European Review for Medical and Pharmacological Sciences 2020; 24: 1970-1978

The potential mechanism of INHBC and CSF1R in diabetic nephropathy

X.-Y. DU[1], B.-T. ZHENG[2], Y. PANG[3], W. ZHANG[1], M. LIU[2], X.-L. XU[4], S.-J. ZHOU[1]

[1]Department of Nephrology, Jining No. 1 People's Hospital, Jining, P.R. China
[2]Affiliated Hospital of Jining Medical University, Jining, P.R. China
[3]Department of Pediatrics, Jining No. 1 People's Hospital, Jining, P.R. China
[4]Department of Neonatology, Maternal and Child Health Care Hospital of Qixia District, Nanjing, P.R. China

Xiaoya Du and Benteng Zheng contributed equally to this study

Abstract. – OBJECTIVE: The aim of this study was to research the potential mechanism of INHBC and CSF1R in diabetic nephropathy.

MATERIALS AND METHODS: 30 SD rats were selected and randomly divided into Con group, Sham group, and DN group. In the DN group, intraperitoneal injection of the streptozotocin-citrate solution was conducted to construct the DN model. In the Sham group, intraperitoneal injection of equal citrate solution was conducted. The Con group did not do anything. After successful modeling, blood glucose, insulin, biochemical indexes, and levels of inflammatory cytokines in blood samples were detected. The expression levels of INHBC, CSF1R, apoptosis-related proteins and IGF-1 were detected by Western blot. MRNA expression levels of INHBC, CSF1R, IGF-1 and inflammatory cytokines were detected by qPCR.

RESULTS: Compared with the Con group, the expression levels of blood glucose, insulin, biochemical indexes, INHBC, CSF1R, IGF-1, IL-6, TNF-α and Bcl2 increased in the DN group, while the expression levels of IL-10, Caspase 3, Caspase 9, and Bax decreased. INHBC mRNA was positively correlated with IGF-1 mRNA. CSF1R was negatively correlated with Caspase 3, Caspase 9, Bax, and IL-10, and positively correlated with IL-6, TNF-α, and Bcl2.

CONCLUSIONS: NHBC and CSF1R induced the secretion of IL-6 and TNF-α, inhibited the production of IL-10, inhibited apoptosis of cells, and promoted the proliferation of renal cells during DN disease. Therefore, INHBC and CSF1R can be used as target objects of DN treatment strategies.

Key Words:
INHBC, CSF1R, Diabetic nephropathy.

Introduction

Diabetic nephropathy (DN) is a diabetic microvessel complication and manifests as chronic disease of renal[1]. DN is defined as the glomerular filtration rate greater than 0.5 g within 24 h[2]. DN is the first cause of end-stage renal disease[3]. There are many causes for DN. The main reason of DN induced by oxidative stress[4]. Hyperglycemia can lead to oxidative damage of biomacromolecule, which induces the increase of oxidative stress to affect the occurrence of DN[5]. Mesangial cell (MC) is closely related to DN. MC determines the overall structure and integrity of the glomerular capillary cluster. Deletion of MC in DN patients will lead to dilatation of glomerular capillary and the formation of microaneurysm[6].

The Inhibin Subunit Beta C (INHBC) protein belongs to the transforming growth factor (TGF)-β superfamily[7]. Although studies[8,9] have discussed the role of INHBC in human endometrium and placenta, the relevant molecular mechanism of INHBC in DN is still unclear. TGF-β family is a major factor in DN pathology. The inhibition of TGF-β pathway can induce apoptosis of DN cells[10]. The increase of TGF-β in DN patients will induce accumulation of extracellular matrix, leading to DN[11]. INHBC is homologous to TGF-β. Its related mechanism in DN may also be related to apoptosis of cells or accumulation of matrix.

Colony stimulating factor 1 receptor (CSF1R) regulates survival, proliferation, differentiation of cells, and topotaxis and plays an important role in innate immune responses[12]. CSFIR/CSF1 contributes to the metastasis of most cancer tumors[13]. Alikhan et al[14] considered that CSF1/CSFIR plays an important nutritional role in the process of kidney growth and repair when studying the relationship between CSF1 and mammalian growth. Therefore, there may be certain relation between CSF1R and DN.

In this paper, a diabetic mouse model was constructed by streptozotocin induction to explore the role of INHBC and CSF1R in the pathogenesis of diabetic nephropathy and what molecular mechanisms are involved to provide a reliable scientific basis for the treatment of diabetic nephropathy.

Corresponding Author: Shiju Zhou, MD; e-mail: zhoushiju@sina.com

Materials and Methods

Materials

30 male SD adult healthy rats, 2 to 3 months old and 305-325 g of weighing were purchased from Hunan SJA Laboratory Animal Co., Ltd (Changsha, Hunan province, China). This study was approved by our Hospital Ethics Committee. The procedure was carried out in strict accordance with the guidelines for the care and use of laboratory animals (NIH Publishing, 1996 revision, No. 85-23). After screening, the rats were determined to have no evident abnormal behavior. According to the randomized principle, it was divided into three groups: control group (Con group), sham group (Sham group), and model group (DN group).

