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Original Research

Up-regulation of receptor interaction protein 140 promotes glucolipotoxicity-induced damage in MIN6 cells

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Abstract: The receptor interaction protein 140 (RIP140) cofactor is a key regulator of metabolic balance, but its function in glucose- and lipid-mediated damage in islet β cells is unknown and was investigated in this study. RIP140 expression and distribution was evaluated in MIN6 cells under high glucose and lipid conditions using real-time Polymerase Chain Reaction (PCR), western blotting and confocal laser scanning microscopy. Cells were separately treated with 500 μM palmitic acid and 25 mM glucose when RIP140 expression was upregulated or downregulated, and cell viability, apoptosis rate, the level of oxidative stress and insulin secretion was assessed, as was the expression of related genes. Increased glucose and palmitic acid elevated RIP140 expression and distribution in nuclei. Overexpression of RIP140 promoted apoptosis but inhibited cell viability in MIN6 cells, and basal insulin secretion and glucose-stimulated insulin secretion levels were altered following treatment with glucose and palmitic acid. In addition, oxidative stress was elevated, phosphorylated extracellular signal-regulated kinases 1/2 and uncoupling protein 2 messenger RNA (mRNA) abundance were increased, B-cell lymphoma-2 protein levels were decreased, and peroxisome proliferators activated receptor gamma co-activator 1 alpha, phosphoenolpyruvate carboxykinase , and pancreatic and duodenal homeobox-1 mRNA levels were downregulated. Furthermore, glucolipotoxicity-induced damage was reversed when RIP140 expression was downregulated by small interfering RNA (SiRNA). RIP140 promotes islet β cells damage caused by glucolipotoxicity.

Key words: Receptor interaction protein 140; Islet β cell; Glucolipotoxicity; Oxidative damage; Expression analysis.

Introduction

Exposure to excessive glucose and lipid, termed glucolipotoxicity, is the most important factor that accelerates the loss of islet β cell function, culminating in diabetes (1). Although the exact mechanism is still not fully understood, recent research suggests the damage caused by glucolipotoxicity involves oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction and proinflammatory cytokines (2). Devising a way to alleviate glucolipotoxicity in islet β cells is a major focus of diabetic-related research around the world (3). The role of cofactors such as coactivators and corepressors in metabolism is under investigation, since many cofactors are seen as potential therapeutic targets to treat or prevent metabolic disturbance (4). Cofactors mainly bind to nuclear receptors such as peroxisome proliferator-activated receptors (PPARs), retinoic acid receptor-related orphan receptors, and estrogen receptors in order to mediate specific chromatin modifications that can either activate or repress the transcription of metabolic pathway-related genes that monitor glucose and lipid levels (5). Different cofactors are recruited to determine the function of nuclear receptors in metabolic pathways. Coactivators mostly increase the expression of genes related to energy consumption, while corepressors mainly upregulate genes associated with energy storage (5).

Receptor interaction protein 140 (RIP140) is a corepressor (5) and multifaceted coregulator of metabolic control (6) that binds to nuclear receptors such as liver X receptor (7), PPARs (8) and estrogen-related receptors (9), thereby regulating downstream gene expression. The expression of genes related to fatty acid oxidation are upregulated in RIP140 knockout mice. Moreover, genes associated with oxidative phosphorylation, mitochondria, and the Krebs cycle are also stimulated (5). RIP140 may control glucose metabolism by regulating gluconeogenesis and glucose uptake-related molecules such as glucose transporter 1 (10), glucose transporter 4 (10,11) and phosphoenolpyruvate carboxykinase (PEPCK) (12). Low levels of RIP140 increases basal fatty acid uptake and insulin-treated fatty acid oxidation (13). RIP140 knockout mice fed a high-fat diet display improved insulin resistance, and subsequent increased insulin stimulates glucose uptake and suppress the abnormal glucose tolerance induced by age and diet (14). Reducing RIP140 expression in macrophages could prevent high-fat diet-induced insulin resistance (15). RIP140 is expressed in islet β cells and regulates β cell function and proliferation (16). However, RIP140 expression and function in islet β cells exposed to high glucose and lipid remain poorly characterized. In this study, we found that expression of RIP140 is increased when islet β cells are treated with high glucose and lipid. In addition, increasing RIP140 expression aggravated glucolipotoxicity-induced islet β cell damage, while

lowering RIP140 expression reversed this damage.

