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Original article

Glucagon-like peptide-1 improves β-cell dysfunction by suppressing the miR-27a-induced downregulation of ATP-binding cassette transporter A1

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ABSTRACT

Lipotoxicity is considered one of the main causes of deterioration in β-cells function. Glucagon-like peptide-1 (GLP-1) has been revealed to protect and improve pancreatic β-cell function against lipotoxicity. However, the mechanism behind these is largely unknown. The aim of this study was to investigate the effects of GLP-1 on cholesterol-induced lipotoxicity in INS-1 cells and examine the underlying mechanisms. The cell viability was determined, and caspase-3 was used to assess the effects of GLP-1 on cholesterol-induced apoptosis. The alterations of miR-27a and ABCA1 resulting from incubation with cholesterol or GLP-1 were detected by real-time PCR and western blot. The inhibition and overexpression of miR-27a were established to explore the effects of a GLP-1-mediated decrease in miR-27a. Further, Oil red O staining and cholesterol measurement were used to detect lipid accumulation. The β-cells function was measured in glucose-stimulated insulin secretion. Our data shows that cholesterol significantly attenuated cell viability, promoted cell apoptosis, facilitated lipid accumulation, and impaired β-cells function, and these effects were significantly reversed by GLP-1. Furthermore, the results demonstrated that GLP-1 decreased miR-27a expression and increased the expression of ABCA1. In conclusion, GLP-1 may affect cholesterol accumulation and β-cells dysfunction by regulating the expression of miR-27a and ABCA1.

1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and a gradual deterioration in β-cell function. Lipotoxicity in which toxic lipids accumulate, is considered one of the major causes for the degradation in β-cell function [1,2]. Most studies examining the link between lipotoxicity and T2DM have focused on free fatty acids (FFAs), but the role of cholesterol in regulating β-cell function and survival is poorly understood [3,4].

ATP binding cassette transporter A1 (ABCA1) is an integral membrane protein, that transports intracellular cholesterol and phospholipid to apolipoprotein acceptors by using ATP as energy [5,6]. Hypercholesterolemic apolipoprotein E (apoE) knockout mice display impairment in insulin secretion associated with decreased islet ABCA1 expression and increased islet cholesterol [7]. Mice with a β-cell-specific inactivation of ABCA1 had notably impaired glucose tolerance and defective insulin secretion but normal insulin sensitivity [3,8]. Consequently, ABCA1-mediated cholesterol efflux is a pivotal determinant of the appropriate maintenance of both cholesterol levels and insulin secretion [9]. In this study, we investigate how GLP-1 contributes to increasing cholesterol efflux by regulating ABCA1.

Glucagon-like peptide 1 (GLP-1) as a new treatment for T2DM, not only has hypoglycemic effect, but also plays a significant role in regulating lipid metabolism [10,11]. GLP-1 plays a unique role in modulating lipid metabolism via lipid assimilation and transport, fat formation and decomposition, hepatic lipid metabolism,and cholesterol transport [12]. The apolipoprotein A-I (apo A-I) gene is considered to encode for the primary anti-atherogenic factor in high-density lipoprotein (HDL) particle. Apo A-I secretion was enhanced in both GLP-1 and exendin-4-treated HepG2 cells, and this was combined with similar changes in the ABCA1 mRNA levels [13]. Activation of the CaMKK/CaMKIV cascade by exendin-4 promoted ABCA1 gene transcription,suggesting that exendin-4 plays a crucial role in cholesterol content and insulin secretion in β-cells [14–16]. However, the mechanism by which GLP-1 improves β-cell dysfunction via ABCA1 in INS-1 cells remains unclear.

MicroRNAs (miRNAs) are a class of small (22 -nt) non-coding RNAs that are involved in the post-transcriptional regulation of their target genes in a sequence-specific manner. MiRNAs are key regulators of lipid synthesis, fatty acid oxidation and lipoprotein formation and secretion.

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Emerging evidence suggests that miRNAs are involved in lipid metabolism, including miR-33, miR-122, miR27a/b, miR378, miR-34a, miR-370 and miR-21 [17–20]. MiR-27a/b targets the 3′UTR of ABCA1 and downregulates the expression of this gene [21]. Many studies have identified significant roles for miR-27a/b in lipid metabolism [22,23], inflammation [24], adipogenesis, oxidative stress and insulin resistance, which play important roles in T2DM [25]. This study investigates the efficacy of GLP-1 on improving β-cells cholesterol metabolism and secretion function through miR-27a/ABCA1 in INS-1 cells.

2. Materials and methods

2.1. Cell culture

The INS-1 pancreatic β-cell line derived from rat insulinoma (purchased from the basic medical institute of Chinese Academy of Medical Sciences,China) was cultured as previously described [26], in RPMI 1640 medium (HyClone, USA) containing 11.1 mM glucose and supplemented with 10% foetal bovine serum (GIBCO, USA), 10 mmol/L HEPES, 2 mmol/L L-glutamine,1 mmol/L sodium pyruvate, 55 mol/L beta-mercaptoethanol, 100 IU/ml penicillin, and 100 g/ml streptomycin. The cells were cultured at 37 °C in a 5% CO_2 environment.

2.2. Lipotoxicity and GLP-1 incubation

The cells were cultured after 12 h, treated or not treated with 5 mmol/L soluble cholesterol (Sigma, USA) medium to exert a lipotoxicity effect for 24 h, and then maintained with or without 10 nmol/L GLP-1 (Sigma, USA) for 24 h.

