

### Immunoblotting

Serum specimens and cell lysates were boiled for 10 min, and immunoblotting analysis was carried out as described previously[19], using primary antibodies against SEMA3A (1:1000, ab23393, Abcam, Cambridge, UK), Bax (1:1000, #2774, Cell Signaling Technology, Danvers, MA, USA), Bcl-2 (1:1000, #15071, Cell Signaling Technology) and loading buffer β-actin (1:5000, ab179467, Abcam).

### Cell transfection

For the TCF7 and SEMA3A silencing studies, podocytes were introduced with siRNA against TCF7 (si-TCF7), siRNA against SEMA3A (si-SEMA3A), or a scrambled negative control sequence (si-NC). For SEMA3A up-regulation, pcDNA-based SEMA3A expression plasmid (pcDNA-SEMA3A) was introduced into podocytes, and an empty pcDNA vector was used as the negative control. MiR-16-5p up-regulation and depletion were carried out using a miR-16-5p mimic, an inhibitor of miR-16-5p (anti-miR-16-5p). Podocytes of 50% confluence in 24-well plates were transfected with 50 nM of oligonucleotides or/and 100 ng of plasmids (all from Ribobio, Guangzhou, China) using Lipofectamine 3,000 (Thermo Fisher Scientific). After 48 h, the transfected cells were acquired for subsequent analyses.

### Cell viability and apoptosis assay

The cell viability of podocytes after various treatments was gauged by a Cell Counting Kit-8 (CCK-8, Dojindo, Munich, Germany) following the instructions of the manufacturers. The optical density was determined using a microplate reader at 450 nm. Cell apoptosis was tested using the Annexin V-FITC/PI Kit (EMD Chemicals, Gibbstown, NJ, USA) following the accompanying protocols. Data analysis was carried out on a NAVIOS flow cytometer (Beckman Coulter, High Wycombe, UK).

### Enzyme-linked immunosorbent assay (ELISA)

Superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, catalase (CAT) activity, and production of reactive oxygen species (ROS) were examined using a SOD Assay Kit, MDA Assay Kit, CAT Assay Kit, and ROS Assay Kit (all from Beyotime, Jiangsu, China), respectively, according to the instructions of the manufacturers. The secretion levels of TNF-α, IL-6, and IL-1β in treated podocytes were evaluated using a corresponding ELISA Assay Kit (R&D Systems, Lille, France) as per the accompanying protocols.

### Bioinformatics

The miRNAs that potentially bind to TCF7 were predicted by the online database miRcode. Analysis for the molecular targets of miR-16-5p was conducted using the starBase v.3 online software.

### Dual-luciferase reporter assay

To construct TCF7 and SEMA3A 3′-untranslated region (3′-UTR) wild-type reporters (TCF7-WT and SEMA3A 3′-UTR-WT), the segments of TCF7 and SEMA3A 3′-UTR harboring the miR-16-5p pairing sites were inserted into the pmirGLO dual-luciferase reporter vector (Promega, Southampton, UK). Site-directed mutations (TCF7-MUT and SEMA3A 3′-UTR-MUT) in the target region were created with the Q5 Site Directed Mutagenesis Kit as per the manufacturing guideline (New England Biolabs, Frankfurt, Germany). The podocytes were cotransfected with TCF7-WT, SEMA3A 3′-UTR-WT, TCF7-MUT, or SEMA3A 3′-UTR-MUT and miRNA mimic. Utilizing the Promega Dual-luciferase Reporter Assay System, we gauged the luciferase activity after 48 h.

### RNA immunoprecipitation (RIP) assay

The RIP assay was carried out using the Magna RNA Immunoprecipitation Kit (Millipore, Molsheim, France). Briefly, cell lysates were incubated with the antibody against Argonaute 2 (anti-Ago2, 1:50, ab186733, Abcam) or IgG (anti-IgG, 1:100, ab172730, Abcam) and protein A/G agarose overnight at 4°C. Total RNA was extracted from the beads to quantify the levels of TCF7, miR-16-5p, and SEMA3A.

### RNA pull-down assay

Biotin-labeled miR-16-5p (Bio-miR-16-5p) and a Bio-NC control were obtained from Ribobio. Cell lysates were incubated with Bio-miR-16-5p or Bio-NC for 3 h at 4°C and then Streptavidin agarose beads (Sigma-Aldrich) were added. After addition, a 2 h incubation was allowed. The total RNA bound to the beads was gauged for the TCF7 level using qRT-PCR.

### Statistical analysis

All assays were performed as 3 biological replicates × 5 technical replicates. Statistical differences ($P < 0.05$ was regarded as significant) were analyzed using ANOVA followed by Tukey's multiple comparisons test (multiple groups) or Student's *t*-test (two groups). Correlation was evaluated using the Pearson test or Spearman test.