The protein extract of rat tissue was purchased from Shanghai Beibo Biological Company (Shanghai, China) and the batch number was BB18011. BCA protein concentration determination kit was purchased from Beyotime Institute of Biotechnology (Shanghai, China) and the batch number was P0012. INHBC, CSF1R, insulin-like growth factor 1(IGF-1), Caspase 3, Caspase 9, Bax, Bcl2, β-actin first antibody (item number: ab73904, ab183316, ab9572, ab197202, ab219590, ab32503, ab32124, ab115777), goat anti rabbit of second antibody (HRP crosslinking, ab205718 of item number) were purchased from Shanghai Abcam Company (Shanghai, China). The tumor necrosis factor (TNF-α), interleukin 6 (IL-6), interleukin 10 (IL-10) enzyme linked immunosorbent assay (ELISA) kit (item number: ab208348, ab100713, ab108870) were purchased from Shanghai Abcam (Shanghai, China). FastKing one-step reverse transcription - fluorescence quantitative kit was purchased by Tiangen Company (Beijing, China; Catalog No. FP314).

Streptozotocin Induced Diabetic Nephropathy Model

All the rats were fed in a constant temperature and humidity animal room, were alternately illuminated with diurnal for 12 hours, were fasted for one night and drank water freely before surgery. In the DN group, streptozotocin was injected into the abdominal cavity (dissolved in 0.1 mmol /L citrate buffer, 180 mg/kg). In the sham group, an equal amount of 0.1 mmol/L citrate buffer was injected into the abdominal cavity. The Con group did not do anything. The rats were fed normally after induction of streptozotocin. After 1 week of modeling, blood samples were collected from the tail vein to detect the blood glucose value. The blood glucose value was higher than 16.7 mmol/L, indicating that the DN model was successfully constructed. After successful modeling, urine was collected for 24 h after 2 weeks of continuous culture. The urine protein content was detected. The blood samples were collected. Blood glucose, insulin, blood urea nitrogen, creatinine, TNF-α, IL-6, and IL-10 were detected. The kidneys were collected and stored in liquid nitrogen at -80°C for subsequent testing.

Detection of INHBC, CSF1R, IGF-1, TNF-α, IL-6, IL-10 mRNA by qPCR

Part of the kidney tissue was ground in TRIzol solution to extract the total RNA. Total RNA concentration and purity were detected by UV-Spectrophotometer at 260-280 nm. OD_{260}/OD_{280}>1.8 were used for next detection. INHBC, CSF1R, IGF-1, TNF-α, IL-6, and IL-10 mRNA were reversed transcribed and PCR was amplified and quantitated by using FastKing one-step reverse transcription – fluorescence quantitative kit (Tiangen Company, Beijing, China, Catalog No. FP314) and ABI PRISM 7000. The primers were designed and synthesized by Sangon Biotech (Shanghai, China) Co., Ltd. Primers sequence table are shown in Table I.

Reaction system (50 μL): 1.25 μL of upstream primers, 1.25 μL of downstream primers, 1.0 uL of probe, 10 pg/μg of RNA template, 50×ROX Reference Dye 5 μl ROX, RNase-Free ddH_2O water supplement to 50 μL.

Table I. Primers sequence table.

Upstream primers	Downstream primers	
INHBC	5'-GCAGCCCGGGTGAGAGTTGG-3'	5'-ACTGCACCCACAGGCCTC-3'
CSF1R	5'-ACAAAGCAGGGACCTCTTGA-3'	5'-ATGGTGTTAAGGTCTTGGGC-3'
IGF-1	5'-TCGCATCTCTTCTATCTGGCCCTGT-3'	5'-GCAGTACATCTCCAGCCTCCTCAGA-3'
TNF-α	5'-GCCACCACGCTCTTCTG-3'	5'-GCAGCCTTGTCCCTTGA-3'
IL-6	5'-TAGTCCTTCCTACCCCAACTTCC-3'	5'-TTGGTCCTTAGCCACTCCTTC-3'
IL-10	5'-AGTGGAGCAGGTGAAGAATG-3'	5'-CCAGCCTTAGGATCGAAGTT-3'
GAPDH	5'-CCCACTAACATCAAATGGGG-3'	5'-ATCCACAGTCTTCTGGGTGG-3'

Reaction program: reverse transcription for 30 min at 50°C, 1 of cycle number, initial denaturation for 3 min at 95°C, 1 of cycle number, PRC cycle, denaturation for 15 s at 95°C, annealing for 30 s at 60°C, 40 of cycle number. The results were analyzed by the ABI PRISM 7000. The internal reference gene was GAPDH. $2^{-\Delta\Delta Ct}$ method was used for calculation.

