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# Regulation of Lnc-NTF3-5 on islet β-cell dysfunction in high glucose environment and related mechanisms

X.-L. ZHANG[1], Y. ZHANG[2], H.-Q. ZHU[3], C.-Y. ZHANG[4], J.-S. JIAO[5], X.-Y. XING[1]

[1]Department of Endocrinology, China-Japan Friendship Hospital, Beijing, China
[2]Department of Endocrinology, Affiliated Hospital of Hebei University, Baoding City, Hebei Province, China
[3]Department of Endocrinology, Emergency General Hospital, Beijing, China
[4]Department of Statistical Teaching and Research, China-Japan Friendship Hospital, Beijing, China
[5]Department of Neurology, China-Japan Friendship Hospital, Beijing, China

*Xuelian Zhang* and *Yao Zhang* contributed equally to this work

**Abstract. – OBJECTIVE: The pathogenesis of diabetes is closely related to islet β-cell dysfunction. Lnc-NTF3-5 participates in the occurrence and development of various diseases. However, Lnc-NTF3-54's effect on islet β-cell dysfunction in high glucose environment remains unclear.**

**MATERIALS AND METHODS: The islet β cell MIN6 cells were cultured in vitro and randomly divided into control group, high glucose group, NTF3-5 siRNA group, and NTF3-5 group, which was respectively transfected with Lnc-NTF3-5 siRNA and Lnc-NTF3-5 plasmid under high glucose condition. Lnc-NTF3-5 expression was measured by real time PCR and cell proliferation was assessed by MTT assay. In addition, Caspase 3 activity, SOD activity, and ROS content were also detected along with the secretion of IL-10 and IL-1 by enzyme-linked immunosorbent assay (ELISA).**

**RESULTS: Compared with control group, Lnc-NTF3-5 expression in MIN6 cells was significantly increased in high glucose environment ($p<0.05$). In high glucose environment, Lnc-NTF3-5 plasmid transfection up-regulated Lnc-NTF3-5 expression, inhibited cell proliferation, increased Caspase 3 activity, and decreased SOD activity. Meanwhile, Lnc-NTF3-5 plasmid also increased ROS content and IL-1 level, and decreased IL-10 level and insulin secretion. Compared with high glucose group, the differences were statistically significant ($p<0.05$). However, transfection of Lnc-NTF3-5 siRNA down-regulated Lnc-NTF3-5 expression under high glucose environment and reversed the above changes. Compared with high glucose group, the differences were statistically significant ($p<0.05$).**

**CONCLUSIONS: Lnc-NTF3-5 expression is increased in high glucose environment. Targeting Lnc-NTF3-5 can inhibit islet cell apoptosis, oxidative stress, and promote islet cell proliferation and insulin secretion.**

*Key Words:*
Diabetes, Lnc-NTF3-5, Islet β cells, Apoptosis, Oxidative stress.

## Introduction

Diabetes mellitus is a common metabolic disease in the endocrine system, with high incidence and several complications. It is one of the problems that plague the world's health[1]. The incidence of diabetes in China is increasing day by day, and the related diseases caused by diabetes exacerbate China's health economic problems[2]. Diabetes is caused by defects in insulin secretion, or islet secretion dysfunction, leading to increased blood glucose[3]. Although diabetes progresses slowly, it can cause a variety of complications due to prolonged hyperglycemia, which causes various tissues and organ damage, and even death[4,5]. Therefore, diabetes is a serious threat to human health. The pathogenesis of diabetes is complex, disorders of glucose and lipid metabolism, inflammation, oxidative stress, and apoptosis are important contributing factors to the occurrence and development of diabetes[6,7]. Diabetes is affected by a variety of factors, including genetic factors, autoimmune system defects, and viral infections. Islet beta cell dysfunction reduces insulin secretion, raises blood glucose levels, and ultimately leads to diabetes[8,9]. Recent studies have shown that diabetes is closely related to islet inflammation caused by immune disorders. Inflammatory factors that regulate inflammatory responses play a key role in the development and

*Corresponding Author:* Xiaoyan Xing, MD; e-mail: zhongmi920598nowqn@126.com

progression of diabetes by inducing islet beta cell apoptosis and insulin secretion defects[10,11]. Abnormal insulin secretion is a key factor in the development of diabetes[12]. Although the pathogenesis of diabetes has not been fully elucidated, the pathogenesis of diabetes is reported to be closely related to islet β-cell dysfunction.

