Rosiglitazone attenuates high glucose-induced proliferation, inflammation, oxidative stress and extracellular matrix accumulation in mouse mesangial cells through the Gm26917/miR-185-5p pathway

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Abstract. Rosiglitazone (RSG) is widely used to reduce the amount of sugar in the blood of patients with diabetes mellitus. Diabetic nephropathy is the most common microvascular complication of diabetes. The role of RSG in diabetic nephropathy is not fully understood. Diabetic nephropathy model was constructed in high glucose (HG)-treated mouse mesangial cells. The effects of RSG on cell viability and cell cycle were investigated using cell counting kit-8 (CCK-8) assay and flow cytometry assay. Oxidative stress was assessed according to ROS production and SOD activity in cells. Inflammatory responses were assessed according to the releases of inflammatory cytokines. Extracellular matrix (ECM) accumulation was determined by the levels of fibronectin and collagen IV using western blot. The expression of Gm26917 and microRNA-185-5p (miR-185-5p) was detected by quantitative real-time polymerase chain reaction (qPCR). The interaction between Gm26917 and miR-185-5p was validated by dual-luciferase reporter assay, RNA immunoprecipitation (RIP) assay and pull-down assay. RSG significantly inhibited HG-induced proliferation, oxidative stress, inflammatory responses and ECM accumulation in mouse mesangial cells. The expression of Gm26917 was induced by HG but weakened by RSG. Gm26917 knockdown alleviated HG-induced proliferation, oxidative stress, inflammatory responses and ECM accumulation in mouse mesangial cells, and Gm26917 overexpression partly abolished the effects of RSG. Moreover, miR-185-5p was a target of Gm26917, and miR-185-5p inhibition recovered proliferation, oxidative stress, inflammatory responses and ECM accumulation in mouse mesangial cells that were alleviated by Gm26917 knockdown. RSG ameliorated HG-induced mouse mesangial cell proliferation, oxidative stress, inflammation and ECM accumulation partially by governing the Gm26917/miR-185-5p pathway.

Key words: Rosiglitazone, Gm26917, miR-185-5p, High glucose, Mesangial cell

DIABETIC NEPHROPATHY (DN) is a multifactorial disease involving different pathogenic molecular processes and histopathological structures [1]. It is characterized by persistent albuminuria (>300 mg/24 h), a gradual decrease in glomerular filtration, and increasing morbidity and mortality of arterial hypertension and cardiovascular disease [2, 3]. DN, affecting approximately one-third of diabetic patients, is the most common cause of the end-stage renal disease (ESRD) worldwide [2]. Hemodynamic changes (such as hypertonia and hyperperfusion) caused by hyperglycemia are considered to be the main cause of kidney damage [4, 5]. In many animal experiments, high glucose (HG) is used to induce DN, and a series of avenues against DN injuries, including oxidative stress, inflammatory responses and extracellular matrix (ECM) deposition, have been shown to be promising strategy [6-8].

Rosiglitazone (RSG) is a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, and it has been demonstrated to have anti-fibrotic and anti-inflammatory effects on various human diseases [9, 10]. For example, RSG repressed experimental subarachnoid hemorrhage-
induced early brain injuries in rats [11]. RSG alleviated oxidative stress and neuroinflammation to exert neuro-protection effects in streptozotocin-induced mice of Alzheimer model [12]. Besides, accumulating studies hinted that RSG might relieve the progression of DN. For instance, Hu et al. reported that RSG inhibited the expression of Chemerin and ChemR23 that was elevated in streptozotocin-treated diabetic rats, thus blocking the development of DN [13]. Tang et al. reported that HG-induced vascular endothelial growth factor (VEGF) and inflammatory cytokine sections were attenuated by RSG in proximal tubular epithelial cells [14]. Therefore, the further elucidation of RSG functional mechanism broadens the insights into PPAR-γ agonist as promising therapeutic agents and substantiates a protective role of RSG in the development of DN.

