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CIDEC silencing attenuates diabetic nephropathy via inhibiting apoptosis and promoting autophagy

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ABSTRACT

Objective: The role of cell death-inducing DFF45-like effector C (CIDEC) in insulin resistance has been established, and it is considered to be an important trigger factor for the progression of diabetic nephropathy (DN). We intend to explore whether CIDEC plays an important role in the regulation of DN and its potential mechanism.

Methods: High-fat diet and low dose streptozotocin were used to establish type 2 diabetic rat model. We investigate the role of CIDEC in the pathogenesis and process of DN through histopathological analysis, western blot and gene silencing. Meanwhile, the effect of *CIDEC* on renal tubular epithelial cells stimulated by high glucose was also verified.

Results: DM group exhibited glucose and lipid metabolic disturbance, with hypertrophy of kidneys, damaged renal function, increased apoptosis, decreased autophagy, glomerulosclerosis and interstitial fibrosis. *CIDEC* gene silencing improved metabolic disorder and insulin resistance, alleviated renal hypertrophy and renal function damage, decreased glomerular and tubular apoptosis, increased autophagy and inhibited renal fibrosis. At the cellular level, high glucose stimulation increased CIDEC expression in renal tubular epithelial cells, accompanied by increased apoptosis and decreased autophagy. *CIDEC* gene silencing can improve autophagy and reduce apoptosis. At the molecular level, *CIDEC* gene silencing also decreased the expression of early growth response factor (EGR)1 and increased the expression of adipose triglyceride lipase (ATGL).

Conclusion: *CIDEC* gene silencing may delay the progression of DN by restoring autophagy activity and inhibiting apoptosis with the participation of EGR1and ATGL.

INTRODUCTION

Diabetic nephropathy (DN) occurs in 30–40% of type 2 diabetic patients[1], with the incidence still is increasing[2,3]. DN is the most common cause of end-stage renal disease in the world, significantly increasing the mortality rate of patients with diabetes, so it is also one of the most dangerous and destructive complications of diabetes[4]. The etiology and pathogenesis of DN are very complicated, involving disorders of glucose and lipid metabolism, apoptosis, circulation obstacle, release of inflammatory mediators and autophagy etc[5,6]. Therefore, it is necessary to explore a new target molecular as a basis for the pathogenesis of DN and to find a new therapy for DN.

Increased apoptosis and autophagy defect induced by insulin resistance are the key mechanisms in the progression of DN. Apoptosis is induced and stimulated by high concentration of glucose in DN in glomerular endothelial cells, mesangial cells, podocytes and tubular cells, however, its underlying mechanism remains unclear[5,7]. Apoptosis can promote tubular cell hypertrophy, glomerular basement membrane dilatation, and accumulation of extracellular matrix, which contribute to progressive tubulointerstitial fibrosis and glomerulosclerosi. Therefore, it is considered to be an important inducer of renal

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remodeling[8]. Furthermore, autophagy is negatively correlated with renal fibrosis including glomerular extracellular matrix accumulation and tubulointerstitial fibrosis[3,9,10], while autophagy deficiency promotes the progression of glomerular lesions and tubulointerstitial damage[3]. Therefore, it is necessary to explore a new therapeutic method to inhibit renal apoptosis and activate autophagy.

Cell death-inducing DFF45-like effector C (CIDEC) may be a key molecule in regulating renal apoptosis and autophagy deficiency induced by insulin resistance. Studie has shown the importance of CIDEC in regulating apoptosis[11–14]. Under the condition of high glucose, early growth response factor (EGR)1 can induce DN by enhancing extracellular matrix to deposit and mesangial cells to proliferate, partly due to TGF-β1 upregulation mediated by EGR1 under high glucose conditions[15]. Singh *et al.*[16] has demonstrated EGR1 may inhibit the transcription of adipose triglyceride lipase (ATGL), while CIDEC may bind to EGR1 to enhance this effect. CIDEC inhibits lipid decomposition, at least partly due to the enhanced effect of EGR1 depressing *ATGL* transcription[16]. *ATGL* gene deficiency in mice can induce lipid heterotopic deposition of kidney and promote apoptosis of podocytes, which thereby lead to glomerular filtration barrier dysfunction and albuminuria[17]. It also has the ability to promote apoptosis of proximal tubular cells so eventually lead to renal fibrosis and renal dysfunction[18]. It is essential and adequate to induce autophagy and lipid decomposition via ATGL[19].

Based on the above studies, we speculate that CIDEC may promote apoptosis and inhibit autophagy in the pathogenesis of DN by increasing EGR1 expression and inhibiting ATGL expression, which may lead to renal fibrosis and decline of renal function under the diabetic condition.

MATERIALS AND METHODS

Experimental design

Thirty-two Sprague-Dawley rats (120–140 g) aged 5 weeks were provided by the Experimental Animal Center of Shandong Traditional Chinese Medicine University (Jinan, China). Rats were randomly divided into two groups: control group (n = 8) and DM group (n = 24). The DM rats was offered with high-fat diet (34.5% fat, 17.5% protein, 48% carbohydrate; Beijing HFK Bio-Technology, Beijing, China), while the controls were given normal food. After 4 weeks, DM rats with insulin resistance were injected with a single dose of streptozotocin intraperitoneally (STZ; Sigma-Aldrich, St. Louis, MO, USA, 27.5 mg/kg), and the controls were given the same dose of citrate buffer. 1 week after STZ treatment, levels of fasting blood glucose and insulin were detected. The insulin sensitivity index (ISI) was figured out as follows: ISI = In [([fasting glucose] × [fasting insulin])$^{-1}$]. The animals with the levels of fasting blood glucose ≥11.1 mmol/L for two consecutive times, decreased insulin sensitivity, polyuria, polydrink and polydiet were considered to be diabetic model. DM group (n = 24) was randomly divided into three groups: DM, DM + vehicle and DM + *CIDEC* shRNA (n = 8, respectively). Each group was fed with foregoing diet for 12 weeks, adenovirus was injected into the tail vein as previously described[20]. Adenovirus with *CIDEC* shRNA was injected into DM + *CIDEC* shRNA group, while vehicle virus was injected into DM + vehicle group. The 5′-AAG ACA UCU UGA AGC UUA AAG-3′ and 5′-GAC UCU CUU CAG UAU GCA AGC-3′ oligoribonucleo-tides were used to inhibit CIDEC synthesis. The *CIDEC* shRNA is located in the region of the CIDEC transcript (GenBank™ accession number NM_001024333) of the sequence. After fed with foregoing diet for 4 weeks, the rats were drawn blood for tests and then killed. Their kidneys were cut off for weight measuring. All rats were treated with humanitarian care, and all of the research steps were carried out according to the animal procedure ratified by the animal nursing committee of Shandong University.

