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Influence of LncRNA UCA1 on glucose metabolism in rats with diabetic nephropathy through PI3K-Akt signaling pathway

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Abstract. – OBJECTIVE: The aim of this study was to investigate the influence of long non-coding ribonucleic acid (lncRNA) urothelial carcinoma associated 1 (UCA1) on glucose metabolism in rats with diabetic nephropathy (DN), and to explore its regulatory mechanism.

MATERIALS AND METHODS: A total of 30 healthy Sprague-Dawley (SD) rats were selected in this study. All rats were randomly divided into three groups, including the control group, the model group, and the lncRNA UCA1 inhibitor group. The rat model of DN was successfully established via intraperitoneal injection of streptozotocin (STZ). The pathological changes in kidney tissues were detected via hematoxylin-eosin (HE) staining. The levels of blood urea nitrogen (BUN), serum creatinine (Scr), and urinary protein (UP) were detected using the biochemical method. Meanwhile, the content of serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) was detected via enzyme-linked immunosorbent assay (ELISA). In addition, the messenger RNA (mRNA) and protein levels of phosphatidylinositol 3-hydroxy kinase (PI3K) and protein kinase B (Akt) in kidney tissues were detected via reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting, respectively.

RESULTS: The model group showed severe pathological damage to the kidney, compared with the control group. Meanwhile, the levels of BUN, Scr and UP, and the content of serum TNF-α and IL-6 increased significantly in the model group. The mRNA and the protein levels of PI3K and Akt in kidney tissues of the model group were significantly up-regulated as well. LncRNA UCA1 inhibitor group exhibited relieved pathological damage to the kidney, compared with the model group. The levels of BUN, Scr and UP, and the content of serum TNF-α and IL-6 remarkably decreased in UCA1 inhibitor group. Furthermore, the mRNA and the protein levels of PI3K and Akt in kidney tissues of UCA1 inhibitor groups were significantly down-regulated.

CONCLUSIONS: LncRNA UCA1 can relieve the pathological damage to the kidney, improve renal function, and alleviate inflammatory response in DN rats. The underlying mechanism may be related to the inhibition of the PI3K-Akt signaling pathway.

Key Words:
Diabetic nephropathy (DN), Inflammatory response, LncRNA, UCA1, PI3K-Akt signaling pathway.

Introduction

The diabetic nephropathy (DN) is a common and severe microvascular complication of diabetes mellitus. In severe cases, DN can lead to end-stage renal disease, seriously reducing the life quality of patients. Currently, DN has become an important killer of human health[1]. With the continuous improvement of the living standards, the morbidity rate of diabetes mellitus and the incidence rate of DN have increased year by year. According to the statistical analysis, the number of patients with diabetes mellitus will reach 3 billion in 2025 worldwide. Among them, 30-40% of type 1 diabetes mellitus and 15-20% of type 2 diabetes mellitus will develop into DN[2]. Due to the slow onset process, DN has already been in the middle and advanced stage, once the symptoms appear. Therefore, the prevention and early diagnosis of DN play important roles in delaying the disease. The pathological manifestations of DN include an elevated urinary protein level, a glomerular damage, and a decline in the glomerular filtration rate[3]. Hemodialysis is dominated in clinical treatment, so as to control the patients' condition. Currently, the pathogenesis of DN has not been fully elucidated. Scholars[4,5] have indicated that DN may be the result of the joint action of multiple factors.

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The main pathogenesis theories include genetic factors, hemodynamic changes, glucose metabolism disorders, oxidative stress, and inflammatory response. In recent years, Tesch[6] has demonstrated that an abnormal inflammatory response plays a key role in the pathogenesis of DN. Therefore, a thorough and in-depth understanding of the abnormal inflammatory response is helpful to the early diagnosis and prevention of DN. Most inflammatory factors in the body are secreted by monocytes, macrophages, and lymphocytes. The release of a large number of inflammatory factors activates fibroblast-like cells in the body. This may cause kidney tissue fibrosis and glomerular sclerosis, eventually inducing and aggravating the occurrence and the development of DN[7].