RSG treatment could affect the expression of long non-coding RNAs (lncRNAs) in mouse models with DN [15], indicating that lncRNAs were involved in RSG-regulated DN progression. LncRNAs are members of non-coding RNAs, with over 200 nucleotides in length. LncRNAs have been regarded to regulate various physiological and pathological processes in human diseases [16], and the studies of lncRNAs provide novel perspectives to disease diagnosis and treatment. Also, lncRNAs are vital players in diabetes and diabetic kidney disease [17]. It was documented that lncRNAs were implicated in DN development by regulating numerous pathologic processes in mesangial cells, such as ECM accumulating, oxidative stress or microRNA (miRNA) suppression [18, 19]. Gm26917 was a predicted lncRNA that was highly expressed in renal cortical tissues from DN mice but downregulated after RSG treatment [15]. However, the function of Gm26917 is still lacking.

The interactions between lncRNAs and miRNAs partly illustrate the mechanism of lncRNA action [20]. LncRNAs may act as endogenous sponges to modulate the expression and function of target miRNAs [20]. Due to the advance of bioinformatics, more putative target miRNAs of lncRNA can be predicted. Herein, miR-185-5p is predicted as a target of Gm26917. However, their interactions remain unknown.

In the present study, we used HG to treat mouse mesangial cells to construct cell models of DN. Then, we investigated the effects of RSG on cell proliferation, cellular oxidative stress, inflammatory responses and ECM accumulation. We also determined the regulation of RSG on Gm26917 expression, and proposed the Gm26917/miR-185-5p axis mediated by RSG. Our study intended to explore the mechanism of RSG in DN from a novel perspective of lncRNA and provided basis for the use of RSG in DN treatment.

Materials and Methods

Cells and agents

Mouse mesangial cells (SV40-MES13) were purchased from Procell (Wuhan, China) and cultured in matched culture medium (71.25% DMEM, 23.75% Ham’s F-12, and 5% FBS; Procell) at 37°C conditions containing 5% CO₂. D-glucose (Solarbio, Beijing, China) was used to treat SV40-MES13 cells (high glucose (HG): 30 mmol/L D-glucose and normal glucose (NG): 5.5 mmol/L D-glucose). Rosiglitazone (RSG) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in DMSO (Sigma-Aldrich). SV40-MES13 cells were treated with RSG at different concentrations (0, 0.1, 1 and 10 μM) to screen dosage.

Cell counting kit-8 (CCK-8) assay

Cells were seeded into a 96-well plate and then cultured for 24 h. Next, at that time, cells were incubated with CCK-8 reagent (Solarbio) for another 2 h. The absorbance at 450 nm was detected to monitor cell viability using a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow cytometry assay

Cell cycle progression was determined using the DNA Content Quantitation Assay (Cell Cycle) (Solarbio). Simply put, cells with treatment or transfection were collected and washed with PBS. A total of 1 × 10⁶ cells were utilized and fixed with 70% precooling ethanol overnight at 4°C. Next, cells were resuspended using 100 μL RNase A for 30 min at 37°C and then probed with 400 μL propidium iodide (PI) for 30 min at 4°C in dark. Finally, cell cycle distribution was examined using a flow cytometer (BD Biosciences, San Jose, CA, USA).

Measurement of ROS level and SOD activity

The production of ROS was measured using the Fluorometric Intracellular Ros Kit (Sigma-Aldrich) according to manufacturer’s instructions. The activity of SOD was checked using a SOD Determination Kit (Sigma-Aldrich) according to the instructions.

Enzyme-linked immunosorbent assay (ELISA)

The releases of inflammatory cytokines (IL-6, IL-1β and TNF-α) were determined using Mouse IL-6 ELISA kit (Abcam, Cambridge, MA, USA), Mouse IL-1β ELISA kit (Abcam) and Mouse TNF-α ELISA kit (Abcam), respectively. The procedures of ELISA were conducted according to the manufacturer’s instructions.

