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Research Article

MicroRNA-451 inhibits inflammation and proliferation of glomerular mesangial cells through down-regulating PSMD11 and NF-κB p65

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The present study aimed to investigate the regulatory roles of microRNA-451 (miR-451) on the inflammation and proliferation of glomerular mesangial cells (GMCs) under high-glucose condition, and reveal the potential mechanisms related to 26S proteasome non-ATPase regulatory subunit 11 (PSMD11) and nuclear factor-κ B (NF-κB) signaling. The interaction between PSMD11 and miR-451 was identified by dual luciferase reporter (DLR) gene assay. GMCs were treated with 5.6 mmol/l (normal, L-GMCs) and 30 mmol/l glucose (high-glucose, H-GMCs), respectively. After transfecting with pcDNA3.1-PSMD11 and/or miR-451 mimics, the expression of miR-451, PSMD11, inhibitor of NF-κB α (IκBα), phosphorylated IκBα (p-IκBα), NF-κB p65, COX-2, and cyclinD1 were detected in H-GMCs by quantitative real-time PCR (qRT-PCR) and/or Western blot. The levels of interleukin (IL)-1β, IL-6, and IL-8, cell cycle, and viability was detected by enzyme-linked immunosorbent assay, flow cytometry, and MTT assay, respectively. MiR-451 was up-regulated in H-GMCs, and negatively regulated its target PSMD11 ($P<0.05$). H-GMCs exhibited significantly higher levels of IL-1β, IL-6, and IL-8, cell viability, and p-IκBα, NF-κB, COX-2, and cyclinD1 expression than L-GMCs ($P<0.05$). The transfection of miR-451 mimics significantly decreased the levels of IL-1β, IL-6, and IL-8, inhibited the cell viability via blocking cells in G_0/G_1 phase, and down-regulated p-IκBα, NF-κB p65, COX-2, and cyclinD1 in H-GMCs ($P<0.05$). The regulatory effects of miR-451 mimics on H-GMCs were reversed by the transfection of PSMD11 ($P<0.05$). The up-regulation of miR-451 inhibits the inflammation and proliferation of H-GMCs through down-regulating PSMD11 and NF-κB p65.

Introduction

Diabetic nephropathy (DN) is a common disease that occurs in approximately 30% of type 2 diabetic patients [1]. DN is mainly characterized by mesangial proliferation, glomerular basement membrane thickening, and podocyte loss [2,3]. In clinical practice, glycemic control, blood pressure control, and renoprotection are the main therapeutic strategies for DN [4]. With increasing knowledge of the pathogenesis of DN, molecular targeting therapy has become a promising therapeutic strategy for DN [5].

MicroRNAs (miRs) are important post-transcriptional regulators in DN [6]. A previous study has proved that miR-192, -216a, -217, -21, -377, -195, -215, -124, -29c, and -135a are up-regulated, and miR-200a, -29a/b, -451, -25, and -93 are down-regulated in DN [7]. MiR-192, -29c, -135a, and -21 are considered as the therapeutic targets for DN [8]. MiR-451 also plays a key regulatory role in DN [9–11]. It has been reported that microRNA-451 (miR-451) expression is decreased in the kidney, peripheral blood

mononuclear cells (PBMCs), as well as glomerular mesangial cells (GMCs) under high-glucose condition [9,10]. The up-regulation of miR-451-5p protects against diabetes-induced kidney fibrosis in DN rats [11]. However, researches on the specific regulatory roles and mechanisms of miR-451 on GMCs are still limited.

Nuclear factor-κ B (NF-κB) is activated in DN patients, experimental animal models of DN, and GMCs under high-glucose condition [12,13]. Various potential therapeutic agents have been identified to ameliorate DN via inhibiting NF-κB, such as caprylic acid-diacylglycerol oil [14], ellagic acid [15], and curcumin [16]. In addition, a previous study has proved that miR-451 inhibits NF-κB activity via targeting large multifunctional protease 7 (LMP7), thereby down-regulating pro-inflammatory molecules in mesangial cells [9]. However, the regulatory mechanisms of miR-451 related to NF-κB are still not fully revealed on GMCs.

The 26S proteasome non-ATPase regulatory subunit 11 (PSMD11) is a multicatalytic proteinase complex that plays a key regulatory role in proteasome activity in embryonic stem cells [17]. Until now, the knowledge on the regulatory roles of PSMD11 on DN is limited. In the present study, a specific interaction between PSMD11 and miR-451 was first identified. Then the regulatory roles of miR-451 on the inflammation and proliferation of GMCs under high-glucose condition were evaluated. Furthermore, the regulatory mechanisms of miR-451 relating with PSMD11 and NF-κB were analyzed. Our findings may reveal a novel therapeutic target for DN, and open up new insights into the underlying mechanisms of DN.

Methods

Cell culture and treatments

GMCs (SV40-MES-13) were purchased from The Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), and maintained in a humidified incubator at 37°C with 5% CO_2. Cells were passaged until 80–90% confluence. GMCs at the third passage were randomly divided into two groups, including L-GMCs (normal physiological environment, 5.6 mmol/l glucose) and H-GMCs (high-glucose environment, 30 mmol/l glucose).

