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Occurrence and development of diabetic nephropathy caused by CD63 by inhibiting Wnt-β-catenin signaling pathway

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Abstract. – OBJECTIVE: This study aimed to investigate the occurrence and development of diabetic nephropathy caused by CD63 by inhibiting Wnt-β-catenin signaling pathway.

PATIENTS AND METHODS: Renal tissues and normal renal tissues distant from renal lesions of patients with diabetic nephropathy treated in The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University from January 2018 to November 2018 were selected. Human renal tubular epithelial cell HKC was purchased. CD63-siRNA group, NC group, blank group, CD63-mimics, CD63-mimics+si-Wnt4, and CD63-inhibitor+sh-Wnt4 were transfected into renal tubular epithelial cell HKC; mRNA expression in the cells was detected by qRT-PCR, and the protein expression in the cells was detected by WB. CCK8 and flow cytometry were used to detect cell proliferation and apoptosis.

RESULTS: CD63, Wnt4, β-catenin, and p-GSK-3β were highly expressed in diabetic nephropathy. Cell experiments showed that inhibiting CD63 and Wnt-β-catenin signaling pathway could promote cell proliferation and reduce cell apoptosis, and the protein expressions of Wnt4, β-catenin, p-GSK-3β, and Bcl-2 were significantly reduced. Rescue experiments showed that after the co-transfection of CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 into EC109 and EC9706, the cell proliferation and apoptosis rates were not different from those of the NC group without transfection sequence.

CONCLUSIONS: CD36 can mediate cell apoptosis by inhibiting the expression of the related proteins in nodal Wnt/β-catenin signaling pathway, and is expected to become a potential therapeutic target for clinical treatment of patients with diabetic nephropathy.

Key Words:
CD63, Wnt-β-catenin, Diabetic nephropathy, Proliferation, Apoptosis.

Introduction

Diabetic nephropathy is one of the complications of diabetic patients, also known as diabetic renal damage[1]. The incidence trend of diabetic nephropathy is not optimistic and is increasing year by year. The morbidity of patients with diabetic nephropathy worldwide is as high as 50%, and the death risk of the end-stage renal disease combined with diabetes is very high[2,3]. In addition, diabetic nephropathy not only brings inconvenience to the life of patients, but also brings heavy economic burden to patients and their families[4]. The pathogenesis of diabetic nephropathy is complex, and the clinical symptoms of renal injury in early diabetic patients are not evident, resulting in patients missing a better treatment opportunity and affecting their prognosis[5]. Therefore, the prevention and treatment situation of diabetic nephropathy is very serious.

Renal tubule absorption function injury, renal interstitial injury, and renal tubulointerstitial transdifferentiation in patients with diabetic nephropathy can all promote renal interstitial fibrosis[6]. Renal tubular epithelial cells play an important role in the progression of diabetic nephropathy. Existing studies have shown that Wnt-β-catenin pathway is closely related to the formation of tubulointerstitial fibrosis by transdifferentiation of renal tubular epithelial cells in diabetic nephropathy[7]. The high glucose environment in diabetic patients will activate Wnt-β-catenin pathway in renal tubular epithelial cells and upregulate the expression of the related proteins, further promoting renal tubulointerstitial fibrosis and cause renal injury in diabetic patients[8].

CD36 is a glycoprotein on the cell surface of group B scavenger receptor family. Previous studies[9,10] have showed that CD36 participates in

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the pathological process of diabetes and other diseases, while the role of CD36 in diabetic nephropathy is unclear. Therefore, this study is to explore the regulation of CD36 expression in renal tissues of patients with diabetic nephropathy, and to observe the effect of Wnt/β-catenin signaling pathway on proliferation and apoptosis of human renal tubular epithelial cells after CD36 changes, so as to provide theoretical basis for the diagnosis and treatment of diabetic nephropathy.

Patients and Methods

Data Collection

Renal tissues and normal renal tissues distant from renal lesions of 52 patients with diabetic nephropathy who were treated in the Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University from January 2018 to November 2018 were selected. The surgically removed diabetic nephropathy tissues and normal renal tissues were collected[11]. Exclusion criteria were as follows: the liver and kidney functions of the patients included were normal, and there were no other malignant tumors. All specimens were stored in liquid nitrogen tanks immediately after resection. The samples of patients with diabetic nephropathy confirmed by postoperative pathology were selected for study. Patients who received chemotherapy, immunotherapy, and radiotherapy before surgery were excluded. Patients and their families were informed before the study was carried out, and an informed consent was signed. The research was approved by the Ethics Committee of The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University.

Main Reagents, Instruments, and Detection Methods

Main Reagents and Instruments

The main reagents and instruments used: human renal tubular epithelial cell HKC (cells derived from Beina Organism, Beijing, China, No. BNCC338628), TRIzol reagent (Invitrogen, Carlsbad, CA, USA), qRT-PCR kit and minScript reverse transcription kit (TaKaRa, Otsu, Shiga, Japan), HBS-1096A enzyme-labelled analyzer (Detie Experimental Equipment, Nanjing, China), real-time quantitative PCR instrument (BioRad, California, USA), Dulbecco's Modified Eagle's Medium (DMEM) culture medium (Gibco, Grand Island, NY, USA), Fetal calf serum (FBS) and trypsin (HyClone, South Logan, UT, USA), Cell Counting Kit-8 (CCK-8; Zhijie Fangyuan, Beijing, China), CyFlow Cube 8 flow cytometry (Partec, Memmingen, Germany), primer sequences of Wnt4, β-catenin, p-GSK-3β, and internal reference β-actin, and miRNA negative control were designed by Shanghai GenePharma Co.,Ltd. More details are shown in Table I.