Materials and Methods

Cell culture and treatment

GFP-MIN6 cells were generated by transfecting MIN6 cells with pEGFP-N1, while O-RIP140-MIN6 cells were produced by transfecting MIN6 cells with pEGFP-N1-RIP140 (16). MIN6, GFP-MIN6 and O-RIP140-MIN6 cells were cultured with low-sugar Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, Utah, USA) containing 10% (v/v) fetal bovine serum (Thermo, Massachusetts, USA), 0.001% (v/v) β-mercaptoethanol, 100 U/mL penicillin and 100 mg/mL streptomycin at 37°C. Targeted RIP140 small interfering RNA (siRNA; RiboBio, Guangzhou, China) (forward 5′CGGCGUUGACAUCAAAGAAdTdT3′, Reverse 3′dTdTGCCGCAACUGUAGUUUCUU 5′) was transfected using Lipofectamine 2000 (Thermo, Massachusetts, USA) according to the manufacturer's protocol(16). Scramble siRNA (RiboBio Co. Ltd, Guangzhou,China) was used as a negative control. Six groups (control, positive control, O-RIP140-MIN6, GFP-MIN6, RIP140 siRNA and Scramble siRNA) were included in the experiment. Cells were seeded into six-well plates for 24 h of pre-culturing. Except for the control group, all other groups were treated with 250 μM palmitic acid and 25 mM glucose, or 500 μM palmitic acid and 25 mM glucose for 24h, depending on the experiment, to mimic the diabetic condition.

Immunocytochemistry

MIN6 cells, which were seeded on poly-D-Lys-covered slides in six-well plates, were treated with 500 μM palmitic acid and 25 mM glucose and washed with cold phosphate buffer saline (PBS). Cells were fixed with 4% paraformaldehyde solution for 10 min at 37C. After further washing with PBS, cells were permeabilized with 0.1% Triton X-100 for 10 min and blocked with 1% bovine serum albumin (BSA) in PBS for 1 h. Primary antibodies against RIP140 (Abcam, ab42126 1:500) were incubated overnight at 4°C. After washing three times with PBS, cells were exposed to secondary antibody for 1 h at room temperature. To visualize nuclei, cells were stained with 4',6-diamidino-2-phenylindole (DAPI). Immunofluorescence images were captured using appropriate filters with a confocal laser scanning microscope.

Detection of oxidative stress and apoptosis

After treatment of 500 μM palmitic acid and 25 mM glucose, cells were centrifuged and collected to detect the oxidation-related enzymes. The activities of total-superoxide dismutase (T-SOD) and malondialdehyde (MDA) were determined using appropriate kits (Jiancheng Bioengineering institute, Nanjing, China). T-SOD activity was assayed using the xanthine/xanthine oxidase method, and cellular MDA was measured using the thiobarbituric acid method. The activities of T-SOD and MDA were separately expressed using U/mg protein and nmol/mg cellular protein, respectively.

For apoptotic cells assays, Annexin V-PE and 7-aminoactinomycin D staining (KeyGen Biotech, China) was used. Briefly, treated cells were resuspended in PBS at a density of 5×105 cells /ml. Samples were stained with Annexin V-PE in the dark for 10 min at room temperature. After washing with PBS, cells were stained with 7-aminoactinomycin D. Finally, samples were analyzed using flow cytometer.

Insulin secretion assay

Cells from each group were separately washed twice with cold PBS, then incubated with krebs ringer bicarbonate buffer (115 mM NaCl, 24 mM NaHCO3, 5 mM KCl, 1 mM MgCl2, 25 mM HEPES, 0.5% BSA, pH 7.4) containing 2 mM glucose at 37°C. After 30 min, cells were incubated with fresh krebs ringer bicarbonate buffer containing 20 mM glucose for 60 min. The supernatant was collected and total cellular protein was extracted. Insulin secretion levels in the supernatant were detected by ELISA (Cusabio, China) and normalized against total cellular protein levels. Cellular protein levels were determined using the bicinchroninic acid method.