Detection of INHBC, CSF1R and Apoptotic Protein-Related Protein by Western Blot

Part of the kidney tissue was cut. Then, the pre-cooled cell protein extract was added, (Cell lysate: proteinase inhibitor: phosphatase inhibitor = 98: 1:1) and centrifuged for 15 min at 1.2×10^4 r/min, and the supernatant was taken. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis was used to distinguish proteins. Then, the protein was transferred to the nitrocellulose (NC) membrane and allowed to stand at room temperature for 1 h (blocking solution was 5% skim milk-PBS solution). Subsequently, INHBC, CSF1R, and apoptotic protein-related protein first antibody were added and allowed to stand overnight at 4°C. They were then washed with phosphate-buffered saline (PBS) solution and this operation was repeated three times. Goat anti rabbit of second antibody (HRP crosslinking) was added and allowed to stand at room temperature for 1 h. Finally, the membrane was washed with PBS solution and visualized by enhanced chemiluminescence (ECL) method. The internal reference protein was β-actin. The relative expression level of the protein to be tested = the gray value of the band to be measured / the gray value of the β-actin band.

Detection of TNF-α, IL-6, IL-10 by ELISA

Blood samples were taken and centrifuged at 3×10^3 × g for 20 min at 4°C. The supernatant was taken and the expression levels of TNF-α, IL-6, and IL-10 were detected by ELISA kit. The operation strictly adheres to the requirements of the kit instructions.

Detection of Biochemical Indexes

The experimental rats were placed in a clean metabolic cage. Urine samples were collected for 24 h. Urine protein was quantitatively detected by Broadford (Isle of Skye, Scotland, UK). Blood samples were taken from the abdominal aorta. Blood urea nitrogen was detected by diacetyl-oxime colorimetry. Creatinine was detected by picric acid method. All steps were conducted in strict accordance with the instructions. Blood glucose level was detected by Advantage electronic sensor blood glucose meter and relevant test paper, and insulin was detected by radioimmunoassay competitive method.

Statistical Analysis

The above index data was input into the SPSS 20.0 software package (IBM Corp., Armonk, NY, USA) for statistical analysis. The measuring data were expressed as Mean±SD. The data comparison method was One-way analysis of variance in groups. Pearson analysis was used for correlation analysis. All the data were tested by two-tailed tests. 95% was taken as the confidence interval and the difference was statistically significant with all $p<0.05$.

Results

DN was Associated with Hyperglycemia and High Insulin

As shown in Figure 1, there were no statistically significant differences in blood glucose and insulin between Con group and Sham group (all $p>0.05$), while blood glucose and insulin in DN group were statistically increased compared with Con group (all $p<0.05$). The blood glucose value of DN group was 22.25±1.26 mmol/L>16.7 mmol/L, indicating successful modeling. These results indicated that DN was associated with hyperglycemia and high insulin.

DN was Associated with Upregulation of INHBC and CSF1R Expression Levels

Western blot and qPCR results showed that there was no difference in INHBC, CSF1R, and mRNA between the Con group and the Sham group (all $p>0.05$). Compared with Con group, the expression of INHBC, CSF1R, and mRNA of kidney cells in DN group was upregulated. The difference was statistically significant (all $p<0.05$). These results indicated that DN was associated with up-regulation of INHBC and CSF1R expression levels. More details are shown in Figure 2.

DN was Associated with the Increase of Urinary Protein, Blood Urea Nitrogen and Creatinine

As shown in Figure 3, compared with the Con group, there was no statistical change in urine protein, blood urea nitrogen, and creatinine in

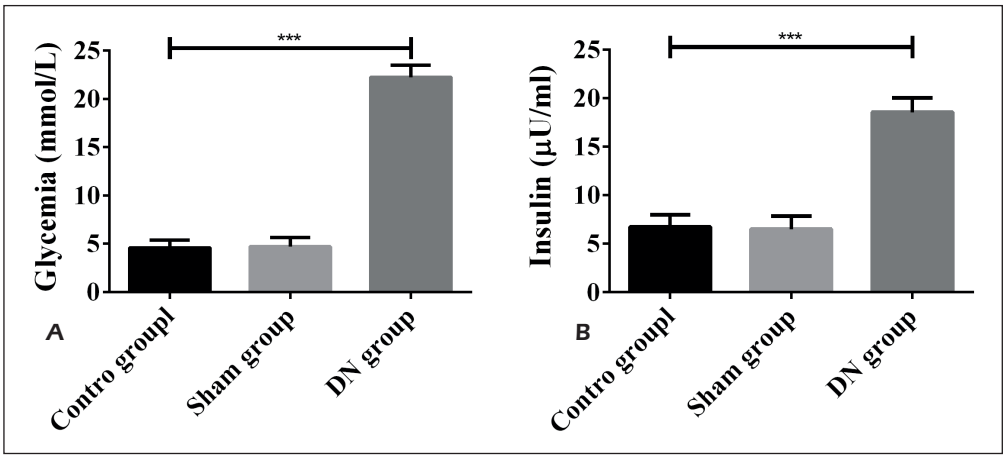

Figure 1. Blood glucose, insulin and insulin resistance index of each group. **A**, Blood glucose content of each group, mmol/L. **B**, Insulin content of each group, μU/ml. Blood glucose and insulin contents were collected from blood samples of rats in each group after modeling for 3 weeks. Compared with Con group, ***p<0.001).

the Sham group (all p>0.05). Compared with the Con group, urine protein, blood urea nitrogen, and creatinine in DN group were statistically increased (all p<0.05). These results indicated that DN destroys renal function and causes the increase of urinary protein, blood urea nitrogen, and creatinine.

