Original Article
LncRNA PTGS2 regulates islet β-cell function through the miR-146a-5p/RBP4 axis and its diagnostic value in type 2 diabetes mellitus

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Abstract: Objective: To investigate the effect of long non-coding RNA (LncRNA) PTGS2 on islet β-cell function via the miR-146a-5p/Retinol binding protein 4 (RBP4) axis and its diagnostic value in type 2 diabetes mellitus (T2DM). Methods: The Gene Expression Omnibus (GEO) was analyzed and LncRNA PTGS2 was identified as a potential regulator of T2DM. Mouse pancreatic β cell INS-1 cells were cultured with high glucose, and the relative expression of LncRNA PTGS2 in the serum of T2DM patients and INS-1 cells was detected by Fluorescence Quantitative PCR (qRT-PCR) and its diagnostic value for T2DM was analyzed. The PTGS2/miR-146a-5p/RBP4 axis in INS-1 cells was intervened to observe the changes in cell function. The proliferation of INS-1 cells was detected by CCK8, and the level of insulin secretion was detected by enzyme linked immunosorbent assay (ELISA). The regulatory relationship among LncRNA PTGS2, miR-146a-5p and RBP4 was determined by dual-luciferase reporter assay. Results: The expression of LncRNA PTGS2 in the serum of T2DM patients increased, and the expression of LncRNA PTGS2 was positively correlated with the fasting blood glucose level of patients (R=0.306, P<0.05). Knockdown of LncRNA PTGS2 could promote the proliferation and insulin secretion of INS-1 cells, while overexpression of LncRNA PTGS2 showed the opposite results (all P<0.05). Knockdown of LncRNA PTGS2 could up-regulate the expression of miR-146a-5p. Overexpression of LncRNA PTGS2 inhibited the proliferation and insulin secretion of INS-1 cells, while miR-146a-5p could partially reverse this effect. RBP4 has been identified as a downstream target gene of miR-146a-5p. Overexpression of miR-146a-5p could inhibit the expression of RBP4, which was positively correlated with LncRNA PTGS2 regulation. The effect of RBP4 on INS-1 cells was the same as that of LncRNA PTGS2. Conclusion: LncRNA PTGS2 can damage islet β-cell function by regulation of miR-146a-5p and up-regulation of RBP4. LncRNA PTGS2 has potential value in the diagnosis of T2DM.

Keywords: Type 2 diabetes mellitus, long non-coding RNA PTGS2, miR-146a-5p, retinol binding protein 4, pancreatic β cells, proliferation

Introduction
Diabetes is a chronic disease seen worldwide. Type 2 Diabetes Mellitus (T2DM) accounts for more than 80% of all diabetes [1]. Different from type 1 diabetes mellitus, T2DM is caused by an elevated blood glucose level due to acquired insulin secretion deficiency or insulin resistance [2]. Islet β-cell dysfunction is the main pathological feature of T2DM. High glucose can lead to islet β-cell failure and glucotoxicity, and glucotoxicity can in turn cause islet β-cell dysfunction, which is frequently seen in T2DM [3, 4]. At present, there is no cure for T2DM, and the disease can only be controlled by medicine [5].

Long non-coding RNA (LncRNA) is a promising marker for disease diagnosis. At present, many studies have found that LncRNA plays an important role in the regulation of various diseases including T2DM [6, 7]. Yang et al. found that LncRNA UCA1 can induce hyperglycemic vascular smooth muscle cell repair by targeting miR-582-5p and alleviate diabetic angiopathy [8]. Shao et al. believed that LncRNA NEAT1 may
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promote the occurrence and development of diabetic retinopathy by activating transforming growth factor-β-1 and vascular endothelial growth factor [9]. LncRNA PTGS2 is also called Lnc-COX2. Previous studies have found that the expression of Lnc-COX2 is significantly up-regulated in rheumatoid arthritis, an autoimmune disease [10]. Diabetes, as a metabolic disease, is also associated with autoimmunity [11].

