Life Sciences 234 (2019) 116755

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Activation of vitamin D receptor attenuates high glucose-induced cellular injury partially dependent on CYP2J5 in murine renal tubule epithelial cell

Yan Liu, Liu Li, Bin Yi, Zhao-Xin Hu, Ai-Mei Li, Cheng Yang, Li Zheng, Hao Zhang*

Department of Nephrology, The Third Xiangya Hospital, Central South University, Changsha, Hunan 410013, China

ARTICLE INFO

Keywords:
Vitamin D receptor
CYP2J5
Diabetic nephropathy
Paricalcitol
Renal tubule epithelial cells

ABSTRACT

Aims: Vitamin D and its receptor, vitamin D receptor (VDR), have renoprotection effect against diabetic nephropathy (DN). But the exact mechanism has not been fully elucidated. Epoxyeicosatrienoic acids (EETs) are cytochrome P450 (CYP) epoxygenase-derived metabolites of arachidonic acid, protecting against diabetes and DN. Herein, we hypothesized that activation of VDR attenuated high glucose-induced cellular injury in renal tubular epithelial cells partially through up-regulating CYP2J5 expression.

Main methods: Streptozotocin (STZ) was injected to induce diabetic in wild type and *Vdr*$^{-/-}$ mice. The effects of VDR knockout and an activator of VDR, paricalcitol, on the renal injury were detected. *In vitro*, a murine kidney proximal tubule epithelial cell line BU.MPT induced by high glucose were treated with or without paricalcitol (30 mM) for 12 h or 24 h.

Key findings: The expression of CYP2J5 was significantly decreased both in wild type and *Vdr*$^{-/-}$ diabetic mice induced by STZ. The STZ-induced kidney architecture damage and apoptosis rate in Vdr$^{-/-}$ mice were more severe. *In vitro*, high glucose treatment strongly reduced the CYP2J5 expression and the synthesis of 14,15-EET in BU.MPT cells. Supplement of 14,15-EET significantly reduced the lactate dehydrogenase (LDH) release induced by high glucose in BU.MPT cells. Furthermore, treatment with paricalcitol attenuated cellular injury and restored the expression of CYP2J5 reduced by high glucose in BU.MPT cells.

Significance: We conclude that activation of VDR attenuates high glucose-induced cellular injury partially dependent on CYP2J5 in murine renal tubule epithelial cells and paricalcitol may represent a potential therapy for DN.

1. Introduction

According to the National Diabetes Statistics Report [1], 9.4% of the population have diagnosed diabetes in the United States. Diabetic nephropathy (DN), a significant complication of diabetes, is the leading cause of end-stage renal disease worldwide [2]. Despite the advance insight into the critical pathogenic mechanisms underlying progressive DN, effective therapies remain elusive [3].

Increasing evidence indicates that vitamin D and its receptor, vitamin D receptor (VDR), play a vital role in the development of DN [4,5]. Patients with DN have a high prevalence of vitamin D deficiency or insufficiency [4]. In addition, we have found that VDR expression is decreased in peripheral blood mononuclear cells and renal tubular epithelial cells from type 2 diabetes mellitus (T2DM) patients [5], and VDR knockout mice developed more severe albuminuria and glomerulosclerosis compared to wild-type (WT) mice after streptozotocin (STZ) injection [6]. While, VDR overexpression in podocytes protects against the development of DN [7]. There are some studies involving mechanisms underlying the protective effect of VDR activation against DN, including suppression of high glucose-induced apoptosis [7], down-regulation of GSK-3β and β-catenin [8], and restoration of nephrin signaling [9]. But the exact mechanism has not been fully elucidated.

Cytochrome P450 (CYP) epoxygenase-mediated metabolism of arachidonic acid (ARA) is involved in diabetes and DN. CYP metabolizes ARA to epoxyeicosatrienoic acids (EETs), which regulate numerous biological processes, including inflammation, vascular tone, and fibrosis [10–12]. Accumulating evidence has demonstrated that EETs exhibit a protective effect against diabetes and DN [13]. Overexpression of CYP2J2, resulting in an increase of EETs, prevents renal tubular epithelial-mesenchymal transition and attenuates renal damage in STZ-induced diabetic mice [14]. Furthermore, genetic disruption of soluble epoxide hydrolase, the primary enzyme hydrolyzing EETs into biologically less active dihydroxyeicosatrienoic acids, protects renal tubular epithelium against TNF-α-induced apoptosis [15]. CYP2J5 is

* Corresponding author.
E-mail address: zhanghaoliaoqing@163.com (H. Zhang).

https://doi.org/10.1016/j.lfs.2019.116755
Received 10 June 2019; Received in revised form 4 August 2019; Accepted 12 August 2019
Available online 12 August 2019

the dominating synthetase in mouse kidney [16]. However, whether CYP2J5/EETs contributes to the renoprotection of vitamin D has not been reported.

Paricalcitol is a synthetic selective VDR activator used by patients with chronic renal failure [17]. It reduces proteinuria and high glucose-induced kidney injury [8], exerting renoprotection effect [5,9]. In this study, we used paricalcitol to activate VDR and tested the hypothesis that activation of VDR would attenuate high glucose-induced cellular injury in renal tubule epithelial cell partially through up-regulating CYP2J5/EETs metabolism. We demonstrate that high glucose markedly suppressed the expression of CYP2J5 in renal tubule epithelial cell *in vitro*, and VDR restored the CYP2J5/EETs, contributing to the renoprotection.

2. Materials and methods

2.1. Animal experiments

Adult male wild type (WT) and $Vdr^{-/-}$ mice were used in this study. All animals were kept in an air-conditioned room with a 12 h light/12 h dark cycle. To induce diabetes, STZ (100 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) was intraperitoneally injected one time in freshly prepared citrate buffer (0.4 M, pH 4.5) after an overnight fast. One week after STZ injection, diabetic mice were confirmed by fasting blood glucose > 216 mg/dl. Three weeks later, diabetic mice received an intraperitoneal injection with paricalcitol (an agonist of VDR, 0.4 μg/kg, Sigma-Aldrich, St. Louis, MO, USA) at 3 times per week. Twelve weeks after the STZ injection, mice were sacrificed under intraperitoneal injection of sodium pentobarbital (80 mg/kg). The blood was collected, and kidneys were collected for histology and immunohistochemistry staining. All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee of Central South University, and all animals received humane care according to the NIH guidelines.

2.2. Histological analysis

Renal tissues were excised and immersed in 4% paraformaldehyde for 24 h. Paraffin-embedded Renal tissues were sectioned at the 4-μm thickness, which was stained with hematoxylin and eosin (H&E) for pathological analysis.

