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Research Article

Caffeic Acid Dimethyl Ether Ameliorates Excessive Glucose and Lipid-Induced Insulin Secretion Dysfunction in Pancreatic Beta-Cells through the miR-378b–PI3K–AKT Pathway

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An important factor in the progression of type 2 diabetes mellitus is the malfunctioning insulin production by β-cells in the pancreas. Caffeic acid dimethyl ether (CADE) reduces resistance to insulin in alcoholic fatty liver disease, but both the therapeutic effects of CADE on excessive glucose and lipid-induced insulin secretion disorders and the underlying mechanisms are unknown. The aim of this research was to (i) explore how CADE impacts insulin production issues caused by a surplus of glucose and lipids β-cells and (ii) elucidate the underlying mechanism. The results of our research demonstrated that insulin production was reduced in the pancreas of mice given a high-fat -diet and streptozotocin, as well as in human 1.1B4 pancreatic β-cells treated with high -glucose and high -fat, with increased activity of miR-378b and decreased expression levels of p110α, p-AKT1/2, insulin receptor, p-FoxO1, and PDX-1. However, treatment with CADE ameliorated the insulin secretion impairment by decreasing the miR-378b level and reversing the inhibitory effects on the aforementioned factors. Overexpression of miR-378b exacerbated the insulin secretion disorder and inhibited the PI3K-AKT signaling pathway, whereas miR-378b deficiency relieved the insulin secretion disorder, activating the PI3K-AKT pathway. In addition, CADE ameliorated the impairment of insulin secretion and reversed the miR-378b overexpression-induced PI3K-AKT pathway inhibition. In conclusion, our study demonstrates that CADE ameliorated insulin secretion dysfunction induced by excess lipid and glucose in β-cells by downregulating miR-378b expression, thus promoting PI3K-AKT activation.

1. Introduction

The prevalence of diabetes is on the rise globally. As per estimates, around 536.6 million people suffered from diabetes in 2021, representing approximately 10.5% of the global population. The numbers are projected to increase to 783.2 million people in 2045, accounting for 12.2% of the global population [1]. Diabetes mellitus and its complications greatly affect patients' quality of life and physical health [2]. Therefore, finding effective ways to prevent diabetes is a crucial public health issue [3]. The main causes of type 2 diabetes mellitus (T2DM) are a decreased number of pancreatic β-cells, insulin secretion problems, and insulin resistance [4].

Previous research has demonstrated that chronic hyperglycemia and hyperlipidemia are key risk factors for impaired insulin secretion by β-cells [5]. Some evidence suggests that palmitate and glucose reduce the dephosphorylation of AKT and FoxO1 in β-cells, thereby inhibiting insulin secretion [6, 7]. Furthermore, the phosphatidylinositol 3-kinase (PI3K)/AKT pathway is a vital pathway for regulating insulin secretion in β-cells [8–10].

Insulin binds to the insulin receptor (IR) on the β-cell membrane [11], activating the downstream PI3K-AKT signaling pathway [12]. It also promotes the phosphorylation of FoxO1 [13] and the expression of pancreatic-duodenal homologous box 1 factor (PDX-1). At the same time, activation of PDX-1 promotes proinsulin mRNA expression [14], thereby increasing insulin secretion.

MicroRNAs (miRNAs) are tiny endogenous RNAs that regulate gene expression at the post-transcriptional level [15]. They play a crucial part in regulating insulin secretion via targeting various proteins participating in the insulin signaling pathway [16]. Several reports have shown that miR-7, miR-29, and miR-802 are important regulators of insulin secretion [17–19]. Moreover, miR-378b improves insulin resistance in the liver by targeting IR and p110α [20, 21]. Nevertheless, the exact function of miR-378b in β-cells remains unknown.

Currently, the main clinically used insulin secretagogues include DPP-4 inhibitors, sulfonylureas, and GLP-1R agonists. They effectively decrease the level of blood glucose to treat T2DM by promoting insulin secretion. However, sulfonylureas increase the risk of cardiovascular disease and hypoglycemia [22, 23]; GLP-1R agonists may cause transient gastrointestinal reactions and pancreatitis [24]; and long-term use of DDP-4 inhibitors may also put patients with heart failure at risk of abnormal cardiac remodeling [25, 26]. Therefore, the search for safe and effective novel drugs to prevent and treat insulin secretion disorders has been a major research focus worldwide. In recent years, natural drugs such as mulberry alkaloids, cordycepin, and berberine have received attention for their potential in improving insulin secretion [27–29]. Caffeic acid dimethyl ether (CADE), also known as methyl ferulic acid, is a bioactive monomer extracted from traditional Chinese herbs [30]. It can improve insulin resistance in mice with alcoholic fatty liver [21] and alleviate hepatic steatosis [31]. However, it remains unclear what effect CADE has on impaired insulin secretion in diabetic patients.

The aim of the present study was twofold: (i) to explore the effect of CADE on the impairment of insulin secretion in β-cells caused by excessive glucose and lipids and (ii) to determine whether CADE ameliorates insulin secretion impairment by modulating the miR-378b-PI3K-AKT pathway. We discovered for the first time that CADE may ameliorate excessive glucose and lipid-induced insulin secretion dysfunction through the miR-378b-PI3K-AKT pathway.

2. Materials and Methods

2.1. Materials and Cell Line. CADE (purity >98%), agarose and glucose were bought from Sigma. Sodium palmitate was purchased from Kunchuang biotechnology (Xi'an, China). miR-378b mimics and miR-378b inhibitor strains, miR-378b and U6 primers, were obtained from Jikai Gene Medical Technology Co., Ltd. (Guangzhou, China). miR-378b and U6 primers were constructed by iGene Biotechnology Co., Ltd. (Guangzhou, China). Primary antibodies against β-actin, IR, p-IR (Y1185), p85, p-AKT2 (S474), PDX-1, and insulin were purchased from Abcam (Cambridge, UK); primary antibodies against p110α, FoxO1, and p-FoxO1 (S256) were purchased from Cell Signaling Technology (Massachusetts, USA); and primary antibodies p-AKT1 (S473), AKT1, and AKT2 were provided by Sangon Biotech (Shanghai, China).

1.1B4 cells were purchased from the Cell Resource Center (Beijing, China). Cells were incubated in RPMI-1640 medium (Gibco, USA) supplemented with 1% penicillin-streptomycin (Solarbio, China) and 10% FBS (Gibco, USA) in a 37°C, 5% CO_2 incubator.

2.2. Animal Models and Treatments. Thirty male C57BL/6J mice (5 weeks old, weight 18–22 g) were provided by SJA Laboratory (Hunan, China). After one week of acclimatization, they were randomly divided into two groups: the control group ($n = 6$) and the diabetic group ($n = 24$). The mice in the control group were fed a normal-fat diet (12% fat, Trophic Animal Feed High-tech Co., Ltd., Nantong, China). The mice in the diabetic group were fed a high-fat diet (HFD; 60% fat, Trophic Animal Feed High-tech Co., Ltd., Nantong, China). After four weeks of high-fat diet, all mice were fasted for 8 h and then intraperitoneally injected with streptozotocin (STZ, 55 mg/kg). STZ was injected for three days. After a week of the final STZ injection, the fasting blood glucose level was examined with a glucometer (Yuyue Medical Equipment and Supply Co., Ltd. Jiangsu, China). Mice with fasting blood glucose >11.1 mM were defined as T2DM mice [32, 33]. The T2DM mice were randomly divided into four groups: the diabetic group (DC), the diabetic + 10 mg/kg glibenclamide group (DC + Gliben), the diabetic + 5 mg/kg CADE group (DC + CADE-L), and the diabetic + 10 mg/kg CADE group (DC + CADE-H) [31]. Mice were gavaged with CADE, glibenclamide or 0.5% CMC-Na once daily for six weeks. Body weight and fasting blood glucose were recorded weekly. At week 5 of treatment, the oral glucose tolerance test (OGTT) was performed on the mice.

After six weeks of treatment, all mice were fasted for 8 h and anesthetized with 1.25% 2,2,2-tribromoethanol (Cat# M2910, Aibei Biotechnology). Serum was taken for insulin analysis. Finally, the mice were euthanized and the pancreas was isolated for subsequent assays. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethical Committee of Guilin Medical University.

2.3. OGTT. Five weeks after CADE administration, all mice were fasted for 8 h and their blood glucose levels were measured. Following this, a glucose solution with 2 g/kg body weight was orally administered to each mouse. The level of blood glucose was then monitored at 15-, 30-, 60-, and 120-minutes post-infusion.

2.4. Hematoxylin and Eosin Staining. Pancreatic tissue was placed in a 4% paraformaldehyde solution for 24 h. Afterward, it was dehydrated in a graded series of alcohol and placed in wax blocks. These blocks were sectioned into 3 μm

thick slices and stained with hematoxylin and eosin (H&E). Pathological changes were observed by light microscopy and images of the islet tissue were taken.

2.5. Immunofluorescence Assay. To investigate insulin expression in pancreatic sections, the paraffin-embedded tissues were first deparaffinized and subjected to antigen retrieval by heating in a 3% citric acid solution for 30 min. After blocking with serum for 30 min, these sections were incubated with anti-insulin (1 : 80) at 4°C overnight. Afterward, the sections were incubated with a fluorescent secondary antibody (1 : 200) for 30 min, followed by DAPI staining at room temperature for 10 min to label nuclei. Finally, the sections were sealed with a water-soluble blocker. Images were captured by fluorescence microscopy.

2.6. MTT Assay. 1.1B4 cells were inoculated in a 96-well plate. After 24 h, the cells were induced by glucose and sodium palmitate with or without CADE for 48 h. Cell viability was analyzed using the thiazolyl blue tetrazolium bromide (MTT) assay (Sigma, Cambridge, UK) according to the instructions provided.

2.7. Cell Treatment. 1.1B4 cells were divided into control, 30 mM glucose (HG) + 0.2 mM sodium palmitate (HF), and three CADE treatment groups. The control group was incubated with RPMI-1640 complete medium containing 10% FBS, while the HG + HF group was incubated with a medium containing 10% FBS, 30 mM glucose, and 0.2 mM sodium palmitate. The three CADE treatment groups were incubated with a complete medium containing 0.2 mM sodium palmitate, 30 mM glucose, and 0.5, 1, or 2 μM CADE, respectively, for 48 hours.