Zhang et al[13] have reported that long-chain non-coding RNAs (LncRNAs) are transcripts with more than 200 nucleotides in length that can participate in the regulation of biological processes through transcriptional and post-transcriptional regulation, but do not have the function of encoding proteins. LncRNAs play key roles in the regulation of diseases and biological processes such as tumors, inflammation, including cell differentiation, proliferation, apoptosis, gene regulation, and tumor development[14]. Lnc-NTF3-5 can participate in the occurrence and development of various diseases such as tumors and orthopedic diseases[15,16]. However, the effect of Lnc-NTF3-54 on islet β-cell dysfunction in high glucose environment and related mechanisms have not been reported. Therefore, this study analyzed the expression of Lnc-NTF3-5 in islet β cells in high glucose environment and assessed the effect of Lnc-NTF3-5 on islet β cells.

## Materials and Methods

### Main Materials and Instruments

The MIN6 cell line was constructed by our laboratory and stored frozen in liquid nitrogen. The eukaryotic expression plasmid pCMV was purchased from Clontech (Mountain View, CA, USA). Fetal bovine serum (FBS) and cyan chain double antibody were purchased from Hyclone Corporation (San Angelo, TX, USA). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole (MTT) powder was purchased from Gibco (Grand Island, NY, USA); trypsin-ethylenediaminetetraacetic acid (EDTA) digest was purchased from Sigma-Aldrich (St. Louis, MO, USA). IL-10 and IL-1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D (Minneapolis, MN, USA). The Lnc-NTF3-5 plasmid and Lnc-NTF3-5 siRNA were designed and synthesized by Shanghai Gemma Gene Co., Ltd (Shanghai, China). The reactive oxygen species (ROS) content detection kit and the superoxide dismutase (SOD) activity detection kit were purchased from Wuhan Boster Co., Ltd (Wuhan, China). The Caspase 3 active kit was purchased from Wuhan Bude Bio Co., Ltd (Wuhan, China). The Labsystem Version 1.3.1 microplate reader was purchased from Bio-Rad Corporation (Hercules, CA, USA). The clean workbench was purchased from Suzhou Purification Equipment Factory in Jiangsu Province (Suzhou, China). The RNA extraction kit and the reverse transcription kit were purchased from ABI (Waltham, MA, USA). The Amp PCR System 2400 DNA Amplifier was purchased from PE Gene (Foster City, CA, USA). Other commonly used reagents were purchased from Shanghai Shenggong Biological Co., Ltd (Shanghai, China).

### MIN6 Islet Cell Grouping and Processing

Liquid nitrogen was used to store human MIN6 cell line, which was thawed in 37°C water bath until the cells were completely thawed, followed by being centrifuged at 1000 rpm for 3 min, resuspended in 1 ml fresh medium, transferred to a 50 ml cell culture flask containing 4 ml fresh medium and cultured at 37°C with 5% $CO_2$ for 24-48 h. MIN6 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 10% heat-inactivated fetal bovine serum (FBS) and 10 U/mL penicillin and 10 μg/mL streptomycin at 37 °C in a 5% CO2 incubator to maintain a cell number of $1 \times 10^6$/bottle. The subcultured MIN6 cells were diluted to $1 \times 10^6$ /ml, inoculated in a 35 mm culture dish, and cultured in serum-free RPMI-1640 medium containing 100 ng/ml phosphomolybdic acid (PMA) and 0.3% bovine serum albumin (BSA) for 24 h. The 3-8 generation logarithmic growth phase cells MIN6 were randomly divided into 3 groups, control group (normal culture cells); high glucose group in which 30 mmol/L glucose was used to prepare high glucose environment to stimulate cultured cells; NTF3-5 siRNA group and NTF3 -5 groups, in which Lnc-NTF3-5 siRNA and Lnc-NTF3-5 plasmid was respectively transfected into MIN6 cells in a high glucose environment.