## RESULTS

### TCF7 and SEMA3A were enhanced in DN serum

Firstly, we determined the expression of TCF7 and SEMA3A in the serum samples of 30 patients with diabetic nephropathy. As shown by the qRT-PCR, TCF7 and SEMA3A mRNA were significantly overexpressed in the serum samples of patients with diabetic nephropathy compared with normal controls and non-diabetic CKD patients (Figure 1a,b). In agreement with mRNA

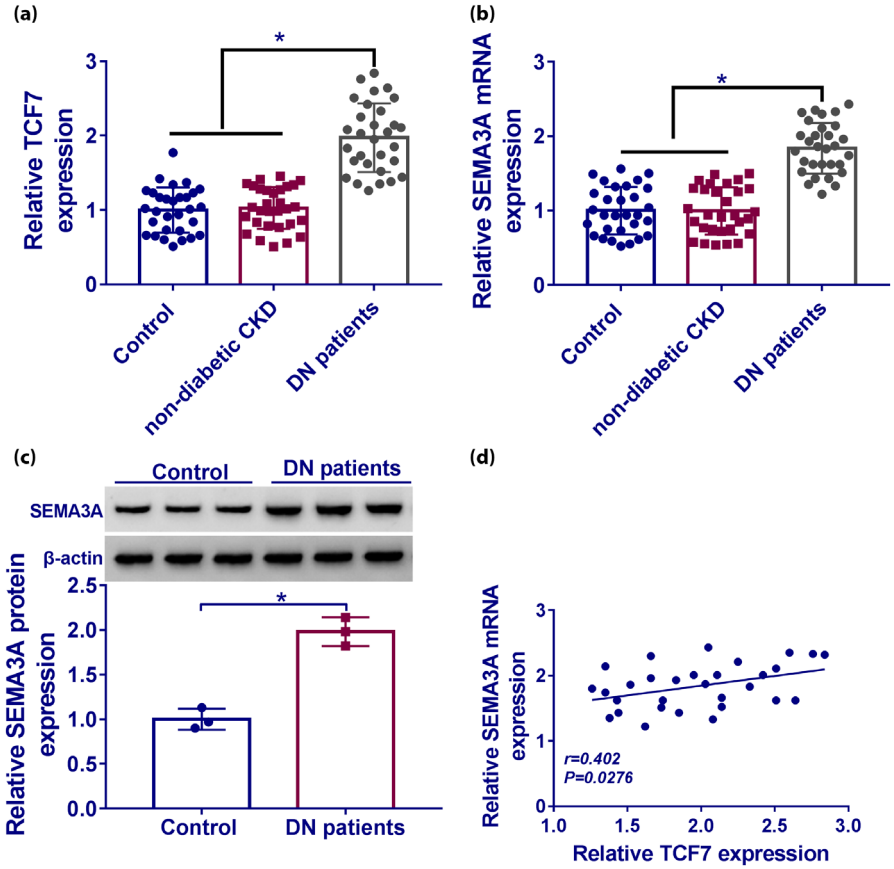



**Figure 1** | TCF7 and SEMA3A levels were elevated in serum of patients with diabetic nephropathy. TCF7 expression (a) and SEMA3A mRNA level (b) in serum samples of 30 patients with diabetic nephropathy, 30 non-diabetic patients with CKD, and 30 healthy volunteers. (c) SEMA3A protein level by immunoblotting in serum samples of 3 patients with diabetic nephropathy and 3 healthy volunteers. (d) Correlation between TCF7 and SEMA3A levels in serum from patients with diabetic nephropathy using the Spearman test. *$P < 0.05$.

expression, the SEMA3A protein level was higher in patients with diabetic nephropathy than that in normal controls (Figure 1c). Furthermore, a positive correlation between TCF7 and SEMA3A expression was observed in serum of patients with diabetic nephropathy (Figure 1d). Additionally, we analyzed the association between the expression of SEMA3A and TCF7 with Glu level, HBA1c level, and UACR level, and confirmed that their expression was positively correlated with the Glu level, the HBA1c level, and the UACR level of patients with diabetic nephropathy (Figure S1a–f).

### Silencing of TCF7 ameliorated HG-triggered injury in podocytes

Relative to the corresponding normal glucose group and the mannitol group, TCF7 expression was significantly augmented in podocytes after treatment with high glucose (Figure 2a and Figure S2a). To observe the role of TCF7 in diabetic nephropathy, we performed "phenocopy" silencing using siRNA against TCF7 (si-TCF7). As expected, in contrast to the negative control, introduction of si-TCF7 prominently reduced the expression of TCF7 in podocytes with or without high glucose treatment (Figure 2b,c). Moreover, high glucose treatment markedly weakened cell viability (Figure 2d) and promoted cell apoptosis (Figure 2e,f) in podocytes. Immunoblotting analysis showed that high glucose treatment resulted in increased pro-apoptotic protein Bax expression and decreased anti-apoptotic protein Bcl-2 level (Figure 2g,h). Additionally, high glucose stimulation led to a significant increase in ROS production (Figure 2i) and MDA consent (Figure 2j), and a distinct reduction in SOD activity (Figure 2k) and CAT activity (Figure 2l), as well as a clear enhancement in IL-1β level (Figure 2m), TNF-α secretion (Figure 2n), and IL-6 production (Figure 2o). Furthermore, TCF7 silencing significantly abolished HG-induced cell viability repression (Figure 2d), apoptosis enhancement (Figure 2e–h), oxidative stress promotion (Figure 2i–l), and inflammation activation (Figure 2m–o) in podocytes.