2.8. Cell Transfection. Plasmids for miR-378b mimics, miR-378b inhibitors, and the negative control (NC) were provided by Gikai Genetics (Shanghai, China). 1.1B4 cells were then transfected with these plasmids by electrotransfection. After 24 h of incubation, all cells were exposed to RPMI-1640 complete medium containing HG and HF; 1 μM CADE was additionally added to the CADE treatment group.

2.9. Measurement of Insulin Secretion. 1.1B4 cells or 1.1B4 cells transfected with miR-378b NC/mimics/inhibitor plasmid were cultured in six-well low-attachment culture plates with RPMI-1640 medium containing 10% FBS. (i) After the cells formed three-dimensional pseudo-islet masses, they were divided into the control, HG + HF, and low-, medium-, and high-dose CADE groups [34, 35]. The control group continued to be cultured in complete medium, the HG + HF group was cultured in complete medium with HG and HF, and the CADE groups were cultured in complete medium with HG, HF and CADE (0.5, 1, or 2 μM). (ii) Similarly, the transfected cells were split into the following groups: the miR-378b mimics group, the miR-378b NC group, the miR-378b NC + CADE group, the miR-378b inhibitor group, the miR-378b mimics + CADE group, and the miR-378b inhibitor + CADE group. Each group was incubated with HG and HF, and 1 μM CADE was additionally added to the CADE groups. Cells were incubated for 48 h. Then, five pseudo-islet masses were isolated under a microscope, placed into EP tubes, and incubated with KRBH buffer with 0.1% bovine serum albumin for 3 h. Afterward, the supernatant was collected. Finally, an ELISA kit (EZ assay Ltd., Guangdong, China) was applied to assess the insulin concentration in the cell supernatant.

2.10. Western Blot Analysis. Total protein was obtained from CADE-treated 1.1B4 cells for Western blot analysis. Proteins from each group were separated by SDS-PAGE and transferred onto nitrocellulose membranes, which were incubated with primary antibodies at 4°C overnight, washed three times, and incubated with secondary antibody (1 : 10,000) for 1 h at room temperature. The expression of target protein was analyzed using a highly sensitive chemiluminescent reagent (Shanghai Liji, Shanghai, China) on a ChemiDoc XRS$^+$ type gel imager (Bio-Rad, CA, USA).

2.11. Real-Time Quantitative PCR. TRIzol reagent (Tiangen, Beijing, China) was applied to extract total RNA from the 1.1B4 cells and the pancreas. To synthesize the initial cDNA chain of miRNA, the cDNA synthesis kit (GeneCopoeia™, Guangzhou, China) was utilized. Afterwards, the cDNA produced was utilized in qRT-PCR for the determination of miR-378b levels. The $2^{-\Delta\Delta Ct}$ method was applied to evaluate the comparative expression of miR-378b, with U6 as the reference gene. The primers used in the PCR experiments were acquired from Sangon Biotech (Shanghai, China) and their sequences can be found in Table 1.

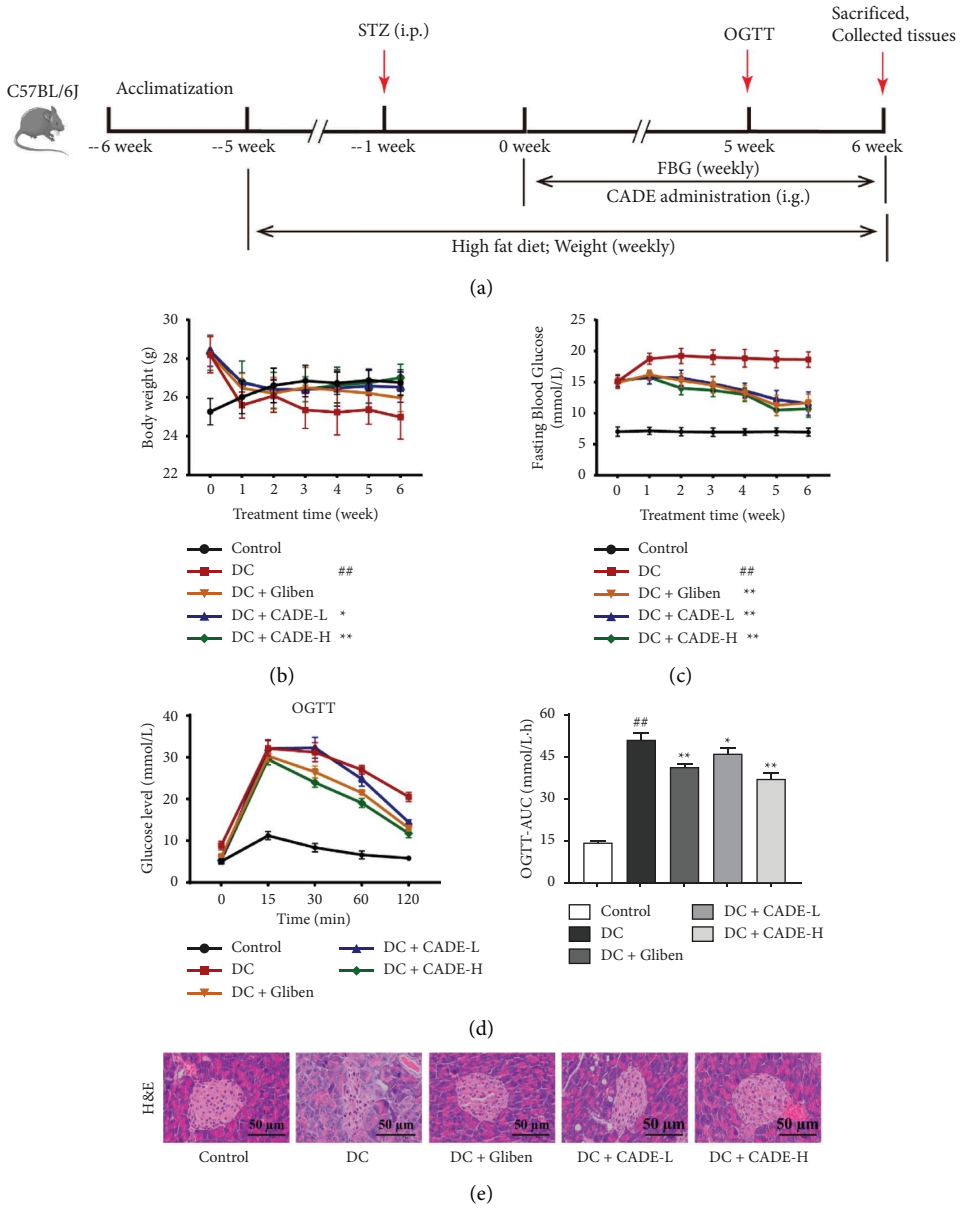
2.12. Statistical Analysis. All experiments were performed at least three times. Unless stated otherwise, the findings are shown as the mean and standard deviation (SD). Data were analyzed by one-way ANOVA using SPSS 25. $P < 0.05$ indicates a statistical significance.

3. Results

3.1. CADE Ameliorated Insulin Secretion Disorder and Downregulated miR-378b in HFD/STZ Mice. To explore the impact of CADE on insulin secretion disorder, we constructed a T2DM mouse model by combined HFD/STZ induction and treated the mice with 0.5% CMC-Na, glibenclamide, or CADE for six weeks (Figure 1(a)). Compared with the control group, the DC group showed significant weight loss, poor glucose tolerance, and elevated fasting glucose. However, after CADE and glibenclamide treatment, the mice had notably higher body weight, lower fasting glucose, and worse glucose tolerance (Figures 1(b)–1(d)). Notably, no statistically significant changes in body weight were observed in the DC + Gliben group.

Table 1: Primers for the RT-PCR assay.

Gene	Forward (5′-3′)	Reverse (5′-3′)
β-actin	CACTCTTCCAGCCTTCCTTCC	CGTACAGGTCTTTGCGGATGTC
IR	AAAACGAGGCCCGAAGATTTC	GAGCCCATAGACCCGGAAG
p110α	AGTAGGCAACCGTGAAGAAAAG	GAGGTGAATTGAGGTCCCTAAGA
Insulin	GCAGCCTTTGTGAACCAACAC	CCCCGCACACTAGGTAGAGA
miR-378b	ACACTCCAGCTGGGAGTGGACTTGGAGTCA	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCTTCTGA



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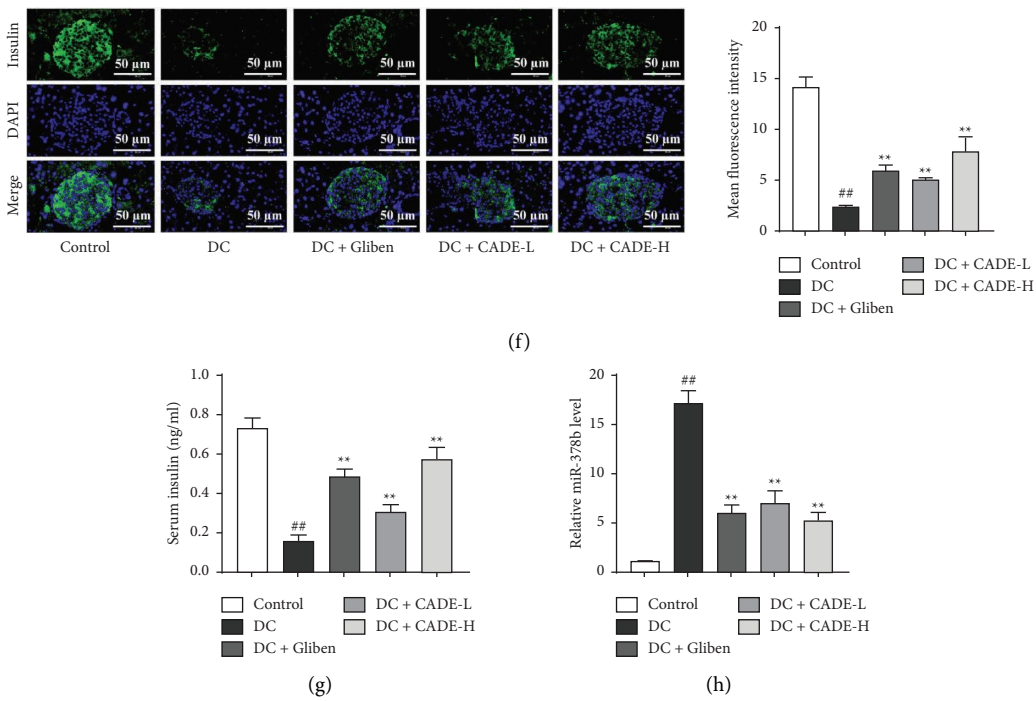


Figure 1: Effects of CADE on impaired insulin secretion in mice. (a) The experimental schedule. (b) Body weight. (c) Blood glucose level after 8 h of fasting. (d) OGTT and area under the curve during OGTT. (e) H&E staining of mouse pancreatic tissue (scale bar: 50 μm). (f) Immunofluorescence of insulin in mouse pancreatic islets (scale bar: 50 μm) and quantification. (g) Insulin secretion levels. (h) miR-378b levels in the pancreas. The results are presented as the mean ± SD from three experiments. STZ, streptozotocin; i.p., intraperitoneal injection; i.g., intragastric administration; FBG, fasting blood glucose; and OGTT, oral glucose tolerance test. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the DC group.