Glucose and insulin tolerance

Intraperitoneal glucose tolerance test (IPGTT). After fasting for 12 h, the rats were assessed levels of blood glucose, at 0, 15, 30, 60 and 120 min, respectively after intraperitoneally injected with glucose (1 g/kg). Intraperitoneal insulin tolerance test (IPITT). After fasting for 4 h, the rats were given intraperitoneal injection of 1 unit/kg insulin to assess insulin tolerance, and blood glucose levels were detected according to the above method. We acquired the blood samples from the caudal vein and detected the levels of plasma glucose by using Johnson's steady blood glucose analyzer (Life Scan, Milpitas, CA, USA). The area under the concentration-time curve (AUC) of glucose was figured out by above results.

Blood analysis

After fasting overnight, all rats were collected of blood samples from the jugular vein. Blood samples were detected by Bayer l650 blood biochemistry analyzer (Bayer, Tarrytown, NY, USA).

Renal weight index

At week 21 (week 0 was seen as the start of a high-fat diet), the rats were weighed after anesthesia. Then the rats were fixed and injected with precooled saline at 4 into the left ventricle until the heart and kidney were pale. The kidneys were quickly removed, cleaned, and weighed. Kidney weight index (KWI) = mean weight of two kidneys (mg)/body weight (g).

Histomorphological analysis

The kidney tissue samples were harvested for pathologic examination, and some sections were fixed overnight in 4% paraformaldehyde and embedded in paraffin. After deparaffinization and rehydration, the paraffin sections (4.5 μm) were stained with Hematoxylin and eosin (HE), periodic acid-Schiff (PAS) and Masson trichrome, respectively, according to the manufacturer's instructions. Images were analyzed by Image-Pro Plus 5.0 automatic image analysis system (Media Cybernatics, Houston, TX, USA).

Immunohistochemical staining

The paraffin enwrapped tissue sections were conventionally dewaxed, heated with microwave for 15 min, incubated with the first antibody of 1:200 diluted CIDEC (Abcam, Cambridge, MA, USA), 1:1000 diluted cleaved caspase 3 (C-caspase3; Cell Signaling Technology, Danvers, MA, USA), 1: 30 diluted cleaved PARP (C-PARP; Boster Biological Technology, Wuhan, China), TGF-β1 (Proteintech, Rosemont, IL, USA), collagen 1 and collagen 4 (Abcam) at 4 overnight, respectively, then incubated with the second antibody for 1h at 37 . The results were observed and photographed under FV1000 laser scanning confocal microscope (Olympus, Tokyo, Japan). The IOD values were measured and analyzed by Image-Pro Plus 5.0 automatic image analysis system (Media Cybernatics, Houston, TX, USA).

Western blot analysis

The proteins were obtained from renal tissues and cells, separated out by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Beyotime Institute of Biotechnology, China). The PVDF membranes were sealed with 5% skimmed milk, incubated with 1:1000 diluted primary antibody of β-actin, CIDEC, ATGL, ATG5, ATG7 (Abcam), cleaved caspase3 (C-caspase3, Cell Signaling Technology), and LC3 (Proteintech), 1:200 diluted cleaved PARP (C-PARP; Boster Biological Technology), EGR1 (Santa Cruz Biotechnology, Dallas, TX, USA) primary antibody overnight, then incubated in 1:5000 diluted horseradish peroxidase labeled second antibody. Immobilon Western HRP substrate (WBKLS0500; Millipore Corporation, Danvers, MA, USA) was used to take pictures and Image J software (National Institutes of Health, Bethesda, MD, USA) was used to analyze the relative strength of the bands.

TUNEL test

TUNEL (Terminal dUTP nick-end labelling) was used to detect renal apoptotic cells. The chromogenic TUNEL cell apoptosis detection kit (QIA33; Calbiochem, Boston, MA, USA) was used. The results were observed and photographed under the FV1000 laser scanning confocal microscope (Olympus).

Cell culture

Normal rat kidney tubular epithelial cell line (NRK-52E) cells were donated by Mrs Zhang Chunmei from the Shandong University (Jinan, China). The cells were cultured in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM; low glucose), supplemented with 10% fetal bovine serum, 4 mM L-glutamine and 1% penicillin/streptomycin. Prior to use, cells were starved for 2 h and then transfected with adenotoxin *CIDEC* shRNA for 8–10 h. The cultured cells were then treated with low glucose (Control), 22.5 mmol/L glucose (HG), HG + Vehicle or HG + *CIDEC* shRNA for 24 h.

Statistical analysis

All results were presented as means and standard deviations. Statistical analysis was performed using SPSS 23.0 (SPSS, Chicago, IL, USA). Data comparison between two groups was performed by Student's *t* test. One-way ANOVA, followed by least significant difference test was used to compare the data between multiple groups. $P < 0.05$ was considered statistically significant.

