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

Huan Wan1,*, Yaping Wang2,*, Qingyun Pan2, Xia Chen3, Sijun Chen4, Xiaohui Li5 and Weiguo Yao6

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.

1Department of Radiotherapy Center, the Fifth Hospital of Wuhan, Wuhan, Hubei
2Department of Endocrinology, the Fifth Hospital of Wuhan, Wuhan, Hubei
3Department of Traditional Chinese Medicine, Yangpu Hospital, Tongji University School of Medicine, Shanghai
4Department of Nephrology, Yangpu Hospital, Tongji University School of Medicine, Shanghai
5Department of Pediatrics, the Fifth Hospital of Wuhan, Wuhan, Hubei
6Department of Pediatrics, Jinshang Branch of Shanghai Sixth People’s Hospital, Shanghai

*Both authors contribute equally to this work.

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Keywords
Quercetin, miR-485-5p, yes-associated protein 1, diabetic nephropathy

Date received: 17 August 2021; accepted: 25 November 2021

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 have 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 on 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: jsxzyy202011). 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 μg 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′-AGAGGCUGGCCGUGAUAUUC-3′;
- miR-485-5p inhibitor: 5′-GAAUUCAUCAUGCCGCA GCCUCU-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).

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 inflammatory 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 quantitation 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−ΔΔCt method. GAPDH was used as the internal reference gene. The primers were synthesized by Sangon Biotechnology Co, Ltd (Shanghai, China) as below: miR-485-5p (F: 5′-ACTTGGAGAGAGGCTGGC-3′, R: 5′-AAAAAGAGAGGAGCCGTGT-3′; YAP1 (F: 5′-TITTACCGCTCTCCCTG ATT-3′, R: 5′-AGAAAACACTGGGCTAGTAGAAA-3′); GAPDH (F: 5′-GACAGT

Wan et al.
CAGCCGCATCTTTCT3′, R: 5′-GCCACCATAGG 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, #7074, 1:1000) or anti-GAPDH (CST, #5174, 1:1000) overnight. The membrane was washed 3 times with TBST with 5% skimmed milk for 1 h, the PVDF membrane was 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 (e)), 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. * \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \).
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.


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
between miR-485-5p and 3′UTR of YAP1 mRNA. 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 plasmids pmiR-GLO-WT-YAP1/pmiR-GLO-MUT-YAP1 as well as Renilla luciferase (Rluc) control plasmids were co-transfected into HMCs for 48 h before luciferase activity detection. The presence of miR-485-5p inhibitor abrogated the effect of quercetin on YAP1 expression (Figure 4(f)). 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 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)).
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. 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.
induced HMCs. Furthermore, YAP1 is implicated in the downstream molecule metalloproteinase 3 (TIMP3).\textsuperscript{58} 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\textsuperscript{44} due to its low toxicity and few side effects. 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.\textsuperscript{31} Quercetin also improves lipid metabolism, thereby reducing early diabetic kidney damage and exerting a protective effect on early DN.\textsuperscript{57} In addition, quercetin has a beneficial effect on lowering blood sugar and serves as an antioxidant in type 2 diabetes animal models.\textsuperscript{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 reduction of glomerular filtration rate, which then transforms into chronic kidney disease (CKD).\textsuperscript{53} Moreover, the activation of YAP1 causes kidney damage in DN\textsuperscript{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.\textsuperscript{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.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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.
ORCID iD
Weiguo Yao  https://orcid.org/0000-0001-5690-5405

