Autotaxin signaling facilitates β cell dedifferentiation and dysfunction induced by Sirtuin 3 deficiency

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ABSTRACT

Objective: β cell cell dedifferentiation may underlie the reversible reduction in pancreatic β cell mass and function in type 2 diabetes (T2D). We previously reported that β cell-specific Sirt3 knockout (Sirt3fl/fl;Cre/+ ) mice developed impaired glucose tolerance and glucose-stimulated insulin secretion after feeding with high fat diet (HFD). RNA sequencing showed that Sirt3-deficient islets had enhanced expression of Enpp2 (Autotaxin, or ATX), a secreted lysophospholipase which produces lysophosphatidic acid (LPA). Here, we hypothesized that activation of the ATX/LPA pathway contributed to pancreatic β cell dedifferentiation in Sirt3-deficient β cells.

Methods: We applied LPA, or lysophosphatidylcoline (LPC), the substrate of ATX for producing LPA, to MIN6 cell line and mouse islets with altered Sirt3 expression to investigate the effect of LPA on β cell dedifferentiation and its underlying mechanisms. To examine the pathological effects of ATX/LPA pathway, we injected the β cell selective adeno-associated virus (AAV-Atx-shRNA) or negative control AAV-scramble in Sirt3−/− and Sirt3fl/fl;Cre/+ mice followed by 6-week of HFD feeding.

Results: In Sirt3fl/fl;Cre/+ mouse islets and Sirt3 knockout MIN6 cells, ATX upregulation led to increased LPC with increased production of LPA. The latter not only induced reversible dedifferentiation in MIN6 cells and mouse islets, but also reduced glucose-stimulated insulin secretion from islets. In MIN6 cells, LPA induced phosphorylation of JNK/p38 MAPK which was accompanied by β cell dedifferentiation. The latter was suppressed by inhibitors of LPA receptor, JNK, and p38 MAPK. Importantly, inhibiting ATX in vivo improved insulin secretion and reduced β cell dedifferentiation in HFD-fed Sirt3fl/fl;Cre/+ mice.

Conclusions: Sirt3 prevents β cell dedifferentiation by inhibiting ATX expression and upregulation of LPA. These findings support a long-range signaling effect of Sirt3 which modulates the ATX-LPA pathway to reverse β cell dysfunction associated with glucolipotoxicity.

Keywords Autotaxin; Lysophosphatidic acid; β cell dedifferentiation; Sirtuin3; Mitogen-activated protein kinases; Type 2 diabetes

1. INTRODUCTION

β cell failure and insulin resistance in peripheral tissues represent the core pathophysiologic defects in type 2 diabetes (T2D) [1]. Glucotoxicity, lipotoxicity, oxidative stress, endoplasmic reticulum stress, and inflammation are known factors which contribute to β cell dysfunction in T2D [2]. β cell dedifferentiation has emerged as a new player in β cell failure, in addition to apoptosis or senescence, under metabolic stress in T2D [3]. Dedifferentiated β cells become progenitor-like cells or transdifferentiate into non-β pancreatic cells, such as α, δ, and polypeptide P (PP) cells with reduced β cell function and contribute to the progression of T2D [4]. During β cell dedifferentiation, marker genes vital for mature β cell function, such as β cell enriched genes (e.g. key transcription factors including Foxo1, Pdx1, Nkx6.1), glucose

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Abbreviations: Sirt3, Sirtuin 3; T2D, Type 2 diabetes; Sirt3fl/fl;Cre/+ , β cell-specific Sirt3 knockout; Enpp2, Ectonucleotide Pyrophosphatase/Phosphodiesterase 2; ATX, Autotaxin; LPA, lysophosphatidic acid; SIRT3 KO, Whole-body deletion of SIRT3; WT, wild-type; LPC, lysophosphatidycoline; AAV, adeno-associated virus; GSIS, glucose-stimulated insulin secretion; KD, knockdown; ChIP, Chromatin immunoprecipitation; OGTT, Oral glucose tolerance tests; ITT, insulin tolerance tests; STD, standard diet; HFD, high fat diet; JNK, c-Jun N-terminal kinase; p38 MAPK, p38 mitogen-activated protein kinase; FBG, Fasting blood glucose; H3K9Ac, Acetyl-Lys 9 of histone H3; H4K16Ac, Acetyl-Lys 16 of histone H4; H3K27Ac, Acetyl- Lys 27 of histone H3; H3K27me, Methylation of lysine 27 on histone H3

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metabolism genes (e.g., Glut2, Idh2, Atp4a), and protein secretory pathway genes (e.g., Glipr, Trpm5, Btg2), are downregulated. On the other hand, progenitor cell markers (e.g., Ngn3, Aldh1a3, Sox9 and Oct4) and β cell “disallowed” genes (e.g., Latha, Sic16a1) are upregulated [5–7].

Recent murine studies suggested the therapeutic potential of controlling β cell dedifferentiation as a strategy to improve β cell function [5,8]. Islets isolated from patients with T2D exhibited marked reduction in expression of SIRT3 protein [10]. In vitro studies confirmed reduced expression of both SIRT3 mRNA and protein in patients’ islets exposed to high glucose condition [11]. On the other hand, overexpression of SIRT3 in rats islets alleviated palmitate-induced β cell dysfunction [12]. Consistent with these studies, we had reported reduced expression of SIRT3 protein in islets from adult male 129Sv mice after 8 weeks of HFD feeding. In Sirt3 global knock-out mice, we revealed that Sirt3 deficiency accelerated HFD-induced impairment of glucose homeostasis and β cell apoptosis. Islets from these mice also showed reduced glucose-stimulated insulin secretion (GSIS) [13].