Measuring mRNA and protein levels

Total RNA from each group was extracted and reverse transcribed to cDNA. Real-time reverse transcription polymerase chain reaction was used to detect mRNA abundance with a SYBN Green PCR kit (Takara, China), and β-actin was used as an internal reference. Differences in mRNA expression in each sample were evaluated by the 2-ΔΔCt method (17). The abundance of uncoupling protein 2 (*UCP-2*) mRNA was tested by a semi-quantitative method. The following primers were used: *RIP140* (NM_173440.2, 207 bp) forward = 5′-GGCAGCAAACCTGAATTCGGC-3′, reverse = 5′-CT CACCGGGCACGGAACATC-3′; *UCP2* (NM_011671.4, 149 bp) forward = 5′-GCATTGGCCTCTACGACTCT-3′, reverse = 5′-CTGGAAGCGGACCTTTACC-3′; peroxisome proliferators activated receptor gamma co-activator 1 alpha (*PGC-1α*) (NM_008904.2, 102 bp) forward = 5′-GTGTTCCCGATCACCATATTCC-3′, reverse = 5′-CG GTGTCTGTAGTGGCTT GATTC-3′; pancreatic and duodenal homeobox-1 (*PDX-1*)(NM_008814.3, 134 bp) forward = 5′-GATGAAATCCACCAAAGCTCA-3′, reverse = 5′-AGAATTCCTTCTCCAGCTCCA-3′; *PEPCK* (NM_011044.2, 199 bp) forward = 5′-ATCTTTGGTGGCCGTAGACCT-3′, reverse = 5′-GCCAGTGGGCCAG GTATTT-3', *β-actin* (NM_007393.3, 314 bp) forward = 5'-TC-TACAAT GAGCTGCGTGTG-3′, reverse = 5′-GGTGAGGATCTTCATGAGGT-3′.

Protein expression was analyzed using western blotting. Equal amounts of protein were separated by 10% sodium dodecyl sulfate polyacrylamide gel electropheresis and transferred onto polyvinylidene difluoride membranes. Membranes were blocked and separately incubated with the following primary antibodies: anti-RIP140 polyantibody (ab42126 Abcam Cambridge, United Kingdom, 1:500), anti-B-cell lymphoma-2 (Bcl-2) polyantibody (bs70205 Bioworld, Minnesota, USA , 1:500), anti-extracellular signal-regulated kinases 1/2 (ERK1/2) polyantibody (#4695 Cell Signaling Technology, Massachusetts, USA, 1:1500), anti-pERK1/2 polyantibody (#4376 Cell Signaling Technology, Massachusetts, USA, 1:1500), anti-fatty acid binding protein

4 (FABP4) polyantibody (sc-18661 Santa Cruz, Texas, USA, 1:1000) and anti-β-actin polyantibody (sc-1615 Santa Cruz, Texas, USA, 1:1000). After incubation with secondary antibody conjugated to horseradish peroxidase, the signal was measured using enhanced chemiluminescence (Thermo, Massachusetts, USA). The intensity of bands was analyzed with AlphaEaseFC 4.0 software (Alpha, California,USA). β-actin densitometric values served as a reference.

Statistical analysis

All data were analyzed by SPSS 17.0 software. Values were presented as means ± standard deviation(S.D.). Significance was detected by two-tailed t test for two-group comparisons. Differences were considered significant at $p<0.05$ or $p<0.01$.

Results

RIP140 expression and distribution in islet β cells following glucolipotoxicity

Following exposure to 250 μM palmitic acid and 25 mM glucose, or 500 μM palmitic acid and 25 mM glucose, the results of real-time PCR and western blotting showed that RIP140 was obviously increased at both mRNA and protein levels compared with controls (Figure 1), and the increase was greater with higher concentrations of glucose and lipid. Accordingly, RIP140 expression in the nucleus was also elevated compared with controls following treatment with 500 μM palmitic acid and 25 mM glucose (Figure 2).

Analysis of islet β cell viability

When MIN6 cells were treated with 250 μM palmitic acid and 25 mM glucose, the results of cell viability showed that the value of absorbance 570nm was decreased compared with controls (Figure 3A). When palmitic acid was increased to 500 μM (and glucose remained at 25 mM glucose), the value of absorbance 570nm was further repressed (Figure 3A). This decline was both time- and dose-dependent. The viability differed significantly at corresponding time points following both treatments. Methyltetrazolium absorption was also decreased significantly in O-RIP140-MIN6 cells after exposure to 500 μM palmitic acid and 25 mM glucose

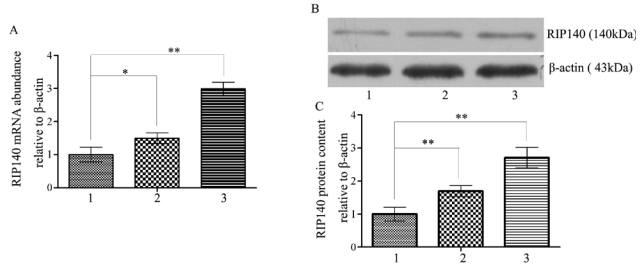

Figure 1. RIP140 expression in MIN6 cells under the condition of excessive glucose and lipid. (A) Analysis of RIP140 mRNA levels using real-time PCR. (B) Analysis of RIP140 protein levels using western blotting. (C) Relative quantification of RIP140 protein. 1, MIN6 cells; 2, MIN6 cells treated with 250 μM palmitic acid and 25 mM glucose; 3, MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose. * $p<0.05$, ** $p<0.01$. Data show mean±S.D. of three independent experiments RIP140 receptor interaction protein 140 S.D. standard deviation.