References
1. Bellomo R, Kellum JA, Ronco C, et al. (2017) Acute kidney injury in sepsis. *Intensive Care Medicine* 43: 816–828.
2. Li S, Liu X, Lei J, et al. (2017) Crocin protects podocytes against oxidative stress and inflammation induced by high glucose through inhibition of NF-κB. *Cellular Physiology and Biochemistry* 42: 1481–1492.
3. Lei J, Zhao L, Zhang Y, et al. (2018) High glucose-induced podocyte injury involves activation of mammalian target of rapamycin (mTOR)-induced endoplasmic reticulum (ER) Stress. *Cellular Physiology and Biochemistry* 45: 2431–2443.
4. Ng KP, Jain P, Gill PS, et al. (2016) Results and lessons from the spironolactone to prevent cardiovascular events in early stage chronic kidney disease (STOP-CKD) randomised controlled trial. *BMJ open* 6: e010519.
5. Liu Y, Long L, Yuan F, et al. (2015) High glucose-induced Galectin-1 in human podocytes implicates the involvement of Galectin-1 in diabetic nephropathy. *Cell Biology International* 39: 217–223.
6. Müller-Krebs S, Nissle K, Tsobaneli J, et al. (2015) Effect of benfotiamine in podocyte damage induced by peritoneal dialysis fluid. *Frontiers in medicine* 2: 10.
7. Lei C-T, Su H, Ye C, et al. (2018) The classic signalling and trans-signalling of interleukin-6 are both injurious in podocyte under high glucose exposure. *Journal of cellular and molecular medicine* 22: 251–260.
8. Ito Y, Hsu M-F, Bettaieb A, et al. (2017) Protein tyrosine phosphatase 1B deficiency in podocytes mitigates hyperglycemia-induced renal injury. *Metabolism* 76: 56–69.
9. Xue R, Gui D, Zheng L, et al. (2017) Mechanistic insight and management of diabetic nephropathy: recent progress and future perspective. *Journal of diabetes research* 2017: 1839809.
10. Rao J, Ye Z, Tang H, et al. (2017) The Rhoa/ROCK Pathway Ameliorates Adhesion and Inflammatory Infiltration Induced by AGEs in Glomerular Endothelial Cells. *Scientific reports* 7: 39727.
11. Chan J and Tay Y (2018) Noncoding RNA:RNA regulatory networks in cancer. *International journal of molecular sciences* 19(5): 1310.
12. Shao L, Sun W, Zhang H, et al. (2020) Long non-coding RNA AGAP2-AS1 increases the invasiveness of papillary thyroid cancer. *Aging* 12: 18019–18032.
13. Bunch H (2018) Gene regulation of mammalian long non-coding RNA. *Molecular Genetics and Genomics* 293: 1–15.
14. Quinn JJ and Chang HY (2016) Unique features of long non-coding RNA biogenesis and function. *Nature reviews Genetics* 17: 47–62.
15. Ambros V (2004) The functions of animal microRNAs. *Nature* 431: 350–355.
16. Salmena L, Polisen L, Tay Y, et al. (2011) A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?. *Cell* 146(3): 353–358.
17. Guttman M, Amit I, Garber M, et al. (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* 458: 223–227.
18. Prensner JR and Chinnaiyan AM (2011) The emergence of lncRNAs in cancer biology. *Cancer Discovery* 1: 391–407.
19. Xian Y, Dong L, Jia Y, et al. (2018) miR-370 promotes high glucose-induced podocyte injuries by inhibiting angiotensin II type 1 receptor-associated protein. *Cell Biology International* 42: 1545–1555.
20. Sun J, Li ZP, Zhang RQ, et al. (2017) Repression of miR-217 protects against high glucose-induced podocyte injury and insulin resistance by restoring PTEN-mediated autophagy pathway. *Biochemical and biophysical research communications* 483: 318–324.
21. Wang Q (2020) XIST silencing alleviated inflammation and mesangial cells proliferation in diabetic nephropathy by sponging miR-485. *Archives of physiology and biochemistry* 15: 1–7.
22. Wu J, Lu K, Zhu M, et al. (2020) miR-485 suppresses inflammation and proliferation of mesangial cells in an in vitro model of diabetic nephropathy by targeting NOX5. *Biochemical and biophysical research communications* 521: 984–990.
