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Quercetin attenuates the proliferation, inflammation, and oxidative stress of high glucose-induced human mesangial cells by regulating the miR-485-5p/YAP1 pathway

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Abstract

Background: Diabetic nephropathy (DN) is a kidney damage caused by diabetes and the main cause of end-stage renal disease. However, the current treatment of DN has many limitations. Quercetin is a bioflavonoid compound with therapeutic benefits in metabolic diseases. This study aims to determine the therapeutic potentials and underlying mechanism of quercetin on DN.

Methods: We collected blood samples from DN patients and healthy controls and treated human mesangial cells (HMCs) with high glucose (HG) to establish an *in vitro* model of DN. Then we assessed the expression difference of miR-485-5p as well as YAP1 in serum of DN patients and healthy controls and between HG-induced HMCs and control cells. qRT-PCR and western blot were performed to assess miR-485-5p and YAP1 expression levels; CCK-8 and ELISAs were used to examine cell proliferation, inflammation, and oxidative stress. Dual luciferase reporter assay was implemented to detect the binding of miR-485-5p and YAP1 mRNA sequence.

Results: Quercetin suppressed proliferation, inflammation, and oxidative stress of HMCs induced by HG. As for mechanism, miR-485-5p directly bound to YAP1 and inhibited YAP1 expression. The downregulation of miR-485-5p and upregulation of YAP1 were also observed in the serum of DN patients. Quercetin modulated miR-485-5p/YAP1 axis to regulate HG-induced inflammation and oxidative stress.

Conclusion: Quercetin inhibits the proliferation, inflammation, and oxidative stress of HMCs induced by HG through miR-485-5p/YAP1 axis, which might provide a novel treatment strategy for DN.

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Introduction

Diabetic nephropathy (DN) is a kidney disease caused by diabetes, which is a common complication of diabetic patients and the main cause of end-stage renal disease in the world. (1) The main characteristics of DN include the progressive decline and loss of renal function, the destruction of glomerular filtration barrier, and glomerular dysfunction. Subsequently, DN leads to a series of symptoms such as proteinuria, hematuria, and hypertension. In severe cases, it is life-threatening by causing renal failure.[2-4] Podocytes, a specialized epithelial cell in outer surfaces of glomerular capillaries, are implicated in the progression of DN.[5,6] High blood sugar often leads to podocyte apoptosis and increased urine protein.[7,8] Current treatments of DN has great limitations, and patients in terminal stage have impaired quality of life and high mortality rate.[9,10] Therefore, exploring the mechanism of DN progression is nontrivial for identifying molecular targets and developing targeted therapy for DN.

Noncoding RNAs (ncRNAs) contain different classes of RNAs that do not encode proteins, including lncRNAs, microRNAs (miRNAs), and small interfering RNAs.[11] The length of miRNAs is only 20–24 nucleotides.[12-14] Although miRNAs have no protein coding ability, they can regulate gene expression in many ways, thereby affecting cell proliferation, death, and the progression of a series of diseases.[15] miRNAs usually inhibit the expression of target genes through binding to 3′UTR of the mRNAs.[16-18] Recent studies found that the dysregulation of miRNAs is implicated in kidney disease. For example, the overexpression of miR-370 and miR-217 cause podocyte damage induced by high glucose (HG), which contributes to the development of DN.[19,20] In contrast, miR-485 is downregulated in serum of DN patients and inhibits the inflammation and proliferation of HG-induced human mesangial cells (HMCs).[21,22] MiR-485-5p also regulates the level of plasma triglycerides in diabetes.[23] However, there is no direct evidence showing the functional role of miR-485-5p in DN. Whether and how miR-485-5p regulates the progress of DN remains to be explored.

Yes-associated protein (YAP), also called YAP1, is a co-activator of gene transcription, which functions as an important molecular switch and downstream effector in Hippo signaling.[24,25] Studies have shown that YAP first binds to transcriptional co-activator with PDZ binding motif (TAZ), and then YAP/TAZ complex interacts with transcription factor TEAD to activate the expression of a series of target genes involved in cell growth, death, differentiation, and organ size.[26-30] For example, in the differentiation of osteoblasts, YAP1 promotes the differentiation of cells in the direction of osteogenesis.[31,32] In tumor biology, YAP1 promotes the development of various tumors, including triple-negative breast cancer[33] and non-small cell lung cancer.[34] In addition, YAP1 also plays a pivotal role in DN. YAP1 promotes kidney damage in type 2 DN, suggesting that targeting YAP1 may be a new strategy for DN treatment.[35] YAP1 also enhances diabetic renal interstitial fibrogenesis.[36] However, the regulatory mechanism of YAP1 expression in DN remains unclear.

Traditional Chinese medicine (TCM) is attracting increasing attention in ameliorating renal diseases due to its low toxicity and few side effects.[37,38] Quercetin is the main component of flavonoids in Chinese medicine,[39,40] which shows beneficial effects on many metabolic disorders, such as the treatment of abnormal glucose metabolism and insulin resistance.[41] Studies have shown that quercetin exhibits therapeutic effects on diabetes and can effectively ameliorate the symptoms of diabetic patients, such as hyperglycemia and dyslipidemia.[42] However, the therapeutic potentials of quercetin on DN and its mechanism in DN treatment need to be investigated.