Western Blot was Used to Detect the Protein Expressions of CD63, Wnt4, β-catenin, p-GSK-3β, and internal Reference β-actin

Renal tissues and normal renal tissues distant from renal lesions of 52 patients with diabetic nephropathy were put into a pre-cooling mortar, and the tissue was grounded into powder in liquid nitrogen. After adding the protein lysate, the total protein in the tissue was separated, and placed into a homogenizer (Shanghai Active Motif Biotechnology, Shanghai, China, item number: 40401/40415). 300 μl of lysate was added, and the tissue mass gradually disappeared through grinding until the lysate was free of impurities and precipitates, and cracked on ice for 30 min. They were centrifuged at 14000 r/min for 20 min, and finally the supernatant was taken as the total cell protein. BCA protein quantification was carried out by 6%-12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane. After selecting the corresponding band according to the target protein, it was sealed with skimmed milk powder with concentration of 5% for 2 hours. After washing the

Table I. Primer sequences of Wnt 4, β-catenin, p-GSK-3β, and internal reference β-actin.

Group	Forward primer	Reverse primer
Wnt4	5'-CCTCGTCTTCGCCGTGTTCT-3'	5'-TGAGCTTCTCGCACGTTTCCT-3'
β-catenin	5′-TGCAGCGACTAAGCAGGA-3′	5′-TCACCAGCACGAAGGACA-3′
p-GSK-3β	5'-CCTTAACCTGGTGCTGGACT-3'	5'-AGCTCTGGTGCCCTGTAGTA-3'
β-actin	5'-GCCAACACAGTGCTGTCT-3'	5'-AGGAGCAATGATCTTGATCTT-3'

membrane, 2 ml of Western primary antibody diluent (Jiangsu Beyotime Biology, Jiangsu, China) with dilution ratio of 1:1,000 was added and stored at 4°C for one night. After the first antibody was re-warmed for 30 min before the start of the experiment on the second day, the Western second antibody (Jiangsu Beyotime Biology, Jiangsu, China) was incubated for 1 h with the same procedure, and the developer was added to the dark room for exposure. PVDF film was imaged by Tocan240 automatic gel imaging system (Shanghai Tocan Biotechnology, Shanghai, China), and the results were analyzed by Image LabTM software.

mRNA Detection of CD63, Wnt4, β-catenin, and p-GSK-3β by qRT-PCR

qRT-PCR was used to detect the expressions of CD63, Wnt4, β-catenin, and P-GSK-3β in diabetic nephropathy tissues and normal renal tissues. All specimens were taken out of the liquid nitrogen tank 30 min in advance, and total RNA of tissues was extracted according to TRIzol reagent operation instructions and dissolved in 20 μl DEPC water. The total RNA was then reversely transcribed using a reverse transcription kit. The reaction system was: M-MLV 1 μl, Olig (dT) 1 μl, RNA enzyme inhibitor 0.5 μl, d NTPs 1 μl, RNase free water made up to 15 μl. They were incubated at 38°C for 60 min. Moreover, 1 μl c DNA was taken, 85°C for 5 s. The synthesized c DNA was used as a template for qRT-PCR amplification. The PCR reaction system was prepared: 10×PCR buffer 2.5 μl, d NTPs 1 μl, upstream and downstream primers 1 μl each, Taq DNA Polymerase 0.25 μl, dd H2O supplemented to 25 μl. The reaction conditions were: pre-denaturation at 95°C for 15 min, denaturation at 95°C for 15 s, annealing at 58°C for 30 s, a total of 35 cycles, and finally extension at 72°C for 15 min. Each sample was provided with 3 multiple wells for 3 repeated tests, CD63, Wnt4, β-catenin, and p-GSK-3β use β-actin as an internal reference. After the reaction was completed, the amplification curve and melting curve of Real Time-PCR were confirmed, and the relative amount of the target gene was calculated according to the result parameters. The relative quantification of the target gene was calculated by $2^{-\Delta CT}$.

Culture and Transfection of Cells

The human renal tubular epithelial cell HKC was placed in a culture medium containing 10% PBS DMEM, transfected in a CO_2 incubator at 37°C. When the transfection reached 50% of cell growth harmony, 25% pancreatin was added for digestion; after digestion was completed, the human renal tubular epithelial cell HKC was placed in the culture medium for continuous culture to complete the passage. The cells in the logarithmic phase were selected for transfection and grouped before transfection. The cells not transfected were set as blank group, negative RNA control (NC group), CD63-siRNA group, CD63-mimics, CD63-mimics+si-Wnt4, and CD63-inhibitor+sh-Wnt 4. The Lipofectamine 2000 and DNA were diluted and mixed according to the instructions of the Lipofectamine 2000 manufacturer's kit (Shanghai Kemin Biotechnology, Shanghai, China, No: 11668-019). NC, CD63-siRNA group, NC group, CD63-mimics, CD63-mimics+si-Wnt4, and CD63-inhibitor+sh-Wnt4 were transfected into human renal tubular epithelial cells HKC using liposome Lipofectamine 2000 respectively, incubated at room temperature for 5 min; finally the mixed solution was mixed with the cells evenly at 37°C. Transfection was carried out under CO_2 conditions. After 48 hours of transfection, qRT-PCR technology was used to detect the mRNA expression of human renal tubular epithelial cells HKC transfected with CD63-siRNA group, NC group, CD63-mimics, CD63-mimics+si-Wnt4, and CD63-inhibitor+sh-Wnt4.