### Lnc-NTF3-5 siRNA and Lnc-NTF3-5 Plasmid Transfection Into MIN6 Islet Cells

Lnc-NTF3-5 siRNA and Lnc-NTF3-5 plasmid were transfected into MIN6 cells in a high glucose environment. The Lnc-NTF3-5 siRNA sequence was 5'-GCTGGGGAGGAATCTTCA-3'; 5'-GCAGGTGACGGTGGTCA-3. The Lnc-NTF3-5 plasmid was designed and synthesized by Hajima Gene Co., Ltd. The cell density was fused to 70-80% in a 6-well plate; the Lnc-NTF3-5

siRNA and Lnc-NTF3-5 plasmid liposomes were separately added to 200 μl of serum-free medium, mixed thoroughly, and incubated at room temperature. The mixed lipo2000 was separately mixed with the corresponding dilution and incubated for 30 min at room temperature. The serum of the cells was removed, phosphate buffered saline (PBS) was gently rinsed, 1.6 ml of serum-free medium was added, and each system was added to each system, cultured in a 5% $CO_2$ incubator, and cultured at 37°C for 48 h for experimental research.

### Real Time-PCR Detection of Lnc-NTF3-5 Expression

RNA of each group of MIN6 islet cells was extracted on ice using TRIzol reagent, and DNA reverse transcription synthesis was performed according to the kit instructions. The primers were designed according to each gene sequence by Primer6.0 and synthesized by Shanghai Yingjun Biotechnology Co., Ltd. (Table I). Real-time PCR detection reaction conditions were as follows: 55°C 1 min, 92°C 30 s, 58-60°C 45 s, 72°C 35 s, a total of 35 cycles. Data was collected using the PCR reactor software and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference. According to the fluorescence quantification, the starting cycle number (CT) of all samples and standards was calculated. Based on the standard CT value, a standard curve was drawn and then the semi-quantitative analysis was carried out by the 2-ΔCt method.

### MTT Assay to Detect Cell Proliferation

The logarithmic growth phase MIN6 cell line was digested, counted, and transferred to the second-generation inoculation in a 96-well plate, and treated as above. The seeding density was $5\times10^3$/well, and the culture plate was transferred to a $CO_2$ incubator for cultivation. Five replicate wells were designed for each group. After adding relevant factors according to group design, 20 μl of 5 g/L MTT solution was added to each group of cells in each well, and continued to culture for 4 hours in the incubator. The supernatant was removed, 150 μl/well of DMSO was added, and shaken for 10 min. After the purple crystals were fully dissolved, the absorbance (A) value was measured at a wavelength of 492 nm by a microplate reader, and the cell proliferation rate was calculated. The experiment was repeated 3 times (n=3).

### Caspase 3 Activity Analysis

The changes in Caspase 3 activity in each group of cells were examined according to the kit instructions. Trypsin digested cells were centrifuged at 600 g at 4°C for 5 min, followed by lysing the lysate on ice for 15 min and being centrifuged at 20000 g at 4°C for 5 min. Then, 2 mM Ac-DEVD-pNA was added and the OD value at 405 nm was measured to calculate Caspase 3 activity changes.

### Analysis of IL-1 and IL-10 Level by ELISA

The supernatants of each group were collected to detect changes in the secretion of inflammatory factors IL-1 and IL-10. The experimental procedure was followed according to the ELISA kit instructions. The measurement should be carried out within 15 minutes after the addition of the stop solution. The linear regression equation of the standard curve was calculated according to the concentration of the standard product and the corresponding OD value, and the corresponding sample concentration was calculated on the regression equation according to the OD value of the sample.

### Oxidative Stress Index Detection

Superoxide dismutase (SOD) activity and changes in ROS content in each group of cells were examined according to the kit instructions. The cell protein was extracted and washed in a 95°C water bath. After 40 min, it was taken out and rinsed with cold water. After cooling, it was centrifuged at 4000 rpm for 10 min. The ethanol phase in the tissue homogenate was extracted using an ethanol-chloroform mixture (5:3, v/v volume ratio 5:3) for detection of total SOD activity.

**Table I.** Primer sequences..

| Gene | Forward 5′-3′ | Reverse 5′-3′ |
|---|---|---|
| GAPDH | AGTAGTCACCTGTTGCTGG | TAATACGGAGACCTGTCTGGT |
| Lnc-NTF3-5 | TCGCTCCCGGATACCATTCC | ACACGAGTTACGAGTTGATT |

Changes in the levels of reactive oxygen species in each group of cells were examined. The treated cells were bathed in a 95°C water bath, and after 40 min, they were taken out and rinsed with cold water, and after cooling, centrifuged at 4000 rpm for 10 min. The tissue homogenate was incubated with 2›, 7›-dichlorofluorescein diacetate (DCF-DA) for 15 min at 37°C, centrifuged at 10,000 rpm for 15 min, and the supernatant was discarded, and pellet was resuspended in sterile PBS phosphate buffer and incubated for 60 min at 37°C. After that, ROS levels were measured by spectrophotometer.