Long non-coding ribonucleic acid (lncRNA) is a kind of non-coding RNA with more than 200 nucleotides in length. They can be divided into five types based on the gene position, including: sense lncRNA, antisense lncRNA, bidirectional lncRNA, intergenic lncRNA, and intronic lncRNA[8]. LncRNAs cannot be directly transcribed and translated into proteins. However, they can be epigenetically modified through their gene promoters, or directly bind to encoding proteins to exert regulatory effects. A large amount of literature research has proved that lncRNAs are involved in regulating various biological processes, such as cell proliferation, differentiation, and apoptosis. The abnormal expression or a series of sequence mutations of lncRNAs can result in various human diseases[9], such as cardiovascular and cerebrovascular diseases, neurodegenerative diseases, pulmonary fibrosis, endocrine metabolic diseases, and malignant tumors. Recent studies have confirmed that lncRNAs are involved in the regulation of pancreatic cell development and insulin secretion. This indicates that lncRNAs play an important role in the pathogenesis and the development of diabetes mellitus and DN. The urothelial carcinoma associated 1 (UCA1) was first discovered in bladder cancer. It has been shown[10] that it can regulate the aerobic glycolysis of tumor cells by regulating its downstream mammalian target of rapamycin (mTOR). Meanwhile, UCA1 is involved in regulating tumor cell cycle through the phosphatidylinositol 3-hydroxy kinase (PI3K) signaling pathway. All the above findings suggest that lncRNA UCA1 plays an important regulatory role in tumors. However, its regulatory role in DN has not been fully elucidated.

In this study, we first established the model of DN in rats via intraperitoneal injection of streptozotocin (STZ). The aim of this study was to investigate the influence of lncRNA UCA1 on glucose metabolism in DN rats and to explore its regulatory mechanism.

Materials and Methods

Reagents

STZ was purchased from Sigma (St. Louis, MO, USA). Hematoxylin-eosin (HE) staining solution, radioimmunoprecipitation assay (RIPA) lysis buffer, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Beijing Solarbio Life Sciences Co., Ltd. (Beijing, China), while phosphorylated PI3K, Akt and β-actin primary antibodies and horse reddish peroxidase (HRP)-labeled secondary antibodies from Cell Signaling Technology (Danvers, MA, USA). PI3K and Akt primers, first-strand complementary deoxyribose nucleic acid (cDNA) synthesis kit and polymerase chain reaction (PCR) amplification kit were purchased from Invitrogen (Carlsbad, CA, USA); lncRNA UCA1 inhibitor from Shanghai Hanbio Biotechnology Co., Ltd. (Shanghai, China), while blood urea nitrogen (BUN), serum creatinine (Scr), and urinary protein (UP) from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Instruments

The electronic balance was purchased from Shanghai Balance Instrument Factory (Shanghai, China), the microplate reader and electrophoresis instrument from Bio-Rad (Hercules, CA, USA), the centrifuge from Sigma (St. Louis, MO, USA), the microscope from Nikon (Tokyo, Japan), the gel imager from Shanghai Clinx Scientific Instruments Co., Ltd. (Shanghai, China), and the ultraviolet spectrophotometer from Varian (Palo Alto, CA, USA).

Animals

A total of 30 healthy clean-grade Sprague-Dawley (SD) rats weighing (200±20) g were purchased from Beijing HFK Bioscience Co., Ltd. [license No.: SCXK (Beijing, China) 2014-0004]. This study was approved by the Ethics Committee of The People's Hospital of Danyang Animal Center.

Establishment of the Rat Model of DN

After the adaptive feeding for 1 week, SD rats were randomly divided into three groups using a random number table, with 10 rats in each group. The rats in the model group and lncRNA UCA1 inhibitor group were fed with high-glucose and high-fat diet for 6 weeks. After that, they were intraperitoneally injected with STZ solution for modeling. Blood glucose level > 16.7 mmol/L indicated the successful establishment of the DN model in rats[11]. Subsequently, the kidney tissues were collected from rats, embedded in paraffin and sliced. After staining using HE staining kit, the results were observed under a microscope.