Cell transfection

MiR-451 mimics, miR-451 mimics negative control (mimics NC), and pcDNA3.1-PSMD11 were purchased from Guangzhou Ruibo Biotechnology Co., Ltd. (Guangzhou, China). GMCs in logarithmic growth phase were seeded in six-well plates, and then transfected with miR-451 mimics, mimics NC, pcDNA3.1-PSMD11, and miR-451 mimics + pcDNA3.1-PSMD11 using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, U.S.A.), respectively. The transfected cells were used for further assays after the transfection for 48 h.

Quantitative real-time PCR

Total RNA was extracted from GMCs using TRIzol, and cDNA was reverse-transcribed using a cDNA Reverse Transcription Kit (Thermo Fisher Scientific) in accordance with manufacturers' instructions. Quantitative real-time PCR (qRT-PCR) was performed by using specific primers (miR-451-F, 3′-CCGAAACCGTTACCATTAC-5′; miR-451-R, 3′-GTGCAGGGTCCGAGGT-5′; PSMD11-F, 3′-AGTTCCAGAGAGCCCAGTCT-5′; PSMD11-R, 3′-TTGCACTGCCTCTTCATCGT-5′) on ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A.). GAPDH was used as an internal control (GAPDH-F, 3′-TGACTTCAACAGAGACACCA-5′; GAPDH-R, 3′-GCTGTTGGGCTGTAGGGA-5′). The PCR program included 95°C for 10 min, 40 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 34 s. The relative expression level was calculated according to the $2^{-\Delta\Delta C_t}$ method [18].

Western blot

GMCs were lysed in RIPA lysis buffer (Beyotime, Shanghai, China). Total proteins were separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, transferred to polyvinylidenefluoride membrane, and blocked with 5% skim milk for 2 h. Then the membrane was incubated with specific primary antibodies (rabbit anti-mouse; anti-PSMD11, #14303; anti-proliferating cell nuclear antigen (PCNA), #13110; anti-inhibitor of NF-κB α (IκBα), #4812; anti- phosphorylated IκBα (p-IκBα), #2859; anti-NF-κB p65, #8242; anti-Histone H3, #9728; anti-COX-2, #4842; anti-cyclinD1, #2922; anti-GAPDH, #5174; 1:1000, Cell Signaling Technology, Boston, U.S.A.) for 12 h at 4°C. After incubating with horseradish peroxidase (HRP)–conjugated secondary antibody (goat anti-rabbit; 1:2000, Cell Signaling Technology) for 1 h at 25°C, the protein brands were visualized using an HRP color development kit (Thermo Fisher Scientific). The protein level was normalized to GAPDH, and standardized to L-GMC group (set as 1).

Dual luciferase reporter gene assay

HEK-293T cells (Cell Bank of Chinese Academy of Sciences) were co-transfected with pGL3 luciferase plasmids (Promega, Madison, WI, U.S.A.) carrying PSMD11-wildtype (PSMD11-WT)/PSMD11-mutant (PSMD11-MUT) and miR-451 mimics/mimics NC for 24 h. The fluorescence was visualized using a Dual Luciferase Reporter (DLR) Assay Kit (Promega, Madison, WI, U.S.A.). The fluorescence intensity was detected by using a Microplate Reader (Molecular Devices, Sunnyvale, CA, U.S.A.).

Enzyme-linked immunosorbent assay

Interleukin (IL)-1β, IL-6, and IL-8 were detected in GMCs by using Enzyme-linked immunosorbent assay (ELISA) kits (Boster, Wuhan, China) in accordance with manufacturers' instructions. The optical density (OD) at 450/550 nm was detected by a Microplate Reader (Molecular Devices).

Flow cytometry

GMCs were fixed in 70% ethanol for 12 h at 4°C, and washed with phosphate buffer saline (PBS). After incubating with Muse Cell Cycle Reagent (Millipore, U.S.A.) in the dark for 30 min, cells were analyzed on MUSE cell analyzer (Millipore).

MTT assay

GMCs were seeded in 12-well plates, and incubated with MTT (Sigma, U.S.A.) for 4 h. Then the medium was removed, and DMSO was added. The OD at 495 nm was detected by Microplate Reader (Molecular Devices).

Immunofluorescence

GMCs were fixed in 4% paraformaldehyde for 20 min at 4°C, and blocked with 5% BSA for 30 min. Then cells were incubated with primary antibody (anti-Ki67, #9449, 1:500, Cell Signaling Technology) overnight at 4°C. After three times of washing with PBS, cells were incubated with Alexa Fluor 594–conjugated secondary antibody (1:500, Cell Signaling Technology) for 1 h at 37°C, and then stained with 4,6-diamino-2-phenylindole (DAPI). Stained cells were observed under fluorescence microscope (Olympus).

Statistical analyses

All data were expressed as mean $\pm$ standard deviation. Comparison between different groups was determined by t test (two groups) or one-way ANOVA (>two groups) using SPSS version 21.0 (SPSS Inc., Chicago, IL, U.S.A.). A P-value < 0.05 represented significant difference.