### Analysis of Insulin Secretion Level

The MIN6 cells in each logarithmic growth phase were counted, digested and counted, and the cell density was adjusted. The cells were seeded in a 48-well plate at 500 μL/well for 24 h to remove the cell culture medium. The supernatant was discarded, and cells were washed with PBS, followed by addition of 300 μL/well of HBSS buffer and incubation for 30 min at 37°C. After that, the supernatant was centrifuged at 800 rpm at 4°C for 5 min, and the supernatant was stored at –20°C. The protein content was measured by the bicinchoninic acid (BCA) assay. The unit mass insulin concentration = insulin content / corresponding protein content / well. The unit was $10^3$ IU·L-1.· g-1.Pro.

### Statistical Analysis

All data was processed by Statistical Package for the Social Sciences (SPSS) 22.0 software (IBM, Armonk, NY, USA) for statistical analysis. Measurement data were described by mean ± standard deviation (SD). One-way ANOVA with Bonferroni post-hoc test was used for comparison of multiple groups of samples. The *t*-test was used for comparison between the two groups. $p < 0.05$ was considered as statistically significant.

## Results

### Expression of Lnc-NTF3-5 in MIN6 Cells in High Glucose Environment

Compared with control group, the expression of Lnc-NTF3-5 in MIN6 cells was increased under high glucose environment, and the difference was statistically significant ($p < 0.05$). The expression of Lnc-NTF3-5 was up-regulated after transfection of Lnc-NTF3-5 plasmid in high glucose environment, and the difference was statistically significant ($p < 0.05$). Lnc-NTF3-5 siRNA transfection into MIN6 cells in high glucose environment down-regulated the expression of Lnc-NTF3-5. Compared with high glucose group, the difference was statistically significant ($p < 0.05$; Figure 1).

### Effects of Lnc-NTF3-5 on the Proliferation of MIN6 Cells

The proliferation of MIN6 cells was decreased in high glucose environment, and the difference was statistically significant ($p < 0.05$). Transfection of Lnc-NTF3-5 plasmid in high glucose environment further inhibited the proliferation of MIN6 cells. Compared with high glucose group, the difference was statistically significant ($p < 0.05$). Lnc-NTF3-5 siRNA transfection into MIN6 cells in high glucose environment promoted the proliferation of MIN6 cells. Compared with high glucose group, the difference was statistically significant ($p < 0.05$; Figure 2).

### Effects of Lnc-NTF3-5 on Caspase 3 Activity in MIN6 Cells

The activity of Caspase 3 in MIN6 cells was increased in high glucose environment, and the difference was statistically significant ($p<0.05$). The expression of Lnc-NTF3-5 was up-regulated by transfection of Lnc-NTF3-5 plasmid in high glucose environment, which could further in-

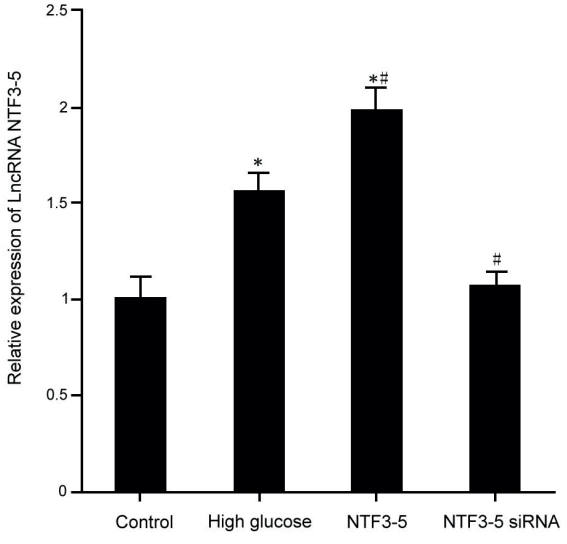


**Figure 1.** Expression of Lnc-NTF3-5 in MIN6 cells in high glucose environment. Compared with control group, * $p < 0.05$; compared with high glucose group, # $p < 0.05$.