RESULTS

Establishment of type 2 diabetic nephropathy model

Glucose and insulin tolerance tests

At 21 weeks, as compared with controls, blood glucose and AUC of the DM group were significantly higher in IPGTT and IPITT test ($P < 0.05$, Figure S1a–d). These findings indicate a model of diabetic rats was successfully established and developed insulin resistance. With *CIDEC* gene silencing, levels of blood glucose in IPGTT and IPITT declined dramatically at all of the time point tested. Similarly, the AUCs in IPGTT and IPITT were both decreased with CIDEC gene silencing ($P < 0.05$, Figure S1a–d).

Pathological features and renal CIDEC expression

Periodic acid-Schiff staining showed that the glomerular sclerotic index and the interstitial sclerotic index in DM group raised markedly as compared with controls ($P < 0.05$, Figure S1e–g). Western blot showed CIDEC expression markedly increased in DM group as compared with controls ($P < 0.05$, Figure S1h,i).

Metabolic parameters

There was no significant difference in body weight among all groups (Table S1). Blood tests showed FINS, FBG, TG, TC and LDL-C in DM group were markedly raised as compared with control group ($P < 0.05$, Table S1). These findings suggest diabetic rats exhibit metabolic disorders. FINS, FBG, TG, TC and LDL-C in DM + *CIDEC* shRNA group were markedly depressed as compared with DM + vehicle group ($P < 0.05$), and total cholesterol also tended to decrease, but there was no statistical difference (Table S1).

KWI, serum creatinine and BUN

The levels of KWI, Scr and BUN in DM group were significantly higher than those in control group ($P < 0.05$, Table S1), indicating the DM group developed renal hypertrophy and decreased glomerular filtration rate. The levels of KWI, Scr and BUN in DM + *CIDEC* shRNA group were significantly lower than those in DM + vehicle group ($P < 0.05$, Table S1).

Histopathological features

Hematoxylin and eosin staining showed the glomerular diameter in the DM group was markedly enlarged as compared with control group ($P < 0.05$), and the glomerular diameter of DM + *CIDEC* shRNA group was markedly lessened as compared with DM + vehicle group ($P < 0.05$, Figure 1a,b). Masson trichrome staining showed the renal fibrotic index in DM group was significantly raised as compared with control group

($P < 0.05$), and the renal fibrotic index in DM + *CIDEC* shRNA group was markedly lower than that in DM + vehicle group ($P < 0.05$, Figure 1a,c). PAS staining indicated the glomerulosclerotic index in DM group was obviously raised as compared with control group ($P < 0.05$), and the glomerulosclerotic index in DM + *CIDEC* shRNA group was markedly lowered as compared with DM + vehicle group ($P < 0.05$, Figure 1a,d). Immunohistochemical (IHC) staining was performed on the kidney tissue of diabetic rats, and the results showed the expression of CIDEC in DM group was significantly raised as compared with control group ($P < 0.05$), and it in DM + *CIDEC* shRNA group was markedly lower than that in DM + vehicle group ($P < 0.05$, Figure 1e,f). Meanwhile, CIDEC was overexpressed in renal tubular cells.

The renal pathological scores demonstrated scores of glomerular hypercellularity, glomerular mesangial matrix dilatation and interstitial fibrosis raised significantly in DM group as compared with the control group ($P < 0.05$, Table S2). As