Results

PSMD11 was a target of miR-451

A binding site of miR-451 was predicted at 3′-UTR of PSMD11 by an online target gene prediction software (TargetScan) (Figure 1A). DLR assay showed that the fluorescence intensity was significantly lower in HEK-293T cells co-transfected with PSMD11-WT + miR-451 mimics (0.589 ± 0.047) than those co-transfected with PSMD11-MUT + miR-451 mimics (0.968 ± 0.051), PSMD11-MUT + mimics NC (0.965 ± 0.065), and COL1A1-WT + mimics NC (1.000 ± 0.064) (P<0.05) (Figure 1B).

miR-451 negatively regulated PSMD11 in H-GMCs

The expression of miR-451 and PSMD11 were detected in H- and L-GMCs. qRT-PCR showed that miR-451 expression was significantly lower in H-GMCs (0.443 ± 0.085) than in L-GMCs (1.000 ± 0.165) at the mRNA level (P<0.05) (Figure 2A). PSMD11 expression was significantly higher in H-GMCs (mRNA, 2.620 ± 0.098; protein, 1.837 ± 0.095) than in L-GMCs (mRNA, 1.000 ± 0.180; protein, 1.000 ± 0.160) at both the mRNA and protein levels (P<0.05) (Figure 2B,C). The transfection of miR-451 mimics significantly up-regulated miR-451 (5.463 ± 0.206), and down-regulated PSMD11 (mRNA, 1.593 ± 0.238; protein, 1.257 ± 0.065) (P<0.05) in H-GMCs. The down-regulated PSMD11 was recovered by the transfection of PSMD11 in miR-451 mimics-transfected H-GMCs (mRNA, 2.55 ± 0.125; protein, 1.837 ± 0.085) (P<0.05). However, miR-451 expression was not significantly influenced by the transfection of PSMD11 in miR-451 mimics-transfected H-GMCs. Both the expression of miR-451 and PSMD11 were not significantly influenced by the transfection of mimics NC (Figure 2A,C).
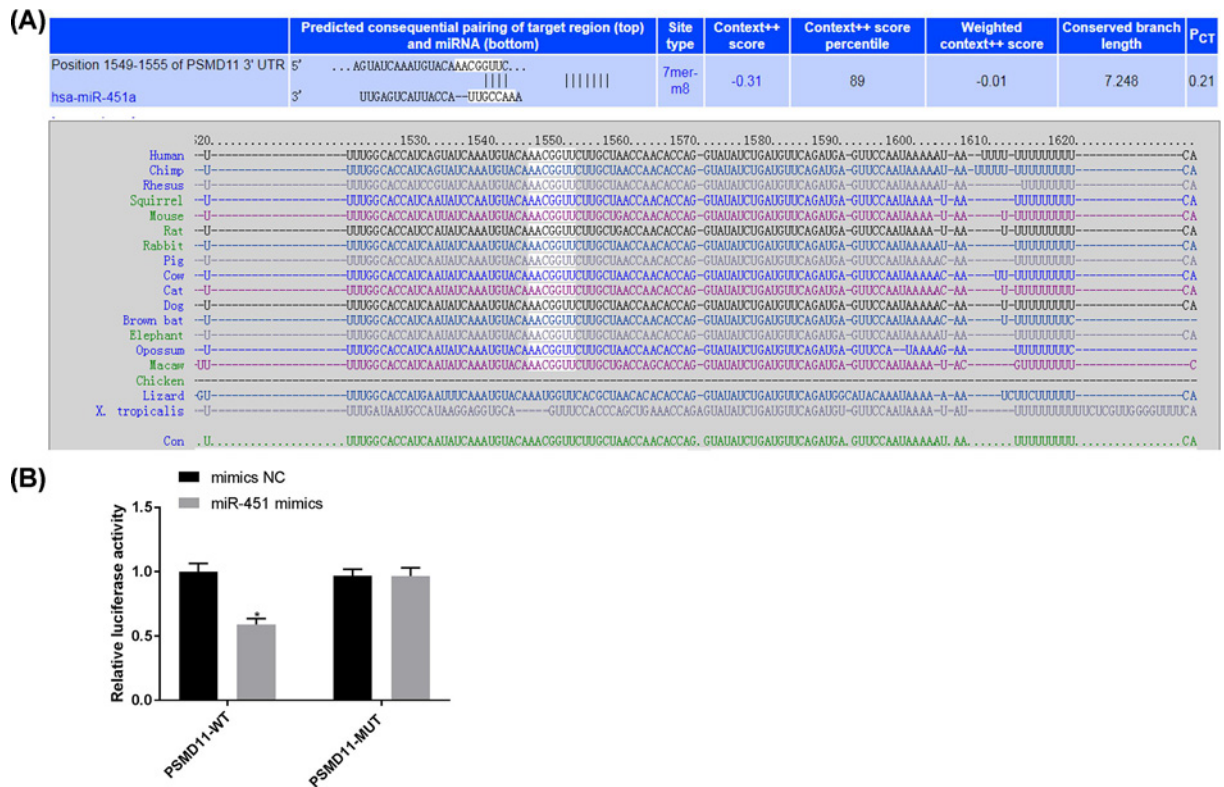
Figure 1. The interaction between miR-451 and PSMD11

(**A**) A binding site of miR-451 at 3′-UTR of PSMD11 predicted by the software of TargetScan. (**B**) Relative fluorescence intensity of HEK-293T cells co-transfected with PSMD11-WT (COL1A1-WT)/ PSMD11-MUT and miR-451 mimics/miR-451 mimics negative control (mimics NC). *$P<0.05$.