MiR-146a-5p has been confirmed to be negatively regulated by LncRNA PTGS2. The role of miR-146 in diabetes and diabetic complications has been widely discussed. For example, it has been shown that miR-146 was decreased in peripheral blood monocytes in patients with type 1 diabetes [12]. Some studies have confirmed that serum RBP4 and leptin levels in patients with diabetes with or without macrovascular complications are elevated, and RBP4 is a risk factor for T2DM macrovascular complications [13].

This study explored the specific mechanism of LncRNA PTGS2 on pancreatic β cells in T2DM and verified the regulation of miR-146a-5p/RBP4 axis by LncRNA PTGS2, RBP4 might be the downstream target gene of miR-146a-5p, providing a new research direction for the prevention and treatment of T2DM.

Methods

Bioinformatics analysis

A microarray called GSE95849 was analyzed in the gene expression database (Gene Expression Omnibus, GEO), which integrates the differentially expressed LncRNAs in type 2 diabetes.

The localization and expression of LncRNAs were predicted by LncLocator (http://www.csbio.sjtu.edu.cn/bioinf/lncLocator/) database. RNA22 (http://cm.jefferson.edu/rna22/) and Starbase (http://starbase.sysu.edu.cn/index.php) online prediction websites analyzed the downstream targets of LncRNA and miRNA. DAVID (http://david.ncifcrf.gov) functional analysis website was used to perform GO analysis. The DisGENET (http://disgenet.org/search) website was used to screen T2DM risk genes. The protein interaction network diagram was drawn by STRING (http://string-db.org/cgi/input?sessionId=bQ1pVjvG0s39&input_page_show_search=on) website.

Clinical case sample collection

A total of 45 T2DM patients treated in our hospital from March 2017 to March 2018 were enrolled. There were 22 males with an average age of (51.2±6.4) years old and 23 females with an average age of (48.6±3.8) years old. According to the World Health Organization in 1999, inclusion criteria of patients with T2DM were: (1) Fasting blood glucose is equal or higher than 7.0 mmol/L. (2) 2 hours postprandial blood glucose is >11.1 mmol/L. (3) Age >32 years old. (4) BMI<40 kg/m². (5) No abnormal glucose regulation or genetic history of diabetes [14]. Exclusion criteria: (1) Patients complicated with hypertension, lung diseases, malignant tumors, autoimmune diseases, metabolic disorders, etc. (2) Patients suffering from chronic inflammation and infectious diseases. (3) Those who participate in other research projects. At the same time, the serum of 32 healthy people who came to our hospital for physical examination was collected as a control (fasting blood glucose level is lower than 6.1 mmol/L, 2 h postprandial blood glucose level is lower than 7.8 mmol/L). All the volunteers enrolled in the study had not received systemic treatment and have signed written informed consent. This study was approved by the Ethics Committee of our hospital (Approval No.20180812).

Cell culture

Mouse pancreatic β cell INS-1 was purchased from Mingzhou Biotechnology Co., Ltd. (Ningbo, China). Normal INS-1 cells were cultured using the mouse pancreatic β cell complete culture medium provided by Mingzhou Biotechnology Co., Ltd. (Ningbo, China). The medium contains 15% fetal bovine serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin. INS-1 cell injury model was established and INS-1 cells were cultured with 25 mM glucose Dulbecco’s modified medium DMEM (Wuhan Punosai Life Science and Technology Co., Ltd., China). The treated cells were called HG-INS-1. The medium was supplemented with 15% fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 50 mmol/L β-mercaptoethanol. All cells were incubated in a 37°C, 5% CO₂ incubator.
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The above detection steps were performed following the instructions of the kit manufacturer. The primers were provided by GenScript Biotechnology Co., Ltd. (Nanjing, China). The detailed sequences are shown in Table 2.

Subcellular isolation

The nucleus and cytoplasm of INS-1 cells were separated using a nuclear/cytoplasmic separation kit (Biyuntian Biotechnology Co., Ltd., Shanghai, China) according to the instructions of the kit. Total RNA was extracted from the nucleus and cytoplasm respectively for qRT-PCR reaction. qRT-PCR steps are shown in the “qRT-PCR” part.

