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Original Article

Promotion of chemerin in rat diabetic kidney disease through enhancement of TGF-β1/Smads/CTGF pathway

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Abstract: Objective: Although increasing evidence shows that the adipokine chemerin is involved in diabetic kidney disease (DKD), it is still unclear whether the chemerin acts as a critical element in renal function through the signaling pathways of transforming growth factor β1/Smads/connective tissue growth factor (TGF-β1/Smads/CTGF) in the context of DKD. Therefore, we sought to determine the role of chemerin and TGF-β1/Smads/CTGF signaling pathway in the development and/or progression of DKD. Methods: We used rat renal mesangial cells (RMCs) and a DKD rat model as study subjects. RMCs and rats were randomly separated into different groups and transfected with the constructed chemerin expression vector pcDNA™ 3.1 (+)-chemerin. Rat renal function and inflammatory cytokines were assessed after treatment with chemerin or CCX832 (ChemR23 antagonist). Real time polymerase chain reverse transcription (RT-QPCR) was used to detect the mRNA expressions of TGF-β1, Smad2, Smad4, and CTGF. Western blot was performed to determine protein expression for semiquantitative analysis. Results: In *in vitro* studies, the mRNA and protein levels of TGF-β1, Smad2, Smad4, and CTGF were significantly increased in the groups of high glucose and chemerin as compared to the normal control and normal glucose groups, most notably in the high glucose chemerin group (all $P<0.05$). In vivo studies revealed that the mRNA and protein levels of TGF-β1, Smad2, Smad4, and CTGF were higher in the DKD group and the normal chemerin group than in the normal control group and the blocking receptor group, while appearing the highest in the DKD chemerin group (all $P<0.05$). Moreover, kidney/body weight ratio, urea, creatinine, and urine protein were increased, and the weight and endogenous creatinine clearance rate decreased in the DKD group and the normal chemerin group (all $P<0.05$). These changes were more pronounced in the DKD chemerin group. At the same time, blood glucose, triglycerides (TGs), and total cholesterol (TC) in the blocked receptor group was lower than those in the DKD group and the DKD chemerin group (all $P<0.05$). In contrast to those in the normal control group and blocked receptor group, tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1 showed higher concentrations in the DKD group and the normal chemerin group. This result was more pronounced in the DKD chemerin group (all $P<0.05$). Conclusion: Chemerin may play a role in DKD by enhancing the signaling pathways of TGF-β1/Smads/CTGF transduction either in vitro or in vivo. Moreover, high glucose accelerates kidney injury by activating fibrotic pathways.

Keywords: Diabetic kidney disease, chemerin, transforming growth factor β1/Smads, connective tissue growth factor

Introduction

With the improvement of living standards in China, the prevalence of diabetes is increasing year by year. Diabetic kidney disease (DKD), one of the complications of diabetes, is becoming a major cause of end-stage renal failure, that not only affects quality of life but also imposes a substantial economic burden [1]. The mechanisms of DKD are complex and not entirely clear. DKD is generally believed to be caused by a combination of metabolic disorders, hemodynamic changes, and cellular inflammatory factors, leading to mesangial membrane dilatation, glomerular basement membrane thickening, podocyte reduction and hypertrophy, renal tubular epithelium atrophy, extracellular matrix (ECM) proliferation, and eventually DKD [2].

New understanding of adipocytes is enabling important research breakthroughs. As an endocrine organ, adipocytes produce and secrete a large amount of adipose-derived cytokines.

Among these cytokines, chemerin, also called tazarotene-induced gene 2 protein (TIG2) or retinoic acid receptor responder 2 (RARRES2), is a 16 kDa cytokine, and was recently found to be primarily produced by white adipose tissue [3]. Chemerin is secreted as prochemerin as its precursor. Due to the different cleavage sites of the carboxyl terminal, inactive prochemerin can form the subtype chemerin with similar structure but different length and biological activity after processing by cathepsin, plasminase, and elastase [4]. Chemerin is a ligand of three G protein-coupled receptors, one of which, chemokine-like receptor 1 (CMKLR1, also known as ChemR23), mediates most of the biologic effects of chemerin and can be blocked by the specific antagonist CCX832. The other two receptors are G protein coupled receptor 1 (GPR1) and C-C chemokine receptor-like 2 (CCRL2), whose roles are currently unclear. Several studies have shown that serum chemerin is significantly elevated and is involved in obesity, metabolic syndrome, diabetes, and coronary heart disease [5-7]. Chemerin expression in the kidneys of diabetic rats was higher than that in normal rats, and serum chemerin was higher in DKD patients than that in healthy controls [8]. These data showed that chemerin may play an important role in DKD, but the mechanisms are unknown [9].