Up-regulation of miR-451 inhibited the inflammatory response of H-GMCs

The inflammatory response of GMCs was evaluated. As shown in Figure 3A–C, IL-1β, IL-6, and IL-8 levels were significantly higher in H-GMCs (IL-1β, 0.686 ± 0.055; IL-6, 0.687 ± 0.053; IL-8, 0.622 ± 0.075) than in L-GMCs (IL-1β, 0.256 ± 0.017; IL-6, 0.240 ± 0.029; IL-8, 0.210 ± 0.028) ($P<0.001$). The transfection of miR-451 mimics significantly decreased IL-1β, IL-6, and IL-8 levels in H-GMCs (IL-1β, 0.511 ± 0.018; IL-6, 0.440 ± 0.049; IL-8, 0.443 ± 0.056) ($P<0.01$). The increased IL-1β, IL-6, and IL-8 levels were recovered by the transfection of PSMD11 in miR-451 mimics-transfected H-GMCs (IL-1β, 0.691 ± 0.055; IL-6, 0.578 ± 0.059; IL-8, 0.630 ± 0.064) ($P<0.05$). IL-1β, IL-6, and IL-8 levels were not significantly influenced by the transfection of mimics NC (Figure 3A–C).

Up-regulation of miR-451 inhibited the proliferation of H-GMCs

The cell cycle and viability of GMCs were detected by flow cytometry and MTT assay, respectively. As shown in Figure 4A, significantly less cells in G_0/G_1 phase, and more cells in S phase were observed in H-GMCs (G_0/G_1, 20.59%; S, 38.05%) than in L-GMCs (G_0/G_1, 18.26%; S, 23.57%) ($P<0.05$). The transfection of miR-451 mimics significantly increased the percentage of cells in G_0/G_1 phase (20.02%), and decreased the percentage of cells in S phase (22.22%) ($P<0.05$) (Figure 4A). In addition, the cell viability was significantly higher in H-GMCs (1.663 ± 0.093) than in L-GMCs (1.000 ± 0.060) ($P<0.05$). The transfection of miR-451 mimics significantly decreased the viability of H-GMCs (1.338 ± 0.054) ($P<0.05$) (Figure 4B). Consistent with cell viability, PCNA (a cell proliferation marker) expression, and the number of Ki67 (a cell proliferation marker) positive cells were both significantly increased in H-GMCs (PCNA, 1.757 ± 0.076; Ki67, 1.913 ± 0.055) when compared with L-GMCs (PCNA, 1.000 ± 0.089; Ki67, 1.000 ± 0.103) ($P<0.05$). The up-regulated PCNA and Ki67 in H-GMCs was significantly decreased by the trans-fection of miR-451 mimics (PCNA, 1.260 ± 0.062; Ki67, 1.403 ± 0.083) ($P<0.05$) (Figure 4C,D). Noteworthily, the effects of miR-451 mimics on the cell cycle (G_0/G_1, 22.22%; S, 37.65%), cell viability (1.683 ± 0.056), as well as PCNA
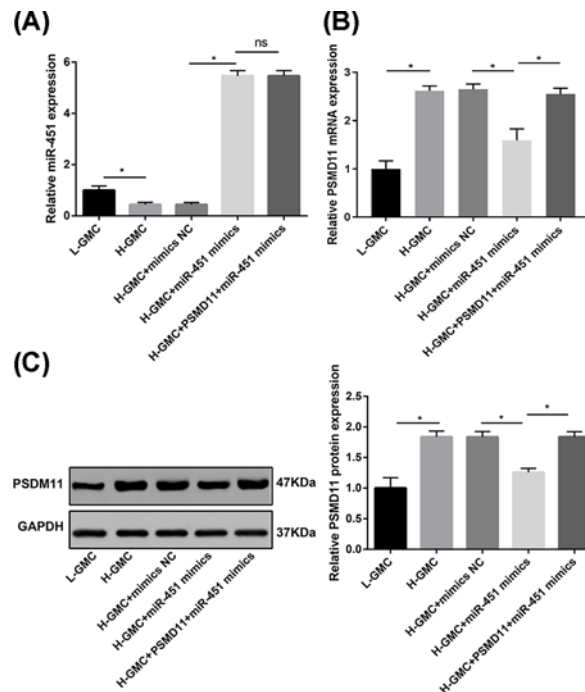
Figure 2. The expression of miR-451 and PSMD11 in GMCs

(**A**) The expression of miR-451 detected by qRT-PCR at the mRNA level. (**B**) The expression of PSMD11 detected by qRT-PCR at the mRNA level. (**C**) The expression of PSMD11 detected by Western blot at the protein level. L-GMCs, GMCs treated with 5.6 mmol/l glucose (normal control); H-GMCs, GMCs treated with 30 mmol/l glucose (high glucose); H-GMCs + mimics NC, H-GMCs transfected with miR-451 mimics negative control; H-GMCs + miR-451 mimics, H-GMCs transfected with miR-451 mimics; H-GMCs + PSMD11 + miR-451 mimics, H-GMCs transfected with pcDNA3.1-PSMD11 and miR-451 mimics. $*P < 0.05$.

and Ki67 expression (PCNA, 1.733 ± 0.123; Ki67, 2.007 ± 0.117) were significantly reversed by the transfection of PSMD11 in miR-451 mimics-transfected H-GMCs ($P < 0.05$) (Figure 4A,D).