To further elucidate the role of SIRT3 in the regulation of pancreatic β cells, our group has established the β cell-selective Sirtuin3 (Sirt3) knockout (Sirt3^flox^flox^) mice by Ins2 Cre driven deletion of the Sirt3 allele. After HFD feeding these Sirt3^flox^flox^mice exhibited a mixed phenotype including impaired glucose tolerance, reduced GSIS and increased hepatic steatosis [14]. Inhibiting 5-hydroxytryptamine synthesis in β cells from Sirt3^flox^flox^mice improved HFD-induced hepatic steatosis but failed to improve glucose tolerance [14]. These findings implicated alternative mechanisms in β cell dysfunction induced by Sirt3 deficiency. RNA-sequencing of islets from Sirt3^flox^flox^mice led to discovery of 5-fold upregulation of Autotaxin (ATX, encoded by Atx) in islets exposed to high glucose condition. ATX knockdown by AAV- mediated reduced cell function induced by ATX knockdown [14,15], we used recombinant AAV8 carrying Atx-shRNA driven by insulin 1 promoter for targeted delivery to mouse islets. PAV- insulin 1 - RFP-mir30- m with 10^12 genome copies of AAV- Atx-shRNA or negative control AAV-scramble in 100 μL of normal saline.

2.2. shRNA lentivirus and adeno-associated virus establishment

We used published method to knock down Sirt3 expression in MIN6 cells [14]. For specific knockdown Enpp2 expression in mouse pancreatic β cells, the Mus Enpp2 shRNA plasmids were purchased from Sun Yuen Technology Limited (Hong Kong) and the shRNA construct sequences are as follows: shRNA- Enpp2-1: 5′-CCGGCCACCAAGATTCATCTGGAATTTGGGTGCTTTTTG-3′; shRNA-Enpp2-2: 5′-CCGGCGCTACAAATCTCAGACATCTCGAGATATGTCAGATTTGGTACTTGGGTGCTTTTTG-3′; shRNA Enpp2-3: 5′-CCGGCCCTCTGTTATAGCTTCTTCCTACTCGGAGAGAAGCACAATTGAAGCTGTTTTT-3′ and expressed scrambled negative shRNA: 5′-TTCTCCAGGAGGGGAATGCTGCTGCGTTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGC
Figure 1: Sirt3 deficiency upregulated ATX expression in β cells. A: The expression levels of Enpp2 from RNA-Seq DEGs analysis (n = 3 mice per group). B: Relative mRNA expression level of Enpp2 was assessed by RT-PCR in Sirt3f/f and Sirt3f/f;Cre/+ mouse islets (n = 3 mice per group). C: ATX level measured in the culture medium from Sirt3f/f and Sirt3f/f;Cre/+ mouse islets (n = 3 mice per group). D: Quantification of relative fluorescence positive areas shown in E (n = 3 mice per group, each containing 6–8 islets). E: Immunofluorescent staining of insulin and ATX in Sirt3f/f and Sirt3f/f;Cre/+ mouse pancreas with STD or HFD feeding (scale bar, 50 μm, green: Insulin, red: ATX, blue: DAPI). F: LPA level measured in culture medium from Sirt3f/f and Sirt3f/f;Cre/+ mouse islets in the presence of different concentrations of LPC (n = 3 mice per group). H2 Representative western immunoblot data of H3K27Ac, H3K27me, and STAT5 from nucleus and cytoplasm of MIN6 cells after knockdown of Sirt3. Three independent experiments were repeated from different samples. H: Quantification of western immunoblot in G. I: Immunofluorescent staining of insulin and H3K27Ac in Sirt3f/f and Sirt3f/f;Cre/+ mouse pancreas with STD or HFD feeding (scale bar, 50 μm, green: Insulin, red: H3K27Ac, blue: DAPI). J: Quantification of relative fluorescence positive areas shown in I (n = 3 mice per group, each containing 6–8 islets). K: Percentage of input in Enpp2 promoter from immunoprecipitated DNA with antibody against H3K27Ac, H3K27me and STAT5 in MIN6 cells after knockdown of Sirt3 were subjected to RT-PCR (n = 3 biological replicates). L: The base pair location of the Enpp2 gene promoter being analyzed by ChIP. Data are presented as Mean ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001 by Student t test, a.u., arbitrary units; ATX, autotaxin; STD, standard diet; HFD, high fat diet; NC, normal control; Sirt3 KD, knockdown of Sirt3; H3K27Ac, acetyl-Lys 27 on histone H3; H3K27me, methylation of lysine 27 on histone H3; STAT5, signal transducer and activator of transcription 5; bp, base pairs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
measured by ELISA from MyBioSource; glucagon level in the mouse serum was measured by ELISA from Millipore; all according to the manufacturers’ instructions.