The most important feature of DKD is epithelial-mesenchymal transition (EMT), which mediates ECM deposition and subsequent interstitial fibrosis. Studies have revealed a much higher concentration of the transformative growth factor β1 (TGF-β1) in both animal models and DKD patients [10-12]. TGF-β, a recognized fibrosis-promoting factor, has received broad attention as a new target for DKD therapy. TGF-β1 activates many signaling pathways, including the the mitogen-activated protein kinase (MAPK) pathway, although the main target is the Smads pathway. Three types of mammalian Smads have been identified based on their physiologic function: (1) receptor-activated Smads (R-Smads), including Smad1, Smad2, Smad3, Smad5, Smad8, and Smad9; (2) common mediator Smads (Co-Smads), with only Smad4 in mammals; and (3) inhibitory Smads (I-Smads), including Smad6 and Smad7. Smad2, Smad3, Smad4, and Smad7 are known to be closely related to the kidneys [13]. For the signaling pathway of TGF-β1/Smads, once TGF-β1 binds to its receptor, TGF-β1 receptor kinase is activated, which promotes Smad2 and Smad3 phosphorylation and activation, and then activated Smad2 and Smad3 form a trimeric complex with Smad4, which moves to the nucleus and controls the transcription of target genes. The oligomeric complex provides negative feedback by increasing Smad7 transcription and expression and inhibiting Smad2 and Smad3 phosphorylation [14]. Downstream, connective tissue growth factor (CTGF) is mainly regulated by TGF-β1/Smads [15]. CTGF promotes ECM production and deposition and is considered as a key factor for eventual organ fibrosis [16]. Recent studies have shown that rosiglitazone reduces TGF-β1 expression levels as well as chemerin in the kidneys of DKD rats and that chemerin and TGF-β1 expression is positively correlated, suggesting that chemerin may promote the development and progression of DKD by regulating TGF-β1 [8]. In our study, we analyzed the expression of relevant factors in rat renal mesangial cells (RMCs) and DKD rats, the role of chemerin in the signaling pathway of TGF-β1/Smads/CTGF, and the effects of chemerin and the TGF-β1/Smads/CTGF pathway in DKD. The primary objective of this research was to indicate the role of chemerin in the pathogenesis of DKD to provide a theoretical basis for early DKD interventions, which may result in a new direction for treatment.

Methods

Construction of a chemerin expression vector (pcDNA™ 3.1 (+)-chemerin)

The coding sequence (CDS) for rat chemerin, with a length of 492 bp, was designed according to GenBank. A HindIII cleavage site (A|AGCTT) and a BamHI cleavage site (G|GATCC) were added to both ends of the CDS (TaKaRa Bio, JAP). The pcDNA™ 3.1 (+) vector (Invitrogen Corporation, USA) (**Figure 1**) and the CDS of chemerin were double-digested and ligated to construct the expression vector pcDNA™ 3.1 (+)-chemerin. Positive clones were screened, identified, and verified by sequencing.

Animal groups and treatments

Altogether, 40 male Sprague Dawley (SD) rats, aged 9-week-old weighing 250±20 g (Beijing

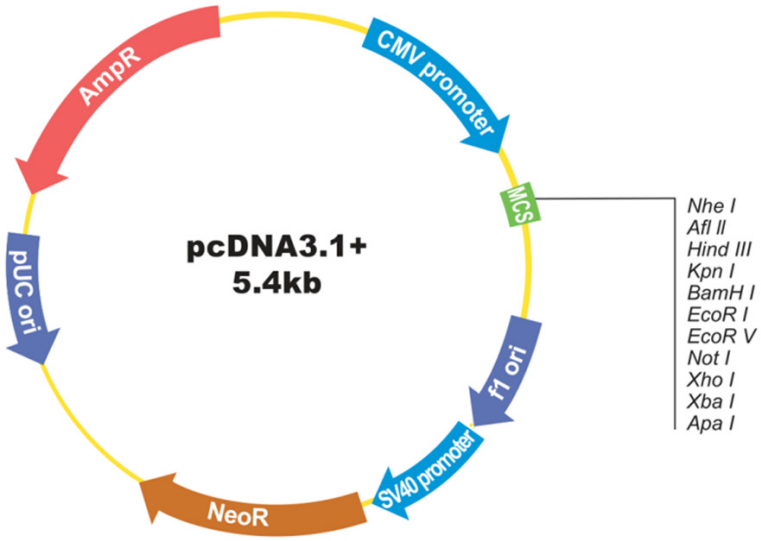

Figure 1. The features of the pcDNA™ 3.1 (+) vector. The complete sequences for pcDNA™ 3.1 (+) were downloaded from www.invitrogen.com.

Vitonlihua Experimental Animal Technology Co., Ltd, Beijing, China) were purchased for our study. After fasting for 12 hours, 24 rats fed with a high sugar and fat diet were randomly selected to acquire a single dose of 40 mg/kg streptozotocin (STZ) (Sigma Inc., USA) by tail vein injection, and 16 rats received an injection of an equal volume of 0.1 mol/L buffer with sterile citric acid-sodium citrate at pH 4.5 by the tail vein. After 72 hours, the blood sample was obtained from tail vein. Blood glucose higher than 16.7 mmol/L was considered as successful diabetic model establishment. After 4 weeks of treatment, the rats showed an increase in proteinuria and renal function deterioration, the pathologic alterations of the kidneys were consistent with the manifestations of DKD as well. The Ethics Committee of our hospital approved this research.

Then the rats were randomly divided into five groups, including: the normal control group of rats fed with conventional feed, the blocked receptor group of DKD rats treated with CCX832 at 75 mg/kg/day over three weeks through oral gavage, the DKD group (DKD rats), the normal chemerin group (normal rats injected with chemerin-RMCs into the renal cortex), and the DKD chemerin group (DKD rats injected with chemerin-RMCs into the renal cortex). CCX832 is a ChemR23 antagonist (ChemoCentryx, Inc., Mountain View, USA). Previous studies found a low nanomolar affinity in the binding of CCX832 to the ChemR23 receptors in humans, mice, and rats [17]. To inject chemerin-RMCs, one kidney was exposed under sterile conditions by ventral medial-lateral incision, and a needle was inserted into the renal cortex to slowly inject 60 μL of chemerin-RMC suspension ($3^{*}10^{6}$/mL) [18]. The other kidney was treated in the same manner. Isoflurane was used to relieve preoperative pain, aseptic procedures were carefully performed, and gentamicin was applied to the wound after surgery. Ten weeks later, all five groups were sacrificed by inhalation of carbon dioxide. Both kidneys were quickly removed, dried with a tissue, and weighed. Some kidney tissue was preserved in liquid nitrogen for RNA and protein extraction.