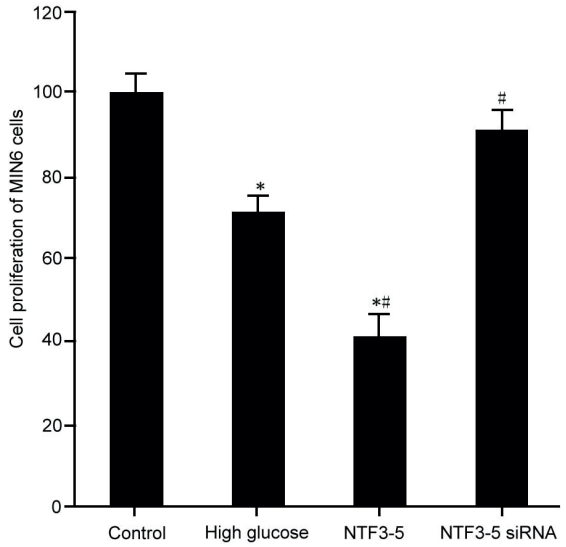


**Figure 2.** Effect of Lnc-NTF3-5 on proliferation of MIN6 cells in high glucose environment. Compared with control group, *$p < 0.05$; compared with high glucose group, #$p < 0.05$.

crease the activity of Caspase 3. Compared with high glucose group, the difference was statistically significant ($p < 0.05$). Lnc-NTF3-5 siRNA transfection into MIN6 cells in high glucose environment inhibited the activity of Caspase 3 in MIN6 cells and the difference was statistically significant ($p < 0.05$; Figure 3).

### *Effects of Lnc-NTF3-5 on Secretion of Inflammatory Factors*

The effect of Lnc-NTF3-5 on the secretion of inflammatory and anti-inflammatory factors in MIN6 cells in high glucose environment was evaluated by ELISA. In high glucose environment, the secretion of IL-10 in the supernatant of MIN6 cells was decreased, and the secretion of IL-1 was increased. Compared with control group, the difference was statistically significant ($p < 0.05$). Transfection of Lnc-NTF3-5 plasmid reduced the secretion of anti-inflammatory factor IL-10 and increased IL-1 secretion. Compared with high glucose group, the difference was statistically significant ($p < 0.05$). Lnc-NTF3-5 siRNA transfection into MIN6 cells increased IL-10 secretion and decreased IL-1 secretion. Compared with high glucose group, the difference was statistically significant ($p < 0.05$; Figure 4).

### *Effects of Lnc-NTF3-5 on Insulin Secretion in MIN6 Cells Under High Glucose*

In high glucose environment, the insulin secretion of pancreatic islet MIN6 cells was decreased, compared with control group, the difference was statistically significant ($p < 0.05$). Transfection of Lnc-NTF3-5 plasmid in high glucose environment inhibited the decrease of insulin secretion in MIN6 cells. Compared with high glucose group, the difference is statistically significant ($p < 0.05$).

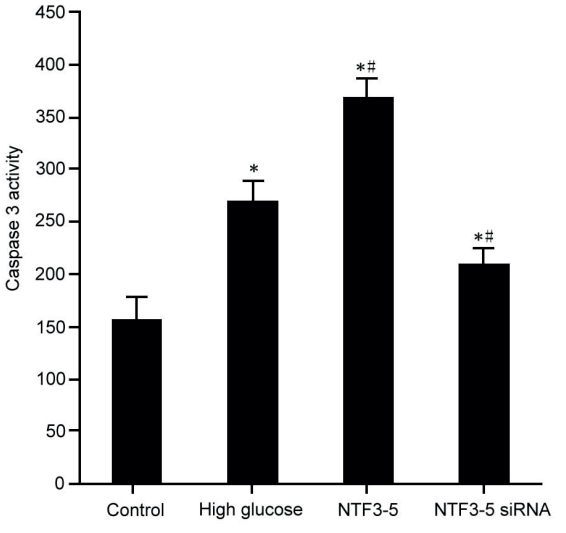


**Figure 3.** Effects of Lnc-NTF3-5 on Caspase 3 Activity in MIN6 Cells under High Glucose Environment. Compared with control group, * $p < 0.05$; compared with high glucose group, # $p < 0.05$.