Detection of Serum Levels of BUN, Scr, and UP in Rats Using Biochemical Method

The levels of BUN, Scr, and UP in rats were detected according to the instructions of the relevant kits. With BUN as an example, 0.02 mL sample was first added into the sample tube. After adding the 0.25 mL enzyme buffer, the mixture was incubated at 37°C for 10 min. Subsequently, 1 mL phenol developing agent and 1 mL alkaline sodium hypochlorite solution were added. Finally, the absorbance was measured at 640 nm to calculate the content.

Detection of Serum TNF-α and IL-6 Content in Rats via ELISA

100 μL standards and samples were first added into each well. Subsequently, they were sealed with sealing films and incubated at 37°C for 2 h. The liquid in each well was discarded, and 100 μL biotin antibodies were added. The mixture was then sealed with sealing films and incubated for another 1 h. After washing for 3 times, the liquid in each well was discarded. Subsequently, 100 μL HRP-labeled solution was added for 1 h of incubation, followed by washing for 3 times. Next, 90 μL tetramethylbenzidine (TMB) was added for incubation for 20 min. The reaction was terminated with 50 μL stop buffer, and the absorbance was measured at 450 nm. Finally, the contents of TNF-α and IL-6 were calculated.

Detection of mRNA Levels of PI3K and Akt in Kidney Tissues via RT-PCR

Total RNA was extracted from kidney tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The concentration of extracted RNA was determined. Subsequently, extracted RNA samples were reverse transcribed into complementary Deoxyribose Nucleic Acid (cDNA) according to the instructions of PrimeScript™ RT MasterMix kit (Invitrogen, Carlsbad, CA, USA). QRT-PCR reaction conditions were as follows: 94°C for 30 s, 55°C for 30 s and 72°C for 90 s, for a total of 40 cycles. The relative expression level of the target gene was expressed by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used are shown in Table I.

Detection of Protein Levels of PI3K and Akt in Kidney Tissues via Western Blotting

The total protein was extracted from kidney tissues with RIPA lysis buffer. The concentration of the protein sample was determined using the Bradford kit. 30 μg proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After sealing with 5% skim milk for 1 h, the membranes were incubated with primary antibodies of PI3K and Akt at 4°C overnight. On the next day, the membranes were washed with TBST for 3 times, followed by incubation with HRP-labeled secondary antibody at room temperature for 1 h. The color was developed using diaminobenzidine (DAB) developing solution. Finally, the gray value was analyzed using the ImageJ software (NIH, Bethesda, MD, USA).

Table I. Primer sequences.

Gene	Type	Sequence
PI3K	Forward	5' CAT CAC TTC CTC CTG CTC TAT 3'
	Reverse	5' CAG TTG TTG GCA ATC TTC TTC 3'
Akt	Forward	5' GGA CAA CCG CCA TCC AGA CT 3'
	Reverse	5' GCC AGG GAC ACC TCC ATC TC 3'
β-actin	Forward	5' GAC TTC AAC AGC AAC TCC CA 3'
	Reverse	5' TGG GTG GTC CAG GGT TTC TT 3'