Postnecropsy analyses

Kidney sections (5 μm) obtained from the cortex and medullary zone to the renal pelvis, were separately stained with periodic acid-Schiff (PAS), hematoxylin-eosin (H&E), and periodic Schiff-methenamine silver (PASM) plus Masson's trichrome. Residual kidney parenchyma was evaluated by light microscopy. Tubulointerstitial and glomerular injury indexes were calculated according to the formula of standard semiquantitative grading scales [19, 20].

Rat general index and renal function test

The rats and their kidneys were weighed, and the kidney-to-body weight ratio was calculated. Urine samples were collected 24 hours prior to sacrifice to analyze 24-hour urine protein and urine creatinine. Moreover, the tail vein was exploited for more blood samples, which were analyzed for levels of serum urea nitrogen, creatinine, blood glucose, triglycerides (TGs), and total cholesterol (TC), and the calculation of endogenous creatinine clearance rate (Ccr = urinary creatinine/serum creatinine*urine volume per minute).

Table 1. Primers used in amplification of target gene mRNA

Target gene (amplified sequence length)	Primer sequence
TGF-β1 (200 bp)	Forward: 5'-CAACAACGCAATCTATGACA-3' Reverse: 5'-CAAGGTAACGCCAGGAAT-3'
Smad2 (175 bp)	Forward: 5'-AGCAGGAATTGAGCCACAGAGT-3' Reverse: 5'-TGGCTGCAAATCCAAGCTGT-3'
Smad4 (191 bp)	Forward: 5'-GCTGAAAGAGAAGAAAGATGAACTGG-3' Reverse: 5'-TTAGTTCGTTCTTGTGTAGATCAGGC-3'
CTGF (247 bp)	Forward: 5'-TGCCTGCCATTACAACTG-3' Reverse: 5'-CACACGGTTCTCACTTCG-3'
β-Actin (282 bp)	Forward: 5'-GAAGTGTGACGTTGACATCCG-3' Reverse: 5'-GCCTAGAAGCATTTGCGGTG-3'

Note: TGF-β1: transforming growth factor β1; CTGF: connective tissue growth factor.

Cell culture and transfection

RMCs were obtained from the Chinese Academy of Sciences/the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were resuspended according to the instructions provided and kept within Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, USA), with supplementation of 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Inc.) and 50 UI/mL penicillin/streptomycin (Gibco, Life Technologies) in a humidified atmosphere of 5% CO_2/95% air at 37°C.

RMCs passaged five to eight times were cultured to 95% confluency, trypsinized, plated, and cultured to 70-80% confluency. Next, the medium was replaced with a medium free from serum and antibiotics to synchronize cells for 24 hours. Lipofectamine 2000 was applied to transfect the pcDNA™ 3.1 (+)-chemerin vector into RMCs and produce chemerin-RMCs, which were incubated at 37°C in 5% CO_2 for 48 hours, followed by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) to determine transfection efficiency. After transfection for 48 hours, the cells were screened with G418 (600 μg/mL; Invitrogen Corporation) to select resistant clones, and RT-qPCR was performed to determine the transfection efficiency of the chemerin vector in stable cell lines.

Cell groups and treatments

RMCs were separated randomly into five groups: the blank control group, the normal glucose group (5.5 mmol/L), the high glucose group (30 mmol/L), the chemerin group (chemerin-RMCs), and the high glucose chemerin group (30 mmol/L glucose + chemerin-RMCs). The cells were incubated in the environment of saturated humidity at 37°C with 5% CO_2 for 48 hours and then harvested.

ELISA

ELISA was used to measure the levels of interleukin (IL)-1 and tumor necrosis factor alpha (TNF-α) based on the kit instructions (BOSTER Biological Technology Co., Ltd, China). The concentrations of chemerin in serum and cell supernatant were measured before and after transfection (LifeSpan BioSciences Co., Ltd, USA).

RT-QPCR

After total RNA was extracted from RMCs and rat kidneys, 2 μg of RNA were reverse transcribed in a reaction mix containing 2 μL of 100 μg/mL oligo-dT, 1 μL of PCR Nucleotide Mix, 4 μL of 5X Reaction Buffer, and 1 μL of Reverse Transcriptase (Promega, USA). RT-qPCR was carried out to confirm the mRNA levels of TGF-β1, Smad2, Smad4, and CTGF. For RT-qPCR system, 2 μL of reverse transcription product were diluted in a reaction buffer containing 12.5 μL of SYBR Premix Ex Taq (TaKaRa Bio, JAP) and 10 μmol/L of primers in a final volume of 25 μL per sample. The reaction conditions were 94°C for 30 s, followed by 40 cycles of 3 steps, denaturation at 95°C for 5 s, annealing at 55-60°C for 30 s, and extension at 72°C for 30 s. β-Actin was used as an internal control and the target genes' relative expression levels were calculated (**Table 1**).