2.3. Periodic acid Schiff (PAS) staining

Renal tissues were fixed in 4% paraformaldehyde and embedded in paraffin. The sections (4 μm in thickness) were used for PAS staining (Baso Diagnostics Inc., Guangdong, China). The sections were examined under an Olympus BX51 microscope (Olympus, Tokyo, Japan) at a magnification of × 40.

2.4. Immunohistochemistry analysis

The sections (4 μm in thickness) were dewaxed, rehydrated, and boiled. The sections were then incubated with 3% hydrogen peroxide (H_2O_2) in PBS for 15 min to quench the endogenous peroxidase activity. The sections were incubated with primary antibodies against VDR (1:500) or CYP2J5 (1:100) overnight at 4 °C, followed by incubation with the horseradish peroxidase (HRP)-conjugated secondary antibody (Sigma) diluted in 1% BSA at 37 °C. n. Immunoreactions were visualized using 3,30-diaminobenzidine. All experiments were repeated three times. The sections were viewed with a microscope (Thermo, USA).

2.5. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay

Renal sections were detected with an ApopTag Peroxidase *in situ* Apoptosis Detection Kit (Chemicon, Millipore, Billerica, MA). Renal tissue sections were dewaxed and hydrated and then quenched by 3% hydrogen peroxide for 5 min. The sections were incubated with the TUNEL reaction mixture containing TdT and biotinylated-dUTP for 1 h in a humidified chamber at 37 °C. The sections were then incubated with anti-digoxigenin peroxidase (Sigma) for 30 min at room temperature and colored with 3,3-diaminobenzidine. Sections were counterstained with hematoxylin and viewed with a microscope (Thermo, USA).

2.6. Cell culture

A conditionally immortalized murine kidney proximal tubule epithelial cell line (BU.MPT) was used in this study. Cells were maintained Dulbecco's modified Eagle's medium-F-12 medium (DMEM/F12) containing 10% fetal bovine serum (FBS) and 2 mM L-glutamine as previously described [18]. Cells were grown at 37 °C in a humidified 5% CO_2 atmosphere. According to the previous report [19], under non-permissive conditions, confluent BU.MPT cells behave like primary cultures of mouse kidney proximal tubular cells.

2.7. Cell treatment

All cells were serum-starved and cultured for 24 h before all experiments. Then cells were incubated with 30 mM glucose (high glucose) or 30 mM mannitol (osmolar control) as a previous study [20]. After treatment for 12 h or 24 h, cells were harvested for gene and protein expressions detection. To investigate the effect of VDR, cells were incubated with paricalcitol (0.01, 0.1, and 1 mM, Sigma-Aldrich, St. Louis, MO, USA) prior to the addition of high glucose for 1 h. Individual treatments were described as following.

2.8. RNA extraction and real-time PCR

Kidney samples or cells were homogenized, and total RNA was extracted using RNAiso Plus (TaKaRa Clontech, Japan) according to the manufacturer's instructions. The total RNA concentration was measured using an ultraviolet spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). One microgram total RNA was used to synthesize the cDNA with a PrimeScript™RT reagent Kit with gDNA Eraser (TaKaRa Clontech, Japan). SYBR® Premix Ex Taq™ II system (TaKaRa Clontech, Japan) was used to quantify mRNA expression by real-time PCR on a deep well Real-Time PCR Detection System (CFX96 Touch™, Bio-Rad, USA). β-actin was used as an internal control. Each experiment was performed three times. Relative gene expression was measured by the $2^{-\Delta\Delta CT}$ method according to a previous study [11]. All primers were synthesized by Sangon Biotech (Shanghai, China), and the sequences of specific primers used in this study are shown in Table 1.

2.9. Western blot

Kidney samples or cells were prepared into homogenate samples

Table 1
The sequences of primers used in this study.

Gene	Sequences of upstream	Sequences of downstream
Cyp2j5	TGATGGGTTCATCAGCAGGC	CTTGGCTCATCTGGGTTCCAAT
Cyp2j6	GGTGCCCTTGTTGTTAGCAC	GGCTAACAAGGAGCCGGTAG
Cyp2c44	CAAGGTACCCCGAGTGAAGAA	CACGGCATCTGTATAGGGCA
Cyp2c29	CCATGGTTGCAGGTAAACCACAT	TCTGTCCCTGCACCAAAGAG
β-actin	TTCCAGCCTTCCTTCTTG	GGAGCCAGAGCA GTAATC

Table 2
The information of antibodies used in this study.

Antibody	Resource	Dilution
CYP2J5	Shanghai Yu Bo biological technology, China	1:500
VDR	Santa Cruz	1:500
β-actin	Sigma-Aldrich, USA	1:5000
Secondary antibody	Cell Signaling Technology, USA	1:1000

using RIPA lysis and extraction buffer (Thermo Fisher Scientific, USA). The protein concentration was determined by bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, USA). Then, 30 μg total protein was separated in 10% or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and was electrotransferred to a polyvinylidene fluoride (PVDF) membrane. After blocked in 5% fat-free milk, the membranes were incubated with specific antibodies, followed by corresponding secondary antibody