Western blot

Western blot was performed to detect the protein level
of ECM deposition-related indicators (fibronectin and collagen IV), using β-actin as an internal reference. Cells were lysed using RIPA lysis buffer (Sigma-Aldrich) to extract total proteins. Equal amount of proteins were separated by SDS-PAGE and then transferred onto PVDF membranes. The membranes were blocked by 5% skim milk and then incubated with the primary antibodies at 4°C overnight, including anti-fibronectin (ab2413; Abcam), anti-collagen IV (SAB4500369; Sigma-Aldrich) and anti-β-actin (ab8227; Abcam). Subsequently, the membranes were incubated with the secondary antibody (ab205718; Abcam) at room temperature for 1.5 h. The protein signals were detected using an enhanced chemiluminescence system (Sigma-Aldrich).

**Cell transfection**

Short hairpin RNA (shRNA) specific to Gm26917 (sh-Gm26917) and matched negative control (sh-NC) were synthesized by IBSBIO (Shanghai, China). Gm26917 overexpression vector (Gm26917) and blank pcDNA vector (control) were also provided by IBSBIO. MiR-185-5p mimic (miR-185-5p), miR-185-5p inhibitor (anti-miR-185-5p and mimic negative control (miR-NC) and inhibitor negative control (anti-miR-NC) were all purchased from Ribobio (Guangzhou, China). Cell transfection was performed using lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) in SV40-MES13 cells according to the manufacturer’s instructions.

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

RNA samples were isolated from cells using Trizol reagent (Sigma-Aldrich), and RNA quality was checked using Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesized using the Superscript III first-strand synthesis system (Life Technologies, Carlsbad, CA, USA) or using the miRNA First-Strand Synthesis Kit (Clontech, Mountain View, CA, USA). Subsequent qPCR procedures were implemented using the SYBR™ Green PCR Master Mix (Clontech). Internal reference was set using β-actin or small RNA U6, and relative expression was calculated using the 2-ΔΔCt method. All sequences of Primers used were exhibited as follows:

Gm26917, F: 5'-CTTCTCTTCCGCCCAACC-3' and R: 5’-CCGACACCTCCTTATCC-3'; β-actin, F: 5’-AC CTTCCTACAATGACTCGG-3' and R: 5’-CTGGATGCTCAGTACATGG-3'; miR-185-5p, F: 5’-CGCGTGAGAAAGGCAGTC-3 and R: 5’-AGTGCAAGGGTCCGAGGT-3';

**Dual-luciferase reporter assay**

The binding sites between Gm26917 and mmu-miR-185-5p were obtained from starbase (http://starbase.sysu.edu.cn/). Then, the wild-type (wt) and mutant-type (mut) sequence fragments of Gm26917 (containing wt binding sites with mmu-miR-185-5p or mut binding sites with mmu-miR-185-5p) were cloned into PGL4 reporter plasmid (Promega, Madison, WI, USA), respectively. Fusion reporter plasmids were named as Gm26917-wt-1, -2, -3 -4 and Gm26917-mut-1, -2, -3, -4. Any one of these reporter plasmids was transfected with miR-185-5p or miR-NC into SV40-MES13 cells. At 48 h post-transfection, luciferase activity in cells was checked using the Dual-Luciferase Reporter Assay System (Promega) following the manufacturer’s instruction.

**RNA immunoprecipitation (RIP)**

The EZ-Magna RIP Kit (Millipore, Billerica, MA, USA) was utilized for RIP assay. Briefly, cells were subjected to RIP lysis buffer, and cell lysates were then incubated with RIP binding buffer supplemented with magnetic beads coupled with antibodies against Ago2 (Millipore) or mouse IgG (control; Millipore). RNAs bound to Ago2 antibody were extracted and detected by qPCR.