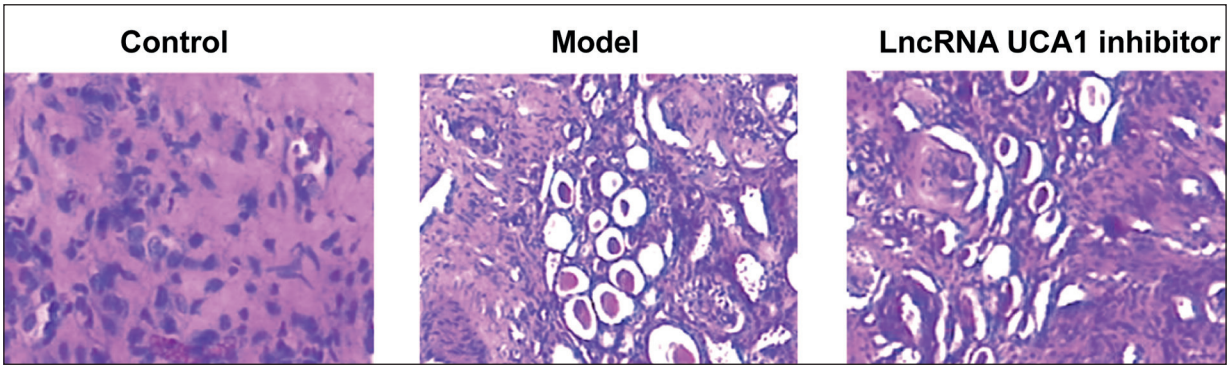

Figure 1. Pathological damage in rats (Magnification × 20).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Statistics for Windows, Chicago, IL, USA) was used for all statistical analysis. The experimental data were expressed as mean ± standard deviation. One-way ANOVA was used to compare the differences among different groups, followed by the post-hoc test (Least Significant Difference). The p values < 0.05 were considered statistically significant.

Results

LncRNA UCA1 Inhibitor Could Improve Pathological Damage in DN Rats

HE staining showed that the kidney tissues had a clear structure and a complete shape in the control group. Meanwhile, no glomerular and stromal hyperplasia, as well as no fibrous tissues, were observed in the control group. In the model group, the extracellular matrix of kidney tissues significantly increased, the hyperemia was observed, and the endothelial cell proliferation was evident. After the treatment with lncRNA UCA1 inhibitor, the pathological damage was significantly improved (Figure 1).

LncRNA UCA1 Inhibitor Could Reduce Serum Levels of BUN, Scr, and UP in DN Rats

Compared with the control group, the serum levels of BUN, Scr, and UP in the model group significantly increased (**p<0.01, **p<0.01, *p<0.05). Compared with the model group, the serum levels of BUN, Scr, and UP were significantly down-regulated after the treatment with lncRNA UCA1 inhibitor ($^{\#}p$<0.05, $^{\#}p$<0.05, $^{\#}p$<0.05) (Table II). The results indicated that the lncRNA UCA1 inhibitor could significantly improve the renal function of DN rats.

LncRNA UCA1 Inhibitor Could Reduce the Content of Serum TNF-α and IL-6 in DN Rats

Compared with the control group, the content of serum TNF-α and IL-6 in the model group remarkably increased (*p<0.05, *p<0.05). Meanwhile, the content of the serum TNF-α and IL-6 significantly declined in the lncRNA UCA1 inhibitor group, when compared with the model group ($^{\#}p$<0.05, $^{\#}p$<0.05) (Table III). These findings suggested that lncRNA UCA1 inhibitor could inhibit the inflammatory response in DN rats.

Table II. Levels of serum BUN, Scr and UP in rats.

Group	BUN (mmol/L)	Scr (mmol/L)	UP (mg)
Control group	6.23 ± 0.67	41.28 ± 4.29	10.98 ± 2.13
Model group	13.92 ± 1.95**	94.28 ± 8.09**	25.67 ± 3.46*
LncRNA UCA1 inhibitor	8.12 ± 2.18$^{\#}$	67.34 ± 4.37$^{\#}$	17.38 ± 3.34$^{\#}$

Note: **p<0.01 & *p<0.05 model group *vs*. control group, $^{\#}p$<0.05 lncRNA UCA1 inhibitor group *vs*. model group.

Table III. TNF-α and IL-6 levels.

Group	TNF-α (pg/mL)	IL-6 (pg/mL)
Control group	18.37 ± 3.17	36.15 ± 4.29
Model group	74.23 ± 6.22*	73.18 ± 6.26*
LncRNA UCA1 inhibitor	37.27 ± 4.35#	52.42 ± 4.72#

Note: *p<0.05 model group *vs*. control group, #p<0.05 lncRNA UCA1 inhibitor group *vs*. model group.