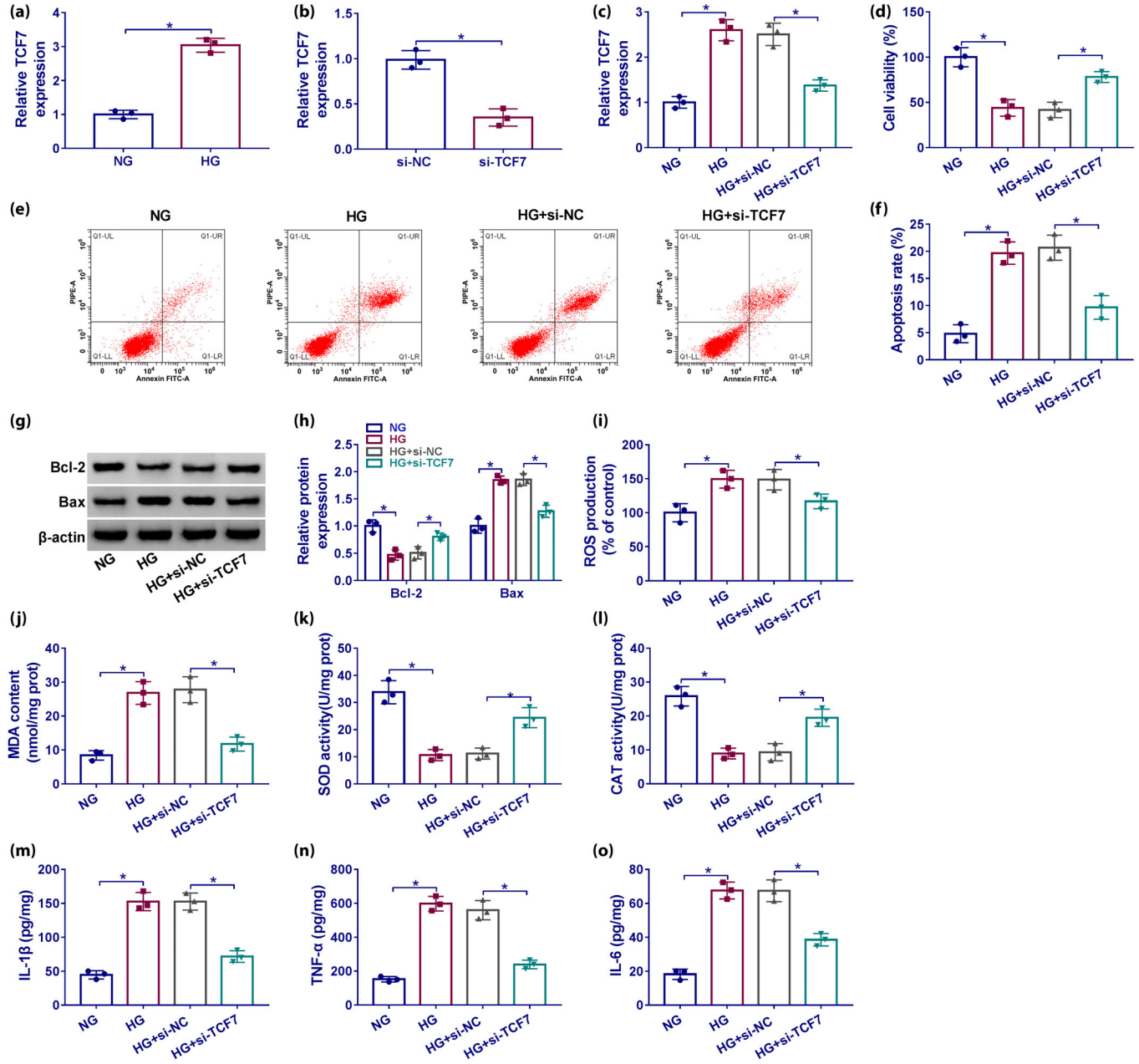



**Figure 2** | TCF7 depletion protected podocytes from cytotoxicity induced by high glucose. TCF7 expression in podocytes after high glucose or normal glucose treatment (a), after transfection by si-TCF7 or si-NC (b). Podocytes were introduced with si-TCF7 or siRNA mimic prior to high glucose treatment and checked for cell viability by CCK-8 assay (d), cell apoptosis by flow cytometry (e, f), Bax and Bcl-2 expression by immunoblotting (g, h), ROS production, MDA consent, SOD activity, and CAT activity using assay kits (i–l), IL-1β, TNF-α and IL-6 secretion by ELISA (m–o). *$P < 0.05$.

### SEMA3A deficiency protected against HG-triggered injury in podocytes

To determine the function of SEMA3A in diabetic nephropathy, we then assessed its level in high glucose-treated podocytes. SEMA3A expression was prominently increased in HG-treated podocytes compared with the normal glucose group and the mannitol group (Figure 3a,b and Figure S2b). Subsequently, we reduced SEMA3A expression using si-SEMA3A. The introduction of si-SEMA3A in podocytes caused a significant decrease in SEMA3A expression (Figure 3c,d). Furthermore, SEMA3A knockdown drastically reversed high glucose-induced anti-viability (Figure 3e), pro-apoptosis (Figure 3f–h), pro-oxidative stress (Figure 3i–l), and pro-inflammation (Figure 3m–o) effects in podocytes.