In this study, we collected the blood samples of DN patients and healthy controls and detected the miR-485-5p expression pattern. In addition, we used HG-induced HMCs as a DN *in vitro* model. We treated HG-induced HMCs with quercetin to explore the effect of quercetin on the cell proliferation, inflammation, and oxidative stress. We also identified YAP1 as a target of miR-485-5p, which was upregulated in the serum of DN patients. Furthermore, we investigated the regulatory role of quercetin in miR-485-5p/YAP1 axis. Our data showed that quercetin modulated miR-485-5p/YAP1 axis to regulate HG-induced inflammation and oxidative stress, which might serve as a novel treatment strategy for DN.

Materials and methods

Patient samples

The blood samples of DN patients (n=25) and healthy controls (n=20) were collected in the Department of Nephrology, Jinshan Branch of Shanghai Sixth People's Hospital. The samples were collected from June 2019 to August 2020, and samples were stored at −80-degree deep freezer until analysis. All the patients and volunteers who provided the samples have been informed about the facts of this study and have signed the informed consent form. This study was approved by the

Medical Ethics Committee of Jinshan Branch of Shanghai Sixth People's Hospital (Approval number: jszxyy202011).

Inclusion criteria: All DN patients (25–65 years old) were type 2 diabetic with end-stage renal disease and diagnosed kidney damage (eGFR <60 mL/min and proteinuria). Their clinical criteria for diagnosis is type 2 diabetes, fasting blood glucose> 126 mg/dL, glycated hemoglobin (HbA1c) >6.5%); kidney damage, overt albuminuria: albumin excreted in urine over 24 h> 300 mg; microalbuminuria: 30–300 mg albumin in 24-h urine.

Exclusion criteria: (1) patients with type 1 diabetes or chronic renal disease; (2) infectious fever or leukocytosis; (3) acute cardiovascular and cerebrovascular events; (4) viral or autoimmune hepatitis or related liver disease; (5) acute diabetic complications; (6) malignancy; (7) patients without complete data; and (8) patients refused to provide informed consent.

Cell culture and transfection

HMCs were cultured in DMEM medium supplemented with 10% fetal bovine serum (Thermo Fisher Scientific/Gibco, Gaithersburg, MD, USA) as well as 100 U/mL penicillin and 100 μg/mL streptomycin. pcDNA3.1-YAP1 overexpression plasmids, miR-485-5p mimic, miR-485-5p inhibitor, or their matched controls were transfected into HMCs via Lipofectamine 2000™ (Invitrogen, Carlsbad, California, USA). 24 h after transfection, glucose and/or quercetin is added to the culture medium for 48 h. The detailed grouping conditions are as follows: NG (normal culture medium with normal glucose), HG (30 mM glucose medium), HG+QL (30 mM glucose medium +10 μM low-dose quercetin), HG+QM (30 mM glucose medium +20 μM medium-dose quercetin), HG+QH (30 mM glucose medium +40 μM high-dose quercetin), HG+QM+miR-485-5p inhibitor, and HG+QM+pcDNA3.1-YAP1 group.

The sequences of miRNA mimic and inhibitor were synthesized by RiboBio (Guangzhou, China) as follows:

miR-485-5p mimic: 5′-AGAGGCUGGCCGUGAUGAAUUC-3';

miR-485-5p inhibitor: 5′-GAAUUCAUCACGGCCAGCCUCU-3'

Cell counting Kit-8 (CCK-8) cell proliferation assay

HMCs were first transfected with pcDNA3.1-YAP1 overexpression plasmids or miR-485-5p inhibitor for 24 h and then treated with different concentrations of glucose (NG or HG) and/or different concentrations of quercetin (10, 20, or 40 μM) for 48 h. Subsequently, 10 μL CCK-8 reaction (Solarbio, CA1210, Beijing, China) solution was added into 96-well plate at the specified time point for 4-h incubation. The absorbance at 450 nm was detected by a Synergy H1 microplate reader (Winooski, Vermont, USA).[43]

Enzyme linked immunosorbent assay (ELISA)

The concentration of secreted protein was assessed by commercial ELISA kits depending on the method proposed by the manufacturer. For the detection of inflammation factors, tumor necrosis factor-α (TNF-α, KHC3011, Invitrogen, CA, USA), interleukin-6 (IL-6, KAC1261, Invitrogen, CA, USA), interleukin-1β (IL-1β, KAC1211, Invitrogen, CA USA), and oxidative stress indexes, malonic dialdehyde (MDA, S0131, Beyotime, Shanghai, China), superoxide dismutase (SOD, S0109, Beyotime, Shanghai, China) and glutathione peroxidase (GSH-px, S0052, Beyotime, Shanghai, China) in culture medium, the culture supernatant of HMCs after different treatments were collected and centrifuged to remove the cell debris. The clear supernatant was used for ELISA based on manufacturer's instructions. Subsequently, the optical density (OD) value was detected using a Synergy H1 microplate reader (Winooski, Vermont, USA). The standard curve was created based on the concentration and OD value of the standard molecule provided in the kit. Finally, the concentration of cytokines and oxidative molecules were calculated based on the standard curve.