Dual-Luciferase reporter assay

The 3’UTR fragments of LncRNA PTGS2 and RBP4 were amplified. Using endonuclease sites SpeI and Hind III, LncRNA PTGS2 and RBP4 fragments were cloned into pmirGLO reporter vector (Haijihaoge Biotechnology Co., Ltd., Shanghai, China), named PTGS2-WT and RBP4-WT. The mutation sites were designed on the 3’UTR fragments of LncRNA PTGS2 and RBP4. After restriction endonuclease digestion, the mutations were inserted into the pmirGLO vector by t4DNA ligase, which was named PTGS2-MUT and RBP4-MUT. The mutant and wild type sequences were co-transfected with miR-146a-5pNC and miR-146a-5pmimic into 293T cells, respectively, and the transfection steps were completed according to the instructions of the Lipofectamine 3000 kit (ThermoFisher, USA). After 48 hours, the transfected cells were collected, lysed and centrifuged for 5 minutes, and the supernatant was collected. Dual-luciferase gene reporter kit (Yisheng Biotechnology Co., Ltd., Shanghai, China) was used to detect the relative luciferase activity of cells in each group, with Renilla luciferase activity as a reference, and the detection steps were carried out under the requirements of the kit instructions.

Western blot

Protein was extracted from cells using RIPA cell lysisate (SevenBiotech, Beijing, China), and protein concentration was detected by a BCA protein determination kit (Solebo, Beijing, China). After adding 12% sodium dodecyl sulfate-polyacrylamide gel for 1-hour electrophoresis, the

<table>
<thead>
<tr>
<th>Table 1. Plasmid sequence</th>
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<tbody>
<tr>
<td>si-PTGS2: GATATCGCGAGGCGCGGCTATTATAGCGCCC</td>
</tr>
<tr>
<td>OE-PTGS2: GAATATATGCGCGCCGCTTTGCGATCC</td>
</tr>
<tr>
<td>OE-RBP4: AAATCCGGCGCAGCGAGCTTCTAGCGACG</td>
</tr>
<tr>
<td>miR-146a-5p mimic: GGGGTTTATATGCGCGCTCTAGC</td>
</tr>
</tbody>
</table>

Note: RBP4: Retinol binding protein 4.

<table>
<thead>
<tr>
<th>Table 2. qRT-PCR primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene name</td>
</tr>
<tr>
<td>LncRNA PTGS2</td>
</tr>
<tr>
<td>miR-146a-5p</td>
</tr>
<tr>
<td>RBP4</td>
</tr>
<tr>
<td>GAPDH</td>
</tr>
<tr>
<td>U6</td>
</tr>
</tbody>
</table>

Note: LncRNA: long non-coding RNA; RBP4: Retinol binding protein 4.
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isolated protein was transferred to PVDF membrane (Solebo, Beijing, China), and the membrane was sealed by 5% skim milk. The membrane was incubated overnight at 4°C with an anti-RBP4 (1chro1000) and an anti-GAPDH (1Drex1000). Horseradish peroxidase-conjugated secondary antibody goat anti-rabbit IgG (1:1000, Abcam, UK) was added to the membrane and incubated for 2 hours. TBST was used to wash the membrane. ECL chemiluminescence reagent (Suolaibao, Beijing, China) was used to visualize the protein and the gel imaging system was used for capture. The protein expression was analyzed by Image J software. The ratio of the gray value of the target protein band to GAPDH protein band was used for comparison.

CCK8

The transfected INS-1 cells were inoculated into a 96-well culture plate and a CCK8 cell proliferation detection kit (SevenBiotech, Beijing, China) was used to carry out the testing. The experimental procedures were carried out following the kit factory instructions. Finally, the OD value was measured at the wavelength of 450 nm with the use of a multifunctional microplate reader (Thermo Fisher, USA).