H&E staining was carried out on pancreatic tissue sections to analyze the pathological changes in mouse islets. The DC group exhibited severe damage to the islet structure compared with the control group, with a significant reduction in islet tissue area and irregularly shaped islets. However, the size and shape of the islets tended to be normal, and the structure was intact with clear borders after CADE or glibenclamide intervention (Figure 1(e)). These findings suggest that CADE might protect against histopathological damage to the islets in HFD/STZ mice.

The synthesis and secretion of insulin in mice were analyzed by tissue immunofluorescence and ELISA. Compared with the control group, a significant reduction in insulin fluorescence and lower insulin levels were observed in the DC group, along with a considerable decrease in serum insulin levels. By contrast, CADE treatment resulted in more intense insulin fluorescence, and both islet and serum insulin levels were substantially increased (Figures 1(f) and 1(g)). Remarkably, the best effect on insulin synthesis and secretion was observed in the DC + CADE-H group. In addition, qRT-PCR analysis demonstrated a dramatic increase in miR-378b levels in the DC group with the control group. However, miR-378b was significantly downregulated in the CADE and glibenclamide groups. The DC + CADE-H group showed the strongest downregulation. The DC + CADE-H group showed the strongest downregulation (Figure 1(h)). Interestingly, the promotion of insulin synthesis and secretion by CADE was closely associated with the expression of miR-378b.

3.2. CADE Enhanced the Viability of HG/HF-Induced 1.1B4 Cells. Cell viability was analyzed using the MTT assay. As illustrated in Figure 2(a), after 48 h of culture of 1.1B4 cells with 0.1–16 μM CADE, there was no statistically significant difference in cell viability compared with the control group. Glucose and sodium palmitate were administered to 1.1B4 cells for 48 h. The viability of 1.1B4 cells treated with 30 mM glucose and ≥0.2 mM sodium palmitate was significantly inhibited compared with that of control cells (Figure 2(b)). To investigate the effects of CADE on HG + HF-induced 1.1B4 cells, 1.1B4 cells were co-cultured with 30 mM glucose, 0.2 mM sodium palmitate, and CADE (0.5, 1, or 2 μM) for 48 h. Cell viability was significantly enhanced by CADE compared with the HG + HG group (Figure 2(c)).

3.3. CADE Improved HG + HF-Induced Insulin Secretion Dysfunction and Reduced Abnormally Elevated miR-378b Levels in 1.1B4 Cells. To measure the impact of CADE on insulin secretion dysfunction in HG + HF-induced 1.1B4 cells, we examined the intracellular expression level of insulin by an immunofluorescence assay. In addition, 1.1B4

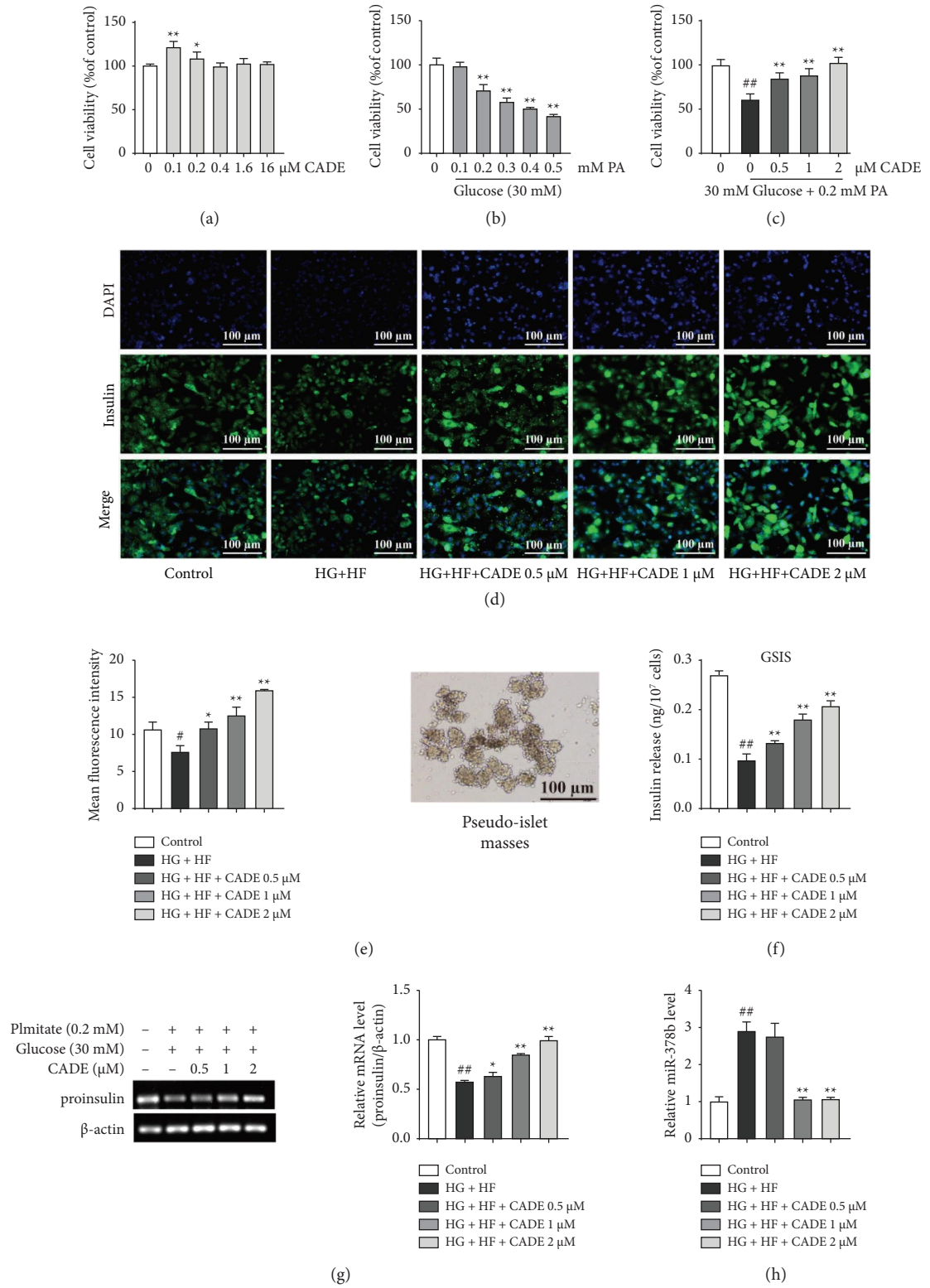


Figure 2: Effects of CADE on HG + HF-induced impairment of insulin secretion in 1.1B4 cells. (a) Effects of CADE on cell viability in 1.1B4 cells. (b) Effects of glucose and sodium palmitate on cell viability in 1.1B4 cells. (c) Effects of CADE on cell viability in glucose and sodium palmitate-induced 1.1B4 cells. (d) Immunofluorescence analysis of insulin (scale bar: 100 μm) and quantification. (e) Pseudo-islet masses (scale bar: 100 μm). (f) Insulin secretion levels of pseudo-islet masses. (g) Proinsulin mRNA levels. (h) Expression levels of miR-378b in 1.1B4 cells. The results are presented as the mean ± SD from three experiments. PA: sodium palmitate. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the HG + HF group.