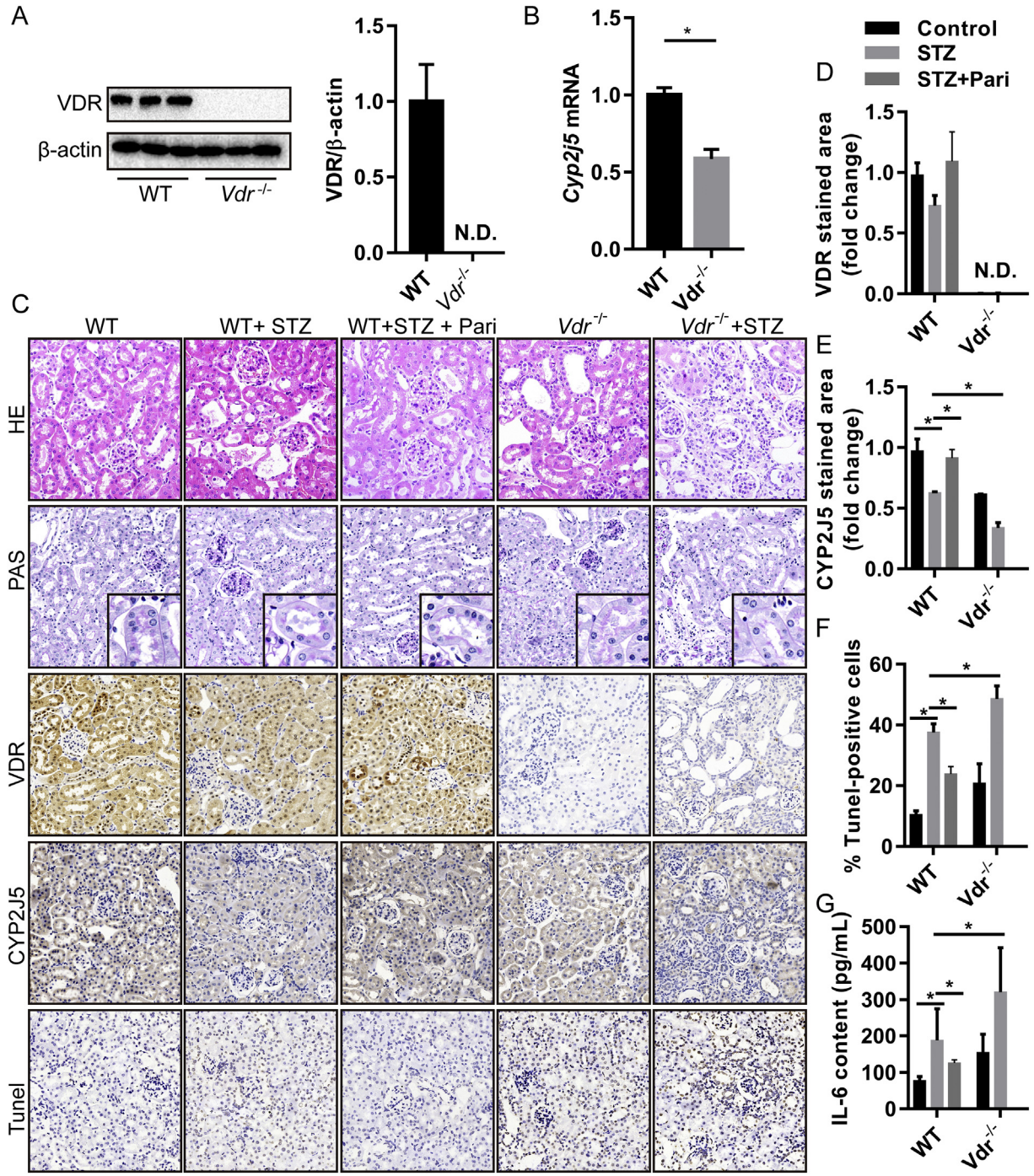

Fig. 1. Aggravated injury and decrease of CYP2J5 expression in the kidney of $Vdr^{-/-}$ mice. WT and $Vdr^{-/-}$ mice were killed 12 weeks after STZ injection. (A) VDR protein levels in kidney of WT and $Vdr^{-/-}$ mice detected by western blot (n = 3). (B) *Cyp2j5* mRNA levels in kidney of WT and $Vdr^{-/-}$ mice detected by real-time PCR (n = 4–6). (C) Histopathological examination, PAS staining, immunostaining of the VDR and CYP2J5 expression, and Tunel staining of the kidney of STZ-induced WT and $Vdr^{-/-}$ mice treated with or without paricalcitol (n = 4–6). (D-E) VDR and CYP2J5 stained area in the kidney of STZ-induced WT and $Vdr^{-/-}$ mice treated with or without paricalcitol (n = 4–6). (F) TUNEL staining with the analyses of the number of cells staining $TUNEL^{+}$ (n = 4–6). (G) IL-6 levels in serum of STZ-induced WT and $Vdr^{-/-}$ mice treated with or without paricalcitol detected by ELISA (n = 4–6). *: $P < 0.05$.

conjugated with horseradish peroxidase. Finally, the bands were visualized using chemiluminescence reagents (Bio-Rad, USA). β-actin was used as an internal control. Each experiment was performed three times. The information of antibodies used in this study is shown in Table 2.

2.10. Enzyme-linked immunosorbent assay (ELISA)

IL-6 levels in serum of mice were detected by an ELISA kit. A stable metabolite of 14,15-EET, 14,15-dihydroxyeicosatrienoic acid (14,15-DHET), in cell culture was measured with a competitive *in vitro* ELISA kit according to the manufacturer's instructions (Abcam, No. ab175811, USA). The 14,15-DHET concentration was calculated from a standard curve.

2.11. Dual-luciferase reporter assay

To verify the direct binding between VDR and promotor of Cyp2j5 in tubule epithelial cells, dual-luciferase reporter assay was performed according to our previous study [5]. BU.MPT cells (1×10^5 per well) were plated into a 24-well plate and transfected with the firefly luciferase expression plasmid pGLC3-basic, wild type (WT)-Cyp2j5 (containing the binding sites of VDR), or mutant-Cyp2j5 (containing mutation in the binding sites of VDR) (provided by Genechem Co., Ltd. Shanghai, China) with the X-treme GENE HP DNA transfection reagent (Roche NimbleGen; Madison, WI, USA) according to the manufacturer's protocol. The Renilla luciferase was used as an internal control. Following 24 h transfection, cells were treated with/without paricalcitol (0.1 mM) for other 24 h. Then the Fly and Renilla luciferase activities were measured with the Dual-luciferase assay system (Promega; Madison, WI, USA) on a Varioskan Flash (Thermo Fisher Scientific, Waltham, MA, USA). The results are shown as the ratio of firefly luciferase intensity and renilla luciferase intensity. All measurements were repeated in triplicate.

2.12. Lactate dehydrogenase (LDH) activity assay

The release of LDH from damaged cells was measured to detect the cell damage following treatments.

After treatment, cell-free supernatant was collected, and the LDH activity was measured using the LDH release assay (Promega, USA).

2.13. Apoptosis assays

Apoptosis was evaluated with an Annexin V-FITC/propidium iodide (PI) kit (BD Biosciences, San Jose, CA, USA). Briefly, BU.MPT cells were plated into 6-well plate and were treated with high glucose (30 mM) for 24 h with/without pre-treatment of paricalcitol (0.1 mM) or 17-ODYA (0.1 mM, Cayman Chemical, Ann Arbor, MI, USA). Then cells were harvested with EDTA free trypsin and washed with PBS twice. Cells were stained with Annexin V-FITC/PI and detected by flow cytometer (Beckman Coulter Life Sciences, Indianapolis, IN, USA). The total of Annexin V-FITC$^+$PI$^-$ and Annexin V-FITC$^+$PI$^+$ cells were considered as the apoptotic cells.