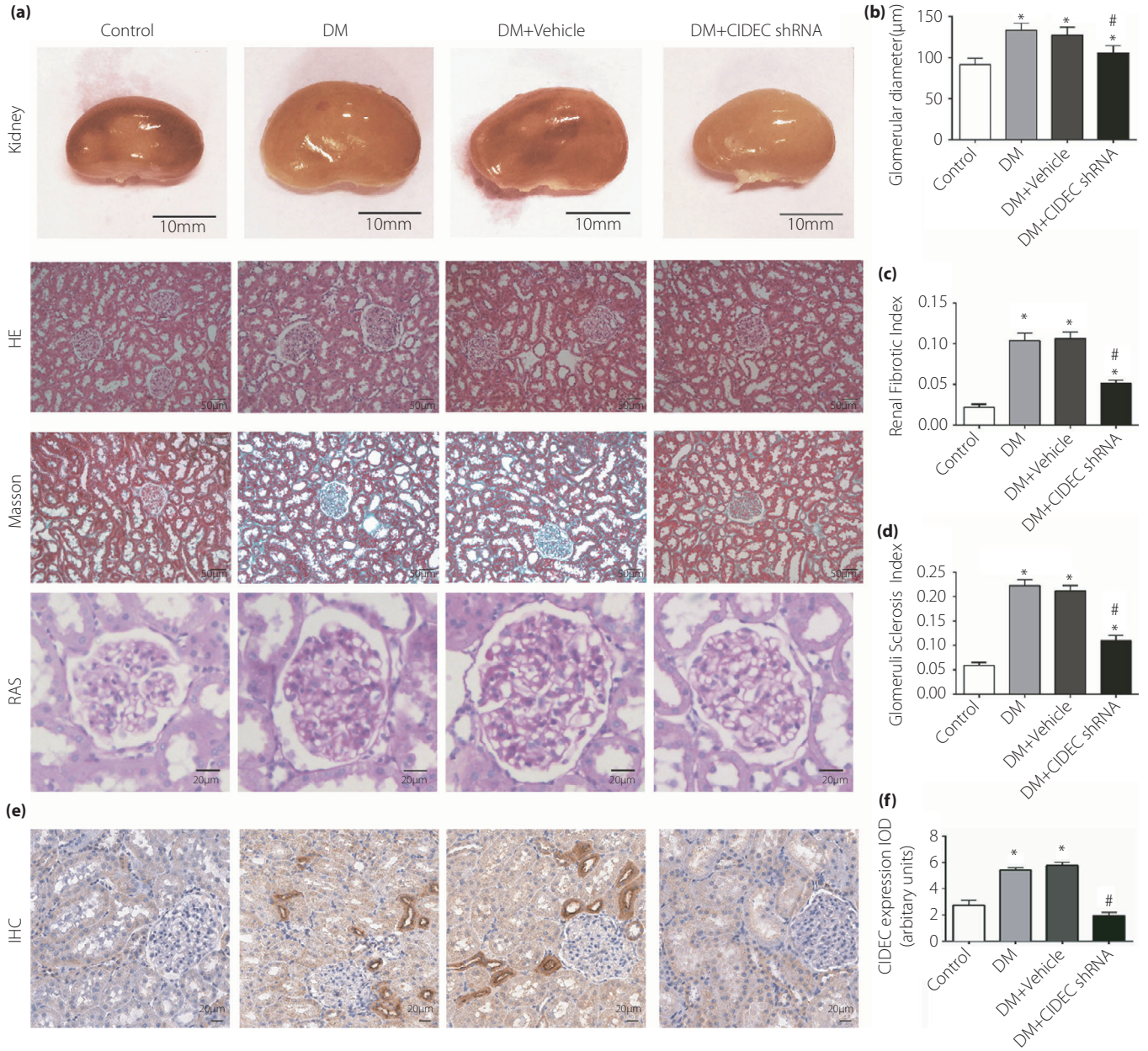

Figure 1 | Renal pathological features in control, diabetes mellitus, diabetes mellitus + vehicle and diabetes mellitus + *CIDEC* shRNA groups. The kidney specimens (a), representative images (a, e) and analysis (b, c, d and f) of renal tissue by HE, Masson, PAS and IHC staining. Data are mean ± SD ($n = 6$). *$P < 0.05$ vs Control; #$P < 0.05$ vs DM + Vehicle.

compared with DM + vehicle group, scores decreased significantly in DM + *CIDEC* shRNA group ($P < 0.05$, Table S2).

Renal fibrotic markers (TGF-β1, collagen I and collagen IV)

Immunohistochemistry indicated the expressions of TGF-β1, collagen I and collagen IV of glomeruli and tubules in DM group were markedly increased as compared with control group ($P < 0.05$), and those expressions above in glomeruli and tubules of DM + *CIDEC* shRNA group were markedly lower than those of DM + vehicle group ($P < 0.05$, Figure 2a–f).

Apoptosis

The expression of cleaved PARP and cleaved caspase3 in DM group was significantly higher than that in control group ($P < 0.05$), and the expression of that in DM + *CIDEC* shRNA group was markedly lower than that in DM + vehicle group ($P < 0.05$, Figure 3a,b). Immunohistochemistry also showed the expression of cleaved PARP and cleaved caspase3 in glomeruli and tubules were markedly raised in DM group as compared with control group ($P < 0.05$), and that decreased significantly in DM + *CIDEC* shRNA group as compared with DM + vehicle group ($P < 0.05$, Figure 3c–e).

TUNEL staining indicated there were relatively few TUNEL positive cells in glomeruli and tubules in control group, but the percentage of TUNEL positive cells of glomeruli and tubules in DM group was significantly higher than that in controls ($P < 0.05$, Figure 3c,f). That in DM + *CIDEC* shRNA group was markedly decreased as compared with DM + vehicle group ($P < 0.05$, Figure 3c,f). These results suggest *CIDEC* gene silencing may improve renal glomerular and tubular apoptosis induced by diabetes.

Kidney autophagy

The expression of ATG5 and ATG7 and the ratio of LC3-II/LC3-I in DM group was markedly decreased as compared with control group ($P < 0.05$, Figure 4a–c). That in DM + *CIDEC* shRNA group was markedly raised as compared with DM + vehicle group ($P < 0.05$, Figure 4a–c). These results indicate *CIDEC* gene silencing may be able to repair diabetic renal autophagy damage.

Mechanism of CIDEC mediating DN in type 2 DCM rats

The expression of CIDEC and EGR1 was markedly higher and the expression of ATGL was markedly lower in DM group, as compared with control group ($P < 0.05$, Figure 4d–f). The expression of CIDEC and EGR1 was significantly lowered and the expression of ATGL was significantly raised in DM + *CIDEC* shRNA group as compared with DM + vehicle group ($P < 0.05$, Figure 4d–f).

Establishment of hyperglycemia model in cells

Animal studies investigated the role and mechanism of CIDEC in DN, as shown in the figure (Figure 4g). We also tested at