2.5. Real time-PCR (RT-PCR) and immunoblotting
RT-PCR was performed with the SYBR Green kit (Promega) on Applied Biosystems 7900HT Fast Real-Time PCR System as previously described [14]. Data were normalized to Gapdh mRNA in each sample. Mouse primers sequences are shown in Supplementary Table. 1. Western immunoblot analysis was performed as previously described; protein (20—30 μg) was loaded into each well of 8—15% SDS-PAGE gel [14]. The protein used in Figure 1B was extracted and separated by nuclear and cytoplasmic, while the others were total extract. Primary antibodies are listed in Supplementary Table. 2. Horseradish peroxidase-linked anti-rabbit and anti-mouse IgG (1:2,000; Cell Signaling Technology) were used as secondary antibodies. Protein bands were developed by Immobilon Western Chemiluminescent HRP Substrate (Millipore).

2.6. Histological staining
We conducted tissue and cell immunofluorescence staining as previously described [13]. Rabbit anti H3K27Ac (1:200, Abcam, catalog #ab4729), PDX1 (1:1,000, Abcam, catalog, #ab47267), RFP (1:200, Thermo Fisher, catalog #PA1-986), ALDH1A3 (1:200, Proteintech, catalog #25167-1-AP), p-JNK (1:50, CST, catalog #4668), p-p38 MAPK (1:100, CST, catalog #4511), glucagon (1:200, Abcam, catalog #ab82517) and guinea pig anti-insulin (1:1,000, DAKO, catalog #A0564) and mouse anti-NKX6.1 (1:1,000, DSHB, catalog # F55A10), NGN3 (1:500, DSHB, catalog # F25A1B3) and ATX (1:200, Abcam, catalog, # ab77104) antibodies were applied to the sections overnight at 4 °C, followed by incubation with secondary antibodies (1:500). The images were captured by LEICA CR6000, using the Leica Qwin image analysis software (Leica) and positive signals were quantified using Image J. Insulin positive cells, indicated by positive staining for β cell identity markers (NKX6.1 or PDX1) and H3K27Ac, were determined by counting the corresponding nuclear staining surrounding the insulin staining area. Quantification of NGN3 was performed in pancreatic sections double-stained for insulin and NGN3, by counting the number of NGN3 positive-insulin negative cells, within the islet area. The ALDH1A3, p-JNK and p-p38 MAPK positive cells were determined as ratio of insulin and these markers positive staining overlapping area expressed against total insulin positive area. Staining, photo acquisition and cell counting was done in a blinded manner and unblinding occurred after counting all samples.

2.7. Chromatin immunoprecipitation
Chromatin immunoprecipitation (ChIP) assays were carried out as previously described [14]. The first antibodies against H3K27Ac (Catalog #ab4729) and H3K27me (Catalog #ab6002) were from Abcam, STAT5 (Catalog #9363) and IgG were from Cell Signaling Technology. ChIP and input DNA was purified and analyzed by quantitative RT-PCR with primers (described in Supplementary Table. 3) directed to the Enpp2 promoter.

2.8. Statistical analysis
Data are expressed as means ± SD. Differences between groups were analyzed by Student’s t test or One-way ANOVA followed by Bonferroni post hoc test where appropriate. Statistical testing was performed using GraphPad Prism 8 software (GraphPad Software). Differences with P values of < 0.05 were considered statistically significant for all tests.

3. RESULTS
3.1. Sirt3 deficiency upregulated ATX expression in β cells
Sirt3−/−/Cre− mice were generated and validated in our previous study [14]. RNA-sequencing indicated that Enpp2 (Ab) was upregulated in islets after Sirt3 deletion, as validated by RT-PCR, secreted ATX from islet into the culture medium, and immunostaining (Figure 1A—E); while the LPA receptors expression levels were not affected in islets (Supplementary Fig. 1A, B). The increased LPA level from culture medium in the presence of different concentrations of LPC was also observed in islets after Sirt3 deletion (Fig. 1F). In view of the previous studies showing a direct regulatory role of SIRT3 in gene transcription by deacetylation histone markers [14,24,25], our previous study has demonstrated the binding of signal transducers and activators of transcription (STAT5) to both tryptophan hydroxylase 1 (Tph1) and Tph2 promoters after Sirt3 knockdown in MIN6 cells [14]. In current study, we measured the expression level of acetyl- Lys 27 of histone H3 (H3K27Ac) and methylation of lysine 27 on histone H3 (H3K27me) in MIN6 cells after knockdown of Sirt3. The results showed that knockdown of Sirt3 increased H3K27Ac and STAT5 expression dramatically with a mild increase of H3K27me, which is in line with previous studies [14,25] (Figure 1G,H). The immunostaining results from mouse pancreases also confirmed that selective deletion of Sirt3 in β cells was accompanied by upregulated expression of H3K27Ac in Sirt3−/−/Cre− mice (Figure 1I,J). More importantly, the ChIP result showed that Sirt3 knockdown was associated with enhancement of the binding of both H3K27Ac and STAT5 to the promoter region of the Enpp2 genes; whereas involvement of H3K27me had minimal effect on the trans-activation of the Enpp2 genes (Figure 1K and Supplementary Fig. 1C). Through Ensembl genome browser (https://asia.ensembl.org), we also identified the binding motifs of STAT5 at the promoter regions of Enpp2. The STAT5 binding base pair location was from −18 to −9 in the Enpp2 gene promoter which being analyzed by ChIP (Fig. 1L). These results suggested that SIRT3 might be involved in suppressing the activation histone marker H3K27Ac. Taken together, in the absence of SIRT3, increased acetylation of H3K27Ac might facilitate STAT5-induced transcription of ATX in β cell.