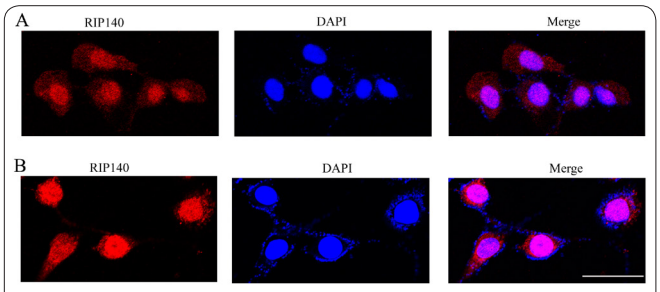

Figure 2. Distribution of RIP140 in MIN6 cells under the condition of excessive glucose and lipid using confocal laser scanning microscopy. (A) MIN6 cells. (B) MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose. Scale bars:20 μm. RIP140 receptor interaction protein 140.

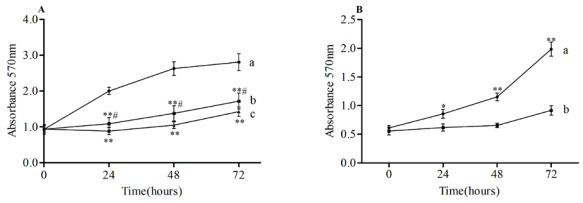

Figure 3. Analysis of MIN6 cell viability under the condition of excessive glucose and lipid. (A) MIN6 cell viability in the presence of different concentrations of palmitic acid and glucose. a, controls; b, MIN6 cells treated with 250 μM palmitic acid and 25 mM glucose; c, MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose. a vs. b or c ** $p<0.01$; b vs. c # $p<0.05$ (B) Effect of overexpression of RIP140 on MIN6 cell viability. a, GFP-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; b, O-RIP140-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose. a vs. b * $p<0.05$ ** $p<0.01$ Data show mean±S.D. of three independent experiments RIP140 receptor interaction protein 140 S.D. standard deviation.

compared with control GFP-MIN6 cells from 0 h to 72 h (Figure 3B). Overexpression of RIP140 therefore appeared to inhibit the viability of MIN6 cells.

Detection of oxidative stress and apoptosis

A high concentration of lipid and glucose can increase oxidative stress (3), and T-SOD and MDA are two classical indicators. In the present study, the 500 μM palmitic acid and 25 mM glucose treatment lowered SOD levels (3.66 ± 0.44 U/mg vs. 14.54 ± 0.95 U/mg, $p<0.01$) but raised MDA levels (9.09 ± 1.06 nmol/mg vs. 5.40 ± 0.64 nmol/mg, $p<0.05$) compared with controls. Increasing RIP140 levels in O-RIP140-MIN6 cells could further prompt oxidative stress. The results showed that SOD was decreased (3.88 ± 0.28 U/mg vs. 6.79 ± 0.17 U/mg, $p<0.05$) and MDA was increased (14.39 ± 1.32 nmol/mg vs. 10.64 ± 1.52 nmol/mg, $p<0.05$) when O-RIP140-MIN6 cells were incubated with 500 μM palmitic acid and 25 mM glucose, compared with GFP-MIN6 cells. Downregulation of RIP140 using RIP140 SiRNA lowered MDA levels (6.38 ± 2.29 U/mg vs. 16.02 ± 1.02 U/mg, $p<0.01$) and raised SOD levels (11.31 ± 0.35 nmol/mg vs. 8.29 ± 0.55 nmol/mg, $p<0.05$) following treatment with 500 μM palmitic acid and 25 mM glucose, compared with control cells treated with scramble siRNA.

Increased oxidative stress is associated with the apoptosis of islet β cells (2). When MIN6 cells were treated with 500 μM palmitic acid and 25 mM glucose, the rate of apoptosis was significantly increased from