2.14. Statistical analysis

Data were presented as means ± SD. Statistical comparisons were performed based on an unpaired, 2-tailed Student's *t*-test or ANOVA followed by the Student-Newman-Keuls *post hoc* test using the SPSS18.0 software (Chicago, IL, USA). All P values < 0.05 were considered to be statistically significant.

3. Results

3.1. Aggravated injury and decrease of CYP2J5 expression in the kidney of $Vdr^{-/-}$ mice

Using $Vdr^{-/-}$ mice (Fig. 1A), STZ was injection to induce diabetes model. We found that the gene and protein levels of CYP2J5 in kidney were significantly decreased in the $Vdr^{-/-}$ mice (Fig. 1B–C, E). Importantly, twelve weeks after STZ injection, we found that the STZ-induced kidney architecture damage and apoptosis rate in $Vdr^{-/-}$ mice was more severe than that in the WT mice (Fig. 1C, F). And the IL-6 levels in serum of STZ-treated $Vdr^{-/-}$ mice were higher than that in the STZ-treated WT mice (Fig. 1G). Furthermore, we found that paricalcitol effectively attenuated the kidney injury induced by STZ (Fig. 1C–E), indicating protection of VDR against the STZ-induced kidney injury. These data indicate the aggravated injury and decrease of CYP2J5 expression in the kidney of $Vdr^{-/-}$ mice.

3.2. Down-regulation of CYP2J5 induced by high glucose aggravates the injury in renal tubular epithelial cells

CYP2J5, CYP2J6, CYP2C44, and CYP2C29 are the resources of EETs in mice. We firstly investigated the expression profile of the CYP generating EETs in the kidney. The real-time PCR results demonstrated that *Cyp2j5* was the dominating subtype generating EETs in the kidney (Fig. 2A). In the kidney of the TIDM mice, the *Cyp2j5* mRNA expression was significantly lower than that in the control group (Fig. 2B). A similar result was found in the protein expression of CYP2J5 (Fig. 2C-D). Then, we treated the renal tubular epithelial cell line BU.MPT, we found high glucose (30 mM) incubation for 12 h or 24 h sharply reduced the *Cyp2j5* mRNA expression (Fig. 2E) and CYP2J5 protein expression (Fig. 2F–G). Additionally, production of CYP2J5, 14,15-DHET, was significantly decreased after high glucose treatment (Fig. 2H). These data indicate that the expression of CYP2J5/EETs was down-regulated in the kidney or renal tubular epithelial cell under hyperglycemia in mice. Interestingly, we found that exogenous 14,15-EET (0.1 μM) significantly attenuated the cellular injury induced by high glucose, characterized as a lower LDH activity in the supernatant (Fig. 2I). Collectively, these results suggest that down-regulation of CYP2J5/EETs induced by high glucose aggravates the cellular injury in renal tubular epithelial cells.

3.3. Paricalcitol eliminates the down-regulation of CYP2J5 induced by high glucose in renal tubular epithelial cells

Next, we wondered whether activation of VDR attenuates high glucose-induced cellular injury through restoring the CYP2J5 expression in renal tubular epithelial cells. We found that high glucose treatment (30 mM) significantly suppressed the gene and protein expressions of CYP2J5 in renal tubular epithelial cells (Fig. 3A–C). While paricalcitol pre-treatment restored the gene expression of *Cyp2j5* in a dose-dependent manner in renal tubular epithelial cells (Fig. 3A). Then, the result of western blot showed that single paricalcitol pre-treatment (0.1 mM) had no effect on the expression of CYP2J5, but it significantly eliminated the down-regulation of CYP2J5 induced by high glucose (Fig. 3B–C). As expected, we found there were two binding sites of VDR, vitamin D response element (VDRE), in the promoter of *Cyp2j5* in mice using LASAGNA-Search 2.0 (http://biogrid-lasagna.engr.uconn.edu/lasagna_search/, Fig. 3D). Consequently, a wild type (WT)- and a mutant-Cyp2j5 luciferase reporter plasmid (containing mutations at the VDR binding site) were constructed (Fig. 3E), and the binding activation of VDR with Cyp2j5 promoter was measured by the dual-luciferase reporter assay. We found that paricalcitol treatment (0.1 mM) significantly increased relative luciferase activity in the WT plasmid, compared with the pGL3 basic plasmid (Fig. 3F). But the increase of relative luciferase activity induced by paricalcitol was weaker in the