23. Caussy C, Charrière S, Marquis C, et al. (2014) An APOA5 3’ UTR Variant Associated with Plasma Triglycerides Triggers APOA5 Downregulation by Creating a Functional miR-485-5p Binding Site. *The American Journal of Human Genetics* 94: 129–134.
24. Yagi R, Chen L-F, Shigesada K, et al. (1999) A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator. *The EMBO journal* 18: 2551–2562.
25. Ramos A and Camargo FD (2012) The Hippo signaling pathway and stem cell biology. *Trends in cell biology* 22: 339–346.
26. Kuser-Abali G, Alptekin A, Lewis M, et al. (2015) YAP1 and AR interactions contribute to the switch from androgen-dependent to castration-resistant growth in prostate cancer. *Nature communications* 6: 8126.
27. Zhang L, Yang S, Chen X, et al. (2015) The hippo pathway effector YAP regulates motility, invasion, and castration-resistant growth of prostate cancer cells. *Molecular and cellular biology* 35: 1350–1362.
28. Salem O and Hansen CG (2019) The hippo pathway in prostate cancer. *Cells* 8(4): 370.
29. Halder G and Johnson RL (2011) Hippo signaling: growth control and beyond. *Development* 138(1): 9–22.
30. Zhao B, Li L, Lei Q, et al. (2010) The Hippo–YAP pathway in organ size control and tumorigenesis: an updated version. *Genes & Development* 24: 862–874.
31. Guo L, Cai T, Chen K, et al. (2018) Kindlin-2 regulates mesenchymal stem cell differentiation through control of YAP1/TAZ. *Journal of Cell Biology* 217: 1431–1451.
32. Pan J-X, Xiong L, Zhao K, et al. (2018) YAP promotes osteogenesis and suppresses adipogenic differentiation by regulating β-catenin signaling. Bone research 6: 18.
33. Wang Y and Liu S (2021) LncRNA GHET1 Promotes Hypoxia-Induced Glycolysis, Proliferation, and Invasion in Triple-Negative Breast Cancer Through the Hippo/YAP Signaling Pathway. Frontiers in cell and developmental biology 9: 643515.
34. Zhu BVM, Lee Y, Wan Y, et al. (2021) miR-582-5p Is a Tumor Suppressor microRNA Targeting the Hippo-YAP/TAZ Signaling Pathway in Non-Small Cell Lung Cancer. Cancers 13(4): 756.
35. Uchmanowicz B, Szymańska-Chabowska A and Jankowska-Polańska B (2019) Assessment of adherence to medication for cardiovascular diseases: measurement tools. Cardiovascular journal of Africa 30: 113–119.
36. Chen J, Wang X, He Q, et al. (2020) YAP activation in renal proximal tubule cells drives diabetic renal interstitial fibrogenesis. Diabetes 69: 2446–2457.
37. Sun GD, Li CY, Cui WP, et al. (2016) Review of Herbal Traditional Chinese Medicine for the Treatment of Diabetic Nephropathy. Journal of diabetes research 2016: 5749857.
38. Wen X, Zeng Y, Liu L, et al. (2012) Zhenqing recipe alleviates diabetic nephropathy in experimental type 2 diabetic rats through suppression of SREBP-1c. Journal of ethnopharmacology 142: 144–150.
39. D’Andrea G (2015) Quercetin: a flavonol with multifaceted therapeutic applications? Fitoterapia 106: 256–271.
40. Khan F, Niaz K, Maqbool F, et al. (2016) Molecular targets underlying the anticancer effects of quercetin: an update. Nutrients 8(9): 529.
41. Peng J, Li Q, Li K, et al. (2017) Quercetin improves glucose and lipid metabolism of diabetic rats: involvement of Akt signaling and SIRT1. Journal of diabetes research 2017: 3417306.
42. Jeong S-M, Kang M-J, Choi H-N, et al. (2012) Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. Nutrition research and practice 6: 201–207.
43. Sun L, Zheng W, Liu Q-D, et al. (2021) Valproic Acid Protects Chondrocytes from LPS-Stimulated Damage via Regulating miR-302d-3p/ITGB4 Axis and Mediating the PI3K-AKT Signaling Pathway. Frontiers in molecular biosciences 8: 633315.
44. Kong P-Z, Yang F, Li L, et al. (2013) Decreased FOXC2 mRNA expression indicates early-onset metastasis and poor prognosis for breast cancer patients with histological grade II tumor. PloS one 8: e61591.