Enzyme linked immunosorbent assay (ELISA)

The transfected INS-1 cells were inoculated in a 96-well culture plate, and the insulin level secreted by INS-1 cells was detected by a mouse insulin ELISA kit (KA3812, Emijie Technology, Wuhan, China). The supernatant of the cell culture medium was centrifuged to remove the precipitate, and the standard well, test sample well and control well were set on the pre-coated plate. Then 50 μL of the standard solution was put into the standard Wells and 50 μL of appropriately diluted cell culture supernatant was added into the sample wells, respectively. In the above wells, 50 μL HRP-labeled anti-M0 insulin antibody was added and incubated at 37°C for 60 minutes. Washing liquid was added to the well and vortex for 2 min. Then 50 μL TMB substrate A and 50 μL TMB substrate B was add to each well in turn, swirl gently and shake for 30 seconds. The plate was incubated in darkness at 37°C for 15 min. At last, 50 μL stop solution was put into each well and mixed. Within 30 minutes after adding the stop solution, OD value at 450 nm was assessed with a microplate reader.

Statistical analysis

The data of this study were statistically analyzed by SPSS23.0 software. The statistical graph was generated using GraphPad Prism 9.0. The data results were expressed as mean ± standard deviation. The Shapiro-Wilk test found that the data of this study were conforming to normal distribution. Independent sample Student’s test was used for data analysis between the two groups, and one-way ANOVA followed with the Tukey method was used comparison among multiple groups. Pearson correlation coefficient test was used to analyze the correlation between genes. The difference was statistically significant when P<0.05.

Results

The expression of LncRNA PTGS2 was elevated in the serum of T2DM patients and INS-1 cells induced by high glucose

The top five differentially expressed LncRNA (CYP1B1, CX3CR1, MYOM2, PTGS2 and EGFR) in the GSE95849 chip were visualized (Figure 1A). The serum of T2DM patients was collected and analyzed by qRT-PCR. The expression of LncRNA PTGS2 in serum of T2DM patients was higher than that of healthy controls (Figure 1B; P<0.0001), and the expression of LncRNA PTGS2 in INS-1 cells cultured with high glucose was also higher than that of normal INS-1 cells (Figure 1C; P<0.05). ROC curve showed that LncRNA PTGS2 was highly sensitive to the diagnosis of T2DM (Figure 1D; P<0.0001). We divided the expression of LncRNA PTGS2 into high and low groups, with 27 people in the high expression group and 18 people in the low expression group. Chi-square test showed that there was a correlation between LncRNA PTGS2 expression and fasting blood glucose (Table 3; R=0.306, P<0.05). The above experimental data suggest that LncRNA PTGS2 may be a potentially effective index for the diagnosis of T2DM.

Knockdown of LncRNA PTGS2 promoted the proliferation and insulin secretion of HG-INS-1 cells

The LncRNA PTGS2 knockdown plasmid was established and transfected into HG-INS-1 cells.
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Compared with the si-NC group, si-PTGS2 significantly inhibited the expression of LncRNA PTGS2 (Figure 2A; \(P<0.05\)). To detect the effect of si-PTGS2 on the proliferation of HG-INS-1 cells, CCK8 experimental data showed that compared with the INS-1 group, the cell proliferation of the HG-INS-1 group was reduced, and compared with the si-NC group, si-PTGS2 significantly increased the proliferation of INS-1 cells induced by high glucose (Figure 2B, all \(P<0.05\)). Compared with the INS-1 group, the decrease of insulin secretion of INS-1 cells induced by high glucose was detected by an ELISA kit. Si-PTGS2 promoted HG-INS-1 cells to secrete insulin (Figure 2C; all \(P<0.05\)). The above experimental data suggest that high glucose can induce functional damage of INS-1 cells, while knockdown of LncRNA PTGS2 can promote INS-1 cell proliferation and insulin secretion, and improve the functional damage of INS-1 cells induced by high glucose.

miR-146a-5p could be used as a target for LncRNA PTGS2

LncLocator database showed the highest expression of LncRNA PTGS2 in the cytoplasm (Figure 3A). The nuclear and cytoplasmic separation of INS-1 cells was performed, and the high expression of LncRNA PTGS2 in the cytoplasm was confirmed by qRT-PCR assay (Figure 3B; \(P<0.05\)). It was suggested that LncRNA PTGS2 could play a role as a competitive endogenous RNA. With the help of an RNA database, it was found that there were specific binding sites between miR-146a-5p and LncRNA PTGS2, and the dual-luciferase report assay showed that there was a targeted relationship between LncRNA PTGS2 and miR-146a-5p (Figure 3C; \(P<0.05\)). Knockdown of LncRNA PTGS2 could promote the expression of miR-146a-5p (Figure 3D; \(P<0.05\)). Compared with healthy human serum, the expression of miR-146a-5p in serum of T2DM patients was