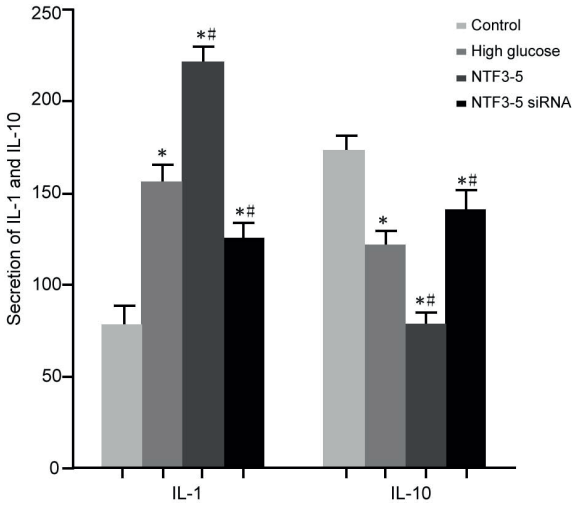


**Figure 4.** Effects of Lnc-NTF3-5 on secretion of inflammatory factors in supernatant of MIN6 cells in high glucose environment. Compared with control group, * $p < 0.05$; compared with high glucose group, # $p < 0.05$.

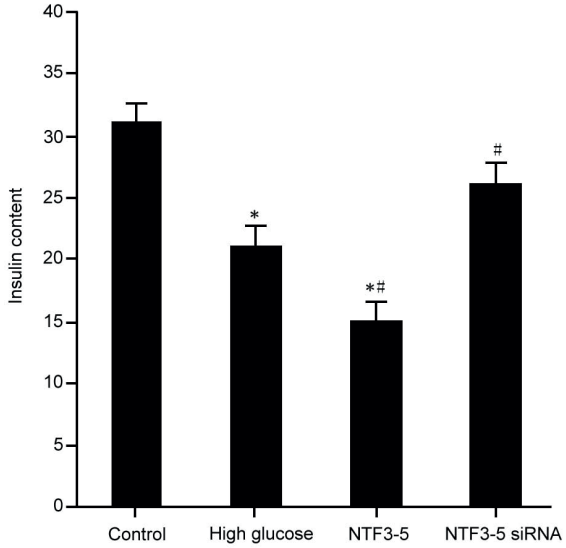


**Figure 5.** Effects of Lnc-NTF3-5 on insulin secretion in MIN6 cells under high glucose. Compared with control group, * $p < 0.05$; compared with high glucose group, # $p < 0.05$.

Transfection of Lnc-NTF3-5 siRNA in high glucose environment promoted insulin secretion of MIN6 cells, compared with high glucose group, the difference was statistically significant ($p < 0.05$; Figure 5).

### *Effects of Lnc-NTF3-5 on the Redox of MIN6 Cells in High Glucose Environment*

In high glucose environment, the ROS content of pancreatic islet MIN6 cells was increased and SOD activity was decreased, compared with control group, the difference was statistically significant ($p < 0.05$). Transfection of Lnc-NTF3-5 plasmid in high glucose environment increased ROS content and decreased SOD activity, compared with high glucose group, the difference was statistically significant ($p < 0.05$). Transfection of Lnc-NTF3-5 siRNA increased SOD activity and decreased ROS content in MIN6 cells (Figure 6).

## Discussion

The pathogenesis of diabetes has not yet been fully elucidated, and insulin resistance and the number and dysfunction of islet β-cells are the two main links in the development of diabetes[17]. As the research progresses, it is confirmed that islet cell damage plays a key role in the occurrence of diabetes[18]. Islet β cells are involved in insulin secretion, and its apoptosis is a key link leading to the onset of diabetes[19]. Islet cells release inflammatory and anti-inflammatory factors, including IL-1, IL-10, and imbalance between inflammatory factors and anti-inflammatory factors secretion may damage islet β-cells, leading to islet β-cell death[20]. Studies have shown that insulin promotes glucose uptake and glycogen synthesis, and the synthesis of glycogen further affects the absorption and metabolism of blood sugar and increases insulin sensitivity. In this report, the islet cell MIN6 cells were cultured in a high glucose environment, and it was found that the proliferation of islet cells MIN6 cells was inhibited and apoptotic activity was increased, with increased secretion of inflammatory factors and decreased secretion of anti-inflammatory factors.