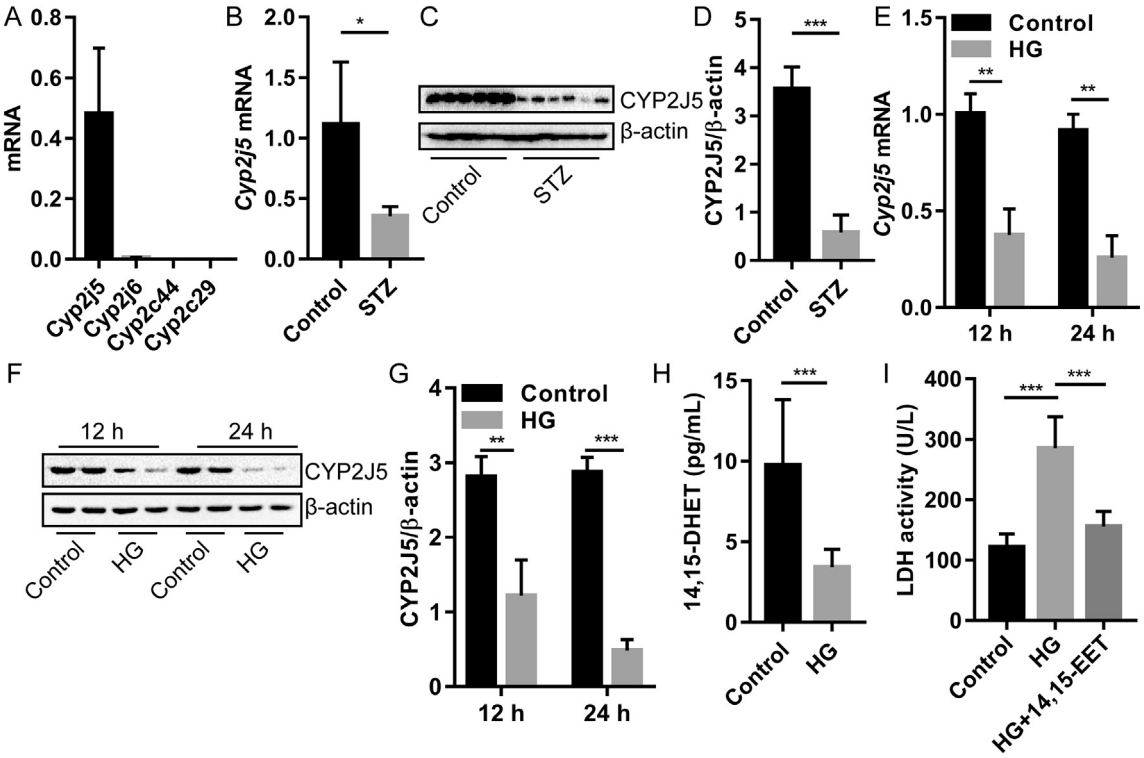

Fig. 2. High glucose reduces the CYP2J5 expression and induces cellular injury in renal tubular epithelial cells. A: the profile of gene expression of *CYP* in healthy mouse kidney detected by real-time PCR ($n = 5$). B–D: The gene and protein expressions of CYP2J5 in the kidney of the mouse were detected by real-time PCR and Western blot, respectively ($n = 5$–6). E–G: The gene and protein expressions of CYP2J5 in renal tubular epithelial cells treated with 30 mM glucose or mannitol for 12/24 h were detected by real-time PCR and Western blot, respectively ($n = 3$ or 6). H: The concentration of 14,15-DHET in the cells culture supernatant was detected using ELISA ($n = 5$). I: The activity of LDH in the cells culture supernatant was detected using the corresponding kit ($n = 6$). Data are shown as means ± SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

mutant-Cyp2j5 plasmid (Fig. 3F). Interestingly, similar results were observed in the cells under high glucose (30 mM) condition (Fig. 3F). Collectively, these data indicate that activation of VDR up-regulates *Cyp2j5* expression *via* binding to VDRE in renal tubular epithelial cells.

3.4. Activation of VDR attenuates cellular injury induced by high glucose via up-regulation of CYP2J5 in renal tubular epithelial cells

Finally, we wondered whether activation of VDR attenuates cellular injury induced by high glucose through up-regulating CYP2J5 expression in renal tubular epithelial cells. High glucose treatment (30 mM) for 24 h significantly increased the LDH release, which was strongly inhibited by paricalcitol treatment in a dose-dependent manner (Fig. 4A). Additionally, paricalcitol treatment (0.1 mM) significantly reduced the apoptosis rate of renal tubular epithelial cells induced by high glucose treatment (30 mM) for 24 h (Fig. 4B–C), indicating that activation of VDR attenuates cellular injury induced by high glucose *in vitro*. To investigate the role of CYP2J5 in the protective effect of paricalcitol against high glucose, an inhibitor of CYP epoxygenase, 17-octadecynoic acid (17-ODYA), was employed to suppress the activity of CYP2J5. Results demonstrated that 17-ODYA pre-treatment (0.1 mM) eliminated the protective effect of paricalcitol against high glucose treatment in renal tubular epithelial cells, as evidenced by higher LDH activity in the supernatant of cell culture (Fig. 4D) and apoptosis rate (Fig. 4E–F). Collectively, these results suggest that activation of VDR attenuates cellular injury induced by high glucose partially *via* CYP2J5 in renal tubular epithelial cells.

4. Discussion

Although the renoprotective role of vitamin D has been well documented in two decades, the underlying mechanism remains elusive. Herein, we provide strong evidence that activation of VDR protects from cellular injury induced by high glucose in renal tubular epithelial cells, and the renoprotection of VDR at least in part is dependent on the upregulation of CYP2J5. These data here actively support the clinical usage of VDR activator for DN.

Firstly, this study demonstrated a potential mechanism for the renoprotection of VDR against DN. A body of literature has documented the renoprotection of vitamin D and its analogs [7,21]. Patients with DN have a high prevalence of vitamin D deficiency or insufficiency [4]. We have reported that VDR signaling suppresses high glucose-induced apoptosis of podocytes and protects against DN [7]. Besides, 1,25(OH) 2D3 transcriptionally stimulated the expression of nephrin, which is an essential slit diaphragm protein synthesized by podocytes [22]. These data indicate a vital role of VDR in podocytes, an integral part of the glomerular filtration barrier. Herein, our results demonstrated that activation of VDR also protects renal tubular epithelial cells, another important target cells during the development of DN, from high glucose-induced cellular injury. A number of reports have confirmed that loss and epithelial-mesenchymal transition (EMT) of renal tubular epithelial cells lead to fibrosis, a significant characteristic of renal failure [23,24]. Our previous studies demonstrated that the renin-angiotensin system is the vital target of vitamin D [24,25]. In this study, we found that the CYP/EETs pathways were significantly down-regulated in $Vdr^{-/-}$ mice and high glucose-stimulated renal tubular epithelia. Activation of VDR by paricalcitol restored the expression of CYP2J5 and reduced the apoptosis induced by high glucose *in vitro*. EETs are endogenous protective lipid mediators with a variety of biological functions, including renoprotection [10]. We provide new evidence linking VDR and DN through CYP/EETs.