Figure 1. LncRNA PTGS2 may be an effective indicator of T2DM diagnosis. A: Heatmap; B: LncRNA PTGS2 is highly expressed in serum of T2DM patients; C: LncRNA PTGS2 is highly expressed in INS-1 cells induced by high glucose; D: LncRNA PTGS2 is highly sensitive to the diagnosis of T2DM. Compared with the normal group, \(* P<0.05\); compared with the INS-1 group, \#P<0.05. LncRNA: long non-coding RNA; T2DM: Type 2 Diabetes Mellitus.
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Table 3. Correlation analysis of the expression of LncRNA PTGS2 with the clinicopathological features of T2DM

<table>
<thead>
<tr>
<th>Variables</th>
<th>LncRNA PTGS2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (n=27)</td>
<td>Low (n=18)</td>
</tr>
<tr>
<td>Gender (n)</td>
<td></td>
<td>0.763</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Age (n)</td>
<td></td>
<td>0.241</td>
</tr>
<tr>
<td>≤50</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>&gt;50</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Weight loss (n)</td>
<td></td>
<td>0.031</td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Fasting blood glucose (n)</td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>High</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>High blood pressure (n)</td>
<td></td>
<td>0.365</td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

Note: LncRNA: long non-coding RNA; T2DM: Type 2 Diabetes Mellitus.
The grouping of fasting blood glucose was sorted according to the fasting blood glucose of each patient from high to low, and divided by the median. The median and above were divided into the hyperglycemia group, and the following was divided into the hypoglycemia group.

The expression of miR-146a-5p in HG-INS-1 cells was also down-regulated compared with normal INS-1 cells (Figure 3F; P<0.05). Pearson correlation analysis showed that there was a negative correlation between miR-146a-5p expression and LncRNA PTGS2 expression in serum of patients with T2DM (Figure 3G; P<0.05). The above experimental data showed that miR-146a-5p, as a target of LncRNA PTGS2, was negatively regulated by LncRNA PTGS2.

miR-146a-5p could partially reverse the damaging effect of LncRNA PTGS2 on INS-1 cells

LncRNA PTGS2 overexpression plasmid and miR-146a-5p mimics were transfected into HG-INS-1 cells, and their effects on INS-1 cells were observed. Compared with the NC group, OE-PTGS2 significantly increased the expression of LncRNA PTGS2 (Figure 4A; P<0.05). Compared with miR-NC group, miR-146a-5p mimic upregulated the expression of miR-146a-5p (Figure 4B; P<0.05). CCK8 experimental data showed that compared with the NC group, the OE-PTGS2 group could significantly inhibit the proliferation of INS-1 cells, while compared with the OE-PTGS2+miR-NC group, the proliferation of cells in the OE-PTGS2+miR-146a-5p mimic group was promoted (Figure 4C; all P<0.05). Insulin secretion levels of cells in each group were detected. Compared with the NC group, the insulin secretion level of the OE-PTGS2 group decreased, while compared with the OE-PTGS2+miR-N group, the insulin secretion level of the OE-PTGS2+miR-146a-5p mimic group increased (Figure 4D; all P<0.05). The above experiments suggest that overexpression of LncRNA PTGS2 can significantly inhibit the proliferation and insulin secretion of HG-INS-1 cells, while overexpression of miR-146a-5p can partially reverse this effect.