DN was Associated with Inflammation

As shown in Figure 4, compared with Con group, there was no significant change in TNF-α, IL-10, IL-6 protein and mRNA in Sham group (all p>0.05). Compared with Con group, contents of TNF-α and IL-6 in DN group increased and expression level of mRNA was upregulated, while IL-10 protein content decreased, and expression of mRNA was downregulated. The difference was statistically significant (all p<0.05). These results indicated that DN causes inflammatory response in rats *in vivo*.

The Occurrence of DN was Associated with the Inhibition of Apoptosis of Cells by CSF1R

Compared with Con group, there were no statistically significant changes in the proteins of Caspase 3, Caspase 9, Bax, and Bcl2 in Sham group (all p>0.05). Compared with the Con group, the expression levels of Caspase 3, Caspase 9, and Bax proteins in the DN group were decreased and the expression level of Bcl2 was increased. The difference was statistically significant (all

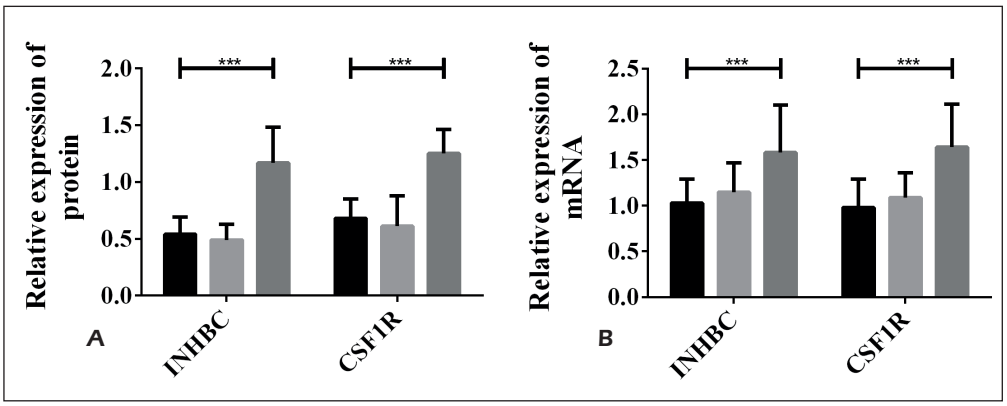

Figure 2. Expression of INHBC, CSF1R and their mRNA in each group. **A**, Expression of INHBC and CSF1R proteins in each group. **B**, Expression of INHBC and CSF1R mRNA in each group. The above data were collected from the kidney tissues of rats in each group after modeling for 3 weeks. Compared with Con group, ***p<0.001).

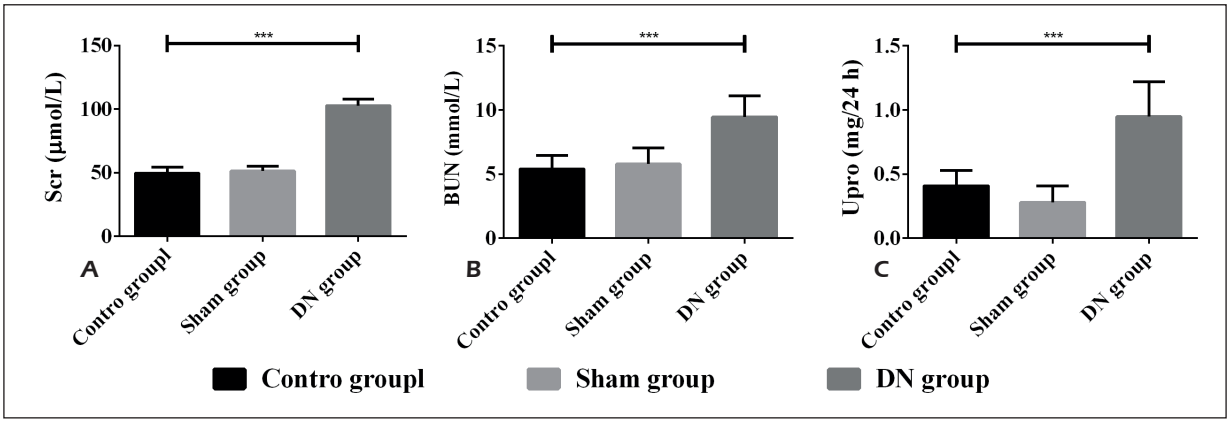

Figure 3. Biochemical indexes of each group. **A**, Creatinine Scr content in each group. **B**, Blood urea nitrogen BUN content in each group. **C**, Upro content of urine protein in each group. The above data were collected from blood samples and urine samples of rats in each group after modeling for 3 weeks. Compared with Con group, ***$p<0.001$).

$p<0.05$). These results indicated that apoptosis of cells was inhibited during the occurrence of DN. Pearson correlation analysis showed that CSF1R was negatively correlated with Caspase 3, Caspase 9, and Bax ($r<0$, $p<0.05$), but positively correlated with Bcl2 ($r>0$, $p<0.05$). These results indicated that apoptosis of cells might be inhibited by CSF1R during the occurrence of DN. More details are shown in Figure 5 and Table II.