45. Chen J, Huang X, Wang W, et al. (2018) LncRNA CDKN2BAS predicts poor prognosis in patients with hepatocellular carcinoma and promotes metastasis via the miR-153-5p/ARHGAP18 signaling axis. Aging 10: 3371–3381.
46. Yu Y, Xiao C-H, Tan L-D, et al. (2014) Cancer-associated fibroblasts induce epithelial-mesenchymal transition of breast cancer cells through paracrine TGF-β signalling. British journal of cancer 110: 724–732.
47. Huang Z, Peng Y, Yu H, et al. (2018) RhoA protects the podocytes against high glucose-induced apoptosis through YAP and plays critical role in diabetic nephropathy. Biochemical and biophysical research communications 504: 949–956.
48. Ma R, Ren JM, Li P, et al. (2019) Activated YAP causes renal damage of type 2 diabetic nephropathy. European review for medical and pharmacological sciences 23: 755–763.
49. Yang T, Heng C, Zhou Y, et al. (2020) Targeting mammalian serine/threonine-protein kinase 4 through Yes-associated protein/TEA domain transcription factor-mediated epithelial-mesenchymal transition ameliorates diabetic nephropathy orchestrated renal fibrosis. Metabolism 108: 154258.
50. Le C, Lin L, Jun D, et al. (2013) The economic burden of type 2 diabetes mellitus in rural southwest China. International journal of cardiology 165: 273–277.
51. Koch EAT, Nakhouli R, Nakhouli F, et al. (2020) Autophagy in diabetic nephropathy: a review. International urology and nephrology 52: 1705–1712.
52. Sun J, Xu Y, Deng H, et al. (2010) Involvement of osteopontin upregulation on mesangial cells growth and collagen synthesis induced by intermittent high glucose. Journal of cellular biochemistry 109: 1210–1221.
53. Wang C, Song J, Ma Z, et al. (2014) Fluctuation between fasting and 2-H postload glucose state is associated with chronic kidney disease in previously diagnosed type 2 diabetes patients with HbA1c ≥ 7%. PloS one 9: e102941.
54. Tong X-L, Dong L, Chen L, et al. (2012) Treatment of diabetes using traditional Chinese medicine: past, present and future. Am J Chin Med 40: 877–886.
55. Shi X, Lu XG, Zhan LB, et al. (2011) The effects of the Chinese medicine ZiBu PiYin recipe on the hippocampus in a rat model of diabetes-associated cognitive decline: a proteomic analysis. Diabetologia 54: 1888–1899.
56. Zhao H-L, Sui Y, Qiao C-F, et al. (2012) Sustained antidiabetic effects of a berberine-containing Chinese herbal medicine through regulation of hepatic gene expression. Diabetes 61: 933–943.
57. Jiang X, Yu J, Wang X, et al. Quercetin improves lipid metabolism via SCAP-SREBP2-LDLr signaling pathway in early stage diabetic nephropathy. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2019; 12: 827-839.
58. Fan H and Zhang W. Overexpression of Linc 4930556M19Rik Suppresses High Glucose-Triggered Podocyte Apoptosis, Fibrosis and Inflammation via the miR-27a-3p/Metalloproteinase 3 (TIMP3) Axis in Diabetic Nephropathy. Medical science monitor : International Medical Journal of Experimental and Clinical Research. 2020; 26: e295361.
59. Fan W, Wen X, Zheng J, et al. LINC00162 participates in the pathogenesis of diabetic nephropathy via modulating the miR-383/HDAC9 signalling pathway. Artificial cells, nanomedicine, and biotechnology. 2020; 48: 1047–1054.
60. Yang X, Yang M, Chen Y, et al. (2021) miR-30a-5p targets Beclin1 to ameliorate high-glucose-induced glomerular
podocyte injury in immortalized rat podocyte cell line. *American journal of translational research* 13: 1516–1525.

61. Yao W, Guo P, Mu Q, et al. (2020) Exosome-derived Circ-PVT1 Contributes to cisplatin resistance by regulating autophagy, invasion, and apoptosis via miR-30a-5p/YAP1 Axis in Gastric Cancer Cells. *Cancer biotherapy & radiopharmaceuticals* 36(4): 347–359.

62. Qian X, He L, Hao M, et al. (2021) YAP mediates the interaction between the Hippo and PI3K/Akt pathways in mesangial cell proliferation in diabetic nephropathy. *Acta Diabetologica* 58: 47–62.