3.2. LPA induced β cell dedifferentiation
To examine the effect of LPA on β cell function, different concentrations of LPA were applied to MIN6 cells for 24 h. GSIS was more severely impaired by higher concentrations of LPA under high glucose treatment in MIN6 cells (Supplementary Fig. 2A), indicating that LPA, but not LPC, impaired β cell function. LPA treatment (from 0.01 to 1 μmol/L) downregulated β cell identity genes, including Foxa1, Nkx6.1, Pdx1 and Neurod1, whereas progenitor cell markers such as Sox9 and Oct4 were upregulated in MIN6 cells in a dose-dependent manner (Supplementary Fig. 2B, C). To confirm the results obtained in MIN6 cells, GSIS was also studied in primary mouse islets treated with 0.1 μmol/L LPA or LPC, a concentration which did not impair β cell viability and induce apoptosis (Supplementary Fig. 2D-G). LPA, but not LPC, diminished the GSIS at 16.7 mmol/L glucose (Figure 2A). Similar to MIN6 cells, LPA, but not LPC, downregulated β cell identity genes, including Foxa1, Nkx6.1 and Pdx1, while increased progenitor cell markers, Aldh1a3, Ngn3, Sox9 and Oct4, as well as α cell enriched genes, such as Arx and Mafa in primary islets (Figure 2B,C). Western immunoblotting also demonstrated PDX1 and NKX6.1 downregulation, accompanied by increased expression of ALDH1A3, NGN3, and ARX after LPA treatment in MIN6 cells (Fig. 2D, E). Thus, these results showed that LPA...
Figure 2: LPA induced β cell dedifferentiation. A: GSIS experiment was performed to evaluate β cell function with 0.1 µmol/L LPA treatment for 24 h in primary islets. B: Relative mRNA expression levels of β cell identity genes (including Foxo1, Nkx6.1 and Pdx1) and Ins2 were assessed by RT-PCR in primary islets with 0.1 µmol/L LPA or LPC treatment for 24 h. C: Relative mRNA expression levels of progenitor cell markers (Aldh1a3, Ngn3, Sox9 and Oct4) and α cell enriched genes (such as Arx and MafB) were assessed by RT-PCR in primary islets with 0.1 µmol/L LPA or LPC treatment for 24 h. D: Representative western immunoblot data (total protein) of β cell identity markers, progenitor cell markers and α cell enriched markers in MIN6 cells with 0.1 µmol/L LPA treatment for 24 h. E: Quantification of western immunoblot in D. F: Primary islets were treated with 0.1 µmol/L LPA for 24 h, followed by 72 h cultured with or without LPA treatment. GSIS was measured after different treatments. G, H: Primary islets were treated with 0.1 µmol/L LPA for 24 h, followed by 72 h cultured with or without LPA treatment. Relative mRNA expression levels of β cell identity genes (including Foxo1, Nkx6.1 and Pdx1), Ins2, progenitor cell markers (Aldh1a3, Ngn3, Sox9 and Oct4) and α cell enriched genes (such as Arx and MafB) were assessed by RT-PCR after different treatments. I: MIN6 cells were treated with 0.1 µmol/L LPA for 24 h, followed by 72 h cultured with or without LPA treatment. Representative western immunoblot data (total protein) of β cell identity markers, progenitor cell markers and α cell enriched markers. J: Quantification of western immunoblot in I. Data are presented as Mean ± SD, **, P < 0.05; ***, P < 0.01; ****, P < 0.001 by One-way ANOVA followed by Bonferroni post hoc test. n = 3 biological replicates. LPA, lysophosphatidic acid; LPC, lysophosphatidylcoline; Foxo1, forkhead box protein a 1; Nkx6.1, NK6 homeobox 1; Pdx1, pancreatic and duodenal homeobox 1; Ins2, insulin 2; Aldh1a3, aldehyde dehydrogenase 1a3; Ngn3, Neurogenin 3; Sox9, SRY-box transcription factor 9; Oct4, octamer-binding transcription factor 4; Arx, aristaless related homeobox; MafB, MAF BZIP transcription factor b; MAFA, MAF BZIP transcription factor A; WD, withdrawing LPA treatment.
was able to trigger β cell dedifferentiation both in MIN6 cells and primary islets. Others [26] have shown that, unlike apoptosis, β cell dedifferentiation can be reversed by a washout treatment to remove the stimuli. Accordingly, to determine the recovery of β cell function after withdrawing LPA treatment, GSIS analysis was performed. GSIS impaired by LPA was restored after withdrawing LPA (Fig. 2f). More importantly, the expression of β cell identity genes, progenitor cell markers, and α cell enriched genes were restored to their basal level after LPA was removed in both MIN6 cells and primary islets (Figure 2g–j and Supplementary Fig. 1H, I), suggesting the LPA induced β cell dedifferentiation could be reversed after LPA was removed.