Western blot

Western blot was applied to analyze protein expressions of TGF-β1, Smad2, Smad4, and CTGF. After treatment, RMCs or rat kidney tissues were lysed in RIPA buffer (Boster Biological Technology, USA), followed by the measurement of protein concentration using the Bradford method (Coomassie brilliant blue G250, Invitrogen Corporation, USA). A 10%

SDS-polyacrylamide gel was used to spread the protein samples of equal amounts, which were then transferred to a nitrocellulose membrane (Invitrogen Corporation, USA), blocked with 1X Tris-buffered saline containing 0.1% Tween® 20 Detergent (TBST) buffer consisting of 5% skim milk, 25 mM Tris-HCl at pH 7.5, 125 mM NaCl, and 0.1% Tween 20. Next, diluted primary antibodies (rabbit anti-rat TGF-β1 polyclonal antibody, 1:500, ab92486; rabbit anti-rat Smad2 polyclonal antibody, 1:500, ab63576; rabbit anti-rat Smad4 monoclonal antibody 1:5000, ab40759; rabbit anti-rat CTGF polyclonal antibody, 1:1000, ab6992; rabbit anti-rat β-Actin monoclonal antibody 1:1000, ab181602; Abcam, UK) were added, and the membrane was incubated at 4°C overnight. After washing, diluted secondary antibody (horseradish peroxidase [HRP]-labeled goat anti-rabbit secondary antibody 1:3000, ab6721) was added, and the membrane was incubated. Finally, electrochemiluminescence (ECL) reagents were added to detect protein levels on the nitrocellulose membrane with a gel imaging system.

Statistical analysis

SPSS 22.0 was used for statistical analysis. The data demonstrated the deviation of mean to standard. The variance's homogeneity was tested. One-way analysis of variance (ANOVA) was achieved through comparisons among the groups. The Student-Newman-Keuls (SNK) method was used to compare the groups under the condition of unviolated homogeneity of variance, while the rank sum test was performed for comparison under the condition of violated homogeneity of variance. $P<0.05$ was considered significant.

Results

General index, renal function, and inflammatory factors in different rat groups

After intervention, the model rats in the DKD group were compared with those in the normal control group, showing a gradually increased urinary protein excretion (**Figure 2I**), deteriorated renal function (**Figure 2F-H**), and renal pathology showed mesangial cell proliferation (**Figure 3A**). The success rate of DKD rat modeling was 83.3% (15/18), and 2 rats died in two weeks after injection; one did not develop stable high blood sugar (≥16.7 mmol/L). The results showed that at 10 weeks after treatment, the kidney/body weight ratio, urea, creatinine, and urine protein were higher, while weight and endogenous creatinine clearance rate (Ccr = urinary creatinine/serum creatinine*urine volume per minute) were lower in the DKD group as well as the normal chemerin group, compared to those in the normal control group and the blocking receptor group (all $P<0.05$). This result was more pronounced in the DKD chemerin group. At the same time, levels of the blood glucose, TG, and TC in the blocking receptor group were lower than those in the DKD group and the DKD chemerin group (all $P<0.05$). In comparison with those in the normal control group and blocking receptor group, the levels of IL-1 and TNF-α were significantly higher in the DKD group and the normal chemerin group (all $P<0.05$). This result was more pronounced in the DKD chemerin group (**Figure 2**).

Effects on kidney tissue architecture

Sections of resected renal tissues were exploited to investigate the major nephron compartments and to calculate glomerular and tubulointerstitial injury scores semi-quantitatively. The result indicated that the renal tubules and glomerular structure of the normal control group were natural. The normal control group showed normal renal tubules and glomeruli. DKD and normal chemerin group kidneys had extensive diffuse glomerular hypertrophy with segmental to systemic glomerular sclerosis, moderate mesangial proliferation, and focal glomerular atrophy. They also presented with inflammatory tubulointerstitial fibrosis to a mild to moderate extent. The above characteristics were more severe in the DKD chemerin group. DKD chemerin group kidneys were distinguished by their severe glomerular and tubulointerstitial injury, whereas blocking receptor group kidneys had proportionally healthier tubules, glomeruli and other nephron structures; and decreased glomerulosclerosis and tubulointerstitial fibrosis (**Figure 3**).

Concentrations of chemerin in rat RMCs

After transfection, the concentrations of chemerin in serum and cell supernatant gradually increased with pcDNA™ 3.1 (+)-chemerin application (**Figure 4**).