Secondly, we provide a new strategy, activation of VDR by

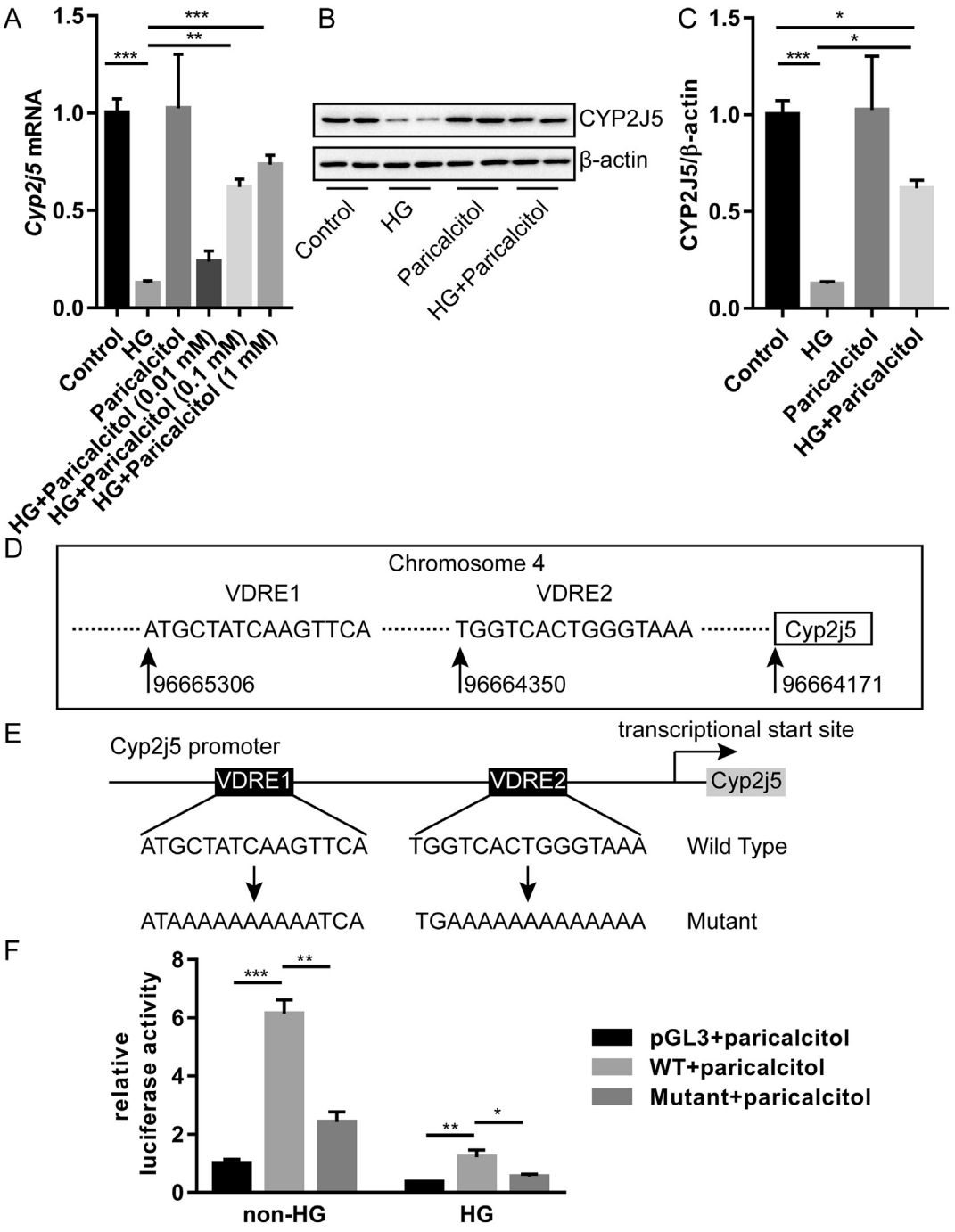

Fig. 3. Paricalcitol eliminates the down-regulation of CYP2J5 induced by high glucose in renal tubular epithelial cells. A: Paricalcitol restored the gene expression of *Cyp2j5* induced by high glucose (30 mM) in a dose-dependent manner in renal tubular epithelial cells ($n = 3$). B–C: The protein expression of CYP2J5 in renal tubular epithelial cells was detected by Western blot ($n = 4$). D: The binding sites of vitamin D receptor in the promoter of Cyp2j5 in mice. E: Schematic structure of the pGL3-Cyp2j5 promoter and the pGL3-Cyp2j5 promoter mutant for vitamin D receptor binding. F: After transfection of the plasmids for 48 h, the luciferase activity of the Cyp2j5 promoter reporters was detected. ($n = 6$). Data are shown as means ± SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

paricalcitol, for the up-regulation of CYP/EETs. It has been well documented that EETs are endogenous eicosanoids with protective effects against various kidney disease, such as ischemia/reperfusion-induced acute kidney injury [26], experimental unilateral ureter obstruction-induced kidney fibrosis [27], and STZ-induced DN [15]. The EETs-enhancing therapies reported mainly include two kinds: pharmacological inhibition of sEH and genetic disruption of sEH [11,15,28]. However, both therapies have a long path to the clinic. Herein, we report that activation of VDR by paricalcitol upregulates the expression of CYP2J5 in the kidney, contributing to the enhancement of EETs. Paricalcitol is a well-documented drug to exert renoprotection [8,17] and cardiovascular protection [29,30]. To the best of our knowledge, this study is the first one reporting that activation of VDR up-regulates Cyp2j5 expression *via* binding to the promoter VDRE in renal tubular epithelial cells. We provide a new option to up-regulation of CYP/EETs.

Lastly, this study furtherly illuminates the interactions between high glucose and CYP/EETs in diabetes. EETs attenuates insulin resistance in HFD-induced obese mice [31] and improves glucose homeostasis [32]. Recently, it has been reported that EETs/PPARs activation mediates the preventive effect of naringenin in high glucose-induced induced