Up-regulation of miR-451 inhibited NF-κB activation in H-GMCs

In order to reveal the regulatory mechanisms of miR-451 related to NF-κB signaling, the expression of IκBα, p-IκBα, NF-κB p65, as well as two NF-kB downstream targets COX-2 and cyclinD1 were detected. As shown in Figure 5A,B, the expression of p-IκB/IκB and NF-κB p65 was significantly higher in H-GMCs (p-IκB/IκB, 1.772 ± 0.125; NF-κB p65, 1.764 ± 0.118) than in L-GMCs (p-IκB/IκB, 1.000 ± 0.058; NF-κB p65, 1.000 ± 0.046) ($P < 0.05$). The transfection of miR-451 mimics significantly down-regulated p-IκBα and NF-κB p65 in H-GMCs (p-IκB/IκB, 1.315 ± 0.083; NF-κB p65, 1.374 ± 0.048) ($P < 0.05$). The down-regulated p-IκBα and NF-κB p65 were recovered by the transfection of PSMD11 in miR-451 mimics-transfected H-GMCs (p-IκB/IκB, 1.662 ± 0.106; NF-κB p65, 1.647 ± 0.114) ($P < 0.05$, Figure 5A,B). Consistent results with p-IκB/IκB and NF-κB p65 were also observed on the expression of COX-2 and cyclinD1 by Western blot (Figure 5C).

Discussion

DN is a serious diabetic complication that may lead to glomerular sclerosis and interstitial fibrosis [19]. Since miRs are closely associated with the regulation of inflammation, miR-targeted therapy exhibits a promising prospect in the treatment of DN [7,20]. MiR-451 is down-regulated in diverse tumors, including non-small-cell lung carcinoma [21], papillary thyroid carcinoma [22], neck squamous cell carcinoma [23], and renal cell carcinoma [24]. Noteworthily, miR-451 is also down-regulated in DN, such as the kidney of DN mice, PBMCs of DN patients, as well as GMCs under high-glucose condition [9,10]. Consistent with previous studies, we found that miR-451 expression was significantly lower in H-GMCs than in L-GMCs. Based on this result, miR-451 was up-regulated in H-GMCs by the transfection of miR-451 mimics, and the specific roles of miR-451 on the inflammation and proliferation of H-GMCs were further analyzed.
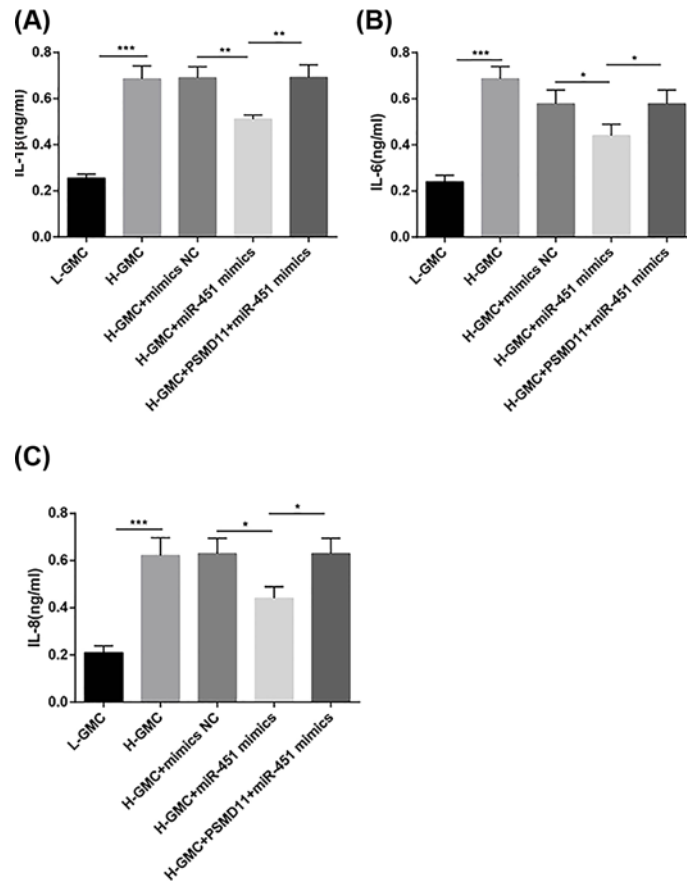
Figure 3. The levels of pro-inflammatory cytokines in GMCs

(**A**) IL-1β; (**B**) IL-6; (**C**) IL-8. L-GMCs, GMCs treated with 5.6 mmol/l glucose (normal control); H-GMCs, GMCs treated with 30 mmol/l glucose (high glucose); H-GMCs + mimics NC, H-GMCs transfected with miR-451 mimics negative control; H-GMCs + miR-451 mimics, H-GMCs transfected with miR-451 mimics; H-GMCs + PSMD11 + miR-451 mimics, H-GMCs transfected with pcDNA3.1-PSMD11 and miR-451 mimics. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