Reverse transcription-quantitative PCR (RT-qPCR)

The extraction of RNA from venous blood was carried out using PAXgeneTM Blood RNA kit (Qiagen, Hilden, Germany), and all extraction steps were performed according to the instructions attached to the kit. The extraction of total RNA from the cells was performed using TRIzol reagent (Thermo Fisher Scientific) method. The extracted total RNA was washed with 75% ethanol and dissolved in DEPC water. NanoDrop™ 2000/2000c spectrophotometer was used to detect the concentration of RNA (DEPC water was used as a negative control). 5 μg of total RNA was reverse transcribed to cDNA using a Superscript III transcriptase kit (Invitrogen, 18080093). The quantification of cDNA was performed on the 7500 Real Time PCR System (Applied Biosystems/Life Technologies, Carlsbad, CA, USA) using SYBR premix EX TAQ II kit (Takara, Dalian, China). Experimental results were analyzed using the $2^{-\Delta\Delta Ct}$ method. GAPDH was used as the internal reference gene.[44] The primers were synthesized by Sangon Biotechnology Co, Ltd (Shanghai, China) as below: miR-485-5p (F: 5′-ACTTGGAGAGAGGCTGGC-3′, R: 5′- AAAAGAGAGGAGAGCCGTGT -3′; YAP1 (F: 5′-TTTTACCGCGTCTCCCTG

ATT -3′, R: 5′-AGAAACACCTGGGCTAGTAGAAA-3′); GAPDH (F: 5′-GACAGT

CAGCCGCATCTTCT-3′, R: 5′-GCGCCCAATACG ACCAAATC-3′).

Dual luciferase reporter experiment

The interaction between miR-485-5p and YAP1 mRNA was tested by dual luciferase reporter experiment. The YAP1 mRNA 3′UTR regions containing wide-type (WT) or mutant (MUT) binding site of miR-485-5p was cloned into pmirGLO luciferase reporter (Promega, Madison, WI, USA) to construct YAP1-WT and YAP1-MUT reporter plasmids. The cells were co-transfected with YAP1-WT or YAP1-MUT and miR-485-5p mimic or miR-NC as well as Renilla luciferase (Rluc) control plasmids. After 48 h, the Dual-Luciferase Reporter Assay Kit (Promega, E1910) was used to detect luciferase activity according to the manufacturer's instructions. The relative luciferase activity was normalized to that of Renilla luciferase.[45]

Western blot

HMCs were lysed by RIPA lysis buffer on ice. 30 min after lysis, the cell lysate was transferred to a 1.5 mL EP tube and centrifuged 15,000 × g at 4°C to remove cell debris. The BCA method (Thermo Fisher Scientific) was used for protein concentration determination. Then 4Xloading buffer was added to the cell lysate, and the protein sample was boiled at 100°C for 10 min. Subsequently, a total of 20 μg proteins in each sample was separated by SDS-PAGE and transferred to the PVDF membrane. After blocking with 5% skimmed milk for 1 h, the PVDF membrane was incubated with the primary antibodies: anti-YAP1 (CST, #14074, 1:1000) or anti-GAPDH (CST,#5174, 1:1000) overnight. The membrane was washed 3 times with TBST for 5 min each and then incubated with the secondary antibody with horseradish peroxidase label (CST, #7074, 1: 2000) for 1 h. The Pierce™ ECL Plus Western Blotting Substrate (Thermo Fisher Scientific, with A and B solution in the ratio of 1:1) was used for protein band development. GAPDH serves as the loading control.[46]

Statistical analysis

Statistical analysis of data was implemented using SPSS 13.0 software. GraphPad Prism 6.0. was used for the preparation of the figures, and data is presented as mean ± standard deviation (SD). The comparison of the difference between the experimental group and the control group was performed using Student's t test. The difference among multiple groups was compared using one-way analysis of variance (ANOVA). $p<0.05$ was considered statistically significant.

Results

Quercetin inhibits the proliferation, inflammation, and oxidative stress of HG-induced HMCs

We first established a HG-induced HMC model to simulate DN. To explore the effect of quercetin on proliferation, inflammation, and oxidative stress of HG-induced HMCs, we treated HMCs with NG (normal culture medium), HG (normal culture medium+30 mM glucose), or HG combined with increasing concentration of quercetin. CCK-8 assay was conducted to analyze cell proliferation. The proliferation of HG-induced HMCs was accelerated, while quercetin can significantly inhibit the growth of HMCs induced by HG in a dose-dependent manner (Figure 1(a)). HG-induced HMCs secreted more inflammatory factors including TNF-α, IL-1β, and IL-6 (Figure 1(b)) and exhibited higher level of oxidative molecule MDA (Figure 1(c)), while SOD and GSH-px levels were suppressed by HG (Figures 1(d) and (e)). Quercetin suppressed the levels of TNF-α, IL-1β, IL-6, and MDA induced by HG (Figure 1(b) and (c)), while promoted the secretion of SOD and GSH-px (Figure 1(d) and (e)). The above results suggest that quercetin exhibits a protective effect on HG-stressed HMCs.

miR-485-5p was upregulated by quercetin

To investigate the underlying mechanism of quercetin in regulating the inflammatory and oxidative response in HG-stressed HMCs, qRT-PCR was employed to detect the miR-485-5p expression in both DN patient samples and HG-stressed cells. The results revealed that the expression of miR-485-5p in peripheral blood of DN patients decreased to a large extent (Figure 2(a)). Consistently, miR-485-5p expression was significantly downregulated in HG-induced HMCs as compared to that in NG condition (Figure 2(b)). Interestingly, quercetin could rescue the miR-485-5p levels under HG stress in a dose-dependent manner (Figure 2(c)). Taken together, the above results indicate that quercetin upregulates the expression of miR-485-5p in HG-induced HMCs.