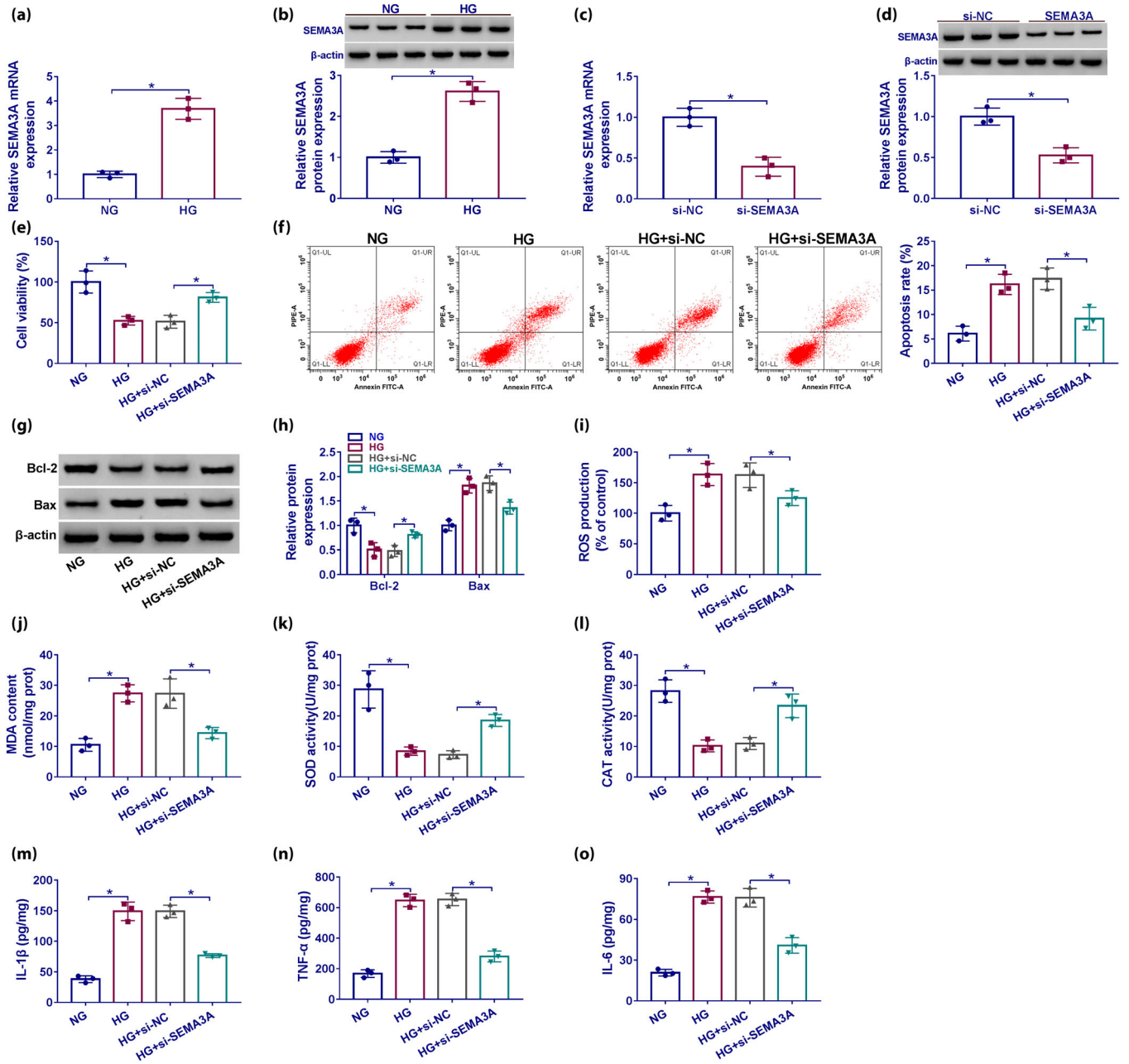
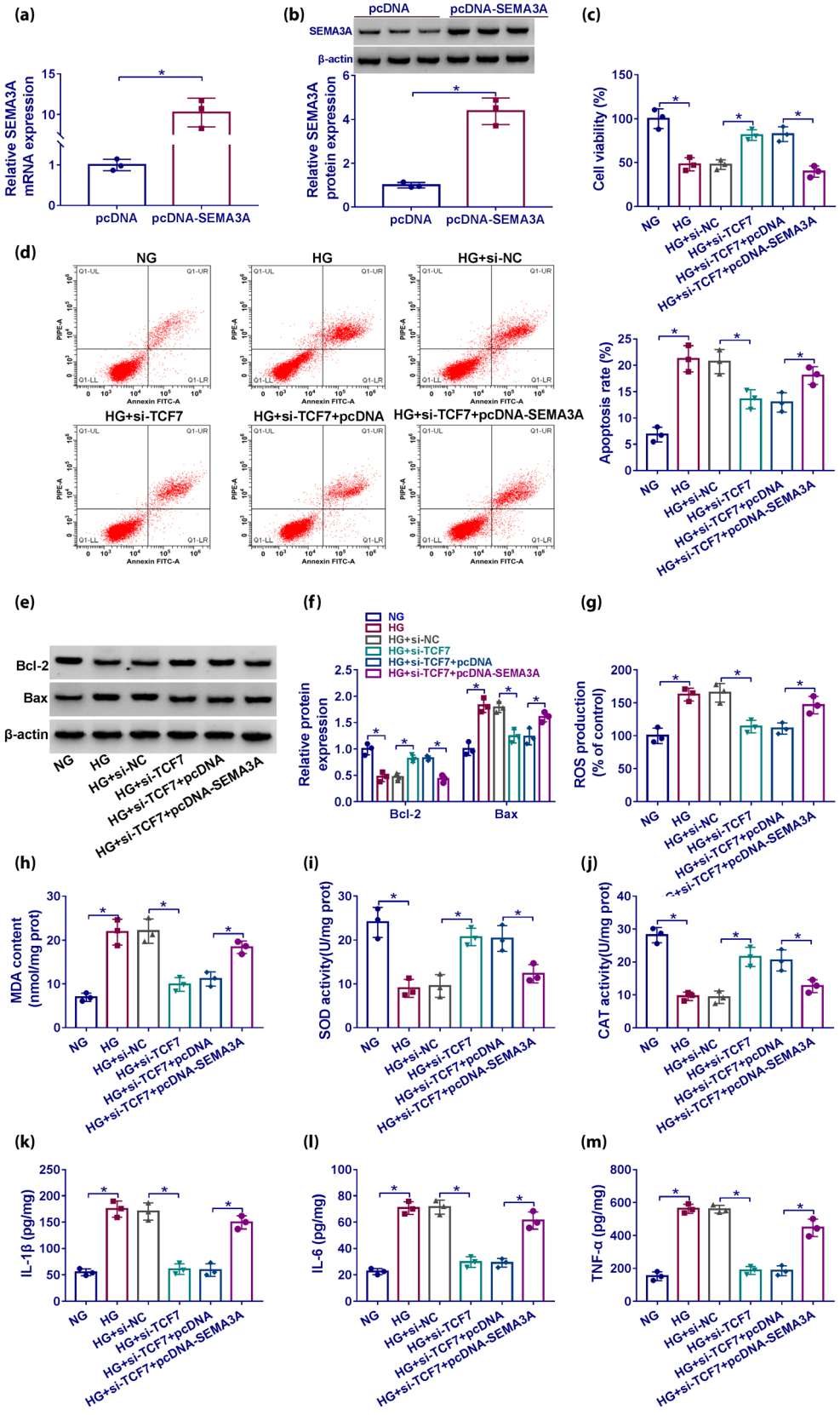