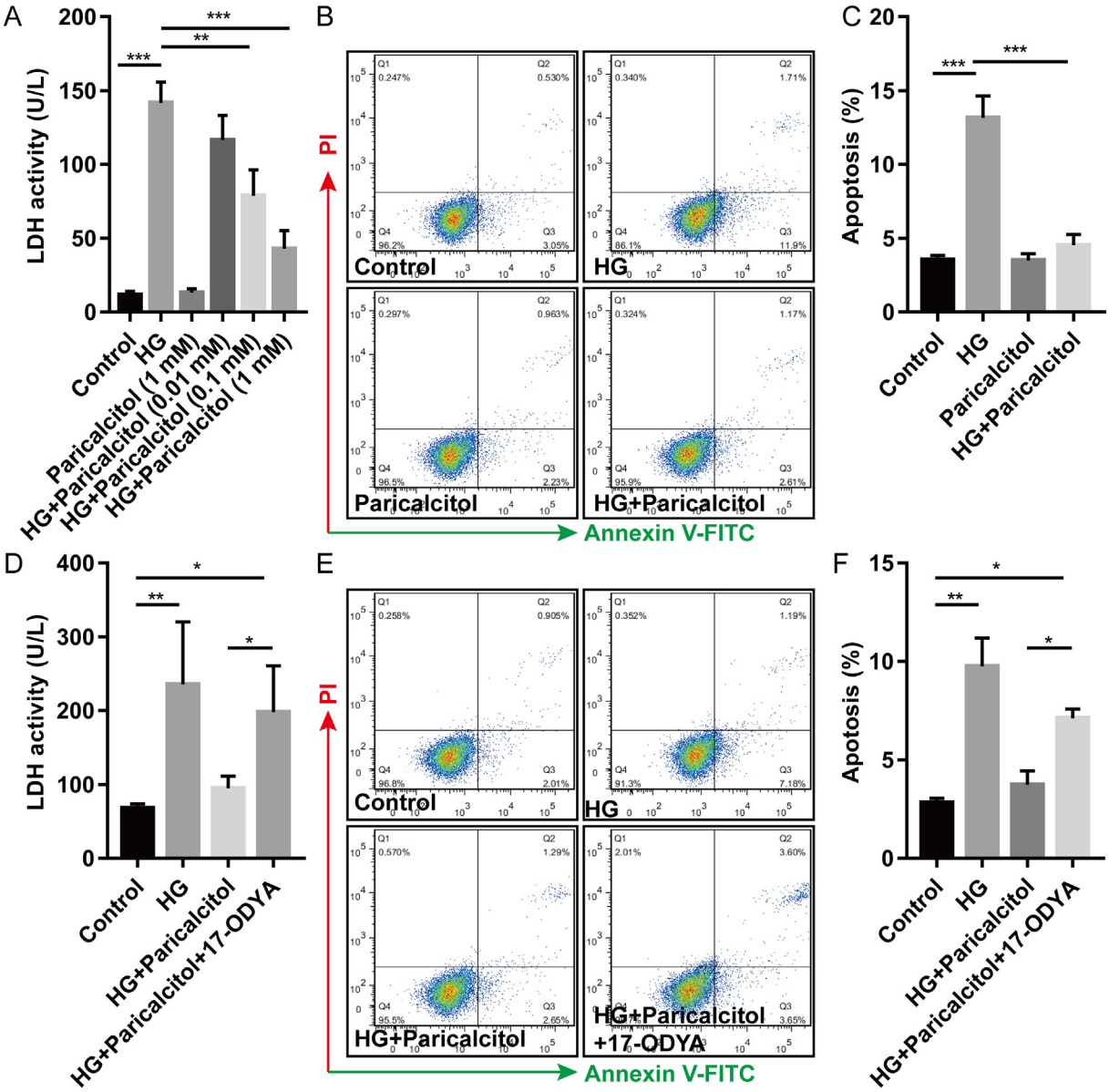

Fig. 4. Paricalcitol attenuates high glucose-induced cellular injury partially dependent on CYP2J5 in renal tubular epithelial cells. A: BU.MPT cells were treated with high glucose (30 mM) for 24 h with/without the series concentration of paricalcitol (0.01, 0.1 and 1 mM), then the LDH activity in the supernatant of cell culture was detected. B–C: BU.MPT cells were treated with high glucose (30 mM) for 24 h with/without paricalcitol (0.1 mM), then the apoptosis rate was detected using Annexin V-FITC/PI kit. D: BU.MPT cells were treated with high glucose (30 mM) for 24 h with/without paricalcitol or 17-ODYA (0.1 mM), then the LDH activity in the supernatant of cell culture was detected. E–F: BU.MPT cells were treated with high glucose (30 mM) for 24 h with/without paricalcitol or 17-ODYA (0.1 mM), then the apoptosis rate was detected using Annexin V-FITC/PI kit. Data are shown as means ± SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

cardiomyocyte hypertrophy [33]. Here, we report that exogenous 14,15-EET significantly attenuates the high glucose-induced cellular injury, which confirms the protective effect of EETs against high glucose-induced injury. On the other hand, high glucose also affects the CYP/EETs metabolism. In a previous study, CYP2C11 and 14,15-EET are up-regulated 6 h after high glucose treatment, while down-regulated 48 h after high glucose treatment [34]. We found that high glucose treatment for 12 and 24 h decreased the CYP2J5 expression and EETs generation. Both CYP2C11 and CYP2J5 mediates the generation of EETs [10,16]. However, CYP2J5 is the dominating synthetase in mouse kidney [16]. So, our study strongly indicates that high glucose impairs the CYP/EETs metabolism in the kidney.

In conclusion, our data suggest that high glucose induces down-regulation of CYP2J5/EETs, aggravating the cellular injury in renal tubular epithelial cells. While activation of VDR attenuates high glucose-induced cellular injury partially dependent on CYP2J5 in murine renal tubule epithelial cells and paricalcitol may represent a potential therapy for DN.

Declaration of competing interest

The authors declared no conflict of interests.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 81870498).

Author contributions

HZ and YL conceived and designed the experiments. YL, LL, BY, AML, CY, and LZ performed the experiments. YL and ZXH analyzed the data. HZ contributed reagents/materials/analysis tools. YL wrote the paper. HZ critically reviewed the manuscript.

References

[1] https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf.

[2] M.E. Molitch, A.I. Adler, A. Flyvbjerg, R.G. Nelson, W.Y. So, C. Wanner, et al., Diabetic kidney disease: a clinical update from kidney disease: improving global outcomes, Kidney Int. 87 (2015) 20–30.

[3] J. Wang, J. Pang, H. Li, J. Long, F. Fang, J. Chen, et al., lncRNA ZEB1-AS1 was suppressed by p53 for renal fibrosis in diabetic nephropathy, Mol Ther Nucleic Acids 12 (2018) 741–750.

[4] M. Herrmann, D.R. Sullivan, A.S. Veillard, T. McCorquodale, I.R. Straub, R. Scott, et al., Serum 25-hydroxyvitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes, Diabetes Care 38 (2015) 521–528.

[5] B. Yi, J. Huang, W. Zhang, A.M. Li, S.K. Yang, J. Sun, et al., Vitamin D receptor down-regulation is associated with severity of albuminuria in type 2 diabetes patients, J. Clin. Endocrinol. Metab. 101 (2016) 4395–4404.

[6] Z. Zhang, L. Sun, Y. Wang, G. Ning, A.W. Minto, J. Kong, et al., Renoprotective role of the vitamin D receptor in diabetic nephropathy, Kidney Int. 73 (2008) 163–171.

[7] Y. Wang, D.K. Deb, Z. Zhang, T. Sun, W. Liu, D. Yoon, et al., Vitamin D receptor signaling in podocytes protects against diabetic nephropathy, J. Am. Soc. Nephrol. 23 (2012) 1977–1986.

[8] J. Guo, C. Lu, F. Zhang, H. Yu, M. Zhou, M. He, et al., VDR activation reduces proteinuria and high-glucose-induced injury of kidneys and podocytes by regulating Wnt signaling pathway, Cell. Physiol. Biochem. 43 (2017) 39–51.

[9] O. Trohatou, E.F. Tsilibary, A. Charonis, C. Iatrou, G. Drossopoulou, Vitamin D3 ameliorates podocyte injury through the nephrin signalling pathway, J. Cell. Mol. Med. 21 (2017) 2599–2609.

[10] M.M. Yeboah, M.A. Hye Khan, M.A. Chesnik, M. Skibba, L.L. Kolb, J.D. Imig, Role of the cytochrome P-450/epoxyeicosatrienoic acids pathway in the pathogenesis of renal dysfunction in cirrhosis, Nephrol. Dial. Transplant. 33 (2018) 1333–1343.

[11] Y. Zhou, T. Liu, J.X. Duan, P. Li, G.Y. Sun, Y.P. Liu, et al., Soluble epoxide hydrolase inhibitor attenuates lipopolysaccharide-induced acute lung injury and improves survival in mice, Shock 47 (2017) 638–645.