Quercetin suppresses the proliferation, inflammation, and oxidative stress of HG-induced HMCs by regulating miR-485-5p

To validate the functional role of miR-485-5p in mediating the protective effect of quercetin, we conducted rescue assays using miR-485-5p inhibitor. qRT-PCR analysis showed that the upregulation of miR-485-5p by quercetin could be suppressed by miR-485-5p inhibitor (Figure 3(a)).

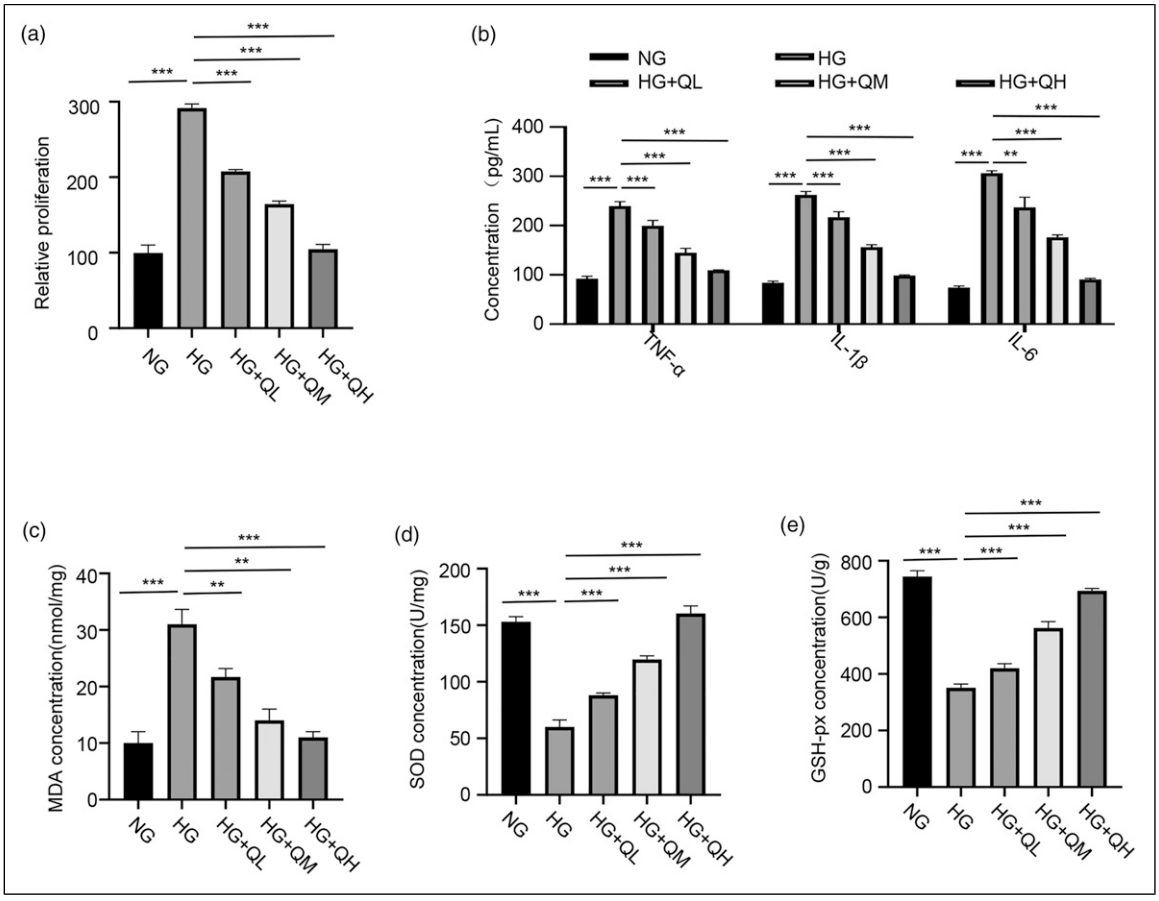

Figure 1. Quercetin suppresses proliferation and alleviates inflammation and oxidative stress of HG-induced HMCs. (a) Cell proliferation ability of HMCs in different groups was detected by CCK-8 cell proliferation assay. (b) The secreted inflammation factors TNF-α, IL-1β, and IL-6 of HMCs in different groups were assessed by ELISA. (c–e) The oxidative stress indexes MDA (c), SOD (d), and GSH-px (e) in cell culture supernatant were detected by ELISA. Data was presented according to three independent assays with three technical replicates. The error bars are defined as s.d. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