Cell Proliferation Detected by CCK8

Each group of human renal tubular epithelial cell HKC transfected for 48 h were inoculated in 96-well plates with 100 μl per well, diluted to 4×10^3 cell/ml after pancreatic digestion. Then, the culture plates were placed in a cell incubator for 24 h; the old culture solution was taken out and discarded; NC, CD63-siRNA, CD63-mimics, CD63-mimics+si-Wnt4, and CD63-inhibitor+sh-Wnt4 were added. The cells transferred for 48 h were collected, diluted to 2×10^4 cell/ml, then inoculated into 96-well plates; each well was inoculated with 100 μl of cells and cultured in 37°C, in 5% CO_2 environment. 10 μl of CCK8 solution was added to each well at 24 h, 48 h, and 72 h after the cells adhered to the wall. After adding reagents, the cells were continuously cultured in an incubator. After 1 h, the absorbance was measured at 450 nm by HBS-1096A enzyme-labeled analyzer (Nanjing Detie Experimental Equipment, Nanjing, China) to detect cell proliferation. The experiment was repeated three times.

Apoptosis in each group detected by flow cytometry

After digestion with trypsin, the cells in the CD63-siRNA group, treated with CD63-mim-

ics, CD63-mimics+si-Wnt4, CD63-inhibitor+sh-Wnt4, and NC for 48 h were respectively collected. The cells were fixed with 75% ethanol at 20°C for 24 h, centrifuged at 4°C at 3000 rpm for 5 min, and then, ethanol was discarded. After rinsing with PBS for one time, they were centrifuged at a constant temperature of 4°C, 3000 rpm for 5 min. The supernatant was discarded. 500 ul of DNA stabilizing solution was added to the samples, and fully mixed. Finally, the prepared solution was transferred to a flow tube, incubated on ice in the dark for 30 min, and then, CyFlow Cube 8 flow cytometer was used for detection.

Results

Expressions of CD63, Wnt-β-Catenin Signal Pathway Related Proteins, and mRNA in Diabetic Nephropathy and Normal Renal Tissues

The expression levels of CD63 protein in diabetic nephropathy tissues and normal renal tissues were (0.62±0.04) and (0.15±0.02) respectively, and the expression levels of CD63 mRNA in diabetic nephropathy tissues and normal renal tissues were (0.70±0.04) and (0.14±0.02) respectively. The expression levels of Wnt4 protein in diabetic nephropathy tissues and normal renal tissues were (23.10±7.15) and (1.00±0.12) respectively, and the expression levels of Wnt4mRNA in diabetic nephropathy tissues and normal renal tissues were (39.20±5.12) and (8.33±1.67), respectively. The expression levels of p-GSK-3β protein in diabetic nephropathy tissues and normal renal tissues were (24.10±6.55) and (3.20±0.13), respectively. The expression levels of p-GSK-3β mRNA in diabetic nephropathy tissues and normal renal tissues were (32.10±4.32) and (10.16±2.52), respectively. The expression levels of β-catenin protein in diabetic nephropathy tissues and normal renal tissues were (17.20±6.13) and (1.47±0.23), respectively. The expression levels of β-catenin mRNA in diabetic nephropathy tissues and normal renal tissues were (39.15±5.00) and (12.00±2.84), respectively. Compared with the two groups, the expression levels of CD63, Wnt4, p-GSK-3β, β-catenin protein, and mRNA in diabetic nephropathy tissues were significantly higher than those in normal renal tissues, with statistically significant difference (p<0.001). More details were shown in Figure 1.

Expressions of Protein and mRNA in Cells After Transfection

The Relative Expression Levels of CD63, Wnt-β-Catenin Signaling Pathway Related Proteins, and mRNA in cells of Each Group After Transfection

The expression levels of CD63 protein in CD63-siRNA group, NC group, and blank group were (0.14±0.01), (0.63±0.03), and (0.62±0.03), respectively. The expression levels of CD63 mRNA in CD63-siRNA group, NC group, and blank group were (0.13±0.02), (0.68±0.04), and (0.69±0.04), respectively. The CD63 expression level of CD63-siRNA was significantly lower than that of NC group and blank group, and the difference in expression was statistically significant (p<0.001). More details were shown in Figure 2.

Expressions of Wnt4, p-GSK-3β, β-Catenin, and mRNA in Transfected Cells of Each Group

The expression levels of Wnt4 protein in CD63-siRNA group, NC group, and blank group were (4.12±0.34), (21.49±7.14), and (21.92±7.32), respectively. The expression levels of Wnt4 mRNA in CD63-siRNA group, NC group, and blank group were (8.15±1.73), (38.44±5.34), and (39.00±5.82), respectively. The expression levels of Wnt4 protein and mRNA in CD63-siRNA group were significantly lower than those in the NC group and blank group (p<0.001). The expression levels of Wnt4 protein and mRNA in NC group and blank group had no significant difference (p>0.05). More details were shown in Figure 3A-B.

The expression levels of p-GSK-3β protein in CD63-siRNA group, NC group, and blank group were (2.79±0.10), (23.15±6.55), and (24.00±6.35), respectively. The expression levels of p-GSK-3β mRNA in CD63-siRNA group, NC group, and blank group were (9.26±2.31), (32.12±4.80), and (31.69±4.05), respectively. The expression levels of p-GSK-3β protein and mRNA in CD63-siRNA group were significantly lower than those in the NC group and blank group (p<0.001). There was no significant difference in the expression levels of p-GSK-3β protein and mRNA between NC group and blank group (p>0.05). More details were shown in Figure 3 (C-D).