3.3. Sirt3 deficiency triggered LPC induced β cell dedifferentiation

To examine how SIRT3 modulated LPA-induced β cell dedifferentiation, knockdown Sirt3 was performed in MIN6 cells by shRNA lentiviral infection (Figure 3a). The upregulation of Enpp2 mRNA expression was observed in Sirt3 knockdown (KD) MIN6 cells (Fig. 3a). Next, Sirt3 KD and scrambled (NC) MIN6 cells were treated with the ATX substrate LPA or the ATX precursor LPC. After treatment, β cell identity genes Nkx6.1, Pdx1, and Ins2 were similarly suppressed by LPA in both NC and Sirt3 KD cells. In particular, LPC treatment suppressed these genes only in Sirt3 KD cells, but not in NC cells (Fig. 3b). Likewise, LPA increased progenitor cell markers including Ngn3 and Oct4, and α cell enriched genes Arx in both NC and Sirt3 KD cells, while LPC only upregulated these genes in both Sirt3 KD cells (Fig. 3c). Furthermore, Western immunoblotting showed that LPA lowered PDX1 and NKX6.1 protein levels and upregulated ALDH1A3, Ngn3, and ARX expression in Sirt3 KD cells, but not NC controls (Figure 3d and Supplementary Fig. 2). These results suggested that the effect of LPC to induce β cell dedifferentiation was dependent on SIRT3 expression, supporting our hypothesis that SIRT3 modulated the ATX expression to facilitate LPC conversion to LPA.

We further validated the different effects of LPA and LPC obtained from MIN6 cells in mouse primary islet isolated from Sirt3f/f and Sirt3f/f;Cre/+ mice. Two β cell identity markers, NKX6.1 and PDX1 were examined by immunofluorescence of the islet treated with LPA or LPC. We found that similar to MIN6 cells, LPA induced downregulation of both NKX6.1 and PDX1 in mouse islet of both groups, while LPC suppressed NKX6.1 and PDX1 only in islet from Sirt3f/f;Cre/+ mice (Figure 3e, f, and h, i). Conversely, the expression of progenitor cell marker ALDH1A3 was upregulated by LPA in both Sirt3f/f and Sirt3f/f;Cre/+ mouse primary islets, but upregulated by LPC only in Sirt3f/f;Cre/+ islets (Figure 3g, j). Collectively, the result from islets also showed that LPC induced β cell dedifferentiation in Sirt3 deficient β cells.

3.4. JNK/p38 MAPK signaling pathways contributed to LPA induced β cell dedifferentiation

Although previous studies examined the effects of LPA in kidney, liver, and other tissues [27], the underlying mechanism of LPA-induced β cell dedifferentiation was still unclear. Earlier works from in vitro cell culture experiments suggested the Mitogen-Activated Protein Kinases (MAPKs) might be involved in the effect of LPA in different cells, like neuronal PC12 cells and ovarian theca cells. In addition, in β cells, there was negative correlation between β cell function and the activity levels of JNK1/2 and p38 MAPK [28]. Based on this evidence, we further examined whether JNK1/2 and p38 MAPK signaling were the downstream effectors of LPA signaling in β cells. In MIN6 cells, LPA increased the phosphorylation of JNK1/2 and p38 MAPK which peaked at 2 h in MIN6 cells (Figure 4a, b). Similar to the results for β cell dedifferentiation markers, LPC also increased the phosphorylation of JNK1/2 and p38 MAPK only in Sirt3 KD MIN6 cells (Figure 4c, d). These results suggested that LPA could activate the JNK and p38 MAPK, whereas the activating effect of LPC on these two signals was dependent on the loss of Sirt3, and most likely via ATX expression. We tested whether the effect of LPA could be blocked by the selective dual receptor 1/3 inhibitor (LPAR1, k16425). LPAR1 treatment dose-dependently decreased LPA-induced phosphorylation of JNK1/2 and p38 MAPK in MIN6 cells (Figure 4e, f and Supplementary Fig. A–C). At a concentration of 10 μM/L, LPAR1 showed effective inhibition and the dose was used in other experiments. As expected, 10 μM/L LPAR1 abolished LPA-induced β cell dedifferentiation (Figure 4g, h).

Next, we used SP600125 as a JNK1/2 inhibitor (JNKi), and SB203580 as a p38 MAPK inhibitor (p38 MAPKi) to examine whether suppression of these signaling pathways would attenuate the effect of LPA on dedifferentiation in MIN6 cells. We found that both JNK and p38 MAPK inhibitors abolished LPA-induced downregulation of PDX1, and NKX6.1. In addition, the combination of both inhibitors showed better recovery of PDX1 and NKX6.1 expression. Regarding the progenitor markers, p38 MAPKi showed slightly more inhibition on ALDH1A3 and ARX upregulation. Similar to the effect on β cell identity markers, the combination of JNK and p38 MAPK inhibitors showed more inhibition on ALDH1A3, ARX, and NGN3 upregulation in MIN6 cells induced by LPA (Figure 4i, j). The mRNA expression levels of other markers (Ins2, Sox9, Oct4 and Mafa) further corroborated the above findings (Figure 4k, l). In Sirt3 KD cells, inhibiting JNK and p38 MAPK abolished the β cell dedifferentiation induced by LPC (Figure 4m, n and Supplementary Fig. 40-d). Taken together, these results indicated that both JNK and p38 MAPK signaling were downstream effectors of ATX-LPA signaling on β cell dedifferentiation.