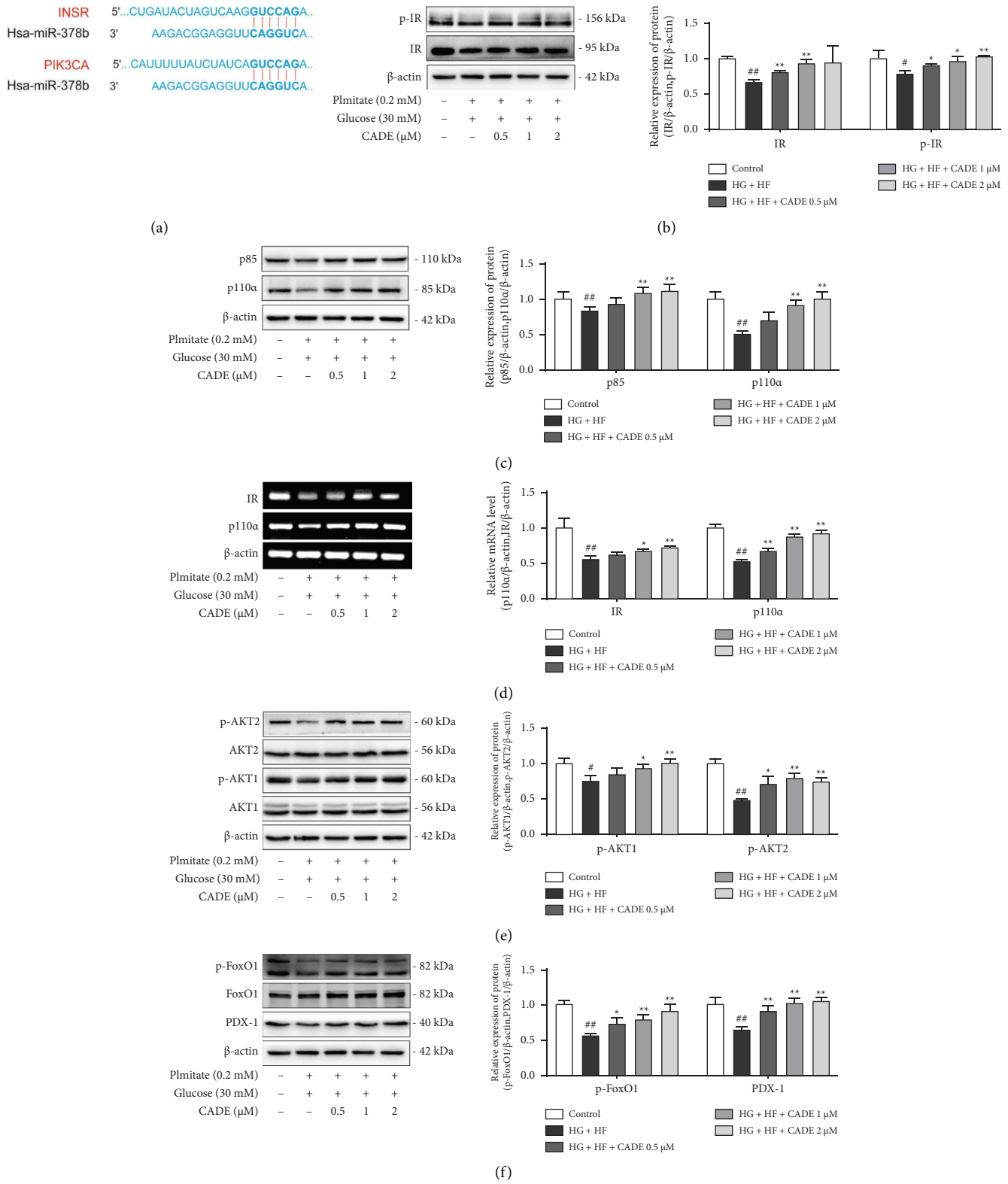


Figure 3: Effects of CADE on the PI3K-AKT signaling pathway in HG + HF-induced 1.1B4 cells. (a) Targets of miR-378b predicted by the TargetScan database. (b, c) Western blot analysis of the expression levels of p-IR, IR, p85 and p110α. (d) RT-PCR analysis of the mRNA expression levels of IR and p110α. (e, f) Protein expression levels of Akt1, p-Akt1, Akt2, p-Akt2, FoxO1, p-FoxO1, and PDX-1. The results are presented as the mean ± SD from three experiments. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the HG + HF group.

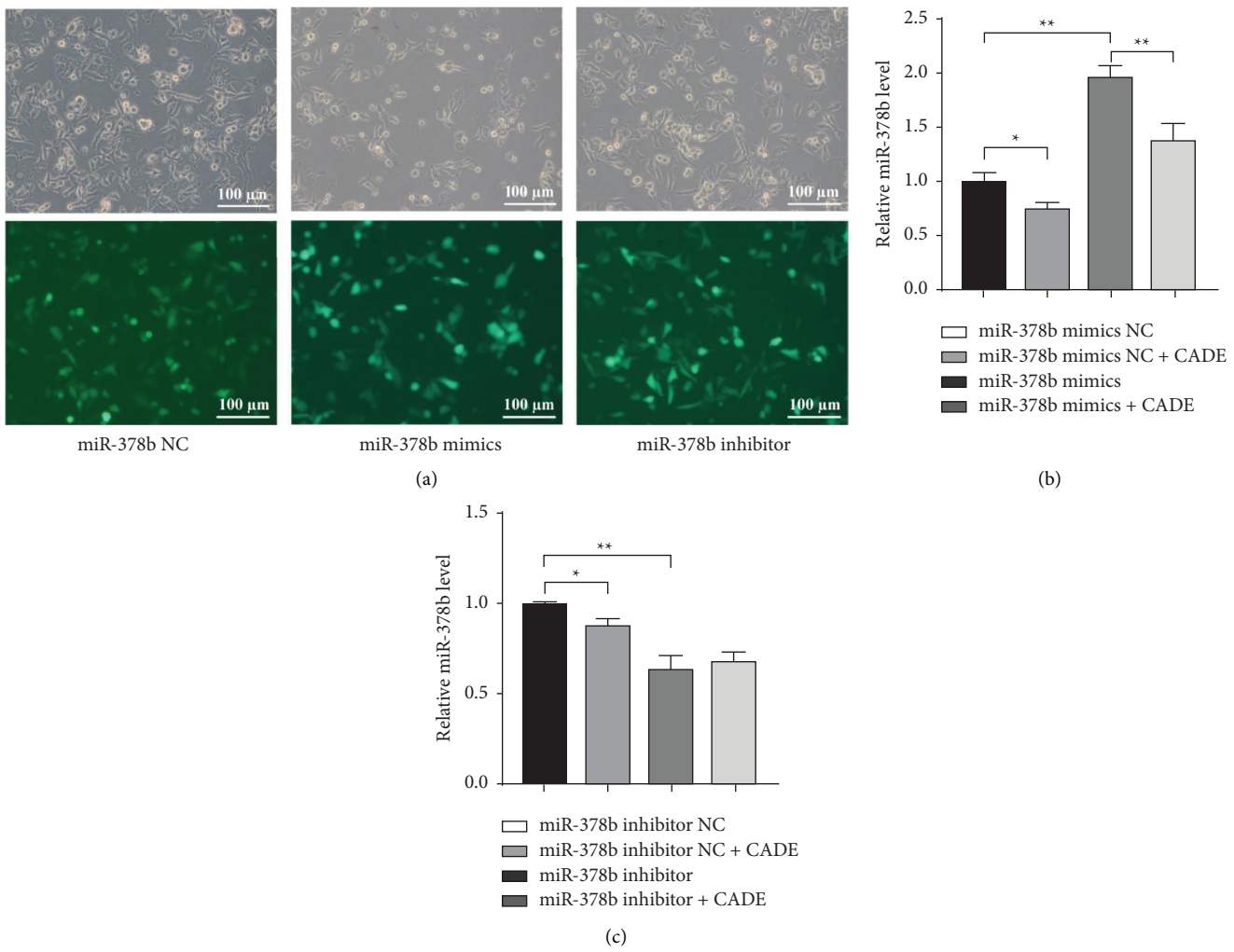
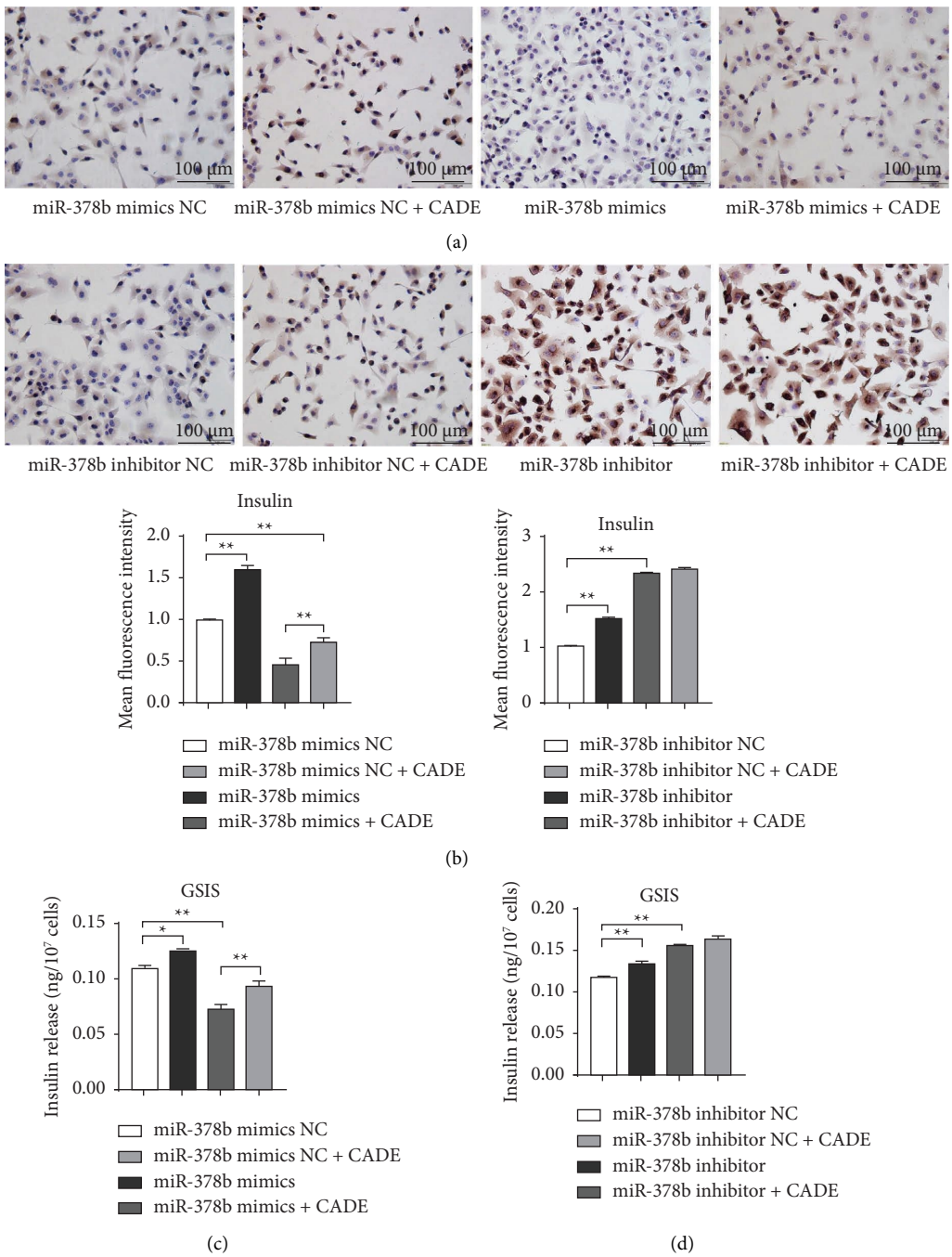


Figure 4: Effects of CADE on the expression of miR-378b in transfected 1.1B4 cells. (a) Efficiency of transfection of miR-378b plasmid into 1.1B4 cells (scale bar: 100 μm). (b, c) Levels of miR-378b in transfected 1.1B4 cells. The results are presented as the mean ± SD from three experiments. $^{*}P < 0.05$ vs. the miR-378b mimics NC or miR-378b inhibitor NC group; $^{**}P < 0.01$ vs. the miR-378b mimics group or miR-378b inhibitor group.

cells were cultured in low-attachment culture dishes to form three-dimensional pseudo-islet masses (Figure 2(e)), and then insulin secretion was analyzed by ELISA in the cell supernatant. Compared with the control group, both insulin synthesis and secretion were decreased significantly in the HG + HF group. CADE enhanced the secretion and synthesis of insulin in a dose-dependent manner (Figures 2(d), 2(f)). Furthermore, the proinsulin mRNA levels in 1.1B4 cells were further analyzed by RT-PCR. The results indicated that proinsulin mRNA levels were decreased by approximately 50% in HG + HF-induced 1.1B4 cells compared with the control group. CADE increased proinsulin mRNA levels in a dose dependence-dependent manner (Figure 2(g)). These results suggest that CADE might promote insulin synthesis and secretion by increasing proinsulin mRNA expression, ultimately improving insulin secretion disorders.