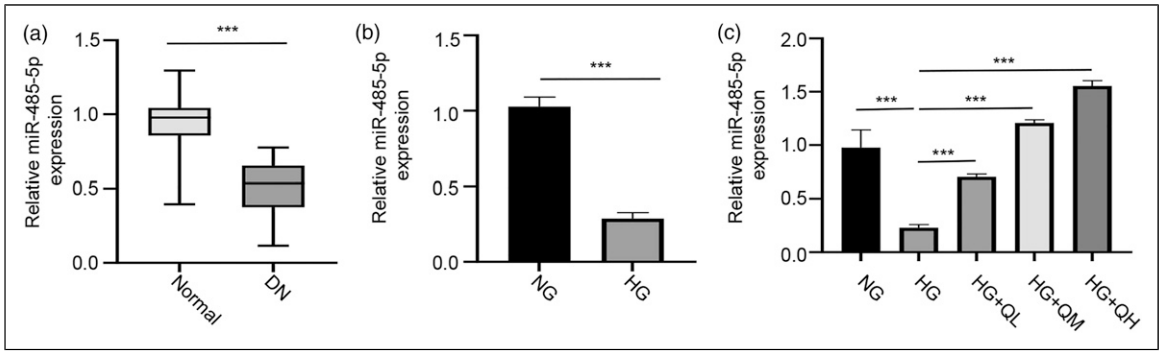

Figure 2. miR-485-5p expression levels were increased by quercetin. (a) miR-485-5p expression levels in DN patients and healthy controls were detected by qRT-PCR. (b and c) miR-485-5p expression levels in HMCs with indicated treatment were detected by RT-qPCR. Data was presented according to three independent assays with three technical replicates. The error bars are defined as s.d. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

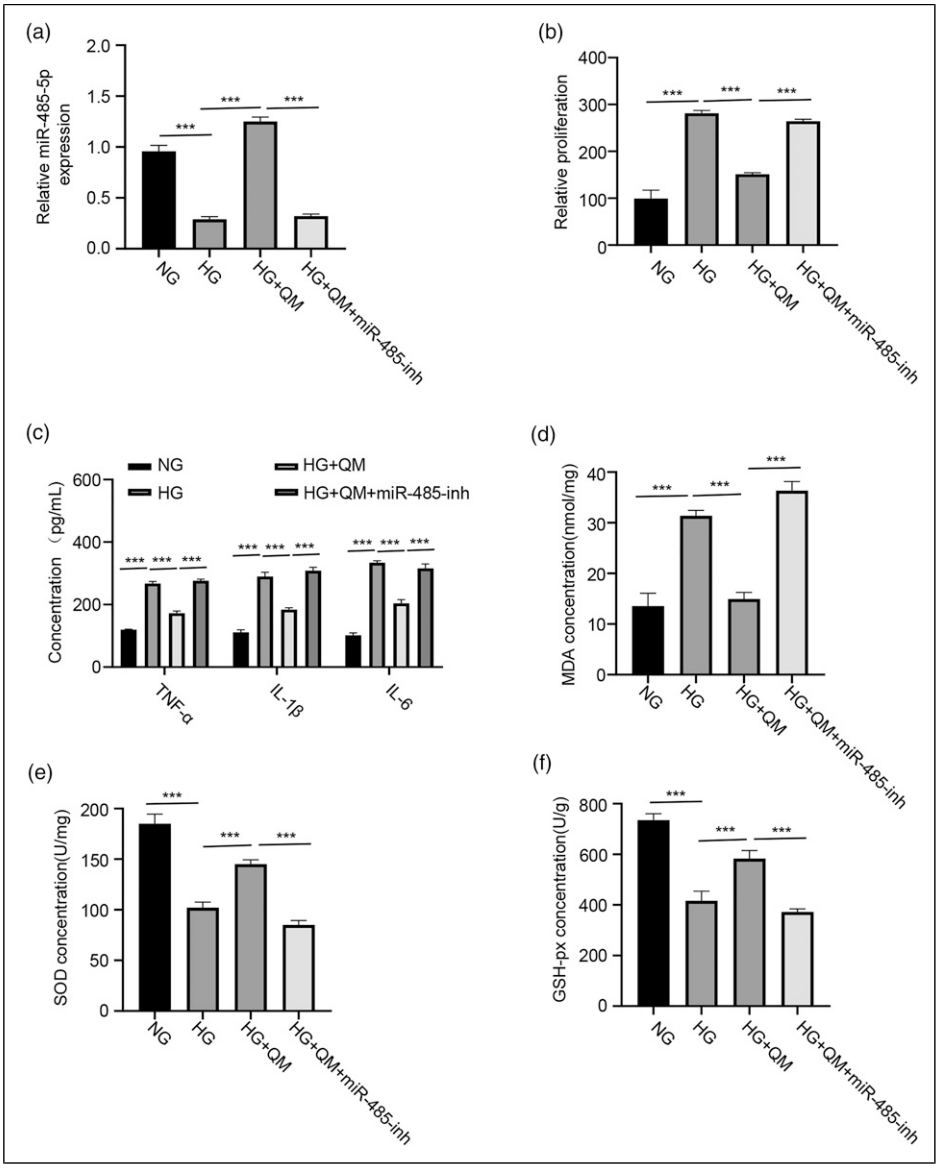

Figure 3. Quercetin exerts an inhibitory effect on proliferation, inflammation, and oxidative stress of HG-induced HMCs by controlling miR-485-5p expression. (a) miR-485-5p expression levels in HMCs treated as indication were detected by RT-qPCR. (b) HMCs proliferation ability in different treatment groups was detected using CCK-8 cell proliferation assay. (c) Inflammation factors secreted by HMCs treated as indication were analyzed by ELISA. (d–f) Oxidative stress indexes MDA (d), SOD (e), and GSH-px (f) in HMCs in different groups were assessed by ELISA. Data was presented according to three independent assays with three technical replicates. The error bars are defined as s.d. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