3.5. Specific knockdown ATX improved HFD-induced glucose intolerance in Sirt3f/f;Cre/+ islets in mice

Then we suspected selective suppression of ATX expression in pancreatic β cells might relieve the HFD-induced glucose intolerance in mice, especially when islet expression of Sirt3 was reduced. In order to achieve selective ATX inhibition in vivo, we used recombinant AAV8 [29] carrying Atx-shRNA driven by insulin 1 promoter for specifically suppressing ATX expression in mouse islets of normal mice and mice with islet specific deletion of Sirt3. After injection of the 1 × 10¹² genome copies per mouse of AAV-Atx-shRNA or negative control AAV-scramble in the Sirt3f/f and Sirt3f/f;Cre/+ mice at 8 weeks, the mice were fed a HFD for 6 weeks for further investigation of whether selective knocking down of ATX in pancreatic β cells might ameliorate HFD-induced glucose intolerance in Sirt3f/f;Cre/+ mice (Figure 5a).

Six weeks after HFD feeding, mice with both genotypes underwent OGTT and ITT tests. The HFD-induced glucose intolerance was attenuated after AAV-Atx-shRNA injection (Figure 5b, c). Serum insulin levels measured in mouse serum collected during OGTT indicated that knockdown of ATX expression in Sirt3f/f;Cre/+ mouse β cells rescued insulin secretion impaired by HFD (Figure 5d, e). Although the lower fasting serum insulin level of the HFD fed Sirt3f/f;Cre/+ mice was augmented after AAV-Atx-shRNA injection, there was no difference of insulin sensitivity, fasting serum glucagon level, fasting blood glucose (FBG), body weight and food intake among these mice after AAV-Atx-shRNA injection (Supplementary Fig. 5 A–G). Then, we validated the efficiency and specificity of ATX knockdown in mouse pancreatic β cells (Supplementary Fig. 5 and Supplementary Fig. 6h, i). As expected, ATX protein expression was effectively suppressed in HFD-fed Sirt3f/f;Cre/+ mice by AAV-Atx-shRNA. These results also demonstrated that the co-localization between ATX and insulin, not
Figure 3: Sirt3 deficiency triggered LPC induced β cell dedifferentiation. A: Relative mRNA expression levels of Sirt3 and Atx were assessed by RT-PCR in MIN6 cells after knockdown of Sirt3. B, C: Relative mRNA expression levels of β cell identity genes, Ins2, progenitor cell markers and α cell enriched genes were measured by RT-PCR in normal and Sirt3 deficiency MIN6 cells after treatment with 0.1 μmol/L LPA or LPC. D: Representative western immunoblot data (total protein) of SIRT3, ATX, β cell identity markers, progenitor cell markers and α cell enriched markers in normal and Sirt3 deficiency MIN6 cells with 0.1 μmol/L LPC treatment for 24 h (n = 3 biological replicates). E-G: Immunocytochemistry of NKX6.1 (E), PDX1 (F) and ALDH1A3 (G) in primary islets isolated from Sirt3f/f and Sirt3f/f;Cre/þ mice with 0.1 μmol/L LPC or LPA treatment for 24 h (scale bar, 25 μm). Green, Insulin; red, NKX6.1; blue, Hoechst. H-J: Quantification of relative fluorescence positive areas shown in E-G (n = 6–8 islets). Data are presented as Mean ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001 by One-way ANOVA followed by Bonferroni post hoc test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Figure 4: JNK/p38 MAPK signaling pathways contributed to LPA induced β cell dedifferentiation. A: Representative western immunoblot data (total protein) of p-JNK (Thr183/Tyr185)/JNK and p-p38 MAPK (Thr180/Tyr182)/p38 MAPK in MIN6 cells with 0.1 μmol/L LPA treatment for different timepoints. B: Quantification of western immunoblot in A. BC: Representative western immunoblot data (total protein) of p-JNK (Thr183/Tyr185)/JNK and p-p38 MAPK (Thr180/Tyr182)/p38 MAPK in normal and Sirt3 KD MIN6 cells with 0.1 μmol/L LPC treatment for 2 h. D: Quantification of western immunoblot in C. E: Representative western immunoblot data (total protein) of p-JNK (Thr183/Tyr185)/JNK and p-p38 MAPK (Thr180/Tyr182)/p38 MAPK in LPA treated MIN6 cells after treatment with different concentrations of LPAR inhibitor. F: Representative western immunoblot data (total protein) of β cell identity markers, progenitor cell markers and α cell enriched markers in MIN6 cells with different treatments. G: Quantification of western immunoblot in F. I: Representative western immunoblot data (total protein) of β cell identity markers, progenitor cell markers and α cell enriched markers in MIN6 cells with different treatments. J: Quantification of western immunoblot in I. K: Relative mRNA expression levels of Ins2, progenitor cell markers (Sox9 and Oct4) and α cell enriched genes (such as Mafb) were assessed by RT-PCR after different treatments. M: Representative western immunoblot data (total protein) of β cell identity markers, progenitor cell markers and α cell enriched markers in Sirt3 KD MIN6 cells with different treatments. N: Quantification of western immunoblot in M. Data are presented as Mean ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001 by One-way ANOVA followed by Bonferroni post hoc test. n = 3 biological replicates in Figure 4C,D, G, H and K-N. n = 4 biological replicates in Figure 4A,B, E, F, I, J. JNK1/2, c-jun N- Terminal Kinases; p38 MAPK, p38- Mitogen Activated Protein Kinase; LPARi, LPA receptor inhibitor, JNKi, JNK1/2 inhibitor; p38 MAPKi, p38 MAPK inhibitor.
glucagon, in Sirt3f/f;Cre/+ mouse islets (Figure 5F,G). Compared to the baseline, which collected before any treatments, serum ATX and total LPA level were also decreased after 6 weeks HFD feeding with AAV-Atx-shRNA injection (Figure 5H,I). These results implicated that selective suppression of ATX expression in pancreatic β cells rescued the HFD-induced glucose intolerance and restored insulin secretion in Sirt3f/f;Cre/+ islets in mice.