The qRT-PCR assay showed that compared with the control group, miR-378b levels were abnormally elevated in HG + HF-induced 1.1B4 cells. The difference was not significant after 0.5 μM CADE treatment. However, 1 and 2 μM CADE effectively prevented miR-378b upregulation in HG + HF-induced 1.1B4 cells (Figure 2(h)). A CADE concentration of 1 μM exerted an optimal therapeutic effect. Therefore, 1 μM CADE was applied in the following cell transfection experiments.

3.4. CADE Ameliorated HG + HF-Induced Insulin Secretion Dysfunction in 1.1B4 Cells via the PI3K-AKT Signaling Pathway. The network bioinformatics analysis revealed that miR-378b has binding sites in the 3′-UTRs of INSR (encoding IR) and PIK3CA (encoding p110α) (Figure 3(a)). Consequently, we reasoned that miR-378b may affect insulin



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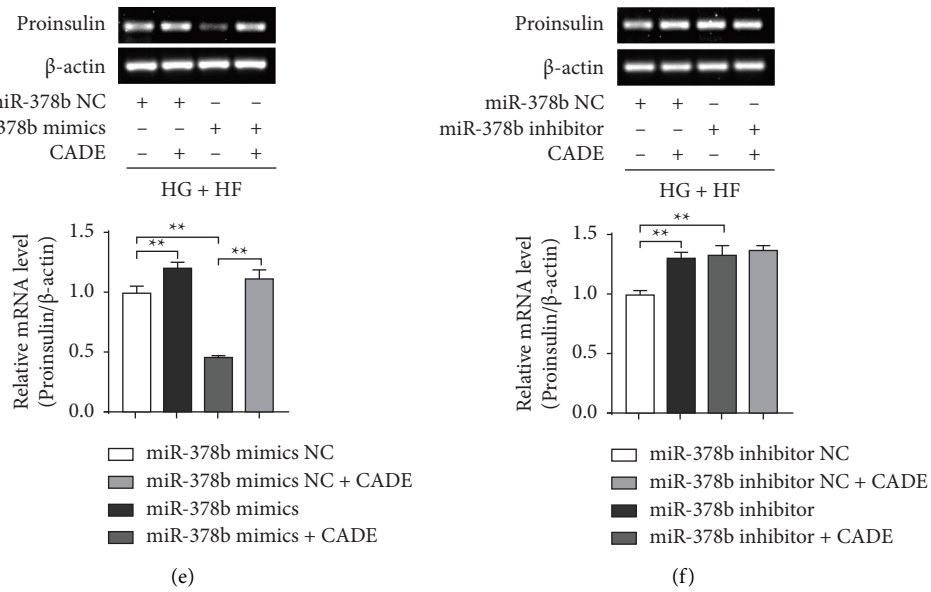
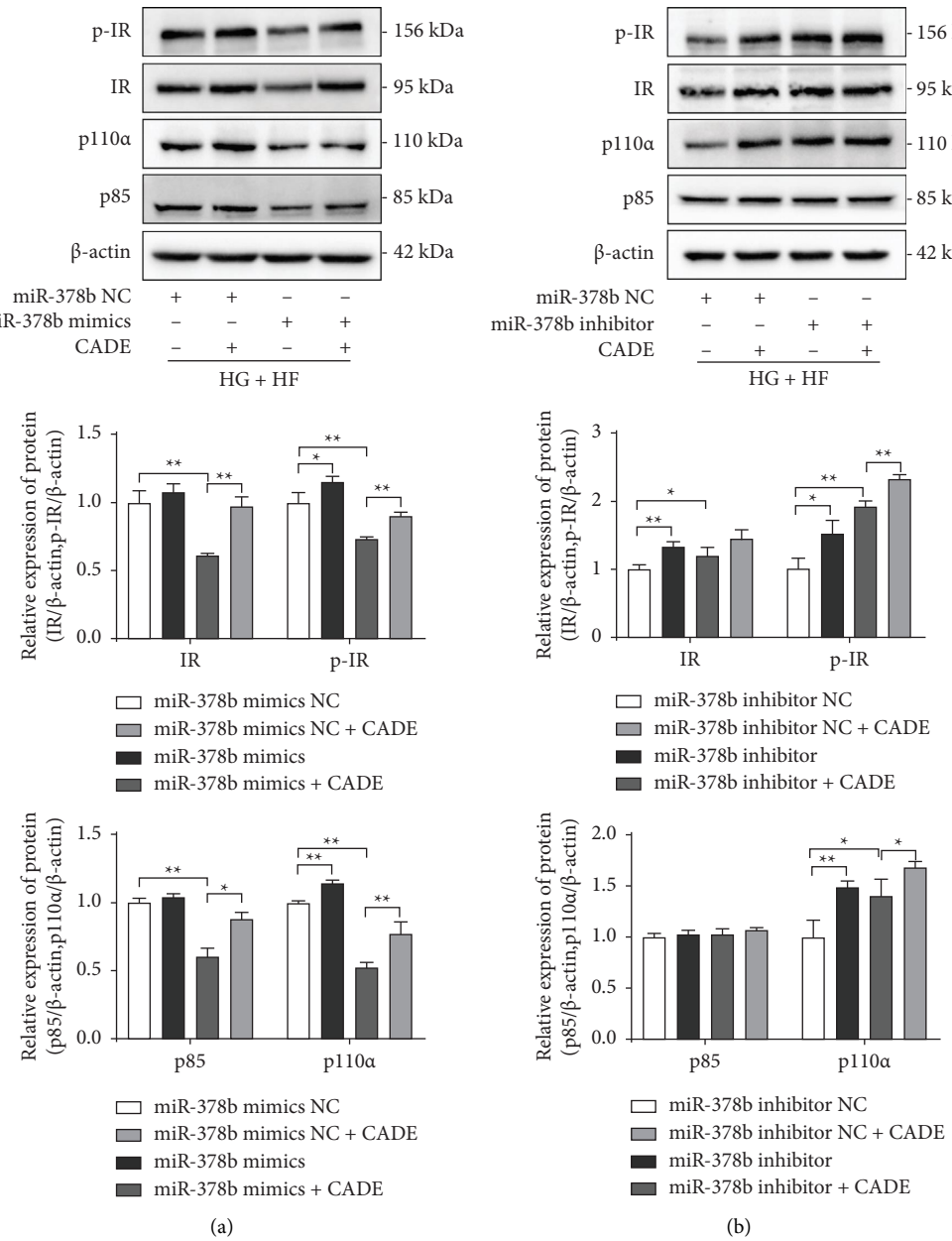


Figure 5: CADE affected impairment of insulin secretion in 1.1B4 cells by miR-378b. (a, b) Immunohistochemistry (scale bar: 100 μm) and quantification of insulin in transfected 1.1B4 cells. (c, d) Insulin secretion levels in transfected 1.1B4 cells. (e, f) Proinsulin expression levels in transfected 1.1B4 cells. The results are presented as the mean ± SD from three experiments. $^{**}P < 0.01$ vs. the miR-378b mimics NC or miR-378b inhibitor NC group. $^{**}P < 0.01$ vs. the miR-378b mimics group or miR-378b inhibitor group.

secretion by regulating IR and p110α expression. To validate the association between miR-378b, IR, and p110α, we first examined the mRNA and protein levels of IR and p110α in HG + HF-induced 1.1B4 cells, as well as the levels of their downstream related factors. The results revealed that the cellular p85, IR, p110α, and p-IR protein levels were greatly reduced in HG + HF-induced 1.1B4 cells compared with the control group, while the mRNA expression levels of IR and p110α were also significantly decreased. Surprisingly, CADE treatment reversed the inhibitory effects of the above factors (Figures 3(b)–3(d)). Further analysis of the downstream factors of IR and p110α revealed that the p-Akt1/Akt1, p-Akt2/Akt2, p-FoxO1/FoxO1, and PDX-1 expression levels were remarkably decreased, whereas the levels of the above proteins were increased after CADE application in a dose-dependent manner (Figures 3(e), 3(f)). These results suggest that the beneficial effects of CADE on insulin secretion dysfunction are closely associated with the regulation of IR and p110α expression by miR-378b.

3.5. CADE Affected HG + HF-Induced Insulin Secretion Dysfunction in 1.1B4 Cells via miR-378b. To determine the direct relationship between CADE and miR-378b in improving insulin secretion dysfunction, 1.1B4 cells were transfected with miR-378b inhibitor or mimics plasmids. The fluorescence intensity of each group was determined by fluorescence microscopy after 12 h. At least 80% of the cells in each group expressed GFP, so the experimental requirements were met (Figure 4(a)). The transfected cells were then treated with 0.2 mM sodium palmitate and 30 mM glucose for 48 h; cells in the CADE treatment group were additionally treated with 1 μM CADE. qRT-PCR analysis showed that miR-378b levels were significantly downregulated in the miR-378b inhibitor group and upregulated in the miR-378b mimic group (Figure 4(b)). In addition, the miR-378b levels were significantly decreased in the miR-378b NC + CADE group compared with the miR-378b NC group. Furthermore, although miR-378b silencing resulted in the downregulation of miR-378b, the difference in miR-378b levels was not significant after CADE intervention (Figure 4(c)). These results suggest that CADE may improve insulin secretion dysfunction by regulating miR-378b expression.