**Pull-down assay**

Biotinylated wt or mut miR-185-5p (bio-miR-185-5p-wt or bio-miR-185-5p-mut) and its negative control (bio-miR-NC) were provided by Ribobio. SV40-MES13 cells were co-cultured with bio-miR-185-5p-wt, bio-miR-185-5p-mut or bio-miR-NC for 48 h and then lysed using lysis buffer from the Pierce™ Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific). Cell lysates were subjected to streptavidin magnetic beads pre-treated with RNase-free BSA and yeast tRNA. RNA complexes bound to the beads were eluted and extracted for qPCR analysis to detect the expression of Gm26917.

**Statistical analysis**

All data were processed using GraphPad Prism 7.0 (GraphPad, La Jolla, CA, USA). The difference between two sets was determined using Student’s t-test, and the difference among more than two sets was determined using analysis of variance (ANOVA) and Tukey post-test. Data were finally displayed as mean ± standard deviation (SD). P-value less than 0.05 was considered to be significant.
Results

RSG suppressed HG-induced proliferation, oxidative stress, inflammation and ECM accumulating in SV40-MES13 cells

SV40-MES13 cells were administered with HG, and we explored the function of RSG in HG-induced SV40-MES13 cells. The chemical construction of RSG was shown in Fig. 1A. Then SV40-MES13 cells were treated with different concentrations of RSG (0, 0.1, 1 and 10 μM), and we found cell viability was strikingly decreased after 10 μM RSG treatment (Fig. 1B). Besides, HG-induced cell viability was significantly weakened by 10 μM RSG treatment in SV40-MES13 cells (Fig. 1C). HG treatment promoted cell cycle progression, while 10 μM RSG treatment largely induced cell cycle arrest at the G0/G1 phase (Fig. 1D). In addition, the level of ROS promoted by HG was notably reduced by 10 μM RSG in SV40-MES13 cells, while the activity of SOD suppressed by HG was notably recovered by 10 μM RSG in SV40-MES13 cells (Fig. 1E and 1F). The releases of inflammatory cytokines (IL-6, IL-1β and TNF-α) were stimulated by HG but largely blocked by 1 μM RSG and 10 μM RSG in SV40-MES13 cells (Fig. 1G). Moreover, the protein levels of fibronectin and collagen IV (markers of ECM deposition) were remarkably increased in HG-treated SV40-MES13 cells, while the addition of 10 μM RSG largely weakened the levels of fibronectin and collagen IV (Fig. 1H). These data illustrated that HG-induced proliferation, oxidative stress, inflammation and ECM accumulation were alleviated by 10 μM RSG in SV40-MES13 cells. Besides, we examined the effects of RSG (0, 0.1, 1 and 10 μM) on these cell phenotypes in SV40-MES13 cells without HG treatment, and we found that only 10 μM RSG notably alleviated SV40-MES13 cell proliferation, oxidative stress, inflammation and ECM accumulation (Fig. S1A–E). In addition, we examined the effects of HG on these cell phenotypes in SV40-MES13 cells, with mannitol as an osmotic control. The data showed that HG, compared to NG or mannitol, notably promoted cell proliferation, oxidative stress, inflammation and ECM accumulation (Fig. S2A–F).

Gm26917 knockdown suppressed HG-induced proliferation, oxidative stress, inflammation and ECM accumulating in SV40-MES13 cells