LncRNA UCA1 Inhibitor Could Inhibit the mRNA Expressions of PI3K and Akt

RT-PCR results (Figure 2A) showed that the mRNA levels of PI3K and Akt in kidney tissues remarkably increased in the model group when compared with the control group (*p<0.05, *p<0.05). However, they were significantly declined in the lncRNA UCA1 inhibitor group, when compared with the model group (#p<0.05, #p<0.05) (Figure 2B).

LncRNA UCA1 Inhibitor Could Inhibit the Protein Expressions of PI3K and Akt

Western blotting (Figure 3A) indicated that the protein levels of PI3K and Akt in kidney tissues of the model group were significantly higher than those of the control group (*p<0.05, **p<0.01). However, the protein expressions of PI3K and Akt were significantly declined in the lncRNA UCA1 inhibitor group when compared with the model group (#p<0.05, #p<0.05) (Figure 3B).

Discussion

DN is the main complication of diabetes mellitus ant it is also the end-stage manifestation of diabetes mellitus. The pathological manifestations of DN include extracellular matrix deposition, podocyte decrease, and continuous

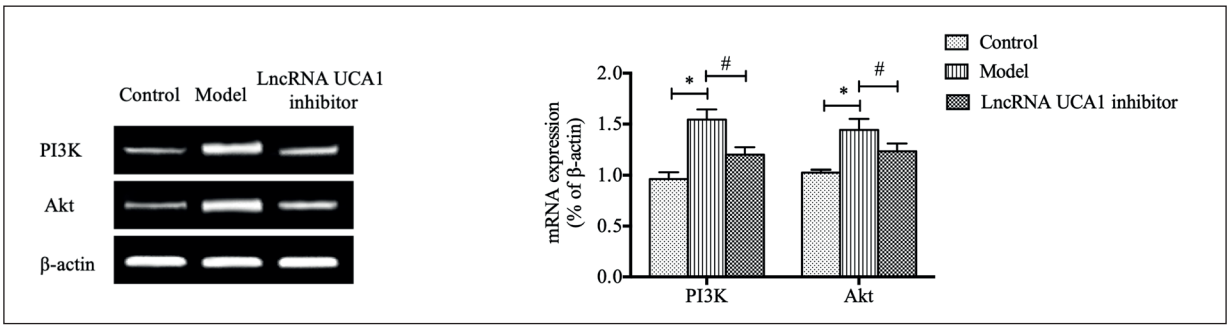

Figure 2. The mRNA levels of PI3K and Akt in kidney tissues detected via RT-PCR. **A,** RT-PCR band, β-actin as an internal reference. **B,** RT-PCR band statistical graph (*p<0.05, #p<0.05).

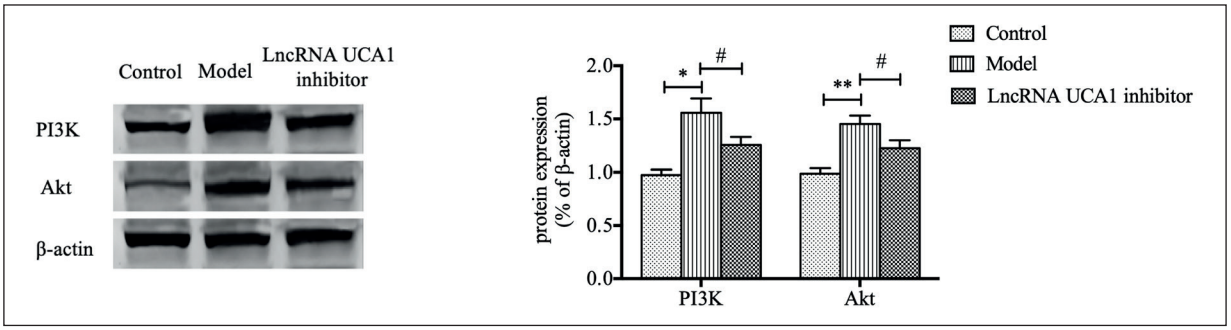

Figure 3. The protein levels of PI3K and Akt in kidney tissues detected via Western blotting. **A,** Western blotting band, with β-actin as an internal reference. **B,** Western blotting band statistical graph (*p<0.05, **p<0.01, #p<0.05).

thickening of the glomerular basement membrane. If the blood glucose cannot be controlled in time, it will accelerate the occurrence and development of DN[12,13]. With the continuous improvement of people's living standards and aging, diabetes mellitus has become an epidemic disease and a problem urgently to be solved worldwide[14]. Therefore, early clinical diagnosis and prevention are of great significance in the control of diabetes mellitus. Kikkawa et al[15] have confirmed that an abnormal inflammatory response plays a key role in the pathogenesis and development of DN.