INHBC Promoted the Expression of IGF-1

Compared with the Con group, there was no statistical change in IGF-1 and its mRNA in the Sham group (all $p>0.05$). Compared with the Con

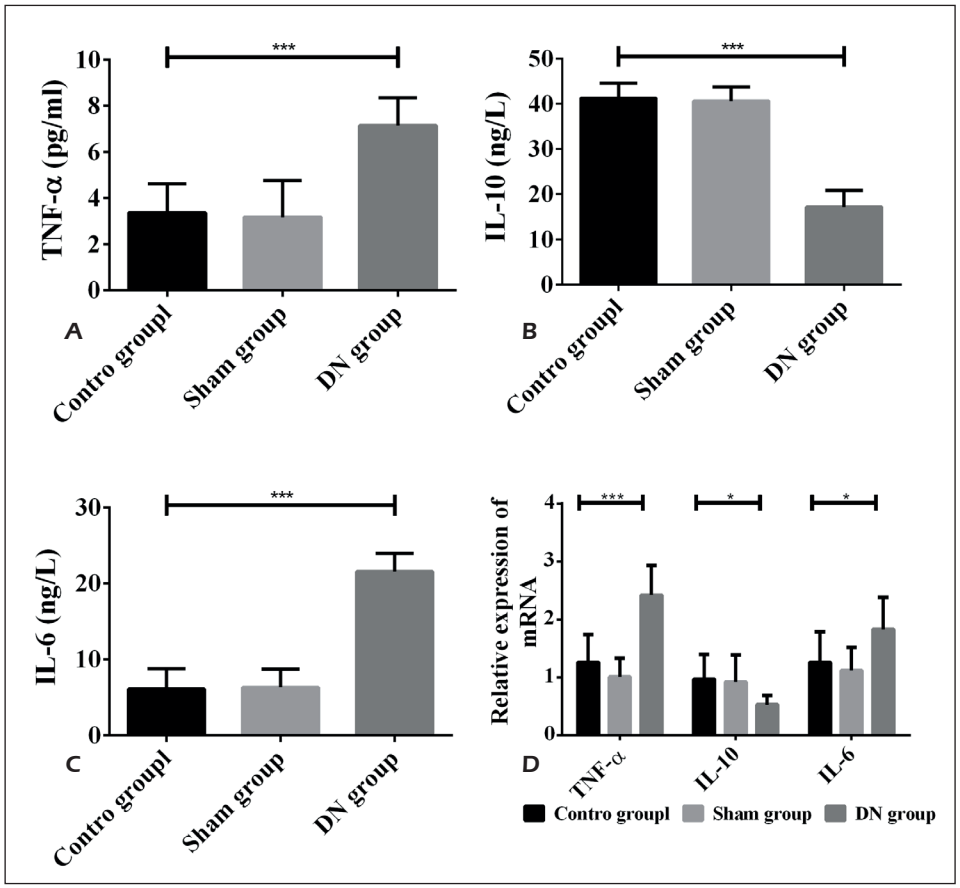

Figure 4. Expression of TNF-α, IL-10, IL-6 and their mRNA in each group. **A**, Serum TNF-α content in each group. **B**, Serum IL-10 content in each group. **C**, Serum IL-6 content in each group. **D**, TNF-α mRNA, IL-10 mRNA and IL-6 mRNA expression in each group. The above data were collected from kidney tissue and blood samples of rats in each group after modeling for 3 weeks. Compared with Con group, *$p<0.05$, ***$p<0.001$)

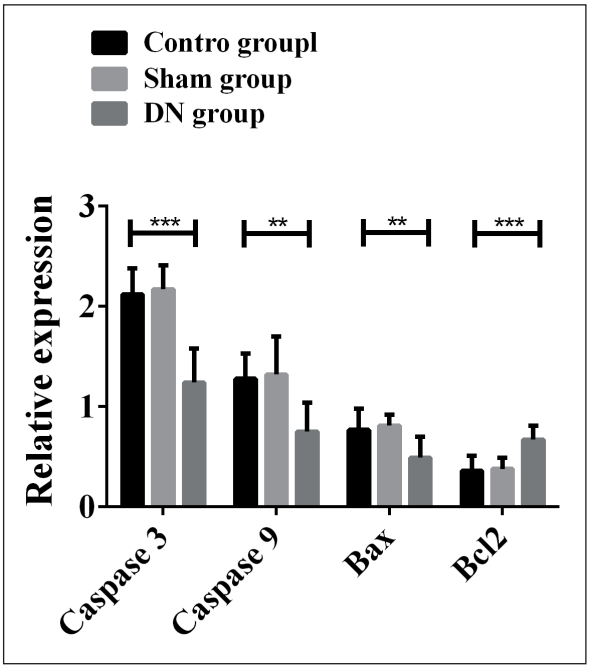

Figure 5. Apoptosis-related proteins in each group. (Compared with the Con group, $^{**}p<0.05$, $^{***}p<0.001$).

group, the expression of IGF-1 and IGF-1 mRNA was upregulated in the DN group. The difference was statistically significant (all $p<0.05$). This indicated the increase of IGF-1 expression during the occurrence of DN. The correlation between INHBC and IGF-1 showed that INHBC was positively correlated with IGF-1 (r=0.6868, $p=0.0282$). This indicated that IGF-1 was upregulated with upregulation of INHBC expression. More details are shown in Figure 6.