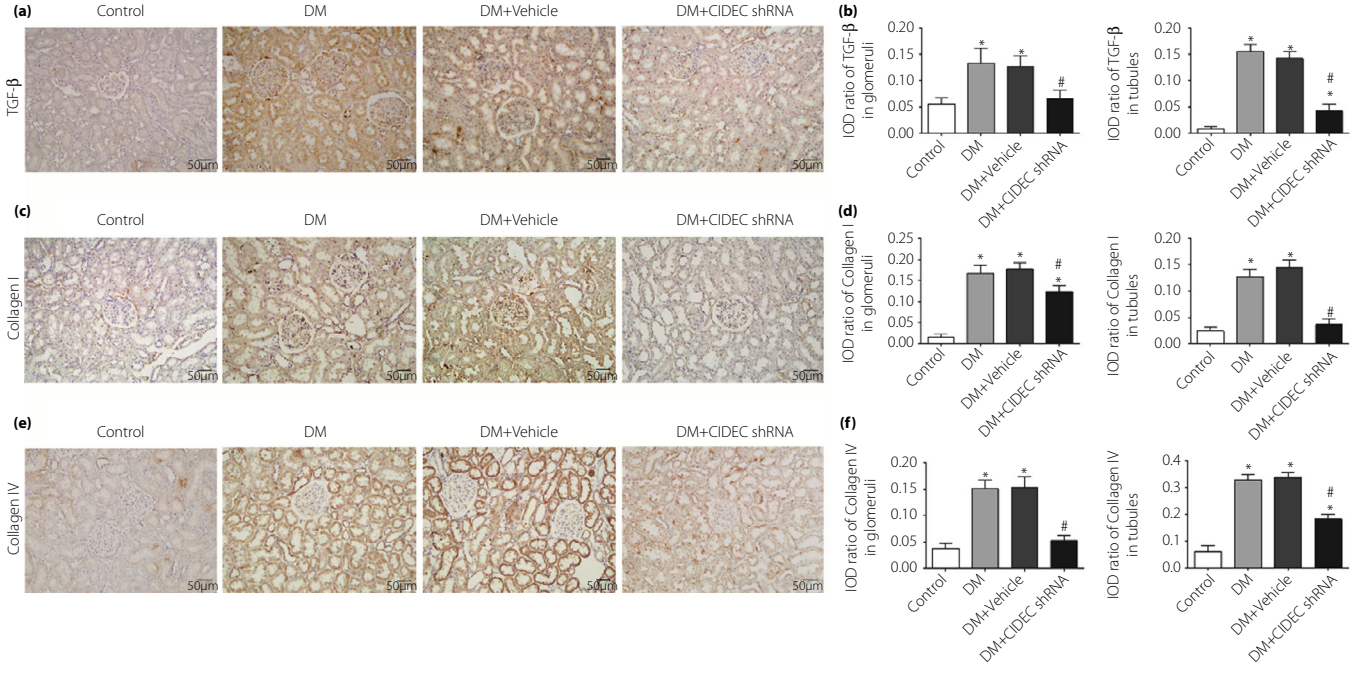

Figure 2 | Renal fibrosis in control, diabetes mellitus, diabetes mellitus + vehicle and diabetes mellitus + CIDEC shRNA groups. Representative immunohistochemical images and analysis of TGF-β1 (a, b), collagen I (c, d) and collagen IV (e, f). Data are mean ± SD ($n = 6$). *$P < 0.05$ vs Control; #$P < 0.05$ vs DM + Vehicle.

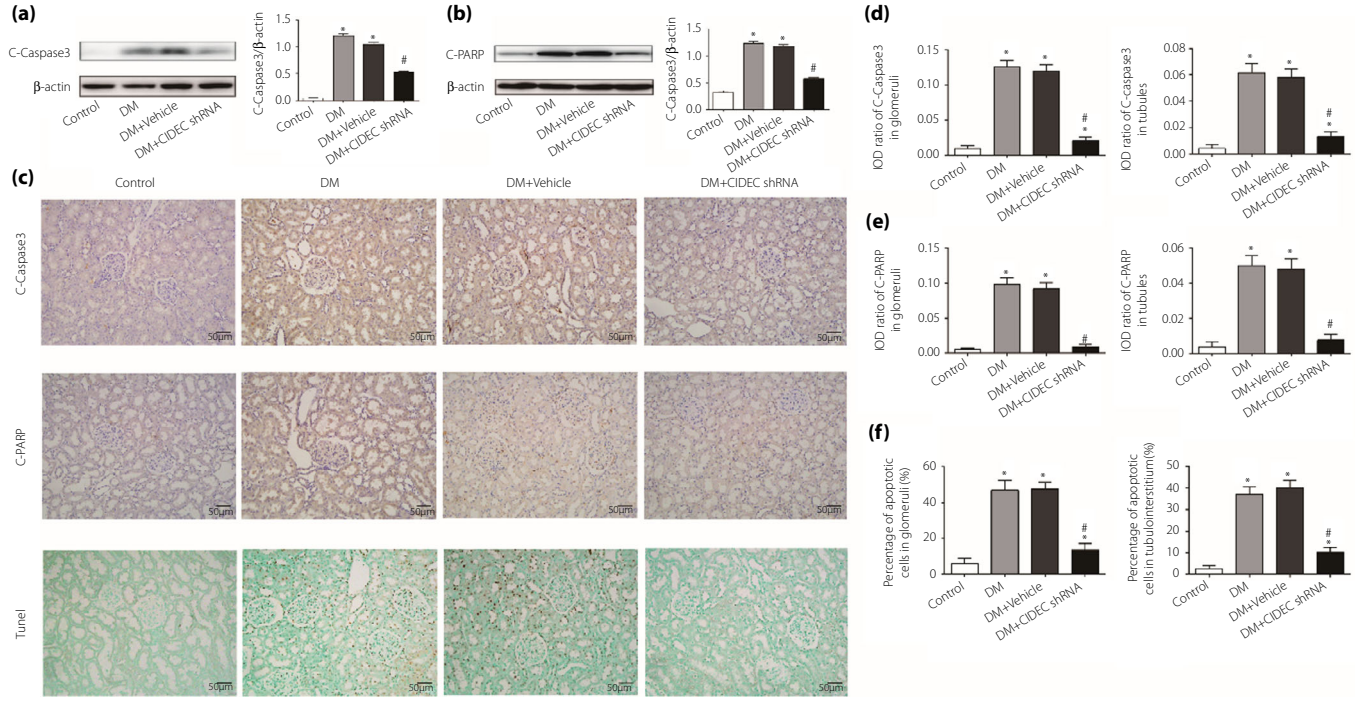

Figure 3 | Renal apoptosis in control, diabetes mellitus, diabetes mellitus + vehicle and diabetes mellitus + CIDEC shRNA groups. Representative western blot bands and analysis of C-caspase3 (a) and C-PARP (b); Representative immunohistochemical images and analysis of C-caspase3 and C-PARP (c–e); Representative images and analysis of TUNEL assay (c and f). Data are mean ± SD ($n = 6$). *$P < 0.05$ vs Control; #$P < 0.05$ vs DM + Vehicle.

the cellular level. Western blot indicated the expression of CIDEC was increased with high glucose stimulation, and the increase was most obvious at 24 h as compared with control group ($P < 0.05$, Figure S2a). Based on this model, we detected autophagy and apoptosis indexes.