[12] Y. Zhou, J. Yang, G.Y. Sun, T. Liu, J.X. Duan, H.F. Zhou, et al., Soluble epoxide hydrolase inhibitor 1-trifluoromethoxyphenyl-3- (1-propionylpiperidin-4-yl) urea attenuates bleomycin-induced pulmonary fibrosis in mice, Cell Tissue Res. 363 (2016) 399–409.

[13] M.H. Gangadhariah, B.W. Dieckmann, L. Lantier, L. Kang, D.H. Wasserman, M. Chiusa, et al., Cytochrome P450 epoxygenase-derived epoxyeicosatrienoic acids contribute to insulin sensitivity in mice and in humans, Diabetologia 60 (2017) 1066–1075.

[14] G. Chen, P. Wang, G. Zhao, G. Xu, A. Gruzdev, D.C. Zeldin, et al., Cytochrome P450 epoxygenase CYP2J2 attenuates nephropathy in streptozotocin-induced diabetic mice, Prostaglandins Other Lipid Mediat 96 (2011) 63–71.

[15] G. Chen, R. Xu, Y. Wang, P. Wang, G. Zhao, X. Xu, et al., Genetic disruption of soluble epoxide hydrolase is protective against streptozotocin-induced diabetic nephropathy, Am. J. Physiol. Endocrinol. Metab. 303 (2012) E563–E575.

[16] K.N. Theken, Y. Deng, M.A. Kannon, T.M. Miller, S.M. Poloyac, C.R. Lee, Activation of the acute inflammatory response alters cytochrome P450 expression and eicosanoid metabolism, Drug Metab. Dispos. 39 (2011) 22–29.

[17] X. Tan, X. Wen, Y. Liu, Paricalcitol inhibits renal inflammation by promoting vitamin D receptor-mediated sequestration of NF-kappaB signaling, J. Am. Soc. Nephrol. 19 (2008) 1741–1752.

[18] V.A. Patel, D.J. Lee, L. Feng, A. Antoni, W. Lieberthal, J.H. Schwartz, et al., Recognition of apoptotic cells by epithelial cells: conserved versus tissue-specific signaling responses, J. Biol. Chem. 285 (2010) 1829–1840.

[19] W. Lieberthal, L. Zhang, V.A. Patel, J.S. Levine, AMPK protects proximal tubular cells from stress-induced apoptosis by an ATP-independent mechanism: potential role of Akt activation, Am J Physiol Renal Physiol 301 (2011) F1177–F1192.

[20] Y. Jia, Z. Zheng, M. Guan, Q. Zhang, Y. Li, L. Wang, et al., Exendin-4 ameliorates high glucose-induced fibrosis by inhibiting the secretion of miR-192 from injured renal tubular epithelial cells, Exp. Mol. Med. 50 (2018) 56.

[21] S. Yang, A. Li, J. Wang, J. Liu, Y. Han, W. Zhang, et al., Vitamin D receptor: a novel therapeutic target for kidney diseases, Curr. Med. Chem. 25 (2018) 3256–3271.

[22] D.K. Deb, Y. Wang, Z. Zhang, H. Nie, X. Huang, Z. Yuan, et al., Molecular mechanism underlying 1,25-dihydroxyvitamin D regulation of nephrin gene expression, J. Biol. Chem. 286 (2011) 32011–32017.

[23] Y. Sun, G. Zhou, T. Gui, A. Shimokado, M. Nakanishi, K. Oikawa, et al., Elevated serum 1,25(OH)2-vitamin D3 level attenuates renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction in kl/kl mice, Sci. Rep. 4 (2014) 6563.

[24] Y. Zhang, J. Kong, D.K. Deb, A. Chang, Y.C. Li, Vitamin D receptor attenuates renal fibrosis by suppressing the renin-angiotensin system, J. Am. Soc. Nephrol. 21 (2010) 966–973.

[25] Y.C. Li, Discovery of vitamin D hormone as a negative regulator of the renin-angiotensin system, Clin. Chem. 60 (2014) 561–562.

[26] B.Q. Deng, Y. Luo, X. Kang, C.B. Li, C. Morisseau, J. Yang, et al., Epoxide metabolites of arachidonate and docosahexaenoate function conversely in acute kidney injury involved in GSK3beta signaling, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 12608–12613.

[27] M. Skibba, M.A. Hye Khan, L.L. Kolb, M.M. Yeboah, J.R. Falck, R. Amaradhi, et al., Epoxyeicosatrienoic acid analog decreases renal fibrosis by reducing epithelial-to-mesenchymal transition, Front. Pharmacol. 8 (2017) 406.

[28] M. Ma, Q. Ren, J. Yang, K. Zhang, Z. Xiong, T. Ishima, et al., Key role of soluble epoxide hydrolase in the neurodevelopmental disorders of offspring after maternal immune activation, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 7083–7088.

[29] S. Mathew, R.J. Lund, L.R. Chaudhary, T. Geurs, K.A. Hruska, Vitamin D receptor activators can protect against vascular calcification, J. Am. Soc. Nephrol. 19 (2008) 1509–1519.

[30] S. Pacini, G. Morucci, J.J. Branca, S. Aterini, M. Amato, M. Gulisano, et al., Effects of vitamin D3 and paricalcitol on immature cardiomyocytes: a novel role for vitamin D analogs in the prevention of cardiovascular diseases, Nutrients 5 (2013) 2076–2092.

[31] M. Dai, L. Wu, P. Wang, Z. Wen, X. Xu, D.W. Wang, CYP2J2 and its metabolites EETs attenuate insulin resistance via regulating macrophage polarization in adipose tissue, Sci. Rep. 7 (2017) 46743.

[32] A. Luria, A. Bettaieb, Y. Xi, G.J. Shieh, H.C. Liu, H. Inoue, et al., Soluble epoxide hydrolase deficiency alters pancreatic islet size and improves glucose homeostasis in a model of insulin resistance, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 9038–9043.

[33] J. Zhang, H. Qiu, J. Huang, S. Ding, B. Huang, P. Zhou, et al., EETs/PPARs activation together mediates the preventive effect of naringenin in high glucose-induced cardiomyocyte hypertrophy, Biomed. Pharmacother. 109 (2019) 1498–1505.

[34] S. Eid, R. Maalouf, A.A. Jaffa, J. Nassif, A. Hamdy, A. Rashid, et al., 20-HETE and EETs in diabetic nephropathy: a novel mechanistic pathway, PLoS One 8 (2013) e70029.