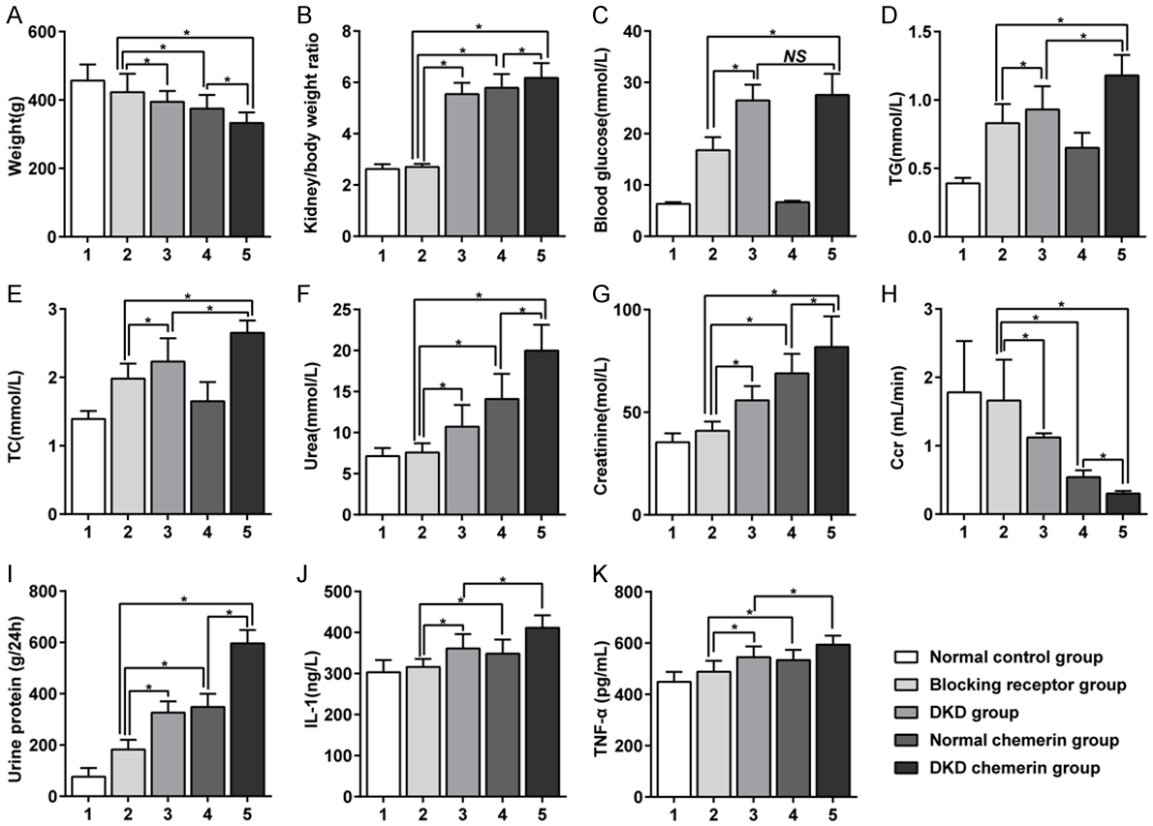

Figure 2. General index, renal function, and inflammatory factors in different rat groups (n=8). Kidney/body weight ratio (B), urea (F), creatinine (G), and urine protein (I) were higher and the weight (A), Ccr (H) were fewer in the DKD group and normal chemerin group than in the normal control group and blocking receptor group. This result was more pronounced in the DKD chemerin group. The blood glucose (C), TG (D), TC (E) of the blocking receptor group were lower than those in the DKD group and the DKD chemerin group. In comparison with those in the normal control group and blocking receptor group, the levels of IL-1 (J) and TNF-α (K) were significantly higher in the DKD group and normal chemerin group. This result was more pronounced in the DKD chemerin group. $^{*}P<0.05$, $^{NS}P>0.05$. TG: triglyceride, TC: total cholesterol, Ccr: endogenous creatinine clearance rate; DKD: diabetic kidney disease.

Expression of the TGF-β1/Smads/CTGF signaling pathway in rat kidneys

The mRNA and protein expression levels of TGF-β1, Smad2, Smad4, and CTGF in the TGF-β1/Smad/CTGF pathway showed no remarkable distinction between the normal control group and the blocking receptor group; nevertheless, these levels were higher in the DKD group, the normal chemerin group, and the DKD chemerin group than those in the normal control group (all P<0.05), especially in the DKD chemerin group (**Figure 5**).

Expression of the TGF-β1/Smads/CTGF signaling pathway in RMCs

The transfection efficiency of rat RMCs was 23.5±1.8%. The mRNA and protein expression levels of TGF-β1, Smad2, Smad4, or CTGF in the TGF-β1/Smads/CTGF pathway showed no remarkable distinction between the blank control group and the normal glucose group; however, the expression levels in the high glucose group, the chemerin group, and the high glucose chemerin group were notably higher than those of the blank control group (all P<0.05), especially in high glucose chemerin group (**Figure 6**).

Discussion

Chemerin is a newly discovered adipose-secreted cytokine with elevated levels in diabetes and DKD patients, indicating that chemerin may have a role in DKD, although the details are unknown [8, 9]. TGF-β1 is considered the "switch" that induces fibrosis and the driver for