Western blot showed the expression of CIDEC and cleaved caspase3 in HG group was significantly higher than that in control group ($P < 0.05$, Figure S2b,d), meanwhile, the ratio of LC3-II/LC3-I decreased significantly ($P < 0.05$, Figure S2c). These results indicated the cell hyperglycemia model was successfully constructed.

Cell signaling pathway

Consistent with animals, we divided the cells into four groups (Control, HG, HG + Vehicle, HG + *CIDEC* shRNA). And we detected the expression of the following indicators, including cleaved PARP and cleaved caspase3 as apoptotic indexes, ATG5, ATG7 and LC3-II/LC3-I as autophagy indexes, and CIDEC, EGR1 and ATGL as signaling pathways. Western blot showed the expression trend of each indicator was consistent with that of animals (Figure 5).

DISCUSSION

In this study, we found diabetic rats developed dilated mesangial matrix, tubulointerstitial fibrosis, increased renal apoptotic cells, autophagy damage and decreased renal function, according with the basic clinical characteristics of DN. Expression of CIDEC in the kidney of diabetic rats was significantly increased with the activation of EGR1 signaling pathway, while *CIDEC* gene silencing inhibited apoptosis of renal cells, activated autophagy of renal cells, and reversed the impairment of renal function and structural remodeling in diabetic rats.

DN due to type 2 diabetes is characterized by progressive albuminuria and gradually decreased glomerular filtration rate, which is closely related to aggravating renal sclerosis or fibrosis, and ultimately leads to chronic renal failure[1,3]. Pathological features of DN include glomerular mesangial dilatation, basement membrane thickening, glomerular and tubular damage, and tubulointerstitial fibrosis[7]. Meanwhile, glomerular and tubular damage in diabetic kidneys may be closely related to apoptosis and autophagy damage. We found increased apoptosis and autophagy injury were positively correlated with renal fibrosis represented by the expression of TGF-β1, collagen I and collagen IV in glomeruli and tubules of diabetic rats. It was also demonstrated the expression of cleaved PARP and cleaved caspase3 in glomeruli and tubules was increased in diabetic rats, which promoted renal glomerular and tubular apoptosis represented by the increased number of TUNEL positive cells. In both rats and patients exhibiting diabetes, excessive tubular cell apoptosis is related to the progress of