**Figure 3** | SEMA3A deficiency attenuated high glucose-induced podocyte damage. (a, b) SEMA3A mRNA expression and SEMA3A protein in podocytes treated with high glucose. (c, d) SEMA3A expression in podocytes transfected as indicated. Podocytes were introduced with si-SEMA3A or siRNA mimic prior to high glucose simulation and assayed by cell viability by CCK-8 assay (e), cell apoptosis by flow cytometry (f), Bax and Bcl-2 expression by immunoblotting (g, h), ROS production, MDA consent, SOD activity and CAT activity using assay kits (i–l), IL-1β, IL-6 and TNF-α secretion by ELISA (m–o). *$P < 0.05$.

**Figure 4** | TCF7 knockdown protected against high glucose-induced podocyte damage through down-regulating SEMA3A. (a, b) SEMA3A mRNA expression and protein level in podocytes transfected as indicated. Podocytes after introduction by si-NC, si-TCF7, si-TCF7 + pcDNA or si-TCF7 + pcDNA-SEMA3A were subjected to high glucose treatment and checked for cell viability by CCK-8 assay (c), cell apoptosis by flow cytometry (d), Bax and Bcl-2 expression by immunoblotting (e, f), ROS production, MDA consent, SOD activity, and CAT activity using assay kits (g–j), IL-1β, IL-6 and TNF-α secretion by ELISA (k–m). *$P < 0.05$.


(a)
Relative SEMA3A mRNA expression
pcDNA
pcDNA-SEMA3A
(b)
pcDNA
pcDNA-SEMA3A
SEMA3A
β-actin
Relative SEMA3A protein expression
(c)
Cell viability (%)
Apoptosis rate (%)
NG
HG
HG+si-NC
HG+si-TCF7
HG+si-TCF7+pcDNA
HG+si-TCF7+pcDNA-SEMA3A
(d)
Annexin FITC-A
PIPE-A
(e)
Bcl-2
Bax
β-actin
(f)
Relative protein expression
(g)
ROS production (% of control)
(h)
MDA content (nmol/mg prot)
(i)
SOD activity(U/mg prot)
(j)
CAT activity(U/mg prot)
(k)
IL-1β (pg/mg)
(l)
IL-6 (pg/mg)
(m)
TNF-α (pg/mg)

### Silencing of TCF7 relieved HG-induced podocyte injury by down-regulating SEMA3A

Having established the alleviative effect of TCF or SEMA3A silencing on HG-induced podocyte injury, we then examined whether SEMA3A was a potential mediator of TCF7 function. pcDNA-SEMA3A transfection markedly elevated SEMA3A expression (Figure 4a,b). Subsequent functional experiments demonstrated that compared with the negative control, the increased expression of SEMA3A highly reversed si-TCF7-mediated viability promotion in podocytes treated with high glucose (Figure 4c). Moreover, the elevated expression of SEMA3A strongly abrogated the suppressive effects of TCF7 depletion on cell apoptosis (Figure 4d–f), oxidative stress (Figure 4g–j) and inflammation (Figure 4k–m) in high glucose-treated podocytes.