The expression levels of β-catenin protein in CD63-siRNA group, NC group, and blank group were (1.32±0.14), (16.28±6.40), and (16.27±6.51), respectively. The expression levels of β-catenin mRNA in CD63-siRNA group, NC group, and blank group were (11.20±2.61), (38.17±4.00), and

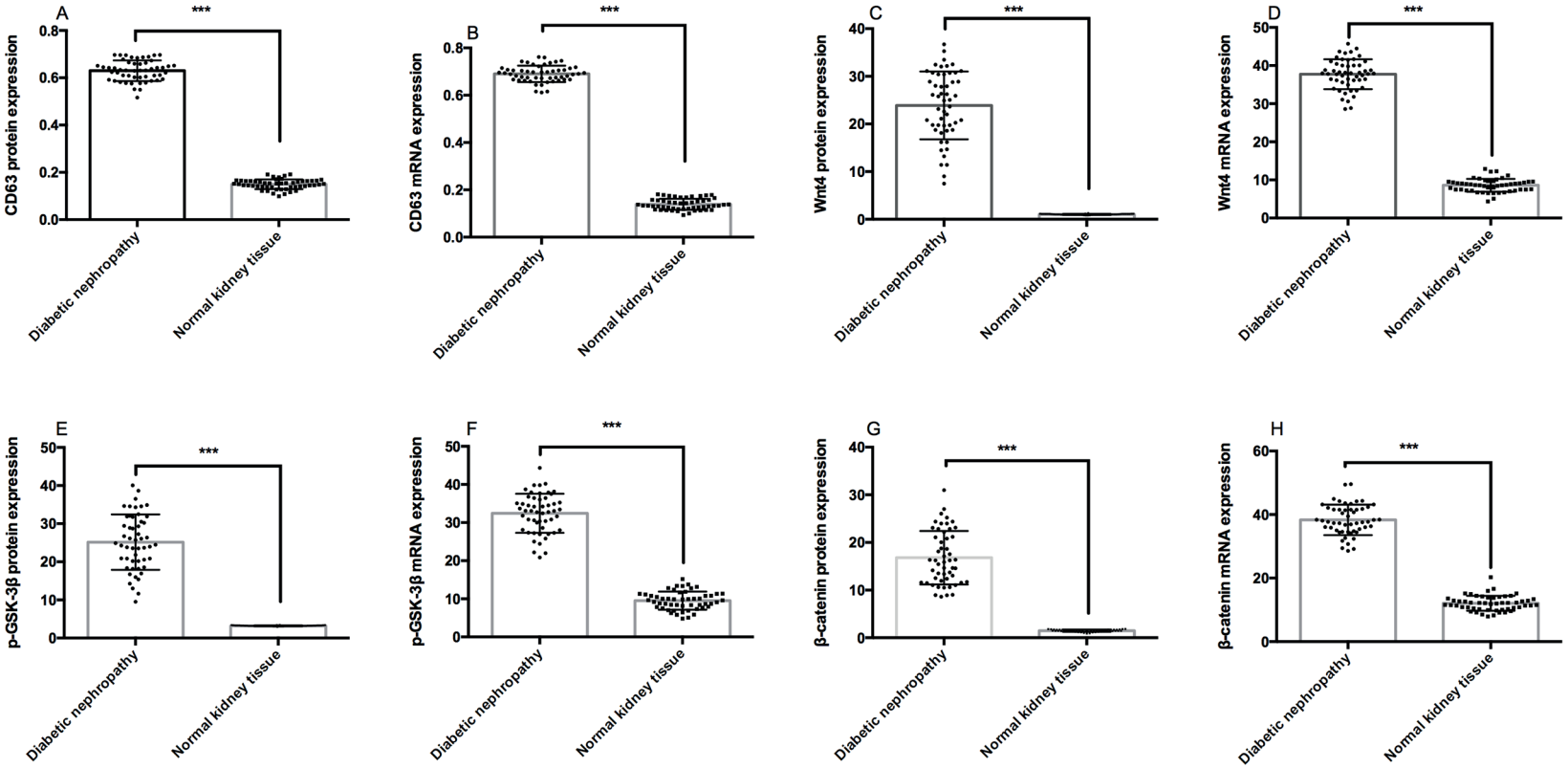

Figure 1. Expressions of CD63, Wnt-β-catenin signaling pathway related proteins, and mRNA in diabetic nephropathy tissues and normal renal tissues. **A,** Expressions of CD63 protein in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of CD63 protein in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001). **B,** Expressions of CD63mRNA in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of CD63 mRNA in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001). **C,** Expressions of Wnt4 protein in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of Wnt4 protein in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001). **D,** Expressions of Wnt4mRNA in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of Wnt4mRNA in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001). **E,** Expressions of β-catenin protein in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of β-catenin protein in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001). **F,** Expressions of β-catenin mRNA in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of β-catenin mRNA in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001). **G,** Expressions of β-catenin protein in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of β-catenin protein in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001). **H,** Expressions of β-catenin mRNA in diabetic nephropathy tissues and normal renal tissues. *** indicates that the expression level of β-catenin mRNA in diabetic nephropathy tissues is significantly higher than that in normal renal tissues (p<0.001).