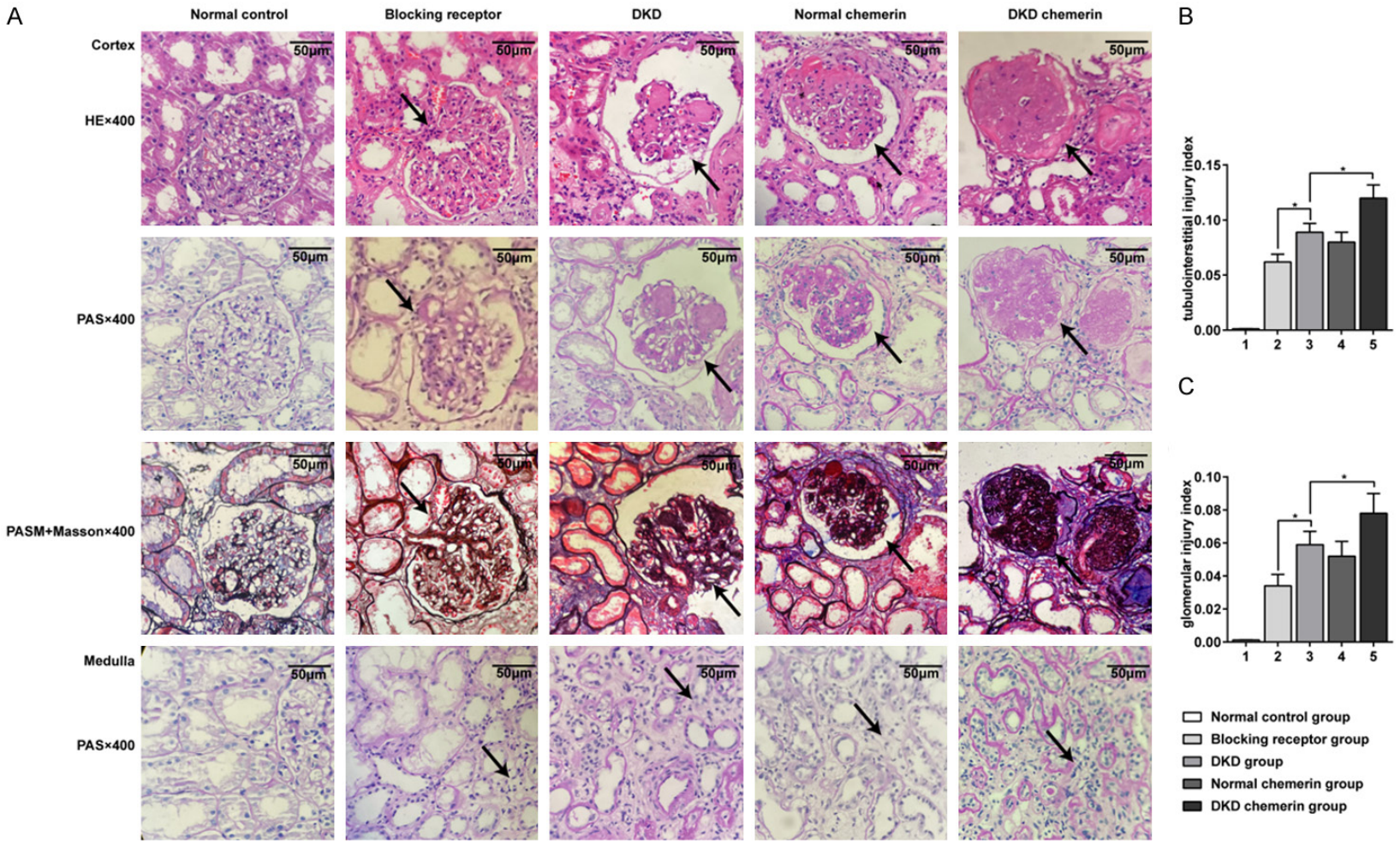

Figure 3. Comparative renal histopathology in different groups of rat kidneys (n=8). (A) The normal control group showed normal renal tubules and glomeruli. Representative sections (stain indicated) from the other four groups of rat kidneys tissues show varying degrees of tubular and glomerular damage evidenced by glomerulosclerosis, tubulointerstitial fibrosis (H&E, PAS, PASM+Massson), and accumulation of protein casts in the tubular lumen (PAS; 200×). (B and C) Standardized semiquantitative methods were used to generate indexes of glomerular injury (B) and tubulointerstitial injury (C). The blocked receptor group had significantly reduced both glomerular and tubulointerstitial injury, while the DKD chemerin group was the worst. *P<0.05. DKD: diabetic kidney disease. The arrows point to the typical location of tubular and glomerular damage.

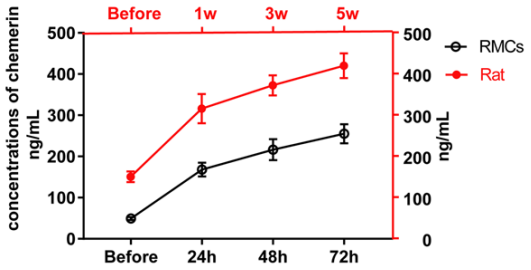

Figure 4. Concentrations of Chemerin in rat and RMCs. RMC: renal mesangial cells.

progressive fibrosis [21, 22]. As a downstream mediator, CTGF is also an important factor in renal fibrosis [16]. Recent studies have found a strong positive association of chemerin with TGF-β1 expression in the kidney of DKD rats [8]. Based on this finding, we found that chemerin may have some association with TGF-β1, an indispensable factor attrinecessary for the induction and growth of DKD. Because glomerular mesangial cell injury is a major mechanism of DKD, we investigated the effect of chemerin on mesangial cell injury using eukaryotic plasmid transfection [1]. This study showed that chemerin alone or in combination with high glucose increased the mRNA and protein levels of TGF-β1, Smad2, Smad4, and CTGF, which are involved in the TGF-β1/Smads/CTGF pathway in RMCs. The same result was found with pcDNA™ 3.1 (+)-chemerin injection into healthy rats and DKD rats. Therefore, both *in vivo* and *in vitro* studies indicated that chemerin may enhance the conduction of TGF-β1/Smads pathway and upregulate the expression of CTGF, thereby promoting the development and progression of DKD (**Figure 7**). On the other hand, we found that rat renal function deterioration was reduced and that the expression of TGF-β1/Smads/CTGF pathway was inhibited after administration of ChemR23 antagonists, which also verified the chemerin's promotion of the signaling pathway. In addition, studies have shown angiotensin II (Ang II) enhanced the Smad pathway through AT1 receptors and MAPK activation independently of TGF-beta, resulting in profibrogenic effects [23]. Whether chemerin can initiate the signaling pathway of Smad through other pathways will be further explored in our subsequent experiments.