### TCF7 directly interacted with miR-16-5p and SEMA3A was a direct target of miR-16-5p

The online database miRcode was used to help identify the miRNAs that potentially bind to TCF7. Of interest, a putative miR-16-5p complementary sequence was predicted in TCF7 (Figure 5a). Cotransfection of the wild-type reporter plasmid and miR-16-5p mimic into podocytes produced lower luciferase activity than cells cotransfected with miR-NC mimic (Figure 5b). We measured miR-16-5p levels and SEMA3A mRNA levels in the cells transfected with TCF7-WT vector and TCF7-MUT vector, and confirmed that miR-16-5p expression was reduced and SEMA3A expression was enhanced in cells transfected with TCF7-WT vector (Figure S3a,b). These data verified that TCF7 could negatively regulate miR-16-5p levels and positively regulate SEMA3A mRNA levels. Ago2 is the core component of the RNA-induced silencing complex (RISC), where miRNAs silence target mRNAs[20]. The data of RIP assays showed that the enrichment levels of TCF7 and miR-16-5p were simultaneously increased by anti-Ago2 antibody (Figure 5c). The RNA pull-down results revealed that compared with the negative control, TCF7 was significantly enriched by Bio-miR-16-5p (Figure 5d). More importantly, the miR-16-5p level was significantly increased in TCF7-silencing podocytes (Figure 5e). Additionally, qRT-PCR data showed a significant down-regulation of miR-16-5p level in the serum samples of patients with diabetic nephropathy and high glucose-induced podocytes (Figure 5f and Figure S2c).

Then, we further searched the molecular targets of miR-16-5p. Using the software starBase v.3, a potential miR-16-5p-binding sequence was identified within SEMA3A 3′-UTR (Figure 5g). Transfection of the 3′-UTR reporter construct in the presence of miR-16-5p mimic triggered a prominent down-regulation of luciferase activity, while this effect was strongly abrogated with the mutation of the miR-16-5p-binding sequence (Figure 5h). The RIP experiments showed that the enrichment levels of miR-16-5p and SEMA3A were synchronously elevated by anti-Ago2 antibody in podocytes (Figure 5i). The data of qRT-PCR revealed a striking reduction of miR-16-5p in anti-miR-16-5p-transfected podocytes (Figure 5j). Furthermore, in contrast to their counterparts, SEMA3A mRNA and protein levels were markedly increased by miR-16-5p knockdown, while they were significantly decreased by miR-16-5p overexpression in podocytes (Figure 5k–m).