CCK-8 assay revealed that the anti-proliferation effect of quercetin upon HG stress could be rescued by miR-485-5p inhibitor (Figure 3(b)). ELISA showed that the protective effect of quercetin in alleviating inflammatory factors (TNF-α, IL-1β, and IL-6) and the MDA level under HG stress was largely abrogated by miR-485-5p inhibitor (Figure 3(c) and (d)). Consistently, miR-485-5p inhibitor could also suppress the elevation of SOD and GSH-px level induced by quercetin upon HG stress (Figure 3(e) and (f)). Taken together, these results indicate that miR-485-5p mediates the beneficial effect of quercetin under HG stress.

miR-485-5p binds and negatively regulates the expression of YAP1 mRNA

To identify the downstream regulatory target of miR-485-5p, we conducted bioinformatics analysis through Starbase database and found that there is a binding sequence

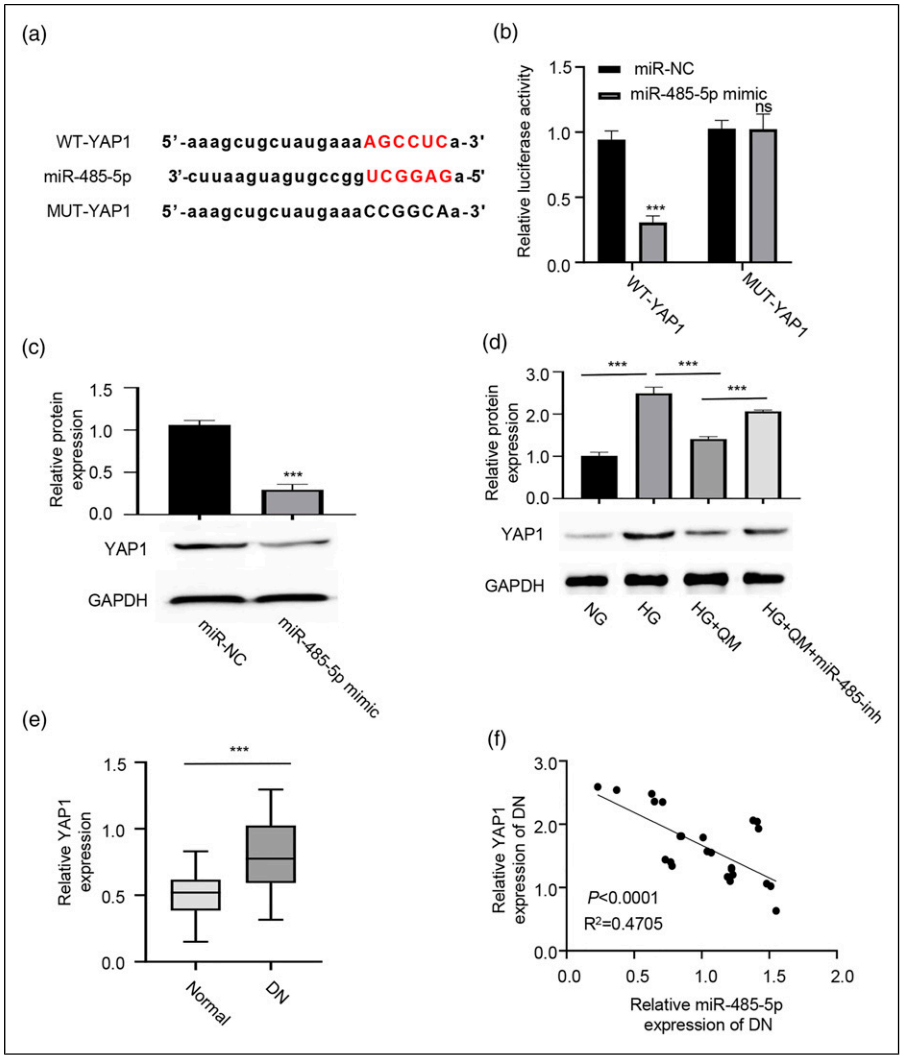

Figure 4. miR-485-5p suppresses YAP1 expression through binding to the 3′UTR of its mRNA. (a) The putative binding site of miR-485-5p and YAP1 was predicted through Starbase database. (b) The binding of miR-485-5p and YAP1 in HMCs were assessed by dual luciferase reporter assay. HMCs were co-transfected with luciferase reporter plasmids pmirGLO-WT-YAP1/pmirGLO-MUT-YAP1 in the presence of miR-485-5p/miR-NC as well as Renilla luciferase (Rluc) control plasmids for 48 h before luciferase activity detection. (c and d) YAP1 expression levels were detected by western blot after indicated treatments. (e) YAP1 mRNA levels in serum of DN patients and healthy controls were detected using qRT-PCR. (f) The correlation between miR-485-5p and YAP1 expression level was analyzed by Spearman's correlation analysis. Data was presented according to three independent assays with three technical replicates. The error bars are defined as s.d. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