In a review on the inflammatory response in DN, Turkmen[16] described that the inflammatory factors can affect the glomerular function by altering the renal vascular flow and vasoconstriction and by regulating extracellular matrix dynamics, endothelial, and vascular proliferation. Meanwhile, the inflammatory response affects the apoptosis and necrosis of smooth muscle cells. Previous studies have found that PI3K signaling pathway plays an important regulatory role in the inflammatory response of DN. Huang et al[17] have indicated that the notoginsenoside R1 (NR1) has a protective effect on podocytes in DN rats. The underlying mechanism may be related to the inhibition of the inflammatory response. Subsequent experimental results have revealed that NR1 inhibits the release of serum TNF-α, TGF-β1, IL-1, and IL-6 in rats. Its inhibiting mechanism of the inflammatory response may be associated with the inactivation of PI3K-Akt-NF-κB signaling pathway. Hong et al[18] have demonstrated that JTD has a protective effect on DN mice, whose mechanism is correlated with the PI3K/Akt/NF-κB signaling pathway. Liu et al[19] have found that miRNA can regulate the renal tubular epithelial cells in the treatment of DN. When PI3K signaling pathway inhibitor (ly294002) is applied, its signaling pathway is inhibited. Moreover, Li et al[20] have indicated that the PI3K signaling pathway plays an important role in the autophagy and DN, and its mechanism may be related to the inhibition of the podocyte adhesion injury. The above results suggest that the PI3K signaling pathway plays a key role in the pathogenesis and development of DN.

In the present study, the rat model of DN was successfully established via intraperitoneal injection of STZ. HE staining showed that the stromal thickening and hemorrhage in kidney tissues were observed in the model group. The pathological damage was significantly improved after the treatment with lncRNA UCA1 inhibitor. Subsequently, the content of BUN, Scr, and UP was detected. Urea is the main product of the protein metabolism in the human body, and it is excreted to the outside of the body with the urine. Meanwhile, the content of serum BUN significantly increases due to kidney failure and nephritis. As a metabolite in the muscle, the creatinine is excreted to the outside of the body through glomerular filtration. The level of creatinine dramatically increases in the case of pathological damage to the kidney. In kidney and renal vascular diseases, the content of UP is significantly elevated. The above three indexes can reflect the renal function. Our results showed that the levels of BUN, Scr, and UP remarkably declined after the treatment with lncRNA UCA1 inhibitor compared with those in the model group. The content of serum TNF-α and IL-6 in rats was detected as well. It was found that the levels of TNF-α and IL-6 were significantly down-regulated by lncRNA UCA1 inhibitor treatment. The above findings indicated that lncRNA UCA1 inhibitor could improve the renal functions and inhibit the inflammatory response in kidney tissues. To further explore the mechanism of lncRNA UCA1 in DN rats, the mRNA and protein levels of PI3K and Akt were detected via RT-qPCR and Western blotting, respectively. The results manifested that lncRNA UCA1 inhibitor could significantly reduce the mRNA and the protein levels PI3K and Akt when compared with those in the model group. These data suggested that lncRNA UCA1 inhibitor could improve the pathological damage of kidney tissues and the renal function in DN rats. Furthermore, it could also inhibit the inflammatory response, whose mechanism might be related to the inhibition of the PI3K-Akt signaling pathway.

Conclusions

LncRNA UCA1 can relieve the pathological damage to the kidney, improve renal function, and alleviate the inflammatory response in DN rats and its mechanism may be related to the inhibition of PI3K-Akt signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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