### TCF7 regulated SEMA3A expression through miR-16-5p

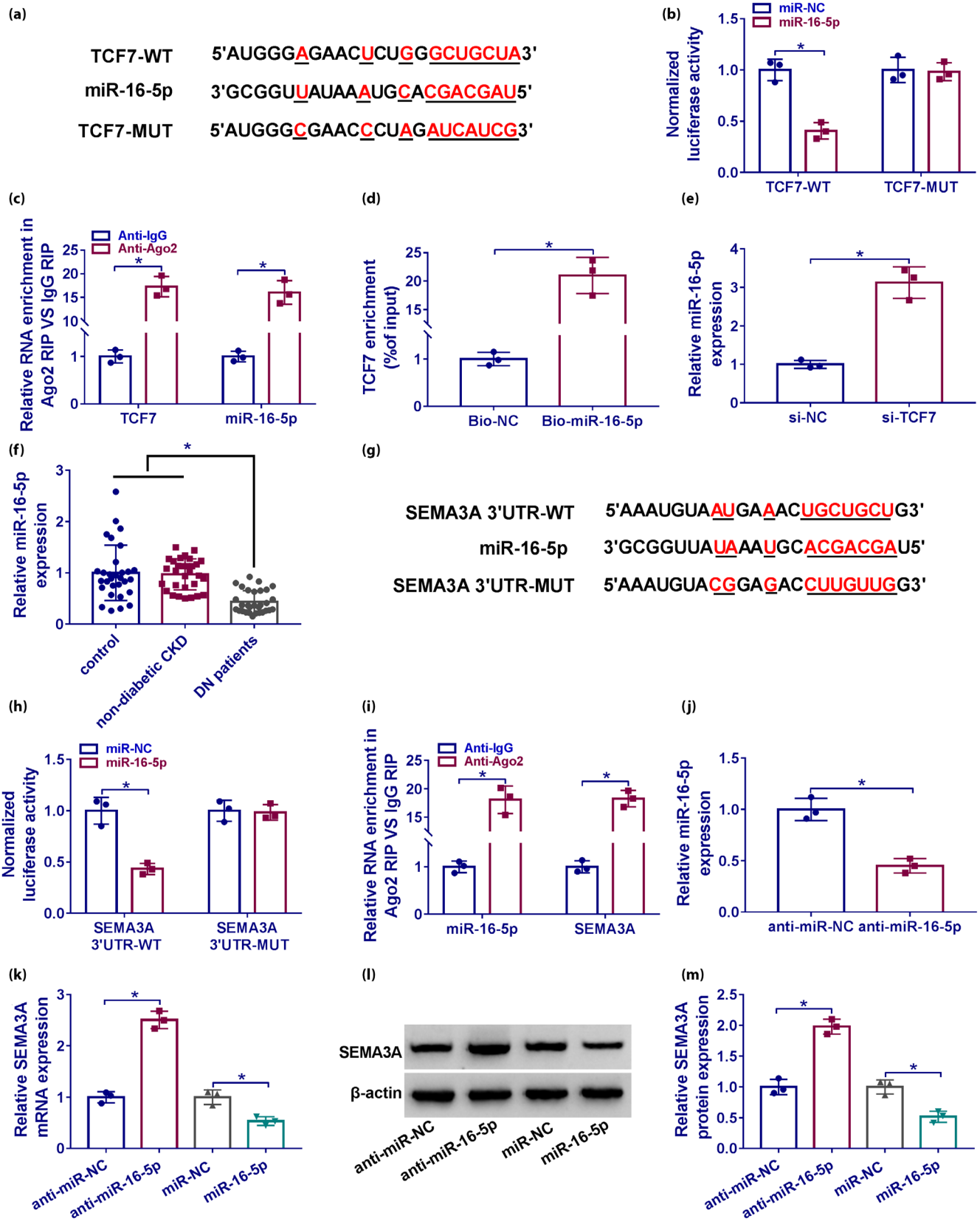
Lastly, we explored how TCF7 regulated SEMA3A expression in podocytes. In podocytes transfected with si-TCF7 and anti-miR-16-5p, we measured miR-16-5p expression and confirmed that anti-miR-16-5p could eliminate the promotion effect of si-TCF7 on miR-16-5p expression (Figure S4). As expected, in comparison with their counterparts, SEMA3A expression was significantly reduced by TCF7 silencing at both mRNA and protein levels (Figure 6a,b). However, the effect was prominently abolished by anti-miR-16-5p cotransfection (Figure 6a, b).

## DISCUSSION

Diabetic nephropathy is a devastating complication in patients with diabetes[21]. LncRNAs have recently been shown to be involved in the progression of diabetic nephropathy[6,22]. Previous reports demonstrated that some lncRNAs, such as GM5524 and plasmacytoma variant translocation (PVT1), regulated HG-induced podocyte injury and apoptosis[23,24]. The present work has led to the identification of TCF7 knockdown that ameliorated HG-induced podocyte injury by regulating SEMA3A expression through sponging miR-16-5p.

LncRNA TCF7 has been identified as a tumor promoter in a series of human tumors, such as hepatocellular carcinoma, non-small cell lung cancer, and glioma[25–27]. Moreover, TCF7 was reported to enhance the proliferation and migration of

**Figure 5** | MiR-16-5p directly interacted with TCF7 and SEMA3A 3′-UTR. (a) The miR-16-5p pairing sequence in TCF7 and the mutant in seed region. (b) Luciferase activity in podocytes after introduction by TCF7-WT or TCF7-MUT. (c) TCF7 and miR-16-5p enrichment in the RISC of podocytes using an anti-Ago2 antibody. (d) TCF7 enrichment in cell lysates of podocytes using Bio-NC or Bio-miR-16-5p. (e) MiR-16-5p expression in podocytes after introduction by si-TCF7 or siRNA mock. (f) Relative miR-16-5p expression in serum samples of 30 patients with diabetic nephropathy, 30 non-diabetic patients with CKD, and 30 healthy volunteers. (g) Schematic model of SEMA3A 3′-UTR illustrating the miR-16-5p-pairing sequence and mutated seed region. (h) Luciferase activity in podocytes after transfection by SEMA3A 3′-UTR-WT or SEMA3A 3′-UTR-MUT. (i) The enrichment of miR-16-5p and SEMA3A in the RISC of podocytes using the anti-Ago2 antibody. (j) MiR-16-5p expression in podocytes after introduction by anti-miR-16-5p or anti-miR-NC. (k–m) SEMA3A level in podocytes transfected as indicated. *$P < 0.05$.