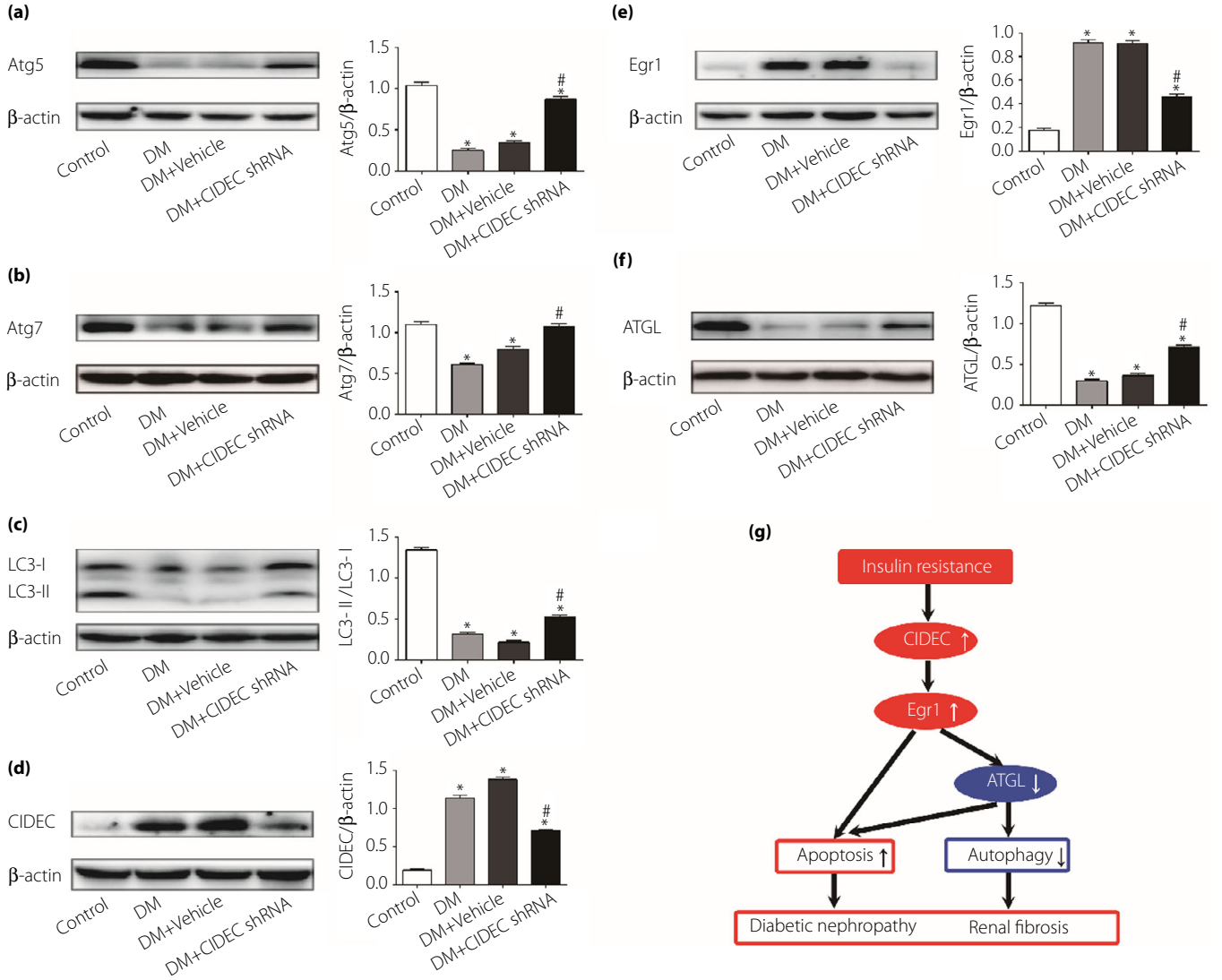

Figure 4 | Renal autophagy, CIDEC and its downstream signaling molecules in control, diabetes mellitus, diabetes mellitus + vehicle and diabetes mellitus + *CIDEC* shRNA groups. Representative western blot bands and analysis of ATG5 (a), ATG7 (b), LC3 (c), CIDEC (d), EGR1 (e) and ATGL (f). Signaling mechanism of CIDEC in diabetic nephropathy (g). Data are mean ± SD (n = 6). *P < 0.05 vs Control; #P < 0.05 vs DM + Vehicle.

renal damage[21]. We also found the expression of ATG5 and ATG7 and the ratio of LC3-II/LC3-I in the kidney of diabetic rats decreased, which indicated the renal autophagy of diabetic rats was impaired. Studies have illustrated impairment of autophagy is associated with the pathomechanism of DN[22]. Numerous evidences suggest key methods of autophagy activation and restoration may have a protective effect on DN for kidney[22,23]. Therefore, it is important to explore a therapeutic method to simultaneously inhibit renal apoptosis and restore autophagy.

As a key regulator in fat and energy metabolism, CIDEC involves in metabolic disorders associated with obesity including type 2 diabetes and insulin resistance[24]. CIDEC is a protein related to lipid droplet and is highly synthetized in either brown or white adipose tissue, with the expression up-regulated during adipogenesis in mice[25]. In vitro human differentiated pre-adipocytes, the expression of CIDEC can be induced by insulin and the level of *CIDEC* mRNA rises in a dose- and time-dependent form[26]. CIDEC deficiency in *CIDEC*$^{-/-}$ mice resultes in decreased white fat pad, reduced obesity, enhanced systemic metabolism, increased insulin sensitivity, and a thin phenotype. Consistently with above studies, we found *CIDEC* gene silencing significantly improved insulin resistance and dyslipidemia in diabetic mice (Table S1), and clarified the biological function of *CIDEC* gene silencing in metabolic regulation. Therefore, we consider *CIDEC* gene silencing can improve systemic metabolic disorders first. In