DN is a chronic low-grade inflammatory process, which is associated with the activation of pro-inflammatory cytokines [25]. In this study, significantly higher IL-1β, IL-6, and IL-8 levels were observed in H-GMCs than in L-GMCs. These results illustrate that the inflammatory response is enhanced by high-glucose in H-GMCs. Previous studies have proved that pro-inflammatory cytokines are negatively regulated by miR-451 [26,27]. For example, the transfection of miR-451 mimics decreases the levels of TNF-α, IL-1β, and IL-6 in synovial fibroblasts isolated from rheumatoid arthritis patients [26]. Overexpression of miR-451 inhibits microglia-induced release of IL-6, IL-1β, and TNF-α [27]. In this study, the transfection of miR-451 mimics significantly decreased IL-1β, IL-6, and IL-8 levels in H-GMCs. Our findings are just consistent with previous studies, and illustrate that the inflammatory response is relieved by the up-regulation of miR-451 in H-GMCs. In addition, it has been proved that some agents exhibit great potential in the treatment of DN via inhibiting pro-inflammatory cytokines, such as resveratrol [28], ligustrazine [29], notoginsenoside R1 [30], and ellagic acid [15]. We suspect that the up-regulation of miR-451 may protect against DN through relieving inflammatory response.

GMCs, the major constituents of the renal glomerulus are important in mesangial matrix homeostasis, glomerular filtration, and phagocytosis [31]. Excessive proliferation of GMCs is a major contributing factor to DN [32]. In this study, we found that H-GMCs exhibited significantly higher cell viability, as well as PCNA and Ki67 expression than L-GMCs. These phenomena illustrate that the proliferation of GMCs is enhanced by high glucose in H-GMCs. In addition, the transfection of miR-451 mimics significantly decreased the cell viability, and down-regulated PCNA and Ki67 in H-GMCs. These findings are just consistent with a previous study that miR-451 overexpression inhibits the proliferation of GMCs both *in vitro* and *in vivo* [33]. Up-regulation of miR-451 may inhibit the proliferation of H-GMCs by blocking cells in G_0/G_1 phase. Besides, previous studies have proved that some therapeutic agents
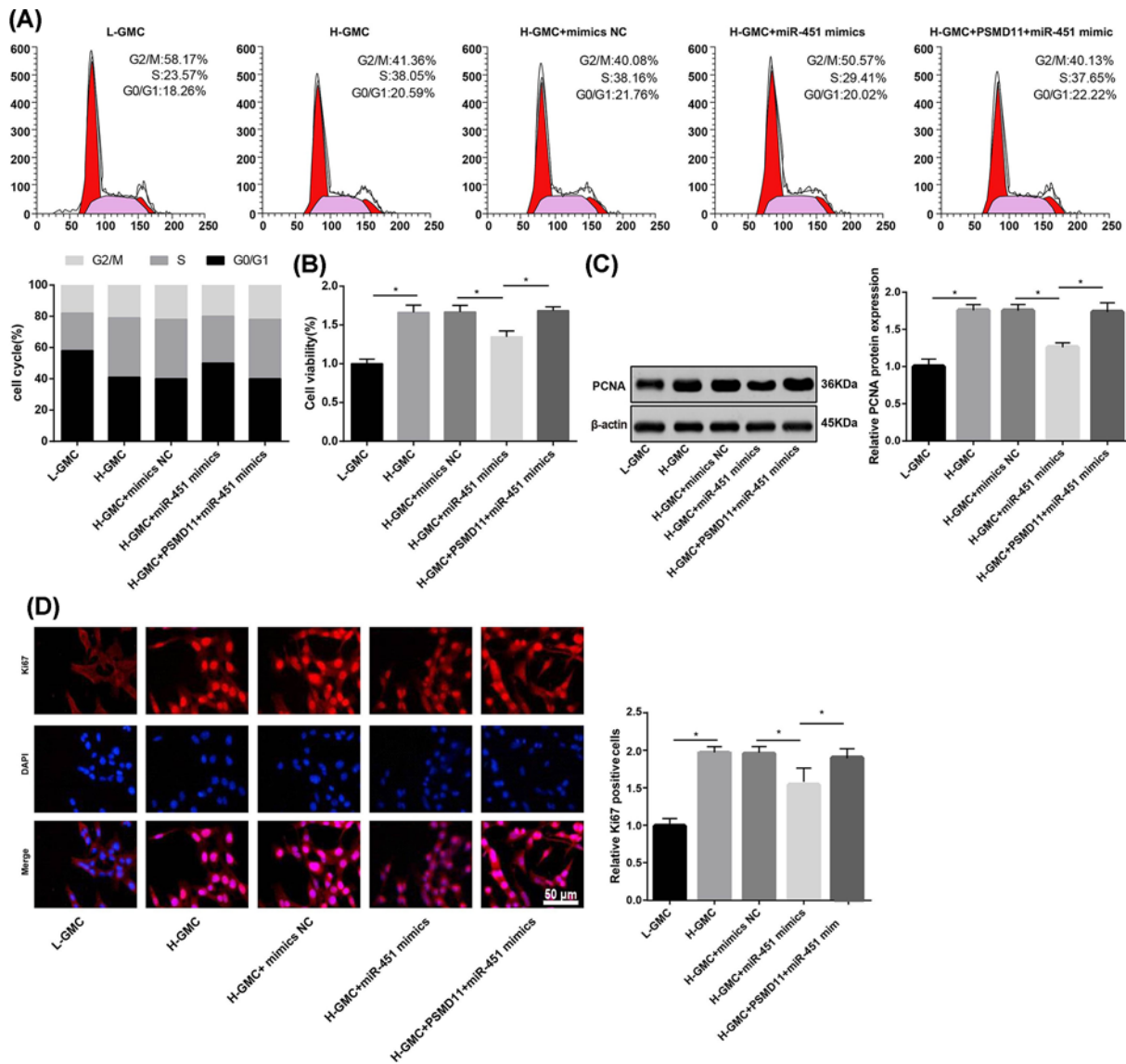
Figure 4. The proliferation of GMCs