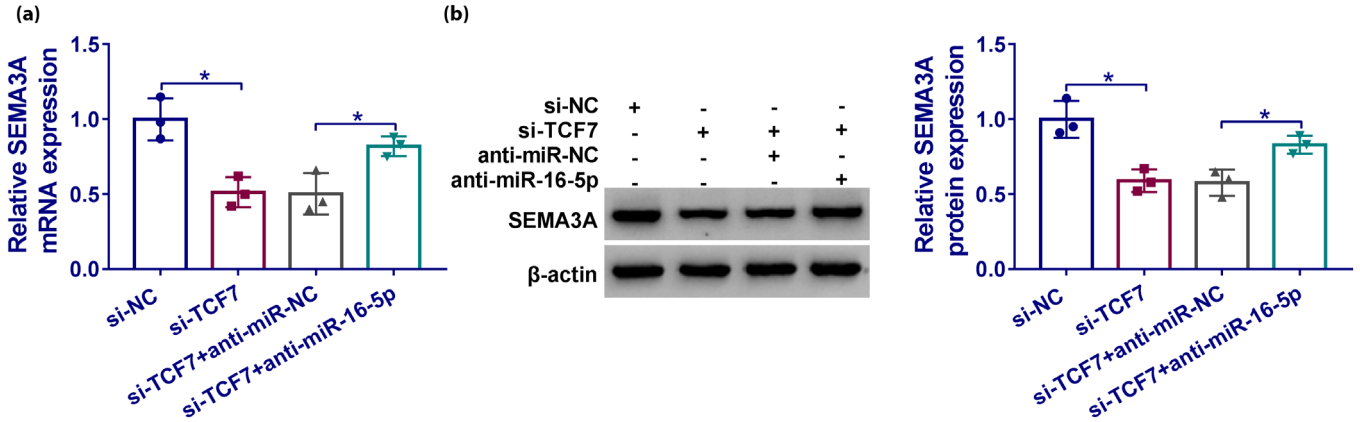



**Figure 6** | TCF7 positively modulated SEMA3A *via* miR-16-5p. SEMA3A mRNA expression (a) and SEMA3A protein level (b) in podocytes transfected as indicated. *$P < 0.05$.

airway smooth muscle cells in asthma[28]. These findings described above provided a possibility for the involvement of TCF7 in the pathogenesis of diabetic nephropathy. Thus, we assumed that TCF7 might regulate the progression of diabetic nephropathy. To address this, we assessed TCF7 expression in diabetic nephropathy, and our data indicated that TCF7 was overexpressed in the serum of patients with diabetic nephropathy and podocytes treated with high glucose, in agreement with a recent study[11]. The activation of oxidative stress and inflammation contributes to the development of diabetic nephropathy[29,30]. Here, we firstly discovered that TCF7 silencing attenuated high glucose-induced damage in human podocytes.

SEMA3A is involved in the pathogenesis of many human diseases, such as acute kidney injury, osteoarthritis, and myocardial infarction[31–33]. Additionally, earlier documents reported that SEMA3A regulated bone microarchitecture and osteogenic differentiation in rats with type 2 diabetes[34,35]. SEMA3A expression was found to be up-regulated under high glucose stimulation in diabetic keratinocytes[36]. Moreover, SEMA3A, secreted by podocytes, was shown to contribute to the initiation and progression of diabetic nephropathy[17,37,38]. In this study, our data first demonstrated that SEMA3A knockdown attenuated HG-induced podocyte damage. Furthermore, we were the first to demonstrate that TCF7 silencing protected against HG-induced podocyte injury *via* down-regulating SEMA3A.

LncRNAs can modulate gene expression through sponging miRNAs. Here, we first confirmed that TCF7 directly targeted miR-16-5p, and that miR-16-5p directly targeted SEMA3A in podocytes. MiR-16-5p has been established as having a critical role in human diseases. For example, miR-16-5p served as a potent anti-tumor factor in astrocytic glioma and malignant mesothelioma[39,40]. MiR-16-5p was also reported to regulate the origin and the progression of arthritis[41,42]. Cao and colleagues showed that plasma miR-16-5p was a new biomarker for the diagnosis of gestational diabetes mellitus[43]. Moreover, recent work has uncovered that exosomal miR-16-5p protected against high glucose-induced podocyte injury in diabetic nephropathy by targeting vascular endothelial growth factor A (VEGFA)[44]. More importantly, our data identified that TCF7 regulated SEMA3A expression through sponging miR-16-5p. Additionally, Ma *et al.*[35] reported that SEMA3A was involved in diabetes-induced damage on bone microarchitecture and strength by the IGF-1/β-catenin pathway in rats. Cerani *et al.*[45] reported that SEMA3A induced vascular permeability in diabetic retinopathy through neuropilin-1. A future challenge will be to identify how SEMA3A regulates HG-induced podocyte damage.

Of course, there are some limitations to our study. Due to the limitation of the conditions, we have not carried out animal *in vivo* experiments, but only used the HG-induced DN cell model to reveal the function and mechanism of TCF7. In the future, *in vivo* experiments are needed to further confirm our conclusions.

In conclusion, our current work indicated that the silencing of TCF7 attenuated HG-induced injury in human podocytes at least in part through regulating SEMA3A expression *via* sponging miR-16-5p. These findings provide a new insight into the molecular basis for podocyte damage triggered by high glucose.

## DISCLOSURE

The authors declare that they have no competing interests.
Approval of the research protocol: The present study was approved by the ethical review committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital.
Informed consent: Written informed consent was obtained from all enrolled patients.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.

## DATA AVAILABILITY STATEMENT

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1 |** The association between the expression of SEMA3A and TCF7 with Glu level, HBA1c level, and UACR level.

**Figure S2 |** The expression of TCF7, SEMA3A and miR-16-5p in podocytes.

**Figure S3 |** The expression of miR-16-5p and SEMA3A in podocytes transfected with TCF7-WT or TCF7-MUT.

**Figure S4 |** The expression of miR-16-5p in podocytes transfected with si-TCF7-WT and anti-miR-16-5p.