Immunohistochemistry and ELISA assays were performed to analyze insulin synthesis and secretion in transfected 1.1B4 cells. The findings revealed that the insulin secretion and synthesis levels were enhanced in the miR-378b NC + CADE group compared with the miR-378b NC group, and the levels were reduced to different degrees when miR-378b was overexpressed, and both were significantly increased after the co-application of CADE and miR-378b mimics (Figures 5(a), 5(c)). On the contrary, insulin synthesis and secretion levels were significantly upregulated in the miR-378b inhibitor group after silencing, and it is worth mentioning that the levels were not statistically significant after the combined application of CADE and miR-378b inhibitor (Figures 5(b), 5(d)). In addition, proinsulin mRNA levels were further analyzed by PCR. The mRNA levels of proinsulin were consistent with the results of insulin synthesis and secretion described above (Figures 5(e), 5(f)). These findings suggest that miR-378b overexpression impairs the secretory function of β-cells, which can be improved by CADE treatment.



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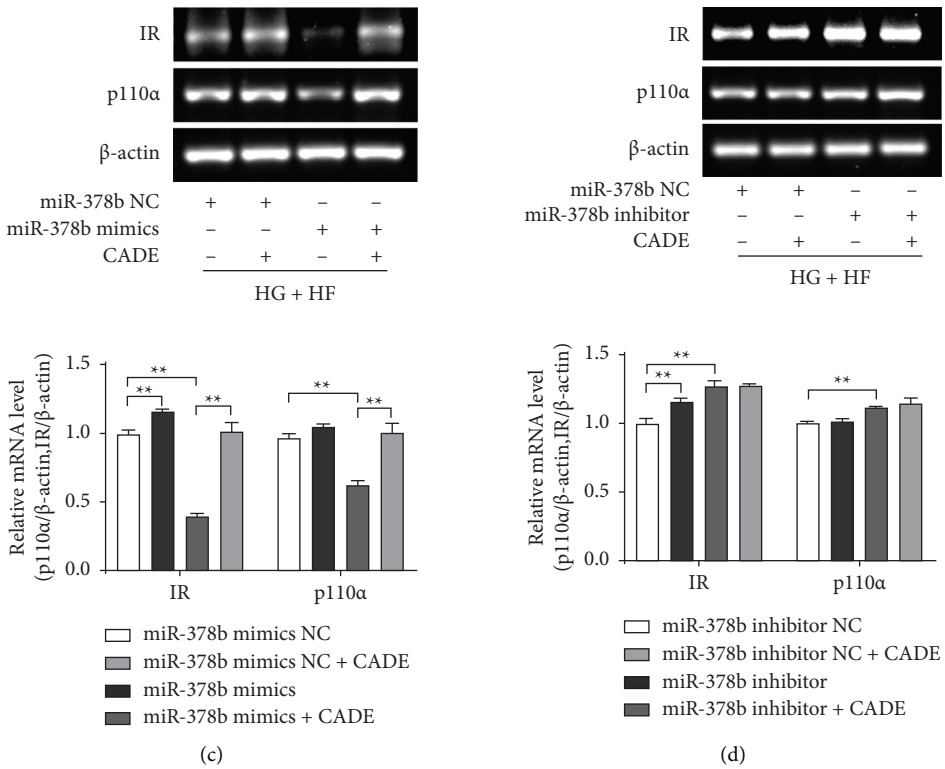

Figure 6: Effects of CADE on the insulin signaling pathway in transfected 1.1B4 cells. (a, b) Protein expression of p-IR, IR, p85, and p110α. (c, d) RT-PCR analysis of IR and p110α mRNA levels. The results shown represent the mean ± SD from triplicate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the miR-378b mimics NC or miR-378b inhibitor NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the miR-378b mimics group or miR-378b inhibitor group.

3.6. CADE Improved HG + HF-Induced Impairment of Insulin Secretion in 1.1B4 Cells via the miR-378b-PI3K-AKT Pathway. Our study has demonstrated that CADE effectively inhibits the impairment of insulin secretory function in T2DM models. We found that the regulatory effect of miR-378b on IR and p110α expression is closely related to the therapeutic effect of CADE on insulin secretion dysfunction. To further investigate the function of the miR-378b-PI3K-AKT pathway in this process, we performed validation experiments on 1.1B4 cells. The results showed that the protein levels of p-IR, p110α, IR, p-Akt1/Akt1, p-Akt2/Akt2, p-FoxO1/FoxO1, and PDX-1 and the mRNA levels of IR and p110α were significantly reduced in the miR-378b mimics group compared with the NC group. However, these protein and mRNA levels were partly restored by CADE treatment. Moreover, the miR-378b inhibitor enhanced the protein levels of IR, p110α, p-IR, p-Akt1/Akt1, p-Akt2/Akt2, p-FoxO1/FoxO1, and PDX-1 and the mRNA levels of IR and p110α. After CADE treatment, p-IR, p110α, and PDX-1 were further upregulated, while the differences in IR and p110α mRNA levels were not significant (Figures 6(a)–6(d) and, 7(a), 7(b)). In summary, CADE improved impairment of β-cell insulin secretion by downregulating miR-378b to facilitate IR and p110α expression, which activated the PI3K-AKT signaling pathway.

4. Discussion

The occurrence of T2DM increases every year [36], resulting in substantial negative effects on patients' quality of life and a huge financial burden on healthcare systems [37]. Hence, it is crucial to find effective and safe insulin secretagogues while gaining insight into the molecular mechanisms underlying diabetes. Our research provides pioneering evidence that (i) CADE improved weight loss and glucose homeostasis in HFD/STZ mice and ameliorated insulin secretion dysfunction and β-cell injury in T2DM models, (ii) The miR-378b-PI3K-AKT signaling pathway is involved in insulin secretion dysfunction in β-cells, and (iii) CADE ameliorated insulin secretion dysfunction in a T2DM model by predominantly suppressing miR-378b and consequently activating the PI3K-AKT pathway.

T2DM is usually associated with fasting glucose >11.1 mM, polyphagia, hyperphagia, and weight loss. In the present study, the symptoms induced by HFD/STZ in C57BL/6J mice were similar to those reported in the above studies, indicating the successful establishment of the T2DM mouse model.

Literature search revealed that CADE alleviates insulin resistance in alcoholic fatty liver disease [38]. However, it is unclear whether CADE attenuates excessive glucose and

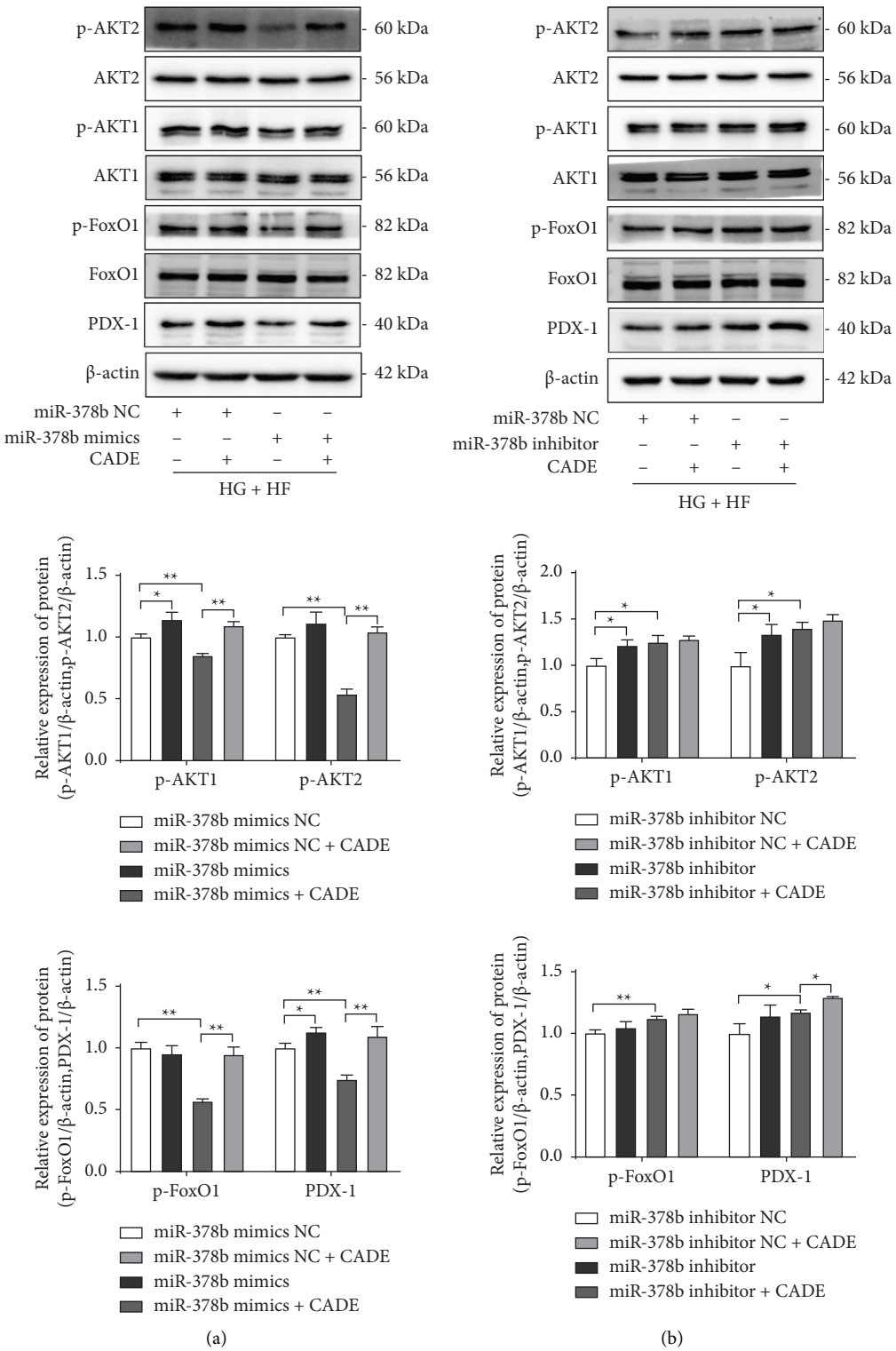

Figure 7: Effects of CADE on the insulin signaling pathway in transfected 1.1B4 cells. (a, b) Protein expression of Akt1, p-Akt1, Akt2, p-Akt2, FoxO1, p-FoxO1, and PDX-1. The results are presented as the mean ± SD from three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the miR-378b mimics NC or miR-378b inhibitor NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the miR-378b mimics group or miR-378b inhibitor group.