RBP4 was proved to be the downstream target of miR-146a-5p

Through the Starbase online prediction website, we found the downstream targets of miR-146a-5p and performed GO analysis on potential downstream targets. Among them, the insulin secretion regulation pathway has attracted our attention, which is enriched in PFKFB2, GLUD1, RBP4, TARDBP genes (Figure 5A). The protein interaction analysis between these four genes and the risk genes related to the onset of T2DM revealed that there was a strong relationship between RBP4 and these proteins (Figure 5B). Some studies have found that RBP4 is upregulated in T2DM nephropathy [15]. Analysis of relative luciferase activity was done using base complementary sequences of RBP4 and miR-146a-5p. The experiment shows that compared with the RBP4-MUT+miR-146a-5p NC group, the relative luciferase activity of the RBP4-WT+miR-146a-5p mimic group was significantly inhibited (Figure 5C; P<0.05). MiR-146a-5p mimic could significantly inhibit the expression of RBP4 (Figure 5D; all P<0.05). The targeting relationship between RBP4 and miR-146a-5p was confirmed. The expression of RBP4 was upregulated in serum of T2DM patients and HG-INS-1 cells (Figure 5E and 5F; all P<0.05). The expression of RBP4 in serum of T2DM patients was negatively correlated with that of miR-146a-5p but positively correlated with that of LncRNA PTGS2 (Figure 5G; all
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Figure 2. Knockdown of LncRNA PTGS2 improved the functional damage of INS-1 cells induced by high glucose. A: Transfection efficiency of LncRNA PTGS2 knockdown cells; B: Comparison of INS-1 cell proliferation in each group; C: Comparison of insulin secretion level of INS-1 cells in each group. Compared with the INS-1 group, #P<0.05; compared with the si-NC group, &P<0.05. LncRNA: long non-coding RNA.

Figure 3. Verification of targeting relationship between LncRNA PTGS2 and miR-146a-5p. A: Results predicted by LncLocator database; B: Results of nuclear-cytoplasmic separation experiment; C: Results of dual luciferase reporter analysis; D: The effect of LncRNA PTGS2 knockdown on miR-146a-5p expression; E: Low expression of miR-146a-5p in serum of T2DM patients; F: Low expression of miR-146a-5p in INS-1 cells induced by high glucose; G: Results of Pearson correlation analysis. Compared with U6, ΔP<0.05; compared with the PTGS2-WT+miR-146a-5pNC group, δP<0.05; compared with the si-NC group, &P<0.05; compared with the normal group, *P<0.05; compared with the INS-1 group, #P<0.05. T2DM: Type 2 Diabetes Mellitus.
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Figure 4. miR-146a-5p could partially reverse the effect of LncRNA PTGS2 on INS-1 cells. A: Detection of OE-PTGS2 transfection efficiency; B: Detection of miR-146a-5p mimic transfection efficiency; C: Comparison of proliferation of INS-1 cells in each group; D: Comparison of insulin secretion of INS-1 cells in each group. Compared with the NC group, ^P<0.05; compared with the miR-NC group, ▲P<0.05; compared with the INS-1 group, #P<0.05; compared with OE-PTGS2+miR-NC group, ☆P<0.05.
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Figure 5. Verification of targeting relationship between 5RBP4 and miR-146a-5p. A: GO analysis; B: Protein interaction network map; C: Results of dual luciferase reporter analysis; D: Effect of miR-146a-5p on the expression of RBP4; E: High expression of RBP4 in serum of patients with T2DM; F: High expression of RBP4 in INS-1 cells induced by high glucose; G: Results of Pearson correlation analysis. Compared with the RBP4-WT+miR-146a-5pNC group, ★P<0.05; compared with the miR-NC group, ▲P<0.05; compared with the normal group, *P<0.05; compared with the INS-1 group, #P<0.05. T2DM: Type 2 Diabetes Mellitus.
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miR-146a-5p could partially reverse the damaging effect of RBP4 on INS-1 cells

The effect of overexpression of RBP4 on HG-INS-1 cells was observed. Compared with the NC group, OE-RBP4 could up-regulate the expression of RBP4 (Figure 6A; all P<0.05). CCK8 test showed that the cell proliferation ability of the OE-RBP4 group was weaker than that in the NC group, while that in OE-RBP4+miR-146a-5p mimic group was higher than that in the OE-RBP4+miR-NC group (Figure 6B; all P<0.05). Compared with the NC group, the insulin secretion level of the OE-RBP4 group was reduced, while compared with the OE-RBP4+miR-NC group, the insulin secretion level in the OE-RBP4+miR-146a-5p mimic group was increased (Figure 6C; all P<0.05). The above experiments showed that overexpression of RBP4 could inhibit the proliferation and insulin secretion of INS-1 cells, while overexpression of miR-146a-5p could partially rescue this effect. RBP4 had the same effect as LncRNA PTGS2.