The expression of Gm26917 was significantly increased in HG-treated SV40-MES13 cells but significantly declined with the addition of RSG, while the reintroduction of GW9662 (PPAR-γ antagonists) largely recovered the expression of Gm26917 (Fig. 2A and Fig. S3). ShRNA targeting Gm26917 was used to decrease the expression of Gm26917 in SV40-MES13 cells. The expression of Gm26917 was notably declined in SV40-MES13 cells transfected with sh-Gm26917 compared to sh-NC (Fig. 2B). Then, we found cell viability promoted by HG was declined in HG-treated SV40-MES13 cells transfected with sh-Gm26917 (Fig. 2C). Gm26917 knockdown significantly induced cell cycle arrest in HG-treated SV40-MES13 cells (Fig. 2D). Besides, ROS level stimulated by HG in SV40-MES13 cells was markedly decreased by sh-Gm26917 transfection compared to sh-NC (Fig. 2E). SOD activity depleted by HG in SV40-MES13 cells was markedly restored by sh-Gm26917 transfection compared to sh-NC (Fig. 2F). Moreover, HG-induced inflammatory cytokines (IL-6, IL-1β and TNF-α) releases were alleviated by Gm26917 knockdown in SV40-MES13 cells (Fig. 2G). Additionally, the protein levels of fibronectin and collagen IV activated by HG were largely weakened by the transfection of sh-Gm26917 in SV40-MES13 cells (Fig. 2H). These data illustrated that Gm26917 downregulation inhibited HG-induced proliferation, oxidative stress, inflammation and ECM accumulating in SV40-MES13 cells. The effects of Gm26917 downregulation on these cell behaviors in SV40-MES13 cells without HG treatment were determined. The data presented that Gm26917 knockdown also suppressed cell viability (Fig. S4A), cell cycle progression (Fig. S4B), oxidative stress (Fig. S4C and S4D), inflammatory responses (Fig. S4E) and ECM accumulation (Fig. S4F).

RSG inhibited the expression of Gm26917 to suppressed HG-induced proliferation, oxidative stress, inflammation and ECM accumulating in SV40-MES13 cells

The expression of Gm26917 was strikingly enhanced in SV40-MES13 cells transfected with Gm26917 overexpression vector compared to blank pcDNA vector (Fig. 3A). Functional experiments showed that HG-induced cell viability was inhibited by RSG, while further Gm26917 transfection recovered RSG-inhibited cell viability (Fig. 3B). Besides, RSG treatment-induced cell cycle arrest was substantially alleviated by Gm26917 overexpression (Fig. 3C and 3D). The level of ROS promoted by HG was depleted by RSG, while further Gm26917 overexpression largely recovered ROS level in SV40-MES13 cells (Fig. 3E). The activity of SOD sequestered by HG was recovered by RSG, while further Gm26917 overexpression pronouncedly reduced SOD activity that was recovered by RSG (Fig. 3F). The releases of inflammatory cytokines (IL-6, IL-1β and TNF-α) suppressed by RSG in HG-treated SV40-MES13 cells were significantly recovered by Gm26917 overexpression (Fig. 3G). The protein levels of fibronectin and collagen IV were inhibited by RSG in HG-treated SV40-
MES13 cells but recovered by additional Gm26917 transfection (Fig. 3H). These data illustrated that RSG inhibited HG-induced proliferation, oxidative stress, inflammation and ECM accumulating in SV40-MES13 cells by depleting Gm26917.
Gm26917 directly bound to miR-185-5p and suppressed miR-185-5p expression

To explore the functional mechanism of Gm26917, we investigated the target miRNAs of Gm26917. Bioinformatics analysis presented that four sites (-1/2/3/4) on Gm26917 sequence fragment harbored binding sites with mmu-miR-185-5p (Fig. 4A), suggesting that miR-185-5p largely might be a target of Gm26917. Fig. 4B showed...
that miR-185-5p mimic could significantly enhance the expression of miR-185-5p in cells. Then, four sequence fragments of Gm26917 (wild-type and mutant-type) were respectively cloned into PGL4 reporter plasmid, and each of them together with miR-185-5p mimic or miR-NC were cotransfected into SV40-MES13 cells for dual-luciferase reporter assay. The results showed that luciferase activity was strikingly decreased in cells.
transfected with miR-185-5p mimic and Gm26917-wt-1/2/4 (Fig. 4C–4F). RIP assay showed that both Gm26917 and miR-185-5p could be enriched by Ago2 binding protein compared to IgG (Fig. 4G). Moreover, pull-down assay presented that bio-miR-185-5p-wt could pull a large amount of Gm26917 down (Fig. 4H). In addition, Gm26917 knockdown notably promoted the expression of miR-185-5p, and Gm26917 overexpression notably depleted the expression of miR-185-5p (Fig. 4I). The evidence indicated that miR-185-5p was a target of Gm26917. Then, we found the expression of miR-185-5p was decreased in HG-treated SV40-MES13 cells but recovered by RSG, while further Gm26917 overexpression largely sequestered miR-185-5p expression (Fig. 4J).