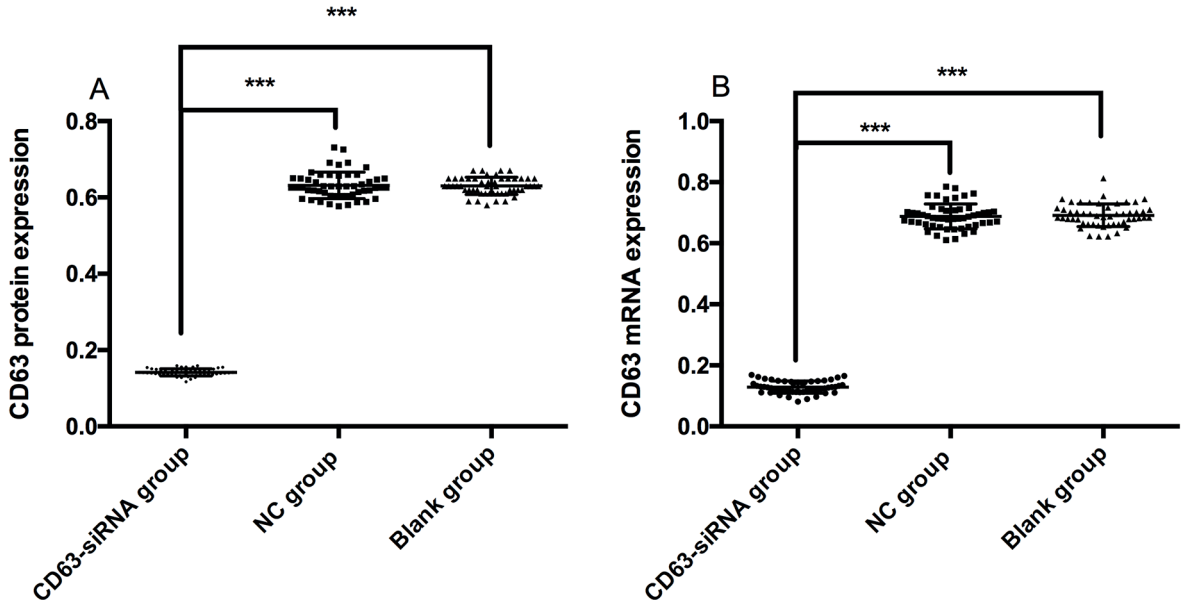

Figure 2. Relative expression levels of CD63 protein and mRNA in each group of cells after transfection. **A,** Expressions of CD63 protein in the transfected cells of each group. ***indicates that the expression level of CD63 protein in CD63-siRNA group is significantly lower than that in NC group and blank group (p<0.001). **B,** Expressions of CD63 mRNA in transfected cells of each group. ***indicates that the CD63mRNA expression level in CD63-siRNA group is significantly lower than that in the NC group and blank group (p<0.001).

(38.05±5.00), respectively. The expression levels of β-catenin protein and mRNA in CD63-siRNA group were significantly lower than those in the NC group and blank group (p<0.001). There was no significant difference in the expression level of β-catenin protein and mRNA between NC group and blank group (p>0.05). More details were in Figure 3 (E-F).

Effects of Biological Function on Renal Tubular Epithelial Cell HKC

Comparison of Proliferation Ability of Renal Tubular Epithelial cell HKC in Each Group After Transfection

The results of intra-group comparison showed that the cell proliferation activity in CD63-siRNA group showed a gradual downward trend from 24 h to 72 h, and the difference was statistically significant at different time points in the groups (p<0.001). There was no significant difference between CD63-siRNA group, NC group, and blank control group in 24-hour cell proliferation activity (p>0.05). At 48 h and 72 h, the cell proliferation activity of CD63-siRNA group was significantly higher than that of the NC group and blank group at the same time point (p<0.001). There was no significant difference between NC group and blank group at 48 h and 72 h (p> 0.05). More details were shown in Figure 4A.

Comparison of Apoptosis Ability in Renal Tubular Epithelial Cell HKC of Each Group After Transfection

The apoptosis rate of CD63-siRNA group was (3.15±0.23)%, significantly lower than that of the NC group (22.79±2.80)% and blank group (21.35±2.69)% (p<0.05). The apoptosis rate of renal tubular epithelial cells in CD63-siRNA group was significantly lower than that of the NC group and blank group (p<0.001). There was no significant difference in the apoptosis rate between the NC group and blank group (p>0.05), as shown in Figure 4B.

Rescue Experiment

Further transfecting CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 into renal tubular epithelial cells HKC to detect cell biological functions found that the proliferation and apoptosis of the transfected CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 were not different from those of the NC group. There was no difference between the two groups (p>0.05), while CD63-mimics+si-Wnt4 and CD63-inhibi-