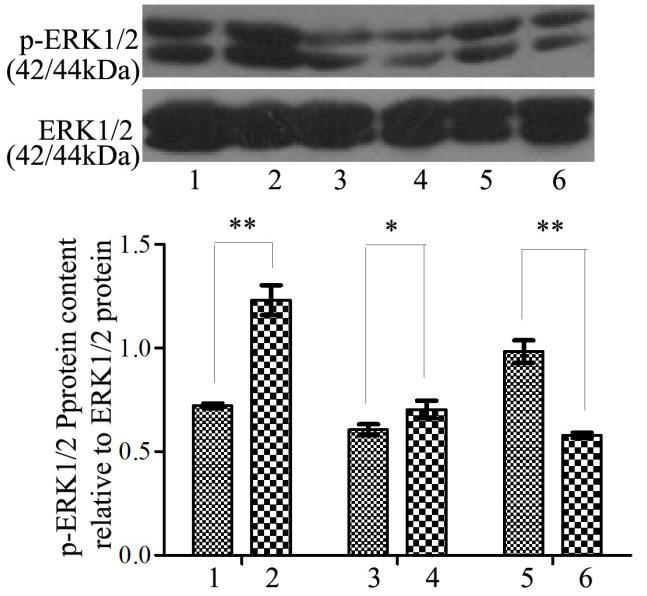

Figure 4. Phosphorylation of ERK1/2 in MIN6 cells under the condition of excessive glucose and lipid. 1, control group; 2, positive control group treated with 500 μM palmitic acid and 25 mM glucose; 3, GFP-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 4, O-RIP140-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 5, Scramble SiRNA group treated with 500 μM palmitic acid and 25 mM glucose; 6, RIP140 SiRNA group treated with 500 μM palmitic acid and 25 mM glucose. * p <0.05, ** p <0.01. up.representative result; down. relative quantification of protein. Data show mean±S.D. of three independent experiments ERK extracellular signal-regulated kinases, RIP140 receptor interaction protein 140, S.D. standard deviation.

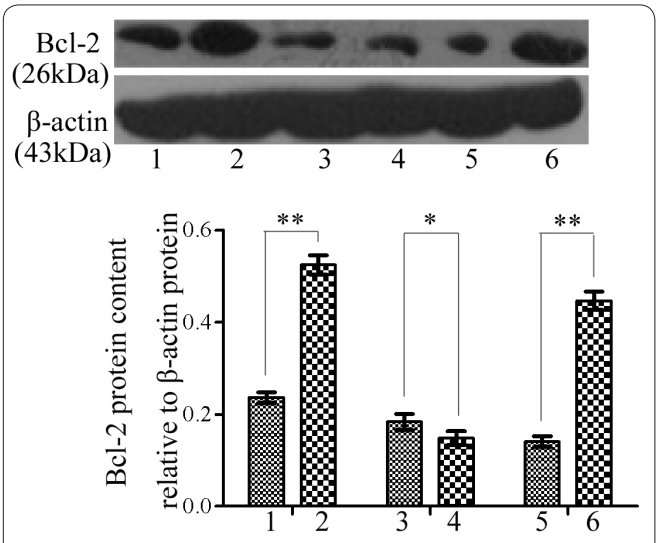

Figure 5. Bcl-2 expression in MIN6 cells under the condition of excessive glucose and lipid. 1, positive control group treated with 500 μM palmitic acid and 25 mM glucose; 2, control group; 3, GFP-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 4, O-RIP140-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 5, Scramble SiRNA group treated with 500 μM palmitic acid and 25 mM glucose; 6, RIP140 SiRNA group treated with 500 μM palmitic acid and 25 mM glucose. * p <0.05, ** p <0.01. up.representative result; down. relative quantification of protein. Data show mean±S.D. of three independent experiments. Bcl-2 B-cell lymphoma-2, RIP140 receptor interaction protein 140, S.D. standard deviation.

8.13 ± 2.29% to 23.37 ± 3.15% (p <0.01). When RIP140 expression was further elevated using O-RIP140-MIN6 cells, apoptosis was increased following treatment with 500 μM palmitic acid and 25 mM glucose (32.07 ± 2.18% vs. 21.84 ± 1.02%, p <0.05). Conversely, downregulation of RIP140 using RIP140 siRNA versed this increase in apoptosis (10.27 ± 2.77% vs. 18.43 ± 2.33%; p <0.05).

Consistent with an increase in apoptosis under conditions of high lipid and glucose, phosphorylated-ERK1/2 levels relative to ERK1/2 were increased (Figure 4), and Bcl-2 protein expression relative to β-actin was decreased (Figure 5) compared with the control group. Phosphorylated-ERK1/2 (Figure 4) was further increased in O-RIP140-MIN6 cells and GFP-MIN6 cells, and Bcl-2 expression was further decreased (Figure 5). Furthermore, a significant down-regulation in phosphorylated- ERK1/2 (Figure 4) and up-regulation in Bcl-2 expression (Figure 5) was observed in MIN6 cells treated with RIP140 siRNA compared with cells treated with scramble siRNA.