lipid-induced impairment of insulin secretion. In the present study, both CADE and the positive control drug glibenclamide were effective in treating T2DM to some extent. Interestingly, 10 mg/kg CADE showed a stronger therapeutic effect than the 10 mg/kg glibenclamide-treated group in terms of body weight and glucose sensitivity, as well as insulin secretion and synthesis. It is worth noting that glibenclamide treatment can lower fasting glucose levels in

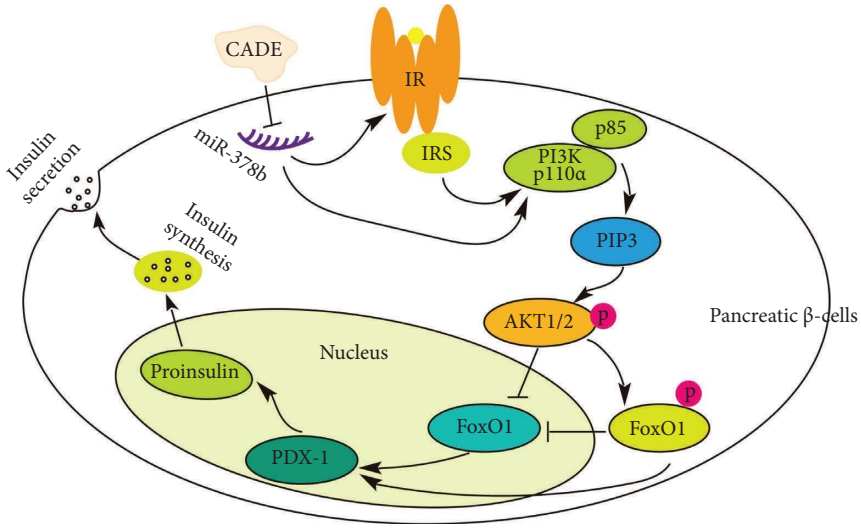

Figure 8: Diagram of the insulin secretion signaling pathway.

patients with T2DM, but it does not significantly alter body weight, plasma insulin levels, or insulin sensitivity [39]. Furthermore, long-term proinsulin secretion with little or no synthesis only depletes intracellular insulin stores. Conversely, CADE had marked effects on both insulin synthesis and secretion. These results indicate that CADE is a promising drug candidate for the long-term treatment of T2DM by promoting insulin synthesis and secretion.

The insulin signaling pathway mediated by PI3K-AKT and the downstream factors FoxO1 and PDX-1 play significant roles in insulin secretion by β-cells [40, 41]. IR and PI3K-AKT are critical factors in mediating insulin signaling and secretion [42]. In addition, FoxO1 participates in cell differentiation, proliferation and apoptosis [43, 44]. PDX-1, a downstream factor of FoxO1, is a critical insulin transcription factor for early pancreatic development, insulin production, and β-cell maturation [45, 46]. Insulin secretion by β-cells is enhanced by phosphorylation of FoxO1 and elevated expression of PDX-1 [47]. There is increasing evidence that the protein levels of IR, p-FoxO1/FoxO1, PDX-1, and p-AKT/AKT in glucose and palmitate-induced β-cells are reduced, impairing insulin secretion [48–50]. In the present study, the abovementioned insulin transduction factors were similarly significantly inhibited in 1.1B4 cells induced by excess glucose and palmitate, whereas CADE treatment reversed these effects and ultimately improved insulin secretion (Figure 8).

Previous research has shown the involvement of miRNAs in insulin secretion from β-cells [51, 52]. It has been reported that excessive miR-378, a major regulatory insulin signaling factor, can impair the insulin signaling pathway [53]. Based on these reports, we found by bioinformatics analysis that there is a miR-378b binding site in the 3′-UTRs of IR and p110α. In addition, we found that CADE ameliorated insulin secretion dysfunction, inhibited the abnormal elevation of miR-378b levels, and promoted the expression of p110α and IR in excessive glucose and lipid-induced β-cells. Thus, we hypothesized that the beneficial effects of CADE on insulin secretion are mediated by the regulation of miR-378b, which in turn affects p110α and IR expression, activating the PI3K-AKT pathway. We confirmed this hypothesis by miR-378b overexpression and silencing assays. The findings indicate that the impaired insulin secretion and the inhibitory effects of the PI3K-AKT pathway were exacerbated in miR-378b overexpressing 1.1B4 cells, while the impaired insulin secretion and the inhibitory effects of the PI3K-AKT pathway were ameliorated in miR-378b-silenced 1.1B4 cells. Co-treatment with CADE and miR-378b mimics alleviated the above aggravated insulin secretion impairment and PI3K-AKT pathway inhibition. Taken together, CADE ameliorates insulin secretion by β-cells through modulation of the miR-378b-PI3K-AKT signaling pathway.

To sum up, we present novel evidence that CADE can effectively alleviate insulin secretion dysfunction caused by excessive glucose and lipids, by modulating the miR-378b-PI3K-AKT signaling pathway in β-cells. However, the clinical application of CADE still poses several challenges since existing research is limited to animal and cellular studies. More clinical studies are required to validate the current findings and to explore new avenues of CADE application for the treatment of T2DM.

5. Conclusions

This study found that CADE has the potential to alleviate insulin secretion dysfunction in β-cells caused by excessive glucose and lipid. CADE, which downregulates miR-378b and activates the PI3K-AKT signaling pathway, is a promising drug candidate for individuals with insulin secretion dysfunction. These findings offer significant support for the development of new therapeutic strategies for T2DM.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Ermei Zhou and Yanqing Liang have contributed equally to this work and share first authorship.

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References

[1] H. Sun, P. Saeedi, S. Karuranga et al., "IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045," *Diabetes Research and Clinical Practice*, vol. 183, Article ID 109119, 2022.

[2] C. X. Ma, X. N. Ma, C. H. Guan, Y. D. Li, D. Mauricio, and S. B. Fu, "Cardiovascular disease in type 2 diabetes mellitus: progress toward personalized management," *Cardiovascular Diabetology*, vol. 21, no. 1, p. 74, 2022.

[3] E. Ahmad, S. Lim, R. Lamptey, D. R. Webb, and M. J. Davies, "Type 2 diabetes," *The Lancet*, vol. 400, no. 10365, pp. 1803–1820, 2022.

[4] J. Inaishi and Y. Saisho, "Beta-cell mass in obesity and type 2 diabetes, and its relation to pancreas fat: a mini-review," *Nutrients*, vol. 12, no. 12, p. 3846, 2020.

[5] N. Shimo, T. A. Matsuoka, T. Miyatsuka et al., "Short-term selective alleviation of glucotoxicity and lipotoxicity ameliorates the suppressed expression of key β-cell factors under diabetic conditions," *Biochemical and Biophysical Research Communications*, vol. 467, no. 4, pp. 948–954, 2015.

[6] O. Kluth, F. Mirhashemi, S. Scherneck et al., "Dissociation of lipotoxicity and glucotoxicity in a mouse model of obesity associated diabetes: role of forkhead box O1 (FOXO1) in glucose-induced beta cell failure," *Diabetologia*, vol. 54, no. 3, pp. 605–616, 2011.

[7] S. K. Marafie, E. M. Al-Shawaf, J. Abubaker, and H. Arefanian, "Palmitic acid-induced lipotoxicity promotes a novel interplay between Akt-mTOR, IRS-1, and FFAR1 signaling in pancreatic β-cells," *Biological Research*, vol. 52, no. 1, p. 44, 2019.

[8] J. L. Beith, E. U. Alejandro, and J. D. Johnson, "Insulin stimulates primary β-cell proliferation via raf-1 kinase," *Endocrinology*, vol. 149, no. 5, pp. 2251–2260, 2008.

[9] B. Maiztegui, C. L. Román, H. C. Barbosa-Sampaio, A. C. Boschero, and J. J. Gagliardino, "Role of islet glucokinase, glucose metabolism, and insulin pathway in the enhancing effect of islet neogenesis-associated protein on glucose-induced insulin secretion," *Pancreas*, vol. 44, no. 6, pp. 959–966, 2015.

[10] Y. Pan, B. Wang, J. Zheng et al., "Pancreatic fibroblast growth factor 21 protects against type 2 diabetes in mice by promoting insulin expression and secretion in a PI3K/Akt signaling-dependent manner," *Journal of Cellular and Molecular Medicine*, vol. 23, no. 2, pp. 1059–1071, 2019.

[11] R. N. Kulkarni, J. C. Brüning, J. N. Winnay, C. Postic, M. A. Magnuson, and C. R. Kahn, "Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes," *Cell*, vol. 96, no. 3, pp. 329–339, 1999.

[12] E. Bernal-Mizrachi, W. Wen, S. Stahlhut, C. M. Welling, and M. A. Permutt, "Islet β cell expression of constitutively active Akt1/PKBα induces striking hypertrophy, hyperplasia, and hyperinsulinemia," *Journal of Clinical Investigation*, vol. 108, no. 11, pp. 1631–1638, 2001.

[13] S. C. Martinez, C. Cras-Méneur, E. Bernal-Mizrachi, and M. A. Permutt, "Glucose regulates Foxo1 through insulin receptor signaling in the pancreatic islet β-cell," *Diabetes*, vol. 55, no. 6, pp. 1581–1591, 2006.

[14] T. Shu, Y. Zhu, H. Wang, Y. Lin, Z. Ma, and X. Han, "AGEs decrease insulin synthesis in pancreatic β-cell by repressing pdx-1 protein expression at the post-translational level," *PLoS One*, vol. 6, no. 4, Article ID e18782, 2011.

[15] T. X. Lu and M. E. Rothenberg, "MicroRNA," *Journal of Allergy and Clinical Immunology*, vol. 141, no. 4, pp. 1202–1207, 2018.