Table II. Pearson analysis of CSF1R and apoptosis-related proteins.

	Ur	p
CSF1R-Caspase 3	-0.5971	0.0254
CSF1R-Caspase 9	-0.6347	0.0314
CSF1R-Bax	-0.6164	0.0273
CSF1R-Bcl-2	0.5869	0.0419

CSF1R Promoted the Expression of TNF-α and IL-6, while Inhibited the Expression of IL-10

As shown in Figure 7, CSF1R mRNA was positively correlated with mRNA of TNF-α and IL-6, but negatively correlated with IL-10 mRNA. These results indicated that the expression of TNF-α and IL-6 mRNA was upregulated with the upregulation of CSF1R mRNA expression, and the expression of IL-10 mRNA was downregulated with the upregulation of CSF1R mRNA expression. Therefore, CSF1R can be considered to promote cellular inflammation in the occurrence of DN.

Discussion

DN is an inflammatory disease caused by metabolic disorders[15]. Renal inflammation is a key factor in the deterioration of DN[16]. The pathogenesis of DN involves many inflammatory cytokines. TNF-α activates NADPH oxidase through PKC and MAPK pathways, thereby promoting the production of reactive oxygen species (ROS)[17]. Then, the enrichment of reactive oxygen species can in-

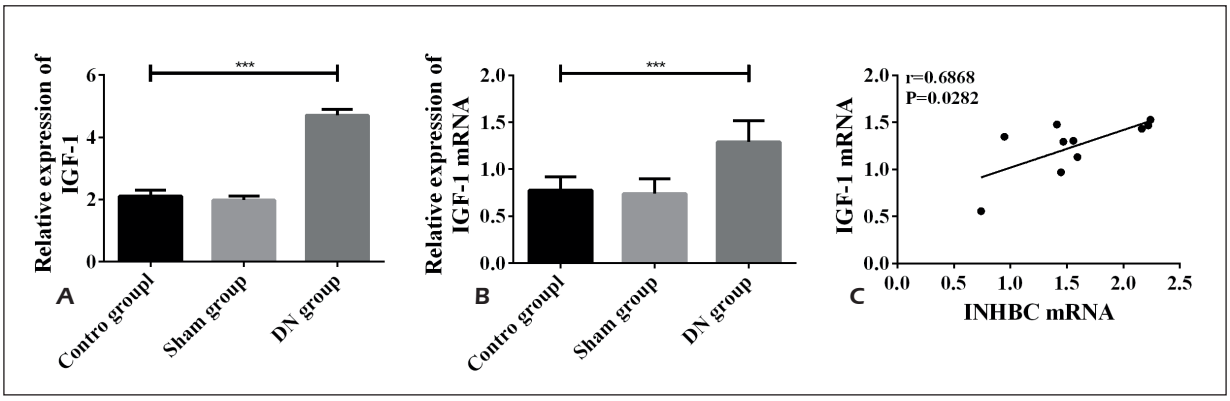

Figure 6. Expression of IGF-1 and IGF-1 mRNA in each group and its correlation with INHBC. **A**, expression of IGF-1 protein in each group. **B**, Expression of IGF-1 mRNA in each group. **C**, correlation analysis of IGF-1 mRNA-INHBC mRNA. The above data were collected from the kidney tissues of rats in each group after modeling for 3 weeks. Compared with Con group, $^{***}p<0.001$. Pearson correlation analysis was used.

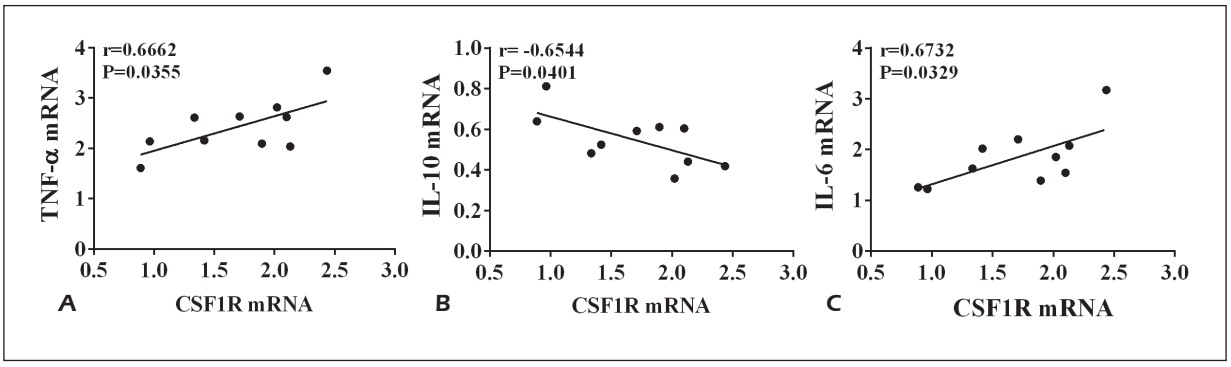

Figure 7. Pearson correlation analysis of the relationship between CSF1R and inflammation. **A**, correlation analysis of CSF1R-TNF-α. **B**, Correlation analysis of CSF1R-IL 10. **C**, Correlation analysis of CSF1R-IL 6. Pearson correlation analysis was used.