**Gm26917 knockdown suppressed HG-induced proliferation, oxidative stress, inflammation and ECM accumulating in SV40-MES13 cells by enriching miR-185-5p**

The inhibitor of miR-185-5p could significantly reduce the expression of miR-185-5p in SV40-MES13 cells (Fig. 5A). Then, HG-treated SV40-MES13 cells were transfected with sh-Gm26917 alone or sh-Gm26917 + anti-miR-185-5p together, using sh-NC or sh-Gm26917 + anti-miR-NC as the corresponding control. The data showed that cell viability was largely decreased in HG-treated SV40-MES13 cells transfected with sh-Gm26917 alone but recovered in HG-treated SV40-MES13 cells transfected with sh-Gm26917 + anti-miR-185-5p (Fig. 5B). In HG-treated SV40-MES13 cells, Gm26917 knockdown induced cell cycle arrest, while further miR-185-5p inhibition relieved cell cycle arrest (Fig. 5C and 5D). The level of ROS was suppressed in HG-treated SV40-MES13 cells transfected with sh-Gm26917 but restored in HG-treated SV40-MES13 cells transfected with sh-Gm26917 + anti-miR-185-5p (Fig. 5E). The activity of SOD was promoted by Gm26917 overexpression in HG-induced SV40-MES13 cells, while additional miR-185-5p deficiency partly impaired SOD activity (Fig. 5F). Moreover, the releases of inflammatory cytokines (IL-6, IL-1β and TNF-α) were suppressed by Gm26917 overexpression in HG-treated SV40-MES13 cells, while further miR-185-5p inhibition promoted the releases of these cytokines (Fig. 5G). The protein levels of fibronectin and collagen IV in HG-treated SV40-MES13 cells were weakened by alone sh-Gm26917 transfection but enhanced by combined sh-Gm26917 + anti-miR-185-5p transfection (Fig. 5H). These data presented that miR-185-5p inhibition reversed the effects of Gm26917 knockdown, hinting that Gm26917 knockdown suppressed HG-induced proliferation, oxidative stress, inflammation and ECM accumulating in SV40-MES13 cells by enriching miR-185-5p.

**Discussion**

Our present study mainly explored the effects of RSG in DN cell models and found that RSG largely alleviated HG-induced mouse mesangial cell (SV40-MES13) proliferation, oxidative stress, inflammatory responses and ECM accumulation. Besides, we found that Gm26917 expression was reinforced by HG but depleted by RSG, and Gm26917 overexpression abolished the effects of RSG, while Gm26917 knockdown also alleviated HG-induced mouse mesangial cell (SV40-MES13) proliferation, oxidative stress, inflammatory responses and ECM accumulation. Furthermore, miR-185-5p was identified to be a target of Gm26917, and miR-185-5p inhibition reversed the effects of Gm26917 knockdown. Our study provided a new mechanism that RSG governed the Gm26917/miR-185-5p pathway to inhibit the progression of DN.

RSG blocked the progression of DN through multiple biological processes. Previous studies recorded that RSG treatment inhibited the expression of the markers of podocyte loss, glomerular fibronectin accumulation and oxidative injuries, thereby preventing the development of DN [21]. RSG treatment reduced the level of serum creatinine and albuminuria, glomerulosclerosis, tubulointerstitial injury and macrophage infiltrating in DN mouse models [22]. RSG was also reported to inhibit DN progression by lessening the production of ROS and the expression of nuclear factor-kappaB and monocyte chemoattractant protein-1 (MCP-1) [23]. Consistent with the result from these findings, we discovered that RSG treatment ameliorated DN development by suppressing HG-induced mesangial cell proliferation, oxidative stress, inflammatory responses and ECM accumulation. Sufficient evidence indicated that RSG therapy prevented the progression of DN.