[16] M. Aghaei, A. Khodadadian, K. N. Elham, M. Nazari, and E. Babakhanzadeh, "Major miRNA involved in insulin secretion and production in beta-cells," *International Journal of General Medicine*, vol. 13, pp. 89–97, 2020.

[17] P. R. Gomes, M. F. Graciano, L. C. Pantaleão et al., "Long-term disruption of maternal glucose homeostasis induced by prenatal glucocorticoid treatment correlates with miR-29 upregulation," *American Journal of Physiology-Endocrinology and Metabolism*, vol. 306, no. 1, pp. E109–E120, 2014.

[18] A. Matarese, J. Gambardella, A. Lombardi, X. Wang, and G. Santulli, "miR-7 regulates GLP-1-mediated insulin release by targeting β-arrestin 1," *Cells*, vol. 9, no. 7, p. 1621, 2020.

[19] F. Zhang, D. Ma, W. Zhao et al., "Obesity-induced overexpression of miR-802 impairs insulin transcription and secretion," *Nature Communications*, vol. 11, no. 1, p. 1822, 2020.

[20] Y. Y. Li, Y. J. Zhong, Q. Cheng et al., "miR-378b regulates insulin sensitivity by targeting insulin receptor and p110α in alcohol-induced hepatic steatosis," *Frontiers in Pharmacology*, vol. 11, p. 717, 2020.

[21] Y. Zhang, J. Lu, Y. J. Zhong et al., "Methyl ferulic acid ameliorates alcohol-induced hepatic insulin resistance via miR-378b-mediated activation of PI3K-AKT pathway," *Biomedicine and Pharmacotherapy*, vol. 145, Article ID 112462, 2022.

[22] S. Bain, E. Druyts, C. Balijepalli et al., "Cardiovascular events and all-cause mortality associated with sulphonylureas compared with other antihyperglycaemic drugs: a Bayesian meta-analysis of survival data," *Diabetes, Obesity and Metabolism*, vol. 19, no. 3, pp. 329–335, 2017.

[23] D. S. Bell, "Do sulfonylurea drugs increase the risk of cardiac events?" *Canadian Medical Association Journal*, vol. 174, no. 2, pp. 185-186, 2006.

[24] D. M. Kendall, R. M. Cuddihy, and R. M. Bergenstal, "Clinical application of incretin-based therapy: therapeutic potential, patient selection and clinical use," *European Journal of Internal Medicine*, vol. 20, no. 2, pp. S329–S339, 2009.

[25] M. Packer, "Is the popularity of dipeptidyl-peptidase-4 inhibitors justified? Insights from mechanistic studies and

clinical trials," *The American Journal of Medicine*, vol. 131, no. 7, pp. e287–e289, 2018.

[26] A. J. Scheen, "DPP-4 inhibitors in the management of type 2 diabetes: a critical review of head-to-head trials," *Diabetes and Metabolism*, vol. 38, no. 2, pp. 89–101, 2012.

[27] L. Lei, Y. Huan, Q. Liu et al., "Morus alba L. (Sangzhi) alkaloids promote insulin secretion, restore diabetic β-cell function by preventing dedifferentiation and apoptosis," *Frontiers in Pharmacology*, vol. 13, Article ID 841981, 2022.

[28] H. Sun, A. Zhang, Y. Gong et al., "Improving effect of cordycepin on insulin synthesis and secretion in normal and oxidative-damaged INS-1 cells," *European Journal of Pharmacology*, vol. 920, Article ID 174843, 2022.

[29] M. M. Zhao, J. Lu, S. Li et al., "Berberine is an insulin secretagogue targeting the KCNH6 potassium channel," *Nature Communications*, vol. 12, no. 1, p. 5616, 2021.

[30] R. Liao, Z. Qi, R. Tang, R. Wang, and Y. Wang, "Methyl ferulic acid attenuates human cardiac fibroblasts differentiation and myocardial fibrosis by suppressing pRB-E2F1/CCNE2 and RhoA/ROCK2 pathway," *Frontiers in Pharmacology*, vol. 12, Article ID 714390, 2021.

[31] J. Lu, Y. Zhang, Y. Z. Wang et al., "Caffeic acid dimethyl ether alleviates alcohol-induced hepatic steatosis via microRNA-378b-mediated CaMKK2-AMPK pathway," *Bioengineered*, vol. 13, no. 4, pp. 11123–11137, 2022.

[32] H. Li, Y. Li, L. Xiang et al., "GDF11 attenuates development of type 2 diabetes via improvement of islet β-cell function and survival," *Diabetes*, vol. 66, no. 7, pp. 1914–1927, 2017.

[33] X. Xia, J. Xu, X. Wang et al., "Jiaogulan tea (Gpostemma pentaphyllum) potentiates the antidiabetic effect of white tea via the AMPK and PI3K pathways in C57BL/6 mice," *Food and Function*, vol. 11, no. 5, pp. 4339–4355, 2020.

[34] A. D. Green, S. Vasu, N. H. McClenaghan, and P. R. Flatt, "Pseudoislet formation enhances gene expression, insulin secretion and cytoprotective mechanisms of clonal human insulin-secreting 1.1B4 cells," *Pfluegers Archiv European Journal of Physiology*, vol. 467, no. 10, pp. 2219–2228, 2015.

[35] H. Guo-Parke, J. T. McCluskey, C. Kelly, M. Hamid, N. H. McClenaghan, and P. R. Flatt, "Configuration of electrofusion-derived human insulin-secreting cell line as pseudoislets enhances functionality and therapeutic utility," *Journal of Endocrinology*, vol. 214, no. 3, pp. 257–265, 2012.

[36] Y. Zheng, S. H. Ley, and F. B. Hu, "Global aetiology and epidemiology of type 2 diabetes mellitus and its complications," *Nature Reviews Endocrinology*, vol. 14, no. 2, pp. 88–98, 2018.

[37] P. Zimmet, Z. Shi, A. El-Osta, and L. Ji, "Epidemic T2DM, early development and epigenetics: implications of the Chinese Famine," *Nature Reviews Endocrinology*, vol. 14, no. 12, pp. 738–746, 2018.

[38] Q. Cheng, Y. W. Li, C. F. Yang, Y. J. Zhong, and L. Li, "Ethanol-induced hepatic insulin resistance is ameliorated by methyl ferulic acid through the PI3K/AKT signaling pathway," *Frontiers in Pharmacology*, vol. 10, p. 949, 2019.

[39] N. M. O'Meara, E. Shapiro, E. Van Cauter, and K. S. Polonsky, "Effect of glyburide on beta cell responsiveness to glucose in non-insulin-dependent diabetes mellitus," *The American Journal of Medicine*, vol. 89, no. 2, 6S pages, 1990.

[40] A. L. Kearney, D. M. Norris, M. Ghomlaghi et al., "Akt phosphorylates insulin receptor substrate to limit PI3K-mediated PIP3 synthesis," *Elife*, vol. 10, Article ID e66942, 2021.

[41] Y. Wang, H. Zhou, O. Palyha, and J. Mu, "Restoration of insulin receptor improves diabetic phenotype in T2DM mice," *JCI insight*, vol. 4, no. 15, Article ID e124945, 2019.

[42] M. L. Hribal, L. Perego, S. Lovari et al., "Chronic hyperglycemia impairs insulin secretion by affecting insulin receptor expression, splicing, and signaling in RIN β-cell line and human islets of Langerhans," *The FASEB Journal*, vol. 17, no. 10, pp. 1340–1342, 2003.

[43] T. Kitamura, J. Nakae, Y. Kitamura et al., "The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic β cell growth," *Journal of Clinical Investigation*, vol. 110, no. 12, pp. 1839–1847, 2002.

[44] S. C. Martinez, K. Tanabe, C. Cras-Méneur, N. A. Abumrad, E. Bernal-Mizrachi, and M. A. Permutt, "Inhibition of Foxo1 protects pancreatic islet β-cells against fatty acid and endoplasmic reticulum stress–induced apoptosis," *Diabetes*, vol. 57, no. 4, pp. 846–859, 2008.

[45] M. Gannon, E. Tweedie Ables, L. Crawford et al., "pdx-1 function is specifically required in embryonic β cells to generate appropriate numbers of endocrine cell types and maintain glucose homeostasis," *Developmental Biology*, vol. 314, no. 2, pp. 406–417, 2008.

[46] T. Gao, B. McKenna, C. Li et al., "Pdx1 maintains β cell identity and function by repressing an α cell program," *Cell Metabolism*, vol. 19, no. 2, pp. 259–271, 2014.

[47] H. Matsuzaki, H. Daitoku, M. Hatta, K. Tanaka, and A. Fukamizu, "Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation," *Proceedings of the National Academy of Sciences*, vol. 100, no. 20, pp. 11285–11290, 2003.

[48] P. V. Babu, D. Liu, and E. R. Gilbert, "Recent advances in understanding the anti-diabetic actions of dietary flavonoids," *The Journal of Nutritional Biochemistry*, vol. 24, no. 11, pp. 1777–1789, 2013.

[49] F. Hao, J. Kang, Y. Cao et al., "Curcumin attenuates palmitate-induced apoptosis in MIN6 pancreatic β-cells through PI3K/Akt/FoxO1 and mitochondrial survival pathways," *Apoptosis*, vol. 20, no. 11, pp. 1420–1432, 2015.

[50] Y. Zhang, L. Li, Y. Zhang, S. Yan, and L. Huang, "Improvement of lipotoxicity-induced islet β cellular insulin secretion disorder by osteocalcin," *Journal of Diabetes Research*, vol. 2022, pp. 1–15, 2022.

[51] H. Ji, L. Fan, A. Shan et al., "Let7b-5p inhibits insulin secretion and decreases pancreatic β-cell mass in mice," *Molecular and Cellular Endocrinology*, vol. 540, Article ID 111506, 2022.

[52] A. D. Mandelbaum, S. Kredo-Russo, D. Aronowitz et al., "miR-17-92 and miR-106b-25 clusters regulate beta cell mitotic checkpoint and insulin secretion in mice," *Diabetologia*, vol. 62, no. 9, pp. 1653–1666, 2019.

[53] W. Liu, H. Cao, C. Ye et al., "Hepatic miR-378 targets p110α and controls glucose and lipid homeostasis by modulating hepatic insulin signalling," *Nature Communications*, vol. 5, no. 1, p. 5684, 2014.