Effect of RIP140 on basal and glucose-stimulated insulin secretion

Previous results showed that RIP140 does not impact insulin secretion following treatment with 5.6 mM glucose (16). Glucolipotoxicity is known to damage the insulin secretion function of islet β cells, and suppress basal insulin secretion (BIS) and glucose-stimulated insulin secretion (GSIS) (2). In the present study, under conditions of high lipid and glucose, insulin levels were inhibited (BIS = 5.47 ± 0.37 vs. 13.74 ± 0.75 nIU/mg protein, p <0.01; GSIS = 6.76 ± 0.39 vs. 19.53 ± 0.75 nIU/mg protein, p <0.01) compared with controls. When O-RIP140-MIN6 and GFP-MIN6 cells were treated with 500 μM palmitic acid and 25 mM glucose, insulin levels were significantly different between the two groups (BIS = 4.68 ± 0.24 vs. 5.57 ± 0.27 nIU/mg protein, p <0.05; GSIS = 6.50 ± 0.40 vs. 7.45 ± 0.40 nIU/mg protein, p <0.05). When RIP140 expression was lowered using RIP140 siRNA, insulin levels clearly improved (BIS = 8.29 ± 0.18 vs. 2.55 ± 0.45 nIU/mg protein, p <0.01; GSIS = 11.56 ± 0.52 vs. 4.56 ± 0.19 nIU/mg protein, p <0.01) compared with the Scramble SiRNA group. Consistent with the changes in insulin secretion following exposure to high lipid and glucose, *PDX-1* mRNA levels relative to β-actin were lower (Figure 6). Furthermore, when RIP140 was overexpressed, *PDX-1* mRNA levels were further diminished compared with GFP-MIN6 cells (Figure 6). *PDX-1* mRNA levels were increased by RIP140 SiRNA-mediated downregulation of RIP140 expression compared with the Scramble SiRNA group (Figure 6).

Regulation of RIP140 influences the expression of metabolic genes

PGC-1α and *UCP2* are mainly involved in the oxidative respiratory chain (10). Following treatment with 500 μM palmitic acid and 25 mM glucose, *UCP2* mRNA expression was significantly increased, but *PGC-1α* mRNA levels were not obviously decreased (Figure 6). When RIP140 expression was elevated, *PGC-1α* and *UCP2* mRNA levels were markedly lower in O-RIP140-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose compared with GFP-MIN6 cells (Figure 6). However, downregulation of RIP140 expression

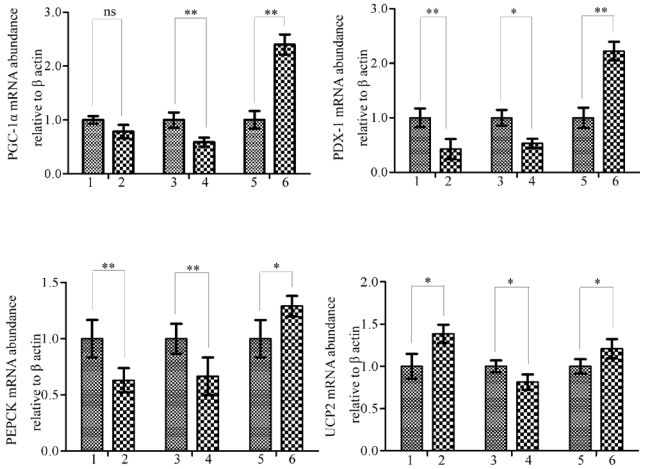

Figure 6. Expression levels of RIP140-related metabolic genes under the condition of excessive glucose and lipid. 1, control group; 2, positive control group treated with 500 μM palmitic acid and 25 mM glucose; 3, GFP-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 4, O-RIP140-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 5, Scramble SiRNA group treated with 500 μM palmitic acid and 25 mM glucose; 6, RIP140 SiRNA group treated with 500 μM palmitic acid and 25 mM glucose. * $p < 0.05$, ** $p < 0.01$. Data show mean±S.D. of three independent experiments. RIP140 receptor interaction protein 140, PGC-1α peroxisome proliferators activated receptor gamma coactivator 1 alpha, PDX-1 pancreatic and duodenal homeobox-1, PEPCK phosphoenolpyruvate carboxykinase, UCP2 uncoupling protein 2, S.D. standard deviation.