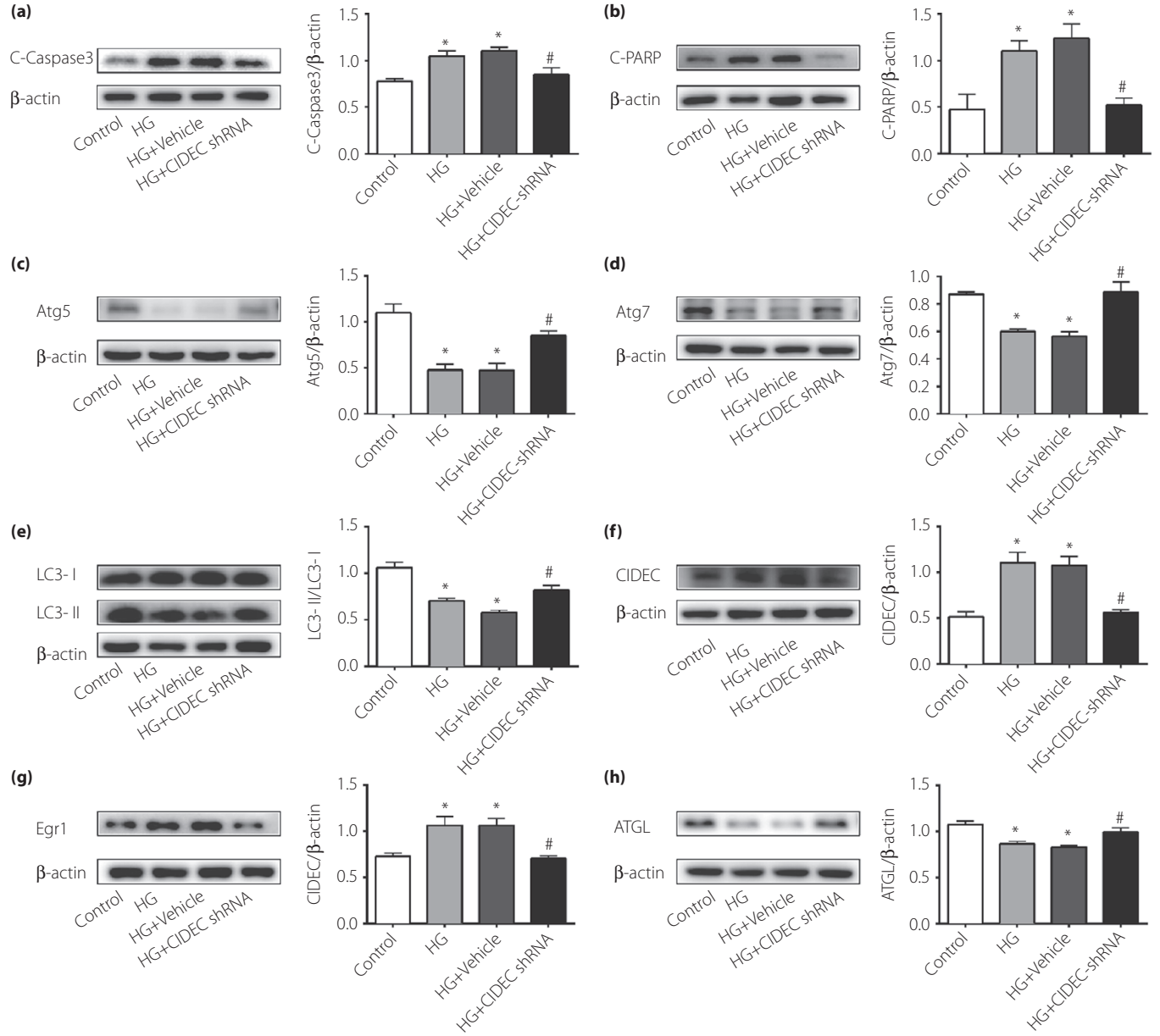

Figure 5 | Detection of cell protein expression in control, HG, HG + vehicle, HG + *CIDEC* shRNA groups. Representative western blot bands and analysis of C-caspase3 (a), C-PARP (b), ATG5 (c), ATG7 (d) and LC3 (E), CIDEC (f), EGR1 (g) and ATGL (h). Data are mean ± SD ($n = 3$). *$P < 0.05$ vs Control; #$P < 0.05$ vs HG + Vehicle.

addition, we revealed the expression of CIDEC in the kidney of diabetic group was higher than that of control group (Figure S1h,i), suggesting the increase of CIDEC expression might be the part of causing renal injury (Figure S1e–g). Our study also confirmed after *CIDEC* gene silencing the glomerular diameter was markedly lessened (Figure 1b) and the renal fibrotic index (Figure 1c) as well as the glomerulosclerotic index (Figure 1d) were significantly lowered. Meanwhile, scores of glomerular hypercellularity, glomerular mesangial matrix dilatation and interstitial fibrosis decreased significantly (Table S2), and the expressions of TGF-β1, collagen I and collagen IV in glomeruli and tubules were obviously lowered, which suggested *CIDEC* gene silencing could reduce kidney damage. CIDEC can promote apoptosis as its ectopic expression in mammalian cells. Consistently with above studies, we found *CIDEC* gene silencing significantly inhibited renal cell apoptosis (Figure 3, Figure 5a,b) and improved autophagy (Figure 4a–c, Figure 5c–e). Therefore, our study infers CIDEC has the ability to reduce renal injury and fibrosis by inhibiting

renal cell apoptosis and improving autophagy. However, the downstream molecular mechanism of the inhibition of renal damage via *CIDEC* gene silencing remains unrevealed.

EGR1 and ATGL are possible downstream signal molecules of CIDEC in the pathologic mechanism of DN. A previous study has shown CIDEC inhibits the expression of ATGL at the transcriptional level by stimulating EGR1, the inhibitory factor for ATGL transcription[16]. CIDEC also inhibits lipid decomposition by preventing the expression, activation and localization of ATGL[27]. The activity of ATGL promoter can be inhibited dose-dependently by CIDEC in vitro and this ATGL promoter domain contains the combinding region of transcription factor EGR1, which directly combines to the ATGL promoter and prevents its activity[16]. Knockout of EGR1 attenuates the inhibitory effect of CIDEC, which indicates CIDEC inhibits fat decomposition medicated by ATGL at any rate partly by enhancing the function of EGR1 to inhibit the gene transcription of ATGL. There is increasing evidence that EGR1 has a crucial effect on DN renal fibrosis[2]. The increasing expression of EGR1 can remarkably raise the expression of fibronectin and collagen I, and increase the deposition of extracellular matrix in glomerular mesangial cells of DN by up-regulating TGF-β1, thereby leading to renal fibrosis. EGR1 in diabetic mice plays a key role in proteinuria and renal injury, and inhibition of EGR1 in diabetic patients may be an available method for the prevention and treatment of DN. Furthermore, ATGL is associated with lipid metabolism and kidney disease. For cultured podocytes treated with *ATGL* shRNA in vitro, *ATGL* gene deficiency induces obvious podocyte apoptosis. Autophagy/lipophage is responsible for the degradation of bulk lipid droplets. ATGL is an autophagy/lipophage inducer promoting the catabolism of lipid droplets induced by autophagy/lipophage. In a word, ATGL plays an essential role in maintaining lipid metabolism and renal function in normal kidney, while restoring ATGL expression may be a possible therapeutic method for DN by preventing renal fibrosis. Consistently with above studies, *CIDEC* gene silencing in vivo could effectively inhibit the increase of EGR1 expression and the decrease of ATGL expression induced by high glucose (Figures 4d–f and 5f–h). Therefore, we believe *CIDEC* gene silencing may improve diabetic renal function by inhibiting EGR1 expression and restoring ATGL expression. The protective effect of *CIDEC* gene silencing on diabetic renal damage suggests CIDEC may be a potential target for the treatment of DN.

In conclusion, studies at the animal level and cellular level shown *CIDEC* gene silencing may prevent apoptosis by inhibiting the expression of EGR1, while restore autophagy activity and inhibit apoptosis by increasing the expression of ATGL, thereby improving renal glomerulosclerosis and tubulointerstitial fibrosis and delaying the progression of DN.

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DISCLOSURE

The authors declare no conflicts of interests. All animal experiments were conducted following the national guidelines and the relevant national laws on the protection of animals.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | To establish the rat model of diabetic renal lesions (control and diabetes mellitus groups): IPGTT (a) and AUC of blood glucose (b), IPITT (c) and AUC of blood glucose (d); representative images and analysis of the renal PAS staining (e); Representative western blot bands and analysis of CIDEC (f).

Figure S2 | To establish the rat model of diabetic renal lesions (control and diabetes mellitus groups): IPGTT (a) and AUC of blood glucose (b), IPITT (c) and AUC of blood glucose (d); representative images and analysis of the renal PAS staining (e); Representative western blot bands and analysis of CIDEC (f).

Table S1 | Physical and biochemical parameters of experimental rats.

Table S2 | Renal scores of the rats at the end of experiment.