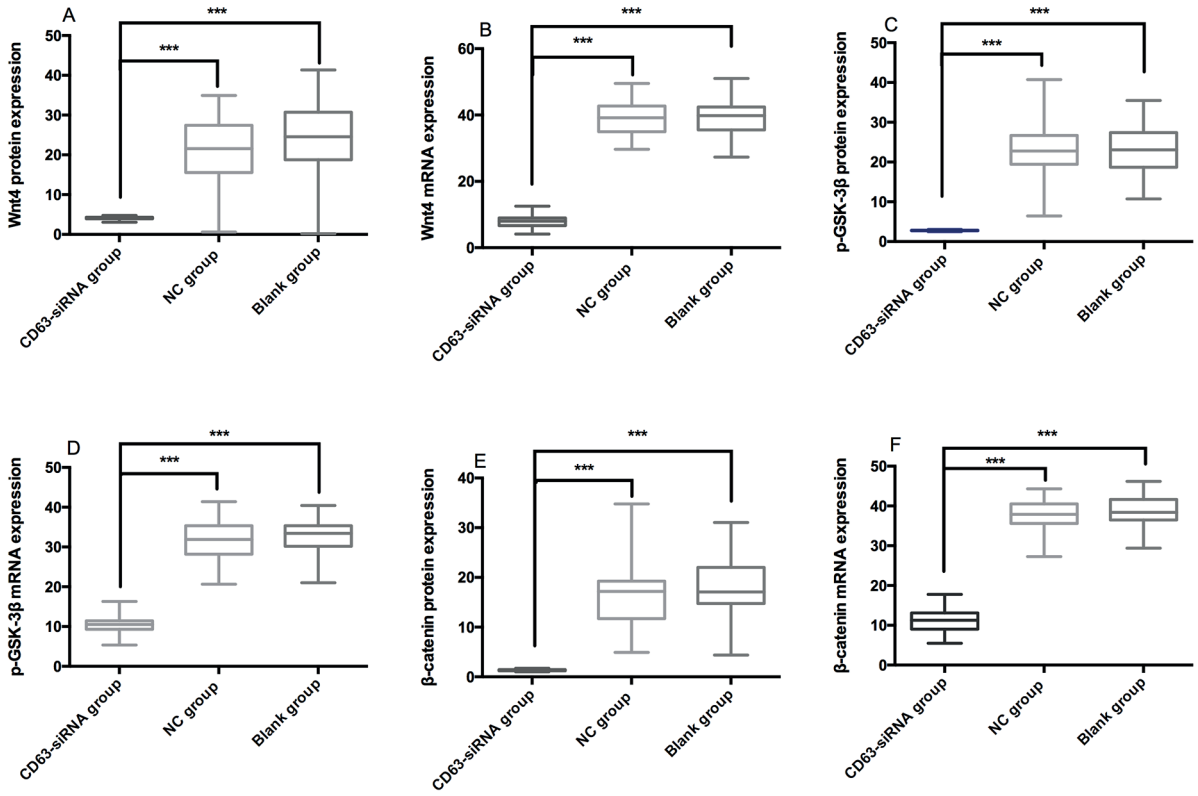

Figure 3. Expressions of Wnt4, p-GSK-3β, β-catenin protein, and mRNA in transfected cells of each group. **A,** Expression of Wnt4 protein in transfected cells of each group. ***indicates that the expression level of Wnt4 protein in CD63-siRNA group is significantly lower than that in the NC group and blank group (p<0.001). **B,** Expression of Wnt4 mRNA in the transfected cells of each group. ***indicates that the expression level of Wnt4mRNA in CD63-siRNA group is significantly lower than that in the NC group and blank group (p<0.001). **C,** Expression of p-GSK-3β protein in the transfected cells of each group. *** indicates that the expression level of p-GSK-3β protein in CD63-siRNA group is significantly lower than that in the NC group and blank group (p<0.001). **D,** Expression of p-GSK-3β mRNA in the transfected cells of each group. *** indicates that the expression level of p-GSK-3β mRNA in CD63-siRNA group is significantly lower than that in the NC group and blank group (p<0.001). **E,** Expression of β-catenin protein in transfected cells of each group. *** indicates that the expression level of β-catenin protein in CD63-siRNA group is significantly lower than that in the NC group and blank group (p<0.001). **F,** Expression of β-catenin mRNA in transfected cells of each group. ***indicates that the expression of β-catenin mRNA in CD63-siRNA group is significantly lower than that in the NC group and blank group (p<0.001).

tor+sh-Wnt4 have significantly upregulated proliferation and downregulated apoptosis compared with CD63-mimics (p<0.05), while compared with CD63-siRNA, the proliferation and upregulated apoptosis were significantly lower (p<0.05). The detection of apoptosis protein found that the expressions of p-GSK-3β and β-catenin protein in CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 had no difference compared with the NC group (p>0.05). However, the expressions of CD63-mimics+si-Wnt4, CD63-inhibitor+sh-Wnt4, and CD63-mimics were significantly lower than those of p-GSK-3β and β-catenin proteins, while the expressions of p-GSK-3β and β-catenin proteins were significantly higher than those of the CD63-inhibitor (p<0.05), as shown in Figure 5.

Discussion

Diabetic nephropathy is one of the common diabetic complications in clinic practice. The number of patients and deaths increases year by year. Bad living habits and their own genetic factors are the causes that trigger the occurrence and progression of diabetic nephropathy[12]. Therefore, effective treatment and early diagnosis are the key to improve the prognosis of patients with diabetic nephropathy[13]. However, the pathogenesis

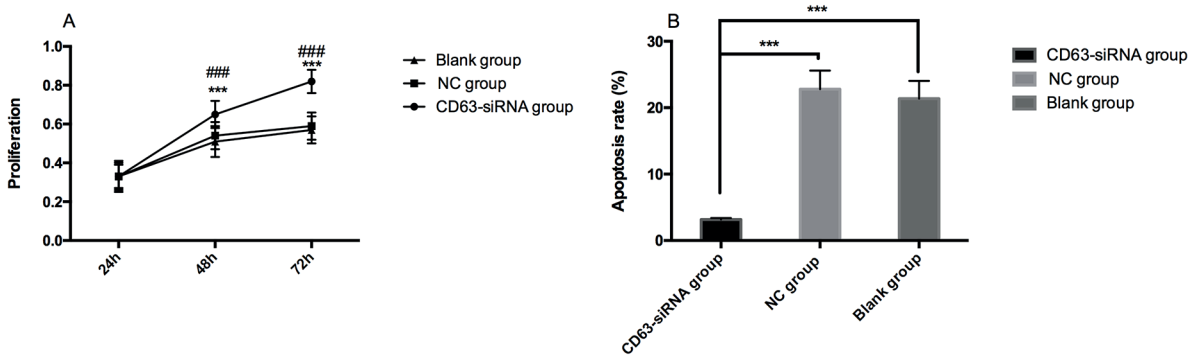

Figure 4. Effects of biological function on renal tubular epithelial cell HKC. **A,** Comparison of proliferation ability of renal tubular epithelial cell HKC in each group after transfection. *** indicates that the difference between different time points in the group is statistically significant ($p<0.001$). ### indicates that at 48 h and 72 h, the cell proliferation activity of CD63-siRNA group is significantly higher than that of the NC group and blank group at the same time point ($p<0.001$). **B,** Comparison of apoptosis ability of renal tubular epithelial cell HKC in each group after transfection. ***indicates that the apoptosis rate of renal tubular epithelial cells in CD63-siRNA group is significantly lower than that in the NC group and blank group ($p<0.001$).