3.6. Specific knockdown ATX improved HFD-induced β cell dedifferentiation in Sirt3f/f;Cre/+ islets in mice

We then examined the molecular effects of ATX/LPA pathway in β cell dedifferentiation in vivo. Consistent with the in vitro results, selective knockdown of ATX in pancreatic β cells attenuated HFD-induced activation of JNK/p38 MAPK signal in Sirt3f/f;Cre/+ mice (Figure 6A,B and Supplementary Fig. 5J, K). The results showed that β cell identity

Figure 5: Specific knockdown ATX improved HFD-induced glucose intolerance in Sirt3f/f;Cre/+ islets in mice. Sirt3f/f and Sirt3f/f;Cre/+ mice were injected with AAV-Atx-shRNA (for specific knocking down ATX in pancreatic β cells) or AAV-scramble respectively followed by HFD feeding for 6 weeks before sacrifice. A: Experimental design of 6 weeks’ HFD feeding, AAV-Atx-shRNA injection and other experiments conducted on Sirt3f/f and Sirt3f/f;Cre/+ mice (n = 5 per group). B: C: OGTT was performed before sacrifice and related AUC was calculated (n = 5 mice per group). D, E: During OGTT, blood samples were collected at each time point, serum was extracted from blood samples for insulin level measurement and related AUC was calculated (n = 5 mice per group). F, G: Immunostaining of insulin and ATX in mouse pancreas (scale bar, 50 μm). For immunofluorescent staining, F: green, ATX; red, insulin; blue, DAPI; G: green, glucagon; red, ATX; blue, DAPI. H, I: Serum ATX and total LPA levels were measured (n = 5 mice per group). *, P < 0.05; **, P < 0.01; ***, P < 0.001 by One-way ANOVA followed by Bonferroni post hoc test. AAV, Adeno-associated viruses; OGTT, oral glucose tolerance test; AUC, Area under curve. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Figure 6: Specific knockdown ATX improved HFD-induced β cell dedifferentiation in Sirt3f/f;Cre/+ islets in mice. Sirt3f/f and Sirt3f/f;Cre/+ mice were injected with AAV-Atx-shRNA (for specific knocking down ATX in pancreatic β cells) or AAV-scramble respectively, followed by feeding with HFD for 6 weeks before sacrifice. A-F: Immunofluorescent staining of insulin and JNK/p38 MAPK pathway or β cell dedifferentiation markers in mouse pancreas (scale bar, 50 μm). Green, p-JNK (A), p-p38 MAPK (B), NKX6.1 (C), PDX1 (D), ALDH1A3 (E) and NGN3 (F); red, insulin; blue, DAPI. G: GSIS was measured in isolated primary islets from four groups of mice (n = 3 mice per group). H, I: Relative mRNA expression levels of β cell identity genes (including Foxo1, Nkx6.1 and Pdx1), Ins2, progenitor cell markers (Aldh1a3, Ngn3, Sox9 and Oct4) and α cell enriched genes (such as Arx and Mafb) were assessed by RT-PCR in isolated primary islets from four groups of mice (n = 3 mice per group). Data are presented as Mean ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001 by One-way ANOVA followed by Bonferroni post hoc test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
4. DISCUSSION

Accumulating evidence suggests that the loss of β cell identity and dedifferentiation is one of the primary processes responsible for impaired β cell function in T2D [4,30]. In the present study, we again demonstrated that Sirt3 expression played a critical role in maintaining normal β cell function. Reduction or absence of Sirt3 expression in β cells induced β cell dysfunction and glucose intolerance in mice. To our best knowledge, this is the first report showing that reduction or absence of Sirt3 expression induced β cell dedifferentiation by activating the ATX/LPA pathway to cause β cell dysfunction (Figure 7).

Like its family members, Sirt3 is classified as a deacetylase. Scher MB et al. first demonstrated that the full-length SIRT3 protein was enzymatically active and deacetylated acetyl-Lys 9 of histone H3 (H3K9Ac) and acetyl-Lys 16 of histone H4 (H4K16Ac) in vivo and in vitro [24]. SIRT3 had been shown to inhibit FOS transcription through specific histone H3 lysine K27 deacetylation (H3K27Ac) at its promoter to prevent inflammation and fibrosis in the mouse heart [31]. Sirt3 knockdown or Sirt3 silencing by siRNA increased the acetylation level of H3K9Ac to promote the transcription of target proteins in primary brown adipocytes [25]. Interestingly, increased transcription at the ENPP2 locus was related to the changes in expression of H3K27me in HEK 293T cell [32].