An extremely close relationship was found between chemerin and renal function. Studies have revealed a negative correlation between serum chemerin and the glomerular filtration rate (GFR) in patients with chronic renal failure and in renal transplants, suggesting that chemerin may be an indicator of renal function [24-26]. Hu and Feng grouped type 2 diabetes patients according to their urinary albumin excretion rate (UAER) and found that serum chemerin was significantly higher in the group of patients with macroalbuminuria (UAER >300 mg/24 h) than in the group of patients with normal albuminuria (UAER <30 mg/24 h) and the microalbuminuria group (UAER 30-300 mg/24 h) [9]. Our study showed that chemerin intervention caused a significant reduction in renal function in rats, which was consistent with literature reports. However, the detailed relationship between elevated chemerin and DKD progression and renal decline is unclear. Studies have shown that oxidative stress may be a mechanism of chemerin [27]. Moreover, chemerin not only has proinflammatory effects but also promotes the release of other inflammatory cytokines, which aggravates the inflammatory response and accelerates kidney damage [28, 29]. This study showed that chemerin may accelerate renal fibrosis and renal deterioration by enhancing the signaling pathway of the TGF-β1/Smads/CTGF, which is a newly described mechanism of chemerin.

The main pathologic features of DKD are renal hypertrophy, microscopically thickened glomerular basement membrane, renal tubule atrophy, and interstitial fibrosis. The early clinical manifestations are proteinuria, and with the progression of disease, a decreased GFR and renal insufficiency may ultimately occur. This study showed similar findings in DKD rats, although the mechanisms are unknown. Acute hyperglycemia may accelerate the apoptosis of intrinsic renal cells by activating oxidative stress and inflammatory responses, causing significant damage to renal structure and function [30]. This study showed that high glucose activated the fibrotic TGF-β/Smads pathway, thereby activating the downstream factors and aggravating renal deterioration, which is consistent with literature reports [31, 32]. On the other hand, the chemerin receptor blockade may prevent the development and/or progression of DKD, as observed in our study.

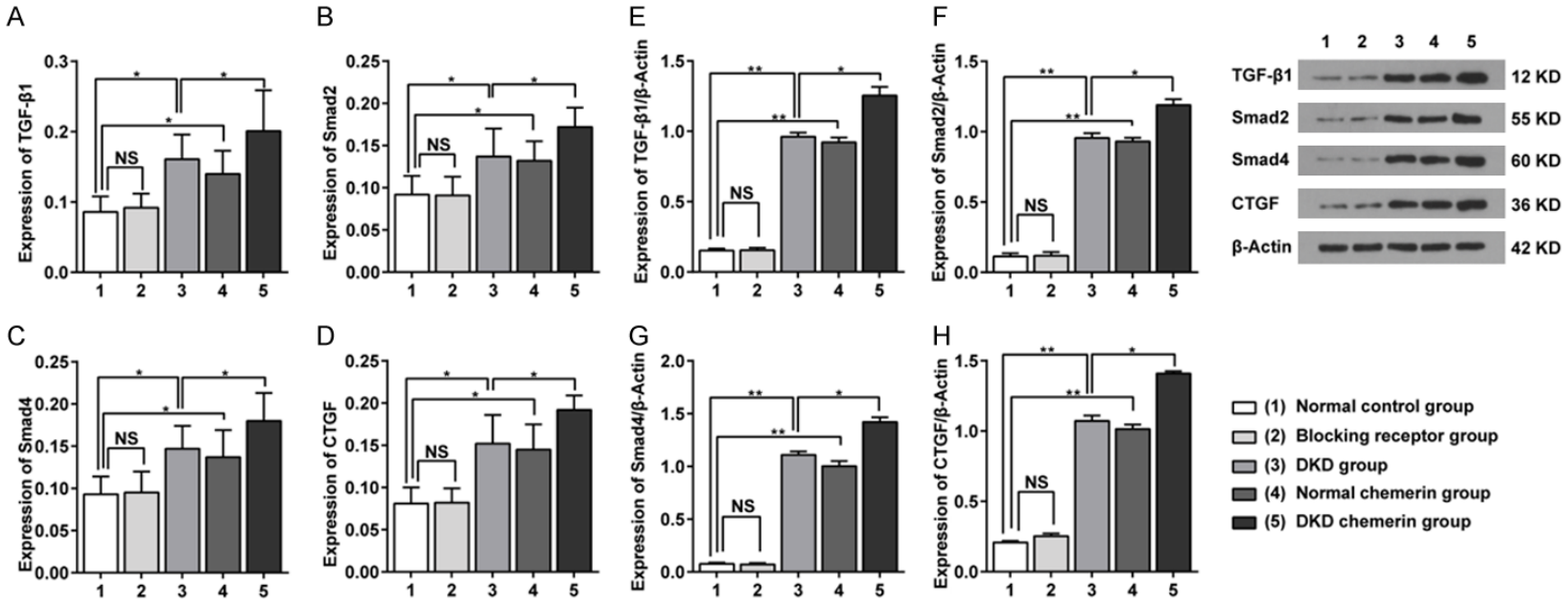

Figure 5. Relative mRNA levels of genes and protein expression in the TGF-β1/Smads/CTGF pathway in different groups of rat kidneys (n=8). The mRNA and protein expression levels of TGF-β1 (A, E), Smad2 (B, F), Smad4 (C, G), and CTGF (D, H) in the TGF-β1/Smad/CTGF pathway showed no remarkable distinction between the normal control group and the blocking receptor group; nevertheless, these levels were higher in the DKD group, the normal chemerin group, and the DKD chemerin group than those in the normal control group, especially in the DKD chemerin group. $^{**}P<0.01$. $^{*}P<0.05$; compared with the normal control group, $^{NS}P>0.05$. DKD: diabetic kidney disease.