duce the occurrence of DN. TNF-α can induce the thickening of F-actin protein bundles and the formation of stress fibers, thereby stimulating the appearance of intercellular space and damaging the permeability of renal endothelial cells[18], and finally causing DN. IL-6 acts on MC and tubular cells and induces MC proliferation and proliferation of tubular cells. In addition, IL-6 may be related to hypertrophy of podocyte[19]. IL-10 can downregulate the production of the pro-inflammatory factors, such as MCP-1 to achieve its anti-inflammatory properties. So, the decrease of IL-10 expression may directly lead to the deterioration of DN[20]. The increased INHBC and CSF1R in DN rats were found in this study, which was similar to the results of Yang et al[21]. Therefore, we suggested the hypothesis that the increased INHBC and CSF1R could promote the occurrence and development of DN.

The potential mechanism of CSF1R in DN may be related to the regulation of the expression of the above inflammatory factors. In this study, we found that, in the DN group, the expression levels of CSF1R, pro-inflammatory factors TNF-α, and IL-6 were increased, while the expression levels of anti-inflammatory factor IL-10 were decreased. The correlation analysis of Pearson showed that CSF1R was positively correlated with TNF-α and IL-6, but negatively correlated with IL-10. The above results implied that CSF1R may promote the expression of TNF-α and IL-6 and inhibit the expression of IL-10, eventually causing the damage of renal cell and the onset of DN. The action pathway of CSF1R and inflammatory factors may involve macrophages. The hyperglycemic environment of diabetes activates the renal p38/JNK pathway, stimulates the increase of downstream CSF1/CSF1R expression, thereby inducing proliferation of macrophages and secretion of IL-6 and TNF-α, eventually causing damage of renal cell[16]. Secretion of IL-6 activates the JAK pathway[22], while the JAK pathway acts on IL-10 with negative feedback. In this study, we also found a positive correlation between INHBC and IGF-1, while IGF-1 induced proliferation, migration, and matrix accumulation of MC[23,24]. Therefore, the underlying mechanism of INHBC in DN may be that INHBC promotes IGF-1 expression, while IGF-1 acts on MC and induces biological changes in MC, and ultimately leading to DN.

In addition, this paper discusses the relationship between CSF1R and apoptosis of cells. The results showed that CSF1R was negatively correlated with Caspase 3, Caspase 9, and Bax protein, and positively correlated with Bcl2. Some scholars[25,26] found that the inhibition of CSF1R can induce apoptosis of cells. Park et al[27] found that the downregulation of inhibin mRNA expression could inhibit proliferation of cells when studying the mechanism of α-solanine action. Combined with previous studies, CSF1R may inhibit apoptosis of renal cells, while INHBC promotes proliferation of renal cells. Therefore, their synergy induce renal hypertrophy and eventually cause DN.

This article focuses on the potential mechanisms of INHBC and CSF1R in the DN model during deterioration of DN. Pearson analysis indicates that INHBC and CSF1R can induce inflammation and apoptosis of cells. In the future experimental design, relevant gene knockout or addition of inhibitors can be further used to provide data support. The direct-action sites of INHBC and CSF1R can be searched to understand its molecular mechanism in the deterioration of DN more, systematically.