(**A**) Cell cycle detected by flow cytometry. (**B**) Cell viability detected by MTT assay. (**C**) The expression of PCNA detected by Western blot at the protein level. (**D**) Relative Ki67 positive cells detected by immunofluorescence staining. L-GMCs, GMCs treated with 5.6 mmol/l glucose (normal control); H-GMCs, GMCs treated with 30 mmol/l glucose (high glucose); H-GMCs + mimics NC, H-GMCs transfected with miR-451 mimics negative control; H-GMCs + miR-451 mimics, H-GMCs transfected with miR-451 mimics; H-GMCs + PSMD11 + miR-451 mimics, H-GMCs transfected with pcDNA3.1-PSMD11 and miR-451 mimics. *$P<0.05$.

targeting DN are effective in the inhibition of GMCs proliferation, such as corosolic acid [31], betulinic acid [31], and triptolide [32]. We suspect that the up-regulation of miR-451 may ameliorate DN through inhibiting GMCs proliferation.

NF-κB signaling plays an important role in diverse biological processes, such as bipolar spindle assembly [34], vertebrate brain development and function [35], as well as cancer initiation and progression [36]. NF-κB is usually activated in DN, and also closely associated with inflammatory response [13]. In this study, the expression of p-IκBα and NF-κB p65 were significantly higher in H-GMCs than in L-GMCs. It is known that NF-κB p65 is activated by the degradation of IκBα, and then induces inflammatory response by binding to specific promoter of the target inflammatory genes [37,38]. Our findings illustrate that NF-κB is activated by high-glucose in H-GMCs. The activation of NF-κB contributes to the high levels of IL-1β, IL-6, and IL-8 in H-GMCs. In addition, miR-451 has been proved
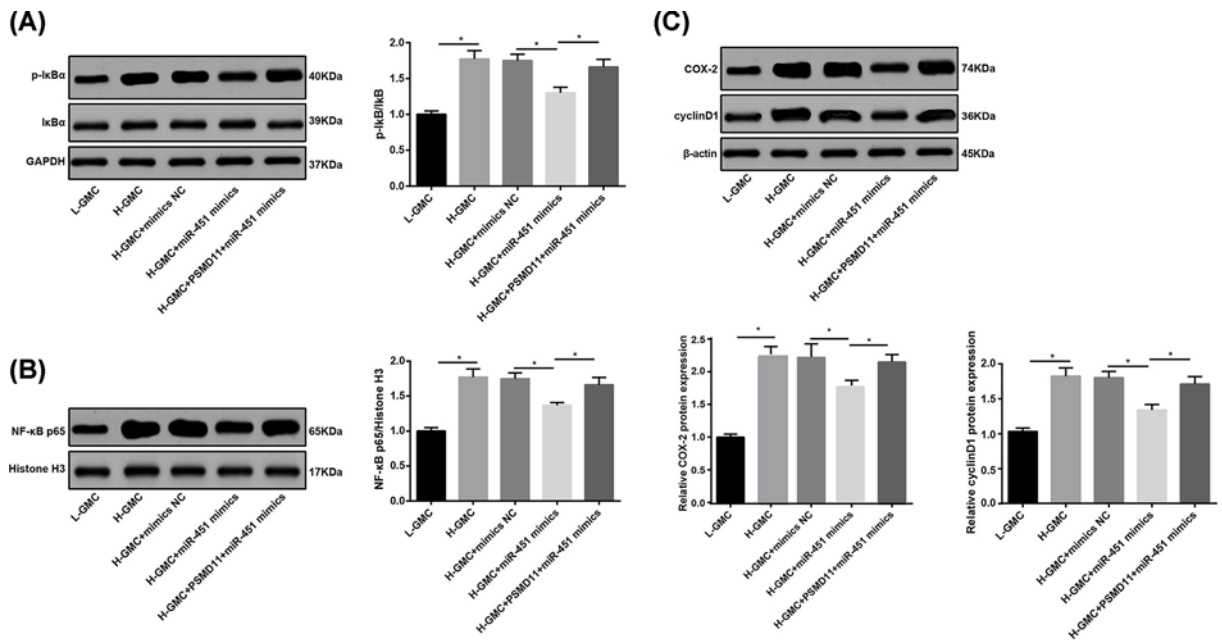
Figure 5. The expression of IκBα, p-IκBα, and NF-κB p65 in GMCs
(**A**) The expression of IκBα and p-IκBα detected by Western blot at the protein level. (**B**) The expression of NF-κB p65 detected by Western blot at the protein level. (**C**) The expression of COX-2 and cyclinD1 detected by Western blot at the protein level. L-GMCs, GMCs treated with 5.6 mmol/l glucose (normal control); H-GMCs, GMCs treated with 30 mmol/l glucose (high glucose); H-GMCs + mimics NC, H-GMCs transfected with miR-451 mimics negative control; H-GMCs + miR-451 mimics, H-GMCs transfected with miR-451 mimics; H-GMCs + PSMD11 + miR-451 mimics, H-GMCs transfected with pcDNA3.1-PSMD11 and miR-451 mimics. *$P<0.05$.