Numerous studies revealed the deregulation of IncRNAs in DN. For example, IncRNA Gm6135 was significantly upregulated in HG-cultured mouse mesangial cells, and Gm6135 downregulation was shown to suppress mesangial cell proliferation [24]. IncRNA GAS5 was downregulated in HG-treated mouse mesangial cells (RAW264.7), and GAS5 overexpression inhibited mesangial cell proliferation and fibrosis [25]. Though several IncRNAs are functionally explored in DN models, there are still a large number of IncRNAs whose functions are unknown. Gm26917 was reported to upregulated in renal cortical tissues from DN mouse models, while its expression was reduced after RSG treatment [15].
proliferation and survival of muscle satellite cells [26]. Our study for the first time investigated the role of Gm26917 in DN cell models, and we found that Gm26917 was highly expressed in HG-induced SV40-MES13 cells, while the treatment of RSG reduced Gm26917 expression. Functional analyses presented that
Fig. 5 Gm26917 knockdown alleviated HG-induced proliferation, oxidative stress, inflammation and ECM accumulation in SV40-MES13 cells by increasing miR-185-5p expression. (A) The efficiency of miR-185-5p inhibitor was checked by qPCR. In SV40-MES13 cells treated with NG, HG, HG + sh-NC, HG + sh-Gm26917, HG + sh-Gm26917 + anti-miR-NC, or HG + sh-Gm26917 + anti-miR-185-5p, (B) cell viability was determined by CCK-8 assay. (C and D) Cell cycle progression was determined by flow cytometry assay. (E and F) ROS production and SOD activity were examined using matched kit to assess oxidative stress. (G) The levels of L-6, IL-1β and TNF-α were detected using ELISA kits. (H) The protein levels of fibronectin and collagen IV were measured by western blot. *p < 0.05.
Gm26917 knockdown suppressed HG-induced proliferation, oxidative stress, inflammation and ECM accumulation, while Gm26917 overexpression partly abolished the effects of RSG and recovered these injuries, suggesting that Gm26917 might contribute to the development of DN.

To explore the mechanism of Gm26917 function in DN cell models, we investigated the target miRNAs of it. We found that miR-185-5p was a target of Gm26917, and its expression was declined in HG-treated SV40-MES13 cells. A previous study reported that miR-185-5p was downregulated in DN mice, HG-induced mouse mesangial cells and HG-induced human kidney tubular cells, and miR-185-5p deficiency promoted ECM accumulation and inflammatory responses [27, 28]. We speculated that miR-185-5p participated in DN-related injuries through Gm26917-mediated manner, which was verified by rescue experiments. The data showed that miR-185-5p inhibition reversed the effects of Gm26917 knockdown and recovered HG-induced proliferation, oxidative stress, inflammation and ECM accumulation in SV40-MES13 cells. In addition, TGF-βG was a special target of miR-185-5p [27], and TGF-β1 was widely reported to be implicated in RSG-alleviated DN injuries [13, 29]. Hence, TGF-β1 might be a key downstream target of the Gm26917/miR-185-5p pathway, and their co-interactions were involved in RSG-mediated DN inhibition. This hypothesis needed to be further confirmed in the future work.

Collectively, RSG attenuated HG-induced proliferation, oxidative stress, inflammatory responses and ECM accumulation in mouse mesangial cells partially by mediating the Gm26917/miR-185-5p pathway (Fig. 6). Our study provided new insights into the role of RSG in DN and expands the application of PPAR-γ agonists as promising interventions for the treatment of DN. However, further in vivo experiments should be conducted to verify these findings.

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Disclosure of Interest

The authors declare that they have no conflicts of interest.

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