Conclusions

This study compared the expression levels of INHBC and CSF1R in Con group, Sham group, and DN group, and found that both of them were highly expressed in the DN group. It was considered that CSF1R induced secretion of IL-6 and TNF-α, inhibited production of IL-10, and inhibited cell apoptosis in the deterioration of DN, while INHBC promoted proliferation of renal cells and IGF-1 expression. Therefore, INHBC and CSF1R can be used as targets object of DN treatment strategies to achieve the purpose of treating DN by inhibiting their expression.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Sagoo MK, Gnudi L. Diabetic nephropathy: is there a role for oxidative stress. Free Radic Biol Med 2018; 116: 50-63.
2) Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 2005; 28: 164-176.
3) Ebrahim N, Ahmed IA Hussien NI, Dessouky AA, Farid AS, Elshazly AM, Mostafa O, Gazzar WBE, Sorour SM, Seleem Y, Hussein AM, Sabry D. Mesenchymal stem cell-derived exosomes ameliorated diabetic nephropathy by autophagy induction through the mTOR signaling pathway. Cells 2018; 7. pii: E226.
4) Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 2008; 57: 1446-1454.
5) Sifuentes-Franco S, Padilla-Tejeda DE, Carrillo-Ibarra S, Miranda-Díaz AG. Oxidative stress, apoptosis, and mitochondrial function in diabetic nephropathy. Int J Endocrinol 2018; 2018: 1875870.
6) Tri Q, Nguyen, Roel G. Chapter 9. The Mesangial Cell in Diabetic Nephropathy. In "Diabetic Nephropathy" JJ Roelofs, L Vogt (eds). Springer International Publishing, 2019. pp: 143-151.
7) H tten G, Neidhardt H, Schneider C, Pohl J. Cloning of a new member of the TGF-β family: a putative new activin βC chain. Biochem Biophys Res Commun 1995; 206: 608-613.
8) Mylonas I, Brüning A, Shabani N, Kunze S, Kupka MS. Evidence of inhibin/activin subunit betaC and betaE synthesis in normal human endometrial tissue. Reprod Biol Endocrinol 2010; 8: 143.
9) Mylonas I, Makovitzky J, Kunze S, Brüning A, Kainer F, Schiessl B. Inhibin-betaC subunit expression in normal and pathological human placental tissues. Syst Biol Reprod Med 2011; 57: 197-203.
10) Wang XB, Zhu H, Song W, Su JH. Gremlin regulates podocyte apoptosis via transforming growth factor-β (TGF-β) pathway in diabetic nephropathy. Med Sci Monit 2018; 24: 183.
11) Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA. Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. Proc Natl Acad Sci U S A 1993; 90: 1814-1818.
12) Moon HG, Kim SJ, Jeong JJ, Han SS, Jarjour NN, Lee H, Abboud-Werner SL, Chung S, Choi HS, Natarajan V, Ackerman SJ, Christman JW, Park GY. Airway epithelial cell-derived colony stimulating factor-1 promotes allergen sensitization. Immunity 2018; 49: 275-287.e5.
13) Okugawa Y, Toiyama Y, Ichikawa T, Kawamura M, Yasuda H, Fujikawa H, Saigusa S, Ohi M, Araki T, Tanaka K, Inoue Y, Tanaka M, Miki C, Kusunoki M. Colony-stimulating factor-1 and colony-stimulating factor-1 receptor co-expression is associated with disease progression in gastric cancer. Int J Oncol 2018; 53: 737-749.
14) Alikhan MA, Jones CV, Williams TM, Beckhouse AG, Fletcher AL, Kett MM, Sakkal S, Samuel CS, Ramsay RG, Deane JA, Wells CA, Little MH, Hume DA, Ricardo SD. Colony-stimulating factor-1 promotes kidney growth and repair via alteration of macrophage responses. Am J Pathol 2011; 179: 1243-1256.
15) Tuttle KR. Linking metabolism and immunology: diabetic nephropathy is an inflammatory disease. J Am Soc Nephrol 2005; 16: 1537-1538.
16) Lim AK, Tesch GH. Inflammation in diabetic nephropathy. Mediators Inflamm 2012; 2012: 146154.
17) Koike N, Takamura T, Kaneko S. Induction of reactive oxygen species from isolated rat glomeruli by protein kinase C activation and TNF-α stimulation, and effects of a phosphodiesterase inhibitor. Life Sci 2007; 80: 1721-1728.
18) WójciakÐStothard B, Entwistle A, Garg R, Ridley AJ. Regulation of TNF-α-induced reorganization of the actin cytoskeleton and cell-cell junctions by Rho, Rac, and Cdc42 in human endothelial cells. J Cell Physiol 1998;176: 150-165.
19) Feigerlová E, Battaglia-Hsu SF. IL-6 signaling in diabetic nephropathy: From pathophysiology to therapeutic perspectives. Cytokine Growth Factor Rev 2017; 37: 57-65.
20) Kung WJ, Lin CC, Liu SH, Chaung HC. Association of interleukin-10 polymorphisms with cytokines in type 2 diabetic nephropathy. Diabetes Technol Ther 2010; 12: 809-813.
21) Yang H, Lian D, Zhang X, Li H, Xin G. Key genes and signaling pathways contribute to the pathogenesis of diabetic nephropathy. Iran J Kidney Dis 2019; 13: 87-97.
22) Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol 2008; 19: 433-442.

23) Vasylyeva TL, Ferry Jr RJ. Novel roles of the IGF–IGFBP axis in etiopathophysiology of diabetic nephropathy. Diabetes Res Clin Pract 2007; 76: 177-186.

24) Ren W, Zhao C, Wang Y, Fang Y, Huang Z, Chen W, Wang L, Hu W, Wang K, Ni L. Ramipril can alleviate the accumulation of renal mesangial matrix in rats with diabetic nephropathy by inhibiting insulin-like growth factor-1. Acta Cir Bras 2019; 34: e20190010000007.

25) Marthyn P, Beuscart A, Coll J, Moreau-Gachelin F, Righi M. DMSO reduces CSF-1 receptor levels and causes apoptosis in v-mycImmortalized mouse macrophages. Exp Cell Res 1998; 243: 94-100.

26) Ullrich K, Wurster KD, Lamprecht B, Köchert K, Engert A, Dörken B, Janz M, Mathas S. BAY 43-9006/ Sorafenib blocks CSF1R activity and induces apoptosis in various classical Hodgkin lymphoma cell lines. Br J Haematol 2011; 155: 398-402.

27) Park S, Park MY, Song G, Lim W. Alpha-solanine inhibits cell proliferation via mitochondrial dysfunction and inhibin synthesis in mouse testis *in vitro* and *in vivo*. Chemosphere 2019; 235: 271-279.