Similar with these studies, we had reported that knockdown of Sirt3 increased H3K9Ac and H4K16Ac with binding of H4K16Ac, CREBP, and STAT5 to the promoter regions of the Tph1 and Tph2 genes by ChIP in sh-Sirt3 MIN6 cells [14]. Collectively, these studies suggested that SIRT3 protein might function as deacetylase of histone and other nuclear proteins in addition to its major role as protein deacetylase in the mitochondria. In our current study, we found that knockdown of Sirt3 increased acetylation of H3K27Ac and binding of STAT5 to the promoter regions of the ATX genes. Taken together, these results suggested that SIRT3 protein might modulate ATX levels via its deacetylase activity to activate ATX/LPA pathway to induce β cell dysfunction.

On the other hand, the key feature of mature β cell is to produce and secrete insulin for maintaining glucose homeostasis [33]. Under physiological conditions, increased blood glucose stimulates insulin secretion in mature β cells to maintain blood glucose within a narrow range without causing hypoglycemia [34]. The characteristics of the mature functional β cell include: high expression of β cell identity genes, as well as low or no expression of progenitor cell marker genes, disallowed genes, and α cell enriched genes. Over-expression of these genes could interfere with normal β cell function [35]. Here, we
showed that LPA was able to suppress GSIS in primary islets and MIN6 cells. Increased LPA levels also induced β cell dedifferentiation in MIN6 cells and primary islets by downregulating expression of β cell identity genes (Foxo1, Mkx6.1 and Pax4), and concomitantly upregulating expression levels of progenitor cell markers (Aldh1a3, Ngn3, Sox9 and Oct4) and α cell enriched genes (Arx and Math5). Interestingly, after withdrawal of LPA, dedifferentiated β cells could redifferentiate to mature insulin positive β cells upon normalization of glucose levels, similar to previous studies using cell lineage tracing [36]. Clinical studies also supported potential reversibility of β cell dedifferentiation process with recovery of β cell function after removal of metabolic stress [37, 38] such as hyperlipidemia and insulin resistance. Several lines of evidence implicated in vitro activation of ATX could lead to increased LPA synthesis with the majority being extracellular [39, 40]. Pharmacological inhibitors of ATX, such as PF8380, decreased plasma LPA levels to more than 95% [41]. Other researchers had also reported involvement of the ATX/LPA pathway in various metabolic disorders including obesity and insulin resistance [40, 42]. In obese patients with insulin resistance and glucose intolerance, mRNA abundance of Atx in adipose tissue and circulating ATX levels were higher than controls with normal glucose tolerance [43, 44]. Notably, despite taking obesogenic diet which induced glucose intolerance, insulin resistance was alleviated in obese patients with lean adipose tissue and circulating ATX levels were higher than controls with normal glucose tolerance [43, 44]. Other researchers had also reported increased ATX and LPA expression in adipose tissue of patients with type 1 diabetes, activation of JNK1/2 and JNK could mediate the effects of pro-inflammatory cytokines to cause β cell apoptosis [49]. There was also evidence of JNK activation in diabetic Zucker fatty rats, INS-1 cells under hyperglycemia and human islets from patients with type 2 diabetes [50, 51]. Other experimental studies also indicated that JNK1/2 could cause cytoplasmic translocation of PDX1 to suppress insulin gene expression under hyperglycemic and oxidative stress conditions [52]. Our previous study also demonstrated that Sirt3 deficiency induced ATX overexpression in islets [14, 46]. In conclusion, we demonstrated that Sirt3 played a critical role in maintaining functional β cells in HFD-fed mice. In conclusion, Sirt3 deficiency promotes palmitate or H2O2-induced JNK phosphorylation in primary islets [13]. Recent work had demonstrated that β cell apoptosis induced by hyperglycemia in vitro and in vivo might be mediated by p38 MAPK [53]. Consistent with these studies, our results showed that LPA treatment induced phosphorylation of JNK and p38 MAPK in MIN6 cells. Pharmacological inhibition of LPAR not only eliminated the LPA-enhanced phosphorylation of JNK and p38 MAPK but also suppressed the LPA-induced β cell dedifferentiation. In the same vein, both JNK and p38 MAPK inhibitor eliminated LPA-induced β cell dedifferentiation. These results suggested JNK and possibly p38 MAPK might be the downstream effectors during LPA-induced β cell dedifferentiation. In conclusion, we demonstrated that Sirt3 played a critical role in maintaining functional β cells in HFD-fed mice. In conclusion, Sirt3 deficiency induced ATX overexpression in pancreatic β cells ameliorated HFD-induced β cell dedifferentiation and glucose intolerance found in Sirt3 KO mice. Similarly, knockdown of ATX in pancreatic β cells relieved the HFD suppression on insulin production in Sirt3 KO mice. These results supported the notion that ATX was the mediator of Sirt3 deficiency-induced β cell dysfunction.

In the present study, we observed impaired GSIS of islets from HFD-fed Sirt3 KO and WT mice [46]. By contrast, in our previous studies, we found glucose intolerance in Sirt3 KO mice on a 129Sv background and Sirt3 KO mice in response to HFD feeding [13, 14, 46]. Of note, the feeding period was lasted for 40 weeks in our previous study compared with the current 12-week study [14, 46]. In our study, glucose intolerance and insulin secretion in response to glucose load were similar between Sirt3 KO and WT mice [13, 14