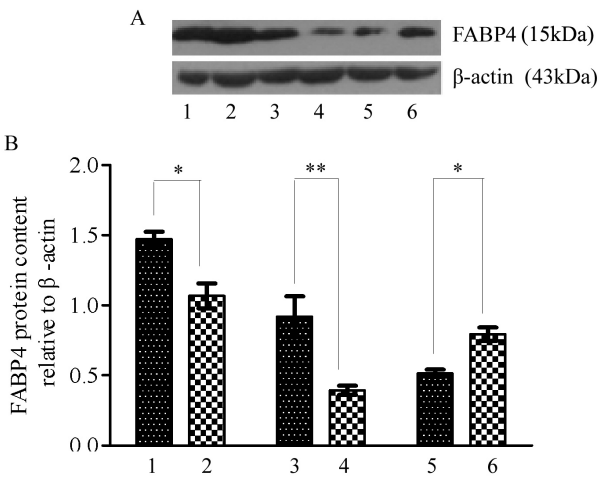

Figure 7. FABP4 expression in MIN6 cells under the condition of excessive glucose and lipid. 1, control group; 2, positive control group treated with 500 μM palmitic acid and 25 mM glucose; 3, GFP-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 4, O-RIP140-MIN6 cells treated with 500 μM palmitic acid and 25 mM glucose; 5, Scramble SiRNA group treated with 500 μM palmitic acid and 25 mM glucose; 6, RIP140 SiRNA group treated with 500 μM palmitic acid and 25 mM glucose. * $p < 0.05$, ** $p < 0.01$. A.representative result B. relative quantification of protein. Data show mean±S.D. of three independent experiments RIP140 receptor interaction protein 140, FABP4 fatty acid binding protein 4, S.D. standard deviation.

increased the expression of *PGC-1α* and *UCP2* (Figure 6). When RIP140 expression was increased in MIN6 cells using the pEGFP-N1-RIP140, FABP4 protein levels and *PEPCK* mRNA levels were further decreased in O-RIP140-MIN6 cells and GFP-MIN6 cells under conditions of high lipid and glucose (Figure 6 and 7). Conversely, the levels of FABP4 protein and *PEPCK* mRNA were increased when RIP140 expression was reduced using SiRNA compared with Scramble SiRNA controls (Figure 6 and 7).

Discussion

Damage to Islet β cells is an important factor in the development of type 2 diabetes (18), and glucolipotoxicity is the main cause of this disease. Glucolipotoxicity-induced damage is mainly associated with oxidative stress, generation of reactive oxygen species, mitochondrial dysfunction, local inflammation, and the accumulation of toxic products of metabolism such as ceramide (1). RIP140 is a nuclear receptor cofactor mainly located in metabolic- and reproductive-related tissues such as liver, adipose tissue, muscle and uterus (6). In myocytes, RIP140 inhibits mitochondrial biogenesis, fatty acid β-oxidation, oxidative phosphorylation, and the TCA cycle (13,19). In hepatocytes, RIP140 is both a corepressor and a coactivator that prompts lipogenesis and inhibits gluconeogenesis by regulating the transcription of LXRα-dependent genes (6,7). Our previous research showed that RIP140 is also located in islet β cells, and it regulates their proliferation (16). Consistent with previous research, our study also demonstrated that stimulation from high lipid and glucose can decrease proliferation and increase apoptosis in islet β cells (20). When islet β cells were treated with high lipid and glucose in the present study, RIP140 expression in MIN6 cells was increased, and its abundance in the nucleus was also significantly increased, compared with controls. The role of intranuclear localization of RIP140 is related to its corepressor activity (21). Therefore, it is possible that increasing the intranuclear distribution of RIP140 plays a more inhibitory role in islet β cells. Subsequent results showed that increasing RIP140 expression further aggravated β cell damage, while decreasing RIP140 expression reversed the damage.

A reduced β cell number can accelerate the development of diabetes and increasing RIP140 expression can trigger the mitochondrial apoptotic pathway (10). When treated with high lipid and glucose, the apoptosis rate of islet β cells was aggravated by increased RIP140 expression. Conversely, downregulation of RIP140 significantly reduced apoptosis. RIP140 inhibits PGC-1α expression in islet β cells, and RIP140 and PGC-1α regulate the expression of genes related to mitochondrial function (6,22). Palmitic acid increases oxidative stress in L6 muscle cells by inhibiting PGC-1α expression (23), and oxidative stress induces islet β cell apoptosis (24). Our results showed that when MIN6 cells were treated with high concentrations of free fatty acids and glucose, oxidative stress and RIP140 were both significantly increased. When RIP140 was up- or down-regulated, oxidative stress was increased or decreased accordingly. RIP140-mediated abnormal oxidative stress is one of the possible reasons for glucolipotoxicity-induced islet β cell apoptosis. ERK1/2 in the MAPK pathway regulates cellular apoptosis, and its activation induces apoptosis (25). RIP140 overexpression can increase phosphorylated ERK1/2 levels (26). Our results showed that RIP140 regulates ERK1/2 activation, and the apoptosis-associated downstream protein Bcl-2 (27)

was changed accordingly. It is therefore reasonable to speculate that RIP140 affects islet β cell apoptosis by regulating ERK1/2 activation and Bcl-2 expression.