between miR-485-5p and 3′UTR of YAP1 mRNA (Figure 4(a)). It was therefore speculated that YAP1 might be a target of miR-485-5p. To verify the binding between miR-485-5p and YAP1 mRNA, miR-485-5p-mimic/miR-NC and dual luciferase reporter plasmid containing YAP1-WT/YAP1-MUT as well as Rluc plasmid were co-transfected into HMCs. The results demonstrated that miR-485-5p mimic significantly reduced the luciferase activity of pmirGLO-YAP1-WT reporter but had no effect on pmirGLO-YAP1-MUT reporter (Figure 4(b)). We also found that the transfection of miR-485-5p mimic suppressed YAP1 expression (Figure 4(c)). In addition, HG induction promoted YAP1 expression, while quercetin suppressed YAP1 upregulation by HG. The presence of miR-485-5p inhibitor abrogated the effect of quercetin on YAP1 expression (Figure 4(d)). qRT-PCR analysis also revealed that YAP1 expression was upregulated in the blood of DN patients (Figure 4(e)). We further analyzed the expression levels of miR-485-5p and YAP1 in serum of DN patients and found a negative correlation between miR-485-5p and YAP1 expression (Figure 4(f)). Collectively, the above results indicate that miR-485-5p

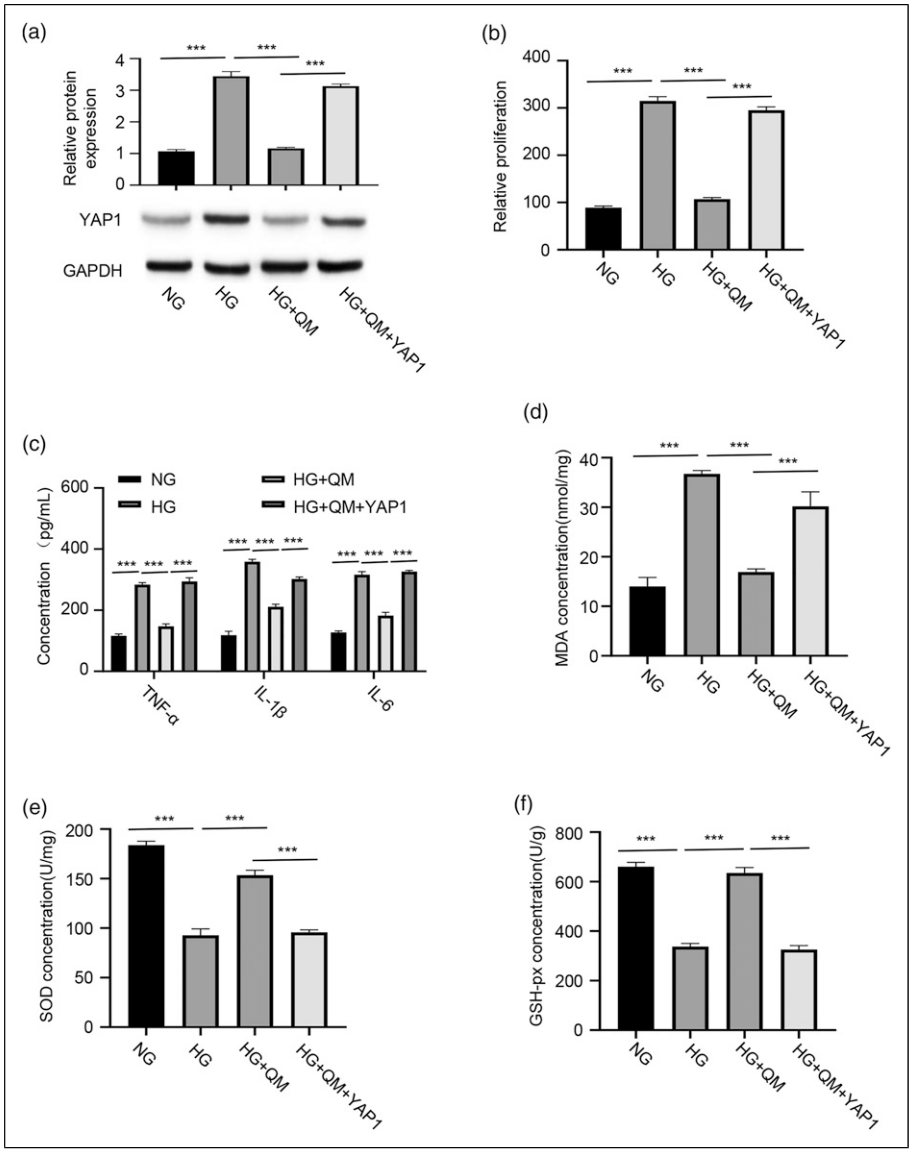

Figure 5. Quercetin suppresses the proliferation, inflammation, and oxidative stress of HG-induced HMCs through inhibiting YAP1 expression. (a) YAP1 protein levels in HMCs with indicated treatment were assessed by western blot. (b) Proliferation ability of HMCs within different groups was assessed by CCK-8 cell proliferation assay. (c) The levels of inflammatory factors secreted by HMCs were evaluated using ELISA. (d–f) The concentrations of oxidative stress indexes MDA (d), SOD (e), and GSH-px (f) in HMCs were evaluated by ELISA. Data was presented according to three independent assays with three technical replicates. The error bars are defined as s.d. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

downregulates YAP1 expression through binding to 3′UTR of YAP1 mRNA.