Although the biological functions of most LncRNAs have not been fully elucidated, LncRNAs have been shown to regulate gene expression patterns by binding key transcription factors to promoters and through the transcription factor itself[21,22]. Lnc-NTF3-5 has been shown to be abnormally expressed in orthopedic diseases, and its role in DR has not been confirmed. This study indicated that the expression of Lnc-NTF3-5 in pancreatic islet β cells was increased in high glucose environment, and it was up-regulated in pancreatic islet β cells transfected with Lnc-NTF3-5 plasmid in high glucose environment, which inhibited pancreatic β-cell proliferation, increased Caspase 3 activity and the secretion of inflammatory factors, and further inhibited the secretion of anti-inflammatory factors. Transfection of Lnc-NTF3-5 siRNA down-regulated the expression of Lnc-NTF3-5 in pancreatic islet β cells under high glucose environment and reversed the above changes. Diabetes is closely related to oxidative stress, which leads to excessive production of free radicals such as reactive oxygen species, causing dynamic imbalance of oxidation and antioxidant systems, leading to inflammation and damage of pancreatic tissue. MPO is highly increased, and SOD activity which is one of important antioxidant enzymes for scavenging oxygen free radicals is decreased, thereby aggravating inflammation and causing apoptosis and damage of MIN6 cells[23]. Further analysis of its mechanism confirmed that high glucose can promote the increase of ROS production in islet β cells and decrease the activity of SOD. Regulation of Lnc-NTF3-5 expression in pancreatic islet β cells under high glucose environment can increase SOD activity, inhibit ROS content, and regulate oxidative

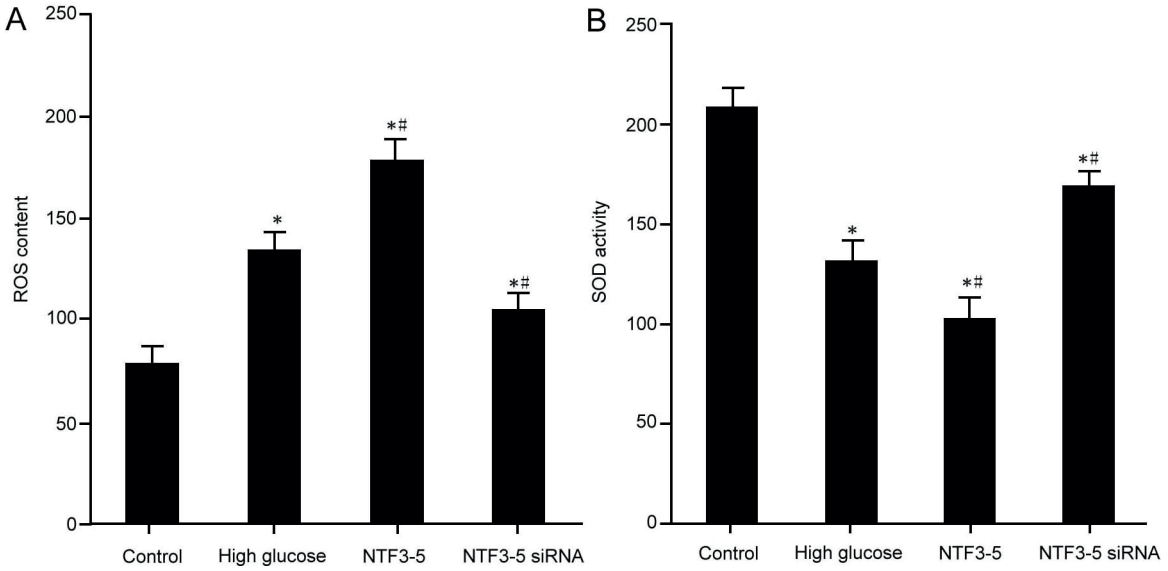


**Figure 6.** Effects of Lnc-NTF3-5 on the redox of MIN6 cells in high glucose environment. **A,** ROS content analysis; **B,** SOD activity analysis, compared with control group, * $p < 0.05$; compared with high glucose group, # $p < 0.05$.

stress. The results of this study confirmed that Lnc-NTF3-5 can affect oxidative stress and secretion of inflammatory factors, thereby affecting pancreatic islet β cell proliferation and insulin secretion in high glucose environment.

## Conclusions

The expression of Lnc-NTF3-5 is increased in high glucose environment. Lnc-NTF3-5 can be used as a target to inhibit islet cell apoptosis, oxidative stress, and inflammation, and promote islet cell proliferation and insulin secretion.

### Conflict of Interests

The Authors declare that they have no conflict of interests.

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