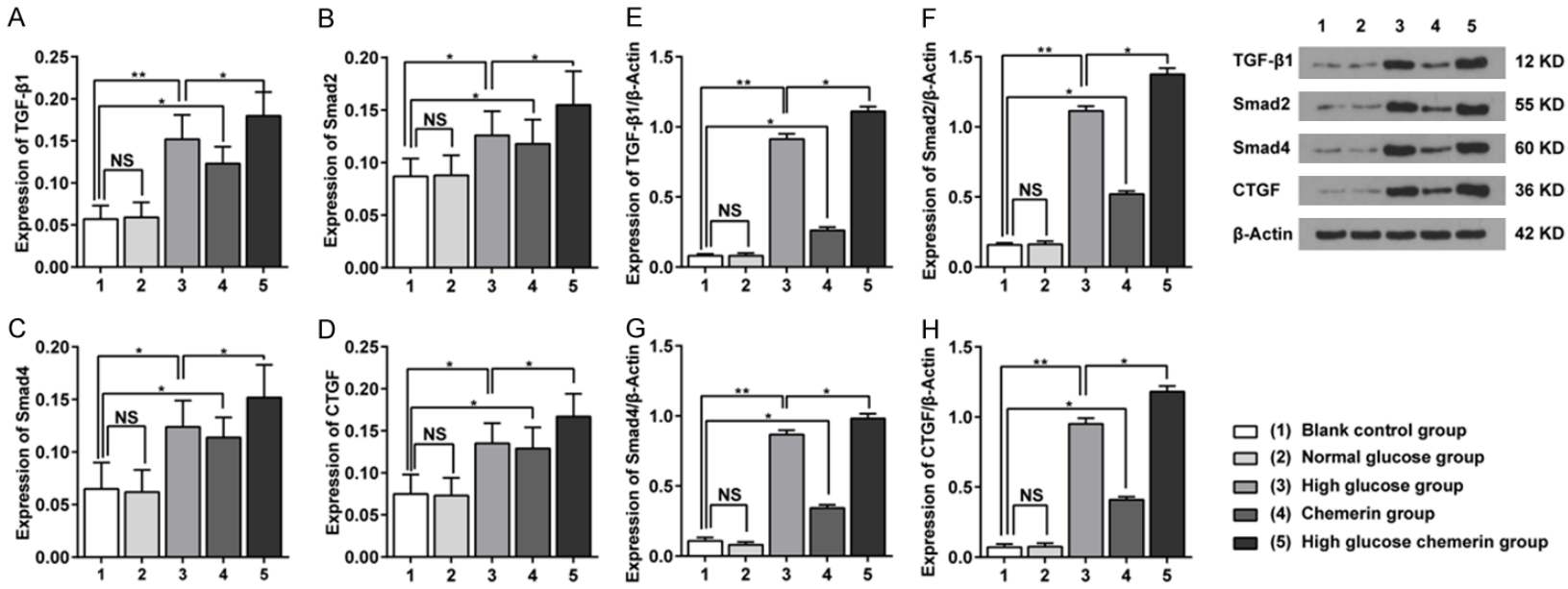

Figure 6. Relative mRNA levels of genes and protein expression in the TGF-β1/Smads/CTGF pathway in different groups of rat mesangial cells (n=6). The mRNA and protein expression levels of TGF-β1 (A, E), Smad2 (B, F), Smad4 (C, G) and CTGF (D, H) in the TGF-β1/Smad/CTGF pathway showed no remarkable distinction between the normal control group and the blocked receptor group; however, the expression levels in the high glucose group, the chemerin group, and the high glucose chemerin group were notably higher than in the blank control group, especially in high glucose chemerin group. $^{**}P<0.01$, $^{*}P<0.05$; compared with the blank control group, $^{NS}P>0.05$. TGF-β1: transforming growth factor β1; CTGF: connective tissue growth factor.

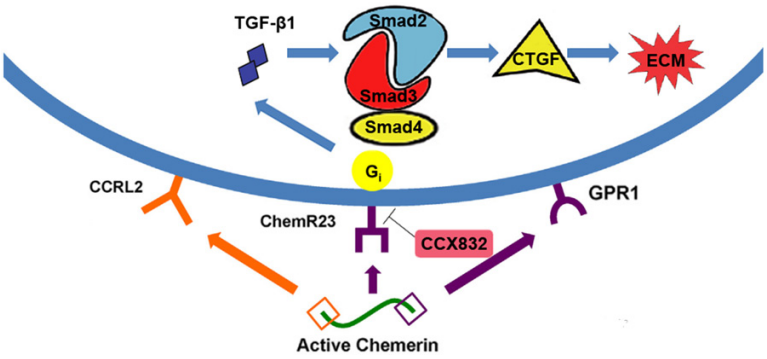

Figure 7. Possible mechanism whereby chemerin administration may affect the TGF-β1/Smads/CTGF pathway to enhance ECM production, which is the key to promote and develop DKD. Three receptors of chemerin: ChemR23, CCRL2, GPR1. TGF-β1: transforming growth factor β1; CTGF: connective tissue growth factor; ECM: extracellular matrix.

Previously, Chinese and foreign researchers investigated the role of chemerin in DKD. This study provides further insight into the mechanism of chemerin and hyperglycemia and analyzes their relationship with TGF-β1, Smad2, Smad4, and CTGF. The results showed that chemerin may promote DKD by enhancing the TGF-β1/Smads/CTGF signaling pathway. Hyperglycemia has similar effects, thereby accelerating kidney injury. However, the specific mechanism of how chemerin acts on TGF-β1 is still unknown, and whether chemerin can activate fibrosis through other pathways requires further studies.

Disclosure of conflict of interest

None.

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