Quercetin inhibits the proliferation, inflammation, and oxidative stress of HG-induced HMCS by YAP1

Previous studies have shown that YAP1 is implicated in the occurrence and progression of DN.[47-49] To further clarify the role of YAP1 in the effect of quercetin under HG stress, we conducted western blot, CCK-8 cell proliferation, and ELISAs, which demonstrated that HG induction promoted YAP1 expression, cell proliferation ability, and inflammation factors as well as MDA (Figure 5(a)-(d)). However, quercetin suppressed the expression of YAP1, which could be restored by overexpressing YAP1 (Figure 5(a)). The protective effects of quercetin on cell proliferation, inflammatory factors (TNF-α, IL-1β, and IL-6), and oxidative molecule MDA were abrogated by YAP1 overexpression (Figure 5(b)-(d)). The overexpression of YAP1 also suppressed the upregulation of SOD and GSH-px by quercetin

(Figure 5(e) and (f)). Collectively, YAP1 mediates the protective effects of quercetin on proliferation, inflammation, and oxidative stress of HG-induced HMCs.

Discussion

Diabetes mellitus (DM) has an extremely high incidence worldwide and has become a public health threat.[50] A common complication of DM is DN, which eventually leads to severe end-stage renal disease.[51] The fluctuation of blood sugar level usually leads to the apoptosis of glomerular mesangial cells[52] and the reduction of glomerular filtration rate, which then transforms into chronic kidney disease (CKD).[53] Therefore, developing novel therapeutics is the key to improving the quality of life of DN patients. TCM is widely used in the treatment of DN[54] due to its low toxicity and few side effects.[38,55,56] Quercetin is a common component of TCM widely used in the treatment of metabolic-related diseases. Quercetin effectively regulates the balance of glucose and lipid metabolism in diabetes, thereby improving the clinical symptoms of diabetic patients.[41] Quercetin also improves lipid metabolism, thereby reducing early diabetic kidney damage and exerting a protective effect on early DN.[57] In addition, quercetin has a beneficial effect on lowering blood sugar and serves as an antioxidant in type 2 diabetes animal models.[42] Similarly, our research also showed that quercetin ameliorates the inflammation and oxidative stress of HG-induced HMCs, which indicates its potential efficacy for the treatment of DN.

In recent years, increasing numbers of ncRNAs are implicated in DN progression. For example, linc4930556M19Rik inhibits podocyte apoptosis by regulating miR-27a-3p and the downstream molecule metalloproteinase 3 (TIMP3).[58] LINC00162 promotes the progression of DN through the miR-383/HDAC9 signaling axis.[59] MiR-30a-5p improves the damage of immortalized rat podocytes.[60] In addition, studies have found that miR-485 was significantly reduced in DN patients' serum, and it can also suppress the proliferation and inflammation of HMCs.[21,22] However, there is little evidence for the potential role of miR-485-5p in DN progression. Here, we found that the expression of miR-485-5p was decreased in the serum of DN patients as well as in HMCs stressed by HG. More importantly, the rescue-of-function assays validated that miR-485-5p is a downstream molecule mediating the protective effect of quercetin in suppressing proliferation, inflammation, and oxidative stress of HG-induced HMCs.

In gastric cancer, miR-30a-5p directly targets YAP1 and inhibits YAP1 expression.[61] Furthermore, YAP1 is implicated in the progression of DN. Studies found that the activation of YAP1 causes kidney damage in DN[48] and accelerates the proliferation of glomerular mesangial cell (MC) and DN progression, as well as the epithelial–mesenchymal transition (EMT) and fibrosis of renal tubular epithelial cells.[49,62] Our study illustrated that YAP1 expression was upregulated in serum of patients with DN and HG-induced HMCs. Importantly, we found that miR-485-5p is an upstream regulator for YAP1, which negatively regulates YAP1 expression. Furthermore, we further performed rescue experiments and confirmed that miR-485-5p/YAP1 axis mediated the suppression function of quercetin on the proliferation, inflammation, and oxidative stress of HG-induced HMCs.

This study also has some limitations. We did not clarify whether quercetin directly targets miR-485-5p/YAP1 axis. Our experiments were only performed in HMCs but not in glomerular endothelial cells and human podocyte cell line. In addition, the functional role of miR-485-5p/YAP1 axis and its interplay with quercetin needs to be validated in mice model of DN. In addition, as the sample size is small, increasing the sample size can increase the statistical power of the analysis.

Conclusion

In summary, our study demonstrated the potentially beneficial effects of quercetin in the treatment of DN and its underlying mechanism. We used HG-induced HMCs as an *in vitro* model to confirm the protective effects of quercetin on the proliferation, inflammation, and oxidative stress upon HG stress. miR-485-5p inhibits the expression of YAP1 by binding to 3′UTR of YAP1 mRNA. The downregulation of miR-485-5p and upregulation of YAP1 was also observed in the serum of DN patients. Quercetin modulates miR-485-5p/YAP1 axis to regulate HG-induced inflammation and oxidative stress. This mechanism enriches the molecular regulation network of kidney damage caused by diabetes and provides new insights for the treatment of DN.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval

Ethical approval for this study was obtained from Jinshan Branch of Shanghai Sixth People's Hospital of Ethics Committee (jszxyy202011).

Informed consent

Written informed consent was obtained from all subjects before the study.

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