2.3. Measurement with the cell counting kit-8 (CCK-8)

This assay was assessed by cultivating INS-1 cells in 96-well plates at a density of 5000 cells/well for 24 h. The cells were exposed to various concentrations of cholesterol (1.0, 2.5, 5 and 10 mmol/L), with or without 10 nmol/L GLP-1 for 24 h. After replacing the RPMI 1640 medium, 10 μl of CCK-8 reagent (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to each well, and the 96-well plate was incubated in the dark at 37 °C for 2 h. The absorbance was measured at 450 nm in a microplate reader.

2.4. Caspase 3 analysis

The expression of caspase-3 is enhanced when cell apoptosis occurs and represents the degree of apoptosis to some extent. The activation level of caspase-3 was detected to explore cholesterol-induced apoptosis using the caspase-3 Activity Kit (Solarbio, China). In brief, after extraction of total cell proteins of each group, 10 μl protein was incubated with 90 μl of provided reaction buffer and 10 μl Ac-DEVD-pNA (2 mM) in 96-well plates at 37 °C for 2 h. Then, the reaction mixtures were measured at 405 nm in a microplate reader.

2.5. MiRNA transfection

The INS-1 cells were plated until they were 50% confluent at the time of transfection. Oligonucleotide analogue and inhibitor which were chemically modified and synthesized were used to increase and decrease the expression of miR-27a in INS-1 cells. The microRNA-27a inhibitor, microRNA-27a mimics, microRNA NC-FAM (GenePharm Co. Ltd, China) and lipofectamine™ 3000 (Invitrogen, USA) were diluted in serum-free RPMI 1640 without antibiotics prior to being incubated at room temperature for 5 min. Then, diluted microRNAs were added to each tube of diluted Lipofectamine™ 3000 Reagent (1:1 ratio) separately. The oligonucleotide-lipo complexes were added to the cells for 24 h. Then cholesterol and GLP-1 were taken every other 24 h before further analysis. The sequences of mimics and inhibitors used are shown below: microRNA-27a mimics, sense 5′- UUCACAGUGGCUAAGUUCCGC -3′; antisense 5′- GGAACUUAGCCACUGUGAAUU -3′; microRNA-27a inhibitor, 5′- GCGGAACUUAGCCACUGUGAA -3′; microRNA NC-FAM, sense 5′- UUCUCCGAACGUGUCACGUTT -3′; antisense 5′- ACGUGACACGUUCGGAGAATT -3′.

2.6. RNA extraction and quantitative PCR

Total RNA was extracted from INS-1 cells using the Trizol reagent (Invitrogen, Waltham, USA). Reverse transcription was performed using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany). qRT-PCR reactions were carried out using the Fast Start Universal SYBR Green Master (Roche, Germany) with CFX96 real time PCR detection system (Bio-Rad, USA). U6 small RNA and GAPDH were used as the reference gene. Each reaction was carried out in triplicate, and the qRT-PCR results were calculated using the $2^{-\Delta\Delta Ct}$ method [27]. The primer sequences were as follows: ABCA1, forward (5′- AATGGTCAATGGGAGGTTCA -3') and reverse (5′- TGGACAGGCTTTAGGTCAGG -3'); GAPDH, forward (5′- GCCAGCCGAGCCACAT -3') and reverse (5′- GGATCTCGCTCC TGGAAGAT -3'); rno-miR-27a, RT primer (5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCGGAA −3'), forward (5′- ATTGGCGGTTCACAGTGGCTAAG −3') and reverse (5′- ATCCAGTGCAGGGTCCGAGG −3'); U6, RT primer (5′- CGCTTCACGAATTTGCGTGTCAT −3'), forward (5′- GCTTCGGCAGCA CATATACTAAAAT -3') and reverse (5′- CGCTTCACGAATTTGCGT GTC AT -3').

2.7. Protein extraction and Western blotting

For protein extraction, the INS-1 cells were lysed with 200 μl of modified RIPA Lysis Buffer (Beyotime institute of Biotechnology, China) containing 1% PMSF (Beyotime institute of Biotechnology, China) on ice. The proteins were quantified using the BCA method. Subsequently, 30 μg of proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes (GE Healthcare, USA). The membrane was incubated with primary antibodies to ABCA1 (1:500, ab18180) or GAPDH (1:10000, ab181602) (Abcam, USA). After the secondary antibody (Cell Signaling Technology, Danvers, USA) was probed for 2 h, the blots were developed using an enhanced chemiluminescence kit (Beyotime institute of Biotechnology, China).

2.8. Oil red O staining

For lipid droplets observation, the INS-1 cells were plated in 6-well plates. After stimulation with cholesterol and GLP-1 for 24 h, each group was rinsed three times in PBS, fixed in 4% paraformaldehyde for 30 min, stained in freshly diluted oil red O for 15 min, decolorized in 70% ethanol solution for 15 s, re-dyed in haematoxylin staining solution for 30 s and rinsed in PBS twice. Finally, the intracellular lipid droplets were observed and photographed with an inverted microscope (Leica, Germany).

2.9. Cholesterol manipulation

The cholesterol content was quantitated in INS-1 cells using a cholesterol quantitation kit (BioVision, USA) according to the manufacturer's instructions. Briefly, 1×10^6 cells were extracted with 200 μl of chloroform: isopropanol: NP-40 (7:11:0.1) by a microhomogenizer. These lipid extracts were dried for 30 min in a vacuum and the residues were dissolved in 200 μl of cholesterol assay buffer by vortexing until the solution became cloudy. The reactions containing the cholesterol probe, enzyme mix, esterase, assay buffer and samples or standards were incubated at 37 °C for 1 h. Then the absorbance of extraction was measured at 570 nm in a microplate reader [28].