to inhibit pro-inflammatory molecules through negatively regulating NF-κB [9,39]. For example, up-regulation of miR-451 inhibits the NF-κB activity by targeting LMP7, thereby inhibiting the transcription of pro-inflammatory molecules in mesangial cells [9]. Overexpression of miR-451 inhibits the translocation of NF-κB p65 into the nucleus, thereby suppressing palmitate-induced production of proinflammatory cytokines in steatotic cells [39]. In consistent with previous studies, the transfection of miR-451 mimics significantly down-regulated p-IκBα and NF-κB p65 in H-GMCs. Our findings illustrate that the up-regulation of miR-451 may relieve the inflammatory response of H-GMCs via inhibiting the activation of NF-κB. In addition, miR-451 mimics-induced down-regulation of COX-2 and cyclinD1 (two NF-kB downstream targets involved in inflammation) further illustrate that miR-451 up-regulation blocks NF-κB singling in H-GMCs. Previous studies have proved that diverse agents targeting NF-κB singling can ameliorate DN *in vivo*, such as irbesartan [40], timosaponin B-II [41], caffeic acid *para*-nitro phenethyl ester [42], Jiangtang decoction [43], and berberine [44]. For example, irbesartan ameliorates metabolic abnormalities, renal dysfunction, and podocyte injury in type 2 DN mice through suppressing the RANKL-RANK-NF-κB pathway [40]. Timosaponin B-II ameliorates the renal histopathological injury and inflammation in alloxan-induced DN mice [41]. We suspect that miR-451 up-regulation may also contribute to the amelioration of DN *in vivo* through blocking NF-κB signaling. However, the present study is still limited in cellular level, and animal-based studies are needed.

PSMD11 is a 26S proteasome non-ATPase regulatory subunit required for proteasome assembly [17]. A previous study has proved that the knockdown of PSMD13 inhibits the production of proinflammatory mediators in lipopolysaccharide-stimulated BV2 microglia via inhibiting IκBα degradation and NF-κB activation [45]. However, the knowledge on the regulatory roles of PSMD11 on DN is greatly limited. In the present study, PSMD11 was identified as a target of miR-451. In contrast with miR-451, the expression of PSMD11 was significantly higher in H-GMCs than in L-GMCs. The transfection of miR-451 mimics significantly down-regulated PSMD11 in H-GMCs. These results indicate that PSMD11 is negatively regulated by miR-451 in H-GMCs. Since the transfection of PSMD11 could not influence miR-451 expression, PSMD11 may not feedback regulate miR-451 in H-GMCs. In addition, we found that the inhibitory effects of miR-451 mimics on the proliferation, inflammation, and NF-κB activation of H-GMCs were significantly reversed by the transfection of PSMD11. We suspect that PSMD11 may exert similar functions with

PSMD13 in H-GMCs. The down-regualtion of PSMD11 may also contribute to the amelioration of DN. However, the roles and regulatory mechanisms of PSMD11 on H-GMCs still need to be studied.

Conclusion

MiR-451 negatively regulated its target PSMD11. The up-regulation of miR-451 significantly inhibited the inflammation and proliferation of H-GMCs through down-regulating PSMD11 and NF-κB p65. The up-regulation of miR-451 may be a promising therapeutic target for DN.

Author Contribution

Hua Wei was involved in the conception and design of the study, and development of the manuscript. Jianzhou Li and Yanhua Li were involved in the conduct of the experiment and collection of data. Jian Song participated in the drafting or critical revision of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

Abbreviations

COL1A1, collagen type 1 alpha 1 chain; COX-2, cytochrome c oxidase subunit 2; DLR, dual luciferase reporter; DN, diabetic nephropathy; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GMC, glomerular mesangial cell; HRP, horseradish peroxidase; IL, interleukin; IκBα, inhibitor of NF-κB α; LMP7, large multifunctional protease 7; miR, microRNA; miR-451, microRNA-451; NF-κB, nuclear factor-κB; OD, optical density; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffer saline; PCNA, proliferating cell nuclear antigen; PSMD11, 26S proteasome non-ATPase regulatory subunit 11; PSMD11-MUT, PSMD11-mutant; PSMD11-WT, PSMD11-wildtype; p-IκBα, phosphorylated IκBα; qRT-PCR, quantitative real-time PCR; RANK, receptor activator of nulear factor κB; RANKL, receptor activator of nulear factor κB ligand.

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