of diabetic nephropathy is still unclear. Recent studies have found a close relationship between CD63 and diabetic nephropathy. CD36 is a glycoprotein located on the cell surface, and its abnormal expression can induce apoptosis[14]. Numerous reports have confirmed that high glucose-induced tubulointerstitial fibrosis is related to Wnt/β-catenin signaling pathway. High glucose induced increased Wnt expression in renal tubular epithelial cells and activated Wnt-β-catenin pathway, resulting in the increased expression of target genes in renal tubular epithelial cells[15,16]. However, inhibition of Wnt/β-catenin signaling pathway can reduce the transdifferentiation of renal tubular cells, but the specific mechanism is still unclear[17]. Therefore, this study further analyzes the role of CD63 in diabetic nephropathy by inhibiting Wnt-β-catenin signaling pathway, providing potential targets for clinical treatment.

We found that CD36 was highly expressed in diabetic nephropathy through TCGA database screening, and further detected the expression of CD36 in diabetic nephropathy cells. It was found that the expression of CD36 in cells was consistent with the database expression. In Shiju et al[18], the expression of CD36 was upregulated in diabetic nephropathy, which was consistent with our results. Subsequently, we further overexpressed and inhibited CD36 expression and transferred it to renal tubular epithelial cell HKC. After detecting the cell biological function, we found that the proliferation ability of renal tubular epithelial cells inhibited expression was significantly alleviated, and the apoptosis rate was significantly decreased. This shows that inhibiting CD36 can alleviate the apoptosis of diabetic nephropathy cells, regulate their proliferation, and may have potential therapeutic targets. However, it is not clear how CD36 is regulated.

Currently, Melnik et al[19] have shown that Wnt/β-catenin signaling pathway can target the downstream genes and play a regulatory role in the cell biological functions. Pathophysiological networks are often used to elucidate the pathogenesis of diseases[20]. In this study, the relationship between related proteins in Wnt/β-catenin signaling pathway and diabetic nephropathy was studied. Wnt/β-catenin signaling pathway is involved in the damage of renal intrinsic cells[21]. Russel and Monga[22] have shown that Wnt/β-catenin signaling pathway regulates proliferation, invasion, migration, and apoptosis in many cell biological functions. In this study, it was found that the related proteins in the Wnt/β-catenin signaling pathway were highly expressed in diabetic nephropathy by detecting TCGA database and detecting the expression of related proteins in diabetic nephropathy cells. Researches[23,24] on related proteins in Wnt/β-catenin signaling pathway are mostly upregulated in esophageal cancer, colorectal cancer, and other diseases, and have the function of regulating cell biology. In this study, we found that the related proteins in Wnt/β-catenin signaling pathway were also elevated in diabetic nephropathy, which suggested that the related proteins Wnt4, p-GSK-3β, and β-catenin in Wnt/β-catenin signaling pathway might also have regulatory effects in diabetic nephropathy. Therefore, we further