Discussion

In this study, we first searched for T2DM-related chips in the GEO database and visualized the chip data, and found that LncRNA PTGS2 was overexpressed in T2DM patients, while the specific role of LncRNA PTGS2 in T2DM has not been revealed. According to previous studies, LncRNA PTGS2, also known as LncRNA COX2, was an important immunomodulatory factor, which could regulate the NF-κB pathway and promote the degradation of IκBα [16]. IκBα is a key regulatory factor in the progression of diabetes mellitus, and inhibiting the degradation of IκBα is beneficial to improve diabetes damage [17, 18]. Further detection of the expression of LncRNA PTGS2 showed that compared with healthy controls and normal INS-1 cells, the expression of LncRNA PTGS2 in T2DM and high glucose-induced INS-1 cells increased. LncRNA PTGS2 may be a potential biomolecule for predicting T2DM. The proliferation and insulin secretion of INS-1 cells induced
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by high glucose was inhibited. After knocking down LncRNA PTGS2, proliferation and insulin secretion were promoted, while the overexpression of LncRNA PTGS2 showed the opposite result.

LncRNA is generally acknowledged as a competitive endogenous RNA, and its activity and function are regulated by competing with miRNA [19]. This study confirmed that LncRNA PTGS2 can bind to miR-146a-5p and positively regulate RBP4 through bioinformatics and dual-luciferase experimental analysis, which may be an important mechanism affecting the function of INS-1 cells.

It has previously been shown that overexpression of miR-146a-5p can reduce premature ovarian failure in mice, and miR-146a can mediate the therapeutic effect of exosomes on the immune response of rheumatoid arthritis [20, 21]. In addition, miR-146 is also an important molecule for insulin regulation [22]. Overexpression of miR-146 can inhibit adenosine deaminase-2 and alleviate diabetic retinal inflammation [23]. In this study, it was proved once again that the expression of miR-146a-5p was low in T2DM samples, and miR-146a-5p could partially inhibit the damaging effect of LncRNA PTGS2 on INS-1 cells.

miRNA can bind to the downstream target mRNA to inhibit the corresponding protein translation and play a regulatory role in the disease [24]. The downstream target genes of miR-146 were explored. This study found that RBP4, as a downstream target gene of miR-146a-5p, had increased expression in T2DM samples, and had a negative correlation with the expression of miR-146a-5p, and a positive correlation with the expression of LncRNA PTGS2. It has been reported that the expression level of RBP4 is related to the function of islet β-cells in diabetic patients [25]. Ram J et al. believe that serum RBP4 levels are independently related to diabetes, and RBP4 may be used as another predictor of diabetes in the future [26]. We have shown that RBP4 and LncRNA PTGS2 have the same effect on INS-1 cells, and both lead to the INS-1 cell dysfunction. MiR-146a-5p could partially inhibit the damaging effect of RBP4 on INS-1 cells.

In our study, miR-146a-5p can regulate multiple target genes, but whether these target genes can be regulated by LncRNA PTGS2/miR-146a-5p axis, like RBP4, and play a role in the function of INS-1 cells need to be further studied. There are still some limitations in this study. Too few clinical samples may cause large bias in the results. The functions of the above factors have not been analyzed in vivo, which needs further investigation.

In summary, this study found that the expression of LncRNA PTGS2 in INS-1 cells increased after high glucose treatment. Knockdown of LncRNA PTGS2 can improve INS-1 cell dysfunction induced by high glucose. LncRNA PTGS2 regulates INS-1 cell function through the miR-146a-5p/RBP4 axis, which provides a new direction for the treatment of T2DM.

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Disclosure of conflict of interest

None.

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