Impairment of insulin secretion is another key element for the development of type 2 diabetes. When pancreatic β cells are exposed to high concentrations of free fatty acids and glucose, BIS and GSIS levels were significantly decreased (28), and when RIP140 is regulated, insulin secretion can be altered. Fatty acid and glucose-induced oxidative stress can inhibit insulin secretion (28). Our results showed that altering RIP140 could change the level of oxidative stress. Consistent with a decrease in oxidative stress, BIS and GSIS levels were increased. Therefore, it is reasonable to speculate that increasing RIP140 expression could inhibit insulin secretion under conditions of high fatty acid and glucose by changing the oxidative stress level. PGC-1α and UCP2 are mainly involved in the oxidative respiratory chain, and UCP2 is important for oxidative stress (29). UCP2 expression in gastrocnemius muscle was increased in RIP140 knockout mice (19). Our results suggest that high free fatty acids and glucose increased the expression of UCP2, and up-regulation of RIP140 decreased UCP2 expression in MIN6 cells.

Some researchers still dispute the role of UCP2 in islet β cells. UCP2 can attenuate GSIS levels (30), and it is a marker of mitohormesis and may be associated with both regulatory and protective roles in islet β cells (29). Our current results also showed that lower RIP140 levels could increase UCP2 expression in MIN6 cells, but an increase in UCP2 in MIN6 cells did not ultimately attenuate insulin secretion or increase islet β cell damage. Other genes that improve insulin secretion may be involved in RIP140-mediated regulation. Indeed, our results suggest PDX-1 may be responsible for the protective effects. PDX-1 is an important factor for pancreas development and maintaining β cell number and function. Reduced β cells mass and decreased insulin secretion is largely responsible for diabetes in PDX-1-haploinsufficient mice (31). Consistent with decreased insulin secretion and increased RIP140 expression, PDX-1 expression was inhibited in MIN6 cells under conditions of high fatty acid and glucose. When RIP140 expression was altered, PDX-1 expression was also altered in the opposite direction; downregulation of RIP140 increased PDX-1 expression. Therefore, regulation of PDX-1 expression may be another factor responsible for the role of RIP140 in the regulation of insulin secretion.

Local abnormal lipid and glucose metabolism is responsible for β cell damage in type 2 diabetes patients (24). Abnormal regulation of metabolic genes is involved, and nuclear receptor cofactors such as the coactivator PGC-1α and the corepressor RIP140 are considered critical transcriptional cofactors for controlling metabolic gene expression. RIP140 could serve as a scaffold or platform for the docking of additional cofactors such as PGC-1α (6,32), PCAF (33), and E2F1 (34) to exert antagonism or synergy (35). RIP140 and PGC-1α are involved in multiple cellular processes, including mitochondrial biogenesis and respiration, fatty acid oxidation, glucose uptake, gluconeogenesis, glycogenolysis, peroxisomal remodeling, oxidative phosphorylation, and muscle fiber-type switching (32). RIP140 is a major stimulator of energy storage, whereas PGC-1α promotes energy expenditure. Consistent with a previous report (35), our current results showed that RIP140 could repress expression of PGC-1α in islet β cells.

Inhibition or deletion of the lipid transport protein FABP4 can increase intracellular free fatty acids (36), while PEPECK is a major enzyme involved in the regulation of gluconeogenesis. Our results demonstrated that RIP140 could regulate both FABP4 and PEPCK, suggesting RIP140 may control local glucose and lipid metabolism through regulating downstream metabolic genes in islet β cells. Some downstream metabolic genes can improve glucolipotoxicity-induced damage, such as *PGC-1α, UCP2,* and *FABP4*. Others may aggravate glucolipotoxicity-induced damage when RIP140 expression is altered in islet β cells. Such specific and major downstream genes will be investigated in future studies of glucolipotoxicity-induced damage in islet β cells.

RIP140 is involved in many important pathways in the regulation of β cell function. Our results indicate that RIP140 is involved in glucolipotoxicity-induced damage of islet β cells and targeting RIP140 could reduce this damage in vitro. However the study lacks the results in vivo and further research is warranted.

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Interest conflict

The authors have declared that no competing interests exist.

Author's contributions

YanCheng Xu conceived the study, designed experiments. Experiments were performed by JunLi Xue, Qi Huang, JiaoE Zeng and Hui Zhu. Analysis of data was performed by JunLi Xue. JunLi Xue and Qi Huang drafted the manuscript and all authors read and approved the final version.

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