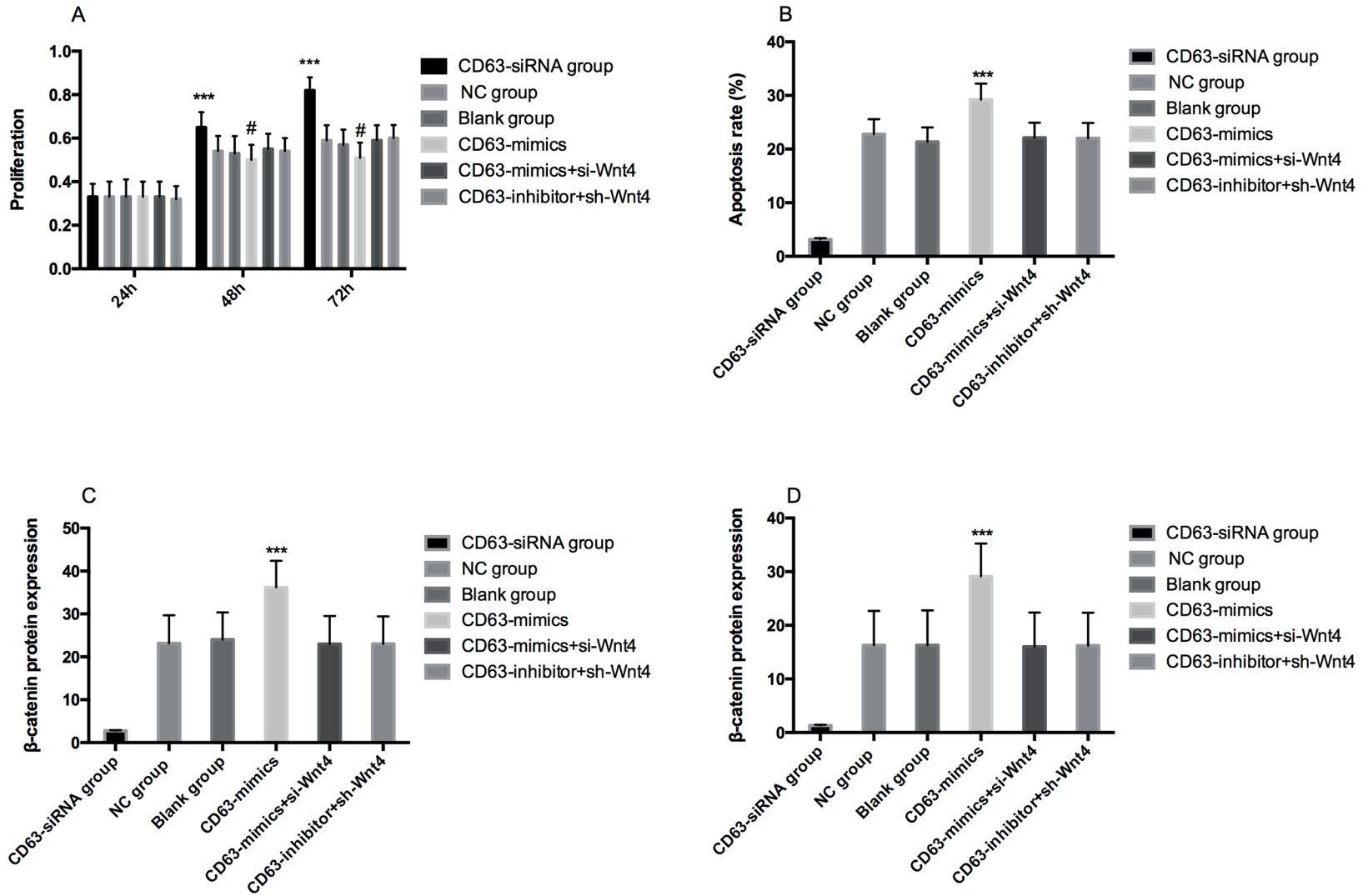

Figure 5. Effects of CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 transfection on cell biological function. **A,** Effects of transfection of CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 on value-added function of renal tubular epithelial cells HKC. ***indicates that $p<0.05$ compared with NC group, #indicates that $p<0.05$ compared with CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4. **B,** Effects of CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 transfection on apoptosis of renal tubular epithelial cell HKC. ***indicates that compared with NC group, $p<0.05$. **C,** Effects of CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 transfection on p-GSK-3β in renal tubular epithelial cell HKC. ***indicates that $p<0.05$ compared with NC. **D,** Effects of CD63-mimics+si-Wnt4 and CD63-inhibitor+sh-Wnt4 transfection on β-catenin in renal tubular epithelial cell HKC. ***indicates that $p<0.05$ compared with NC.

transfected Wnt4, p-GSK-3β, and β-catenin-related protein sequences into renal tubular epithelial cell HKC and observed the biological function of the cells. It was found that the proliferation of renal tubular epithelial cells was significantly increased, and the apoptosis rate was significantly decreased after the downregulation expression of Wnt4, p-GSK-3β, and β-catenin. This shows that the related proteins in Wnt/β-catenin signaling pathway also have the function of regulating cell biological function in diabetic nephropathy.

At the end of this study, we found through rescue experiments that after the relevant proteins in CD36-mimics+si-Wnt/β-catenin signaling pathway, Wnt4, and CD36-inhibitor+SH-Wnt/β-catenin signaling pathway were co-transfected into renal tubular epithelial cell HKC, and the cell proliferation and apoptosis rates were not different from the NC group of unrelated sequences. Further, compared with CD36-inhibitor, the proliferation ability was significantly increased and the apoptosis rate was decreased; while compared with CD36-mimics, the proliferation ability was significantly decreased and the apoptosis rate was increased, which indicated that we could adjust CD36 to downregulate the related proteins in the Wnt/β-catenin signaling pathway. Finally, by detecting the expressions of Wnt4, p-GSK-3β, β-catenin, and mRNA in the transfected renal tubular epithelial cell HKC, we found that inhibiting CD36 could slow down the apoptosis of renal tubular epithelial cells by inhibiting the expressions of the related proteins Wnt4, p-GSK-3β, β-catenin, and mRNA in Wnt/β-catenin signaling pathway. The activation of the Wnt/β-catenin signaling pathway induces renal fibrosis in diabetic nephropathy[25]. Wnt4, a member of Wnt family, has great influence on maintaining the healthy development of kidney. Wnt4 can induce renal interstitial cells to transform into renal tubular epithelial cells and induce the formation of renal tubules[26]. Wnt4, p-GSK-3β, and β-catenin proteins can participate in the formation of renal tubules by activating classical Wnt signaling pathways. The Wnt4 gene in healthy kidney is silent, and the expression content of p-GSK-3β and β-catenin in cytoplasm is also very small. However, when diabetic patients suffer from related kidney injury, Wnt4, p-GSK-3β, and β-catenin proteins in renal tubular epithelial and interstitial cells can be highly expressed, thus activating Wnt/β-catenin signaling pathway and inducing fibrosis[27]. However, Dawodu et al[28] found that CD36 could target the proliferation and apoptosis of renal tubular epithelial cells and podocytes mediated by related proteins in Wnt/β-catenin signaling pathway.

Through the analysis of the data in this study, we found that CD36 expression was upregulated in diabetic nephropathy, and silencing CD36 expression could inhibit apoptosis mediated by related proteins in Wnt/β-catenin signaling pathway. But there are certain limitations in this study. First, we have not conducted clinical experiments and the clinical value of the related proteins in CD36 and Wnt/β-catenin signaling pathway is unclear. Secondly, there is no relevant animal model in this study, and the relevant influence of CD36 on renal injury degree of diabetic patients is unclear. Further research is needed. Therefore, we hope to carry out clinical studies and establish animal models in the future research and explore the regulatory network of CD36 through bioinformatics analysis to provide more bases for our experiments.

Conclusions

In summary, CD36 can mediate apoptosis by inhibiting the expression of related proteins in nodal Wnt/β-catenin signaling pathway, and is expected to become a potential therapeutic target for clinical treatment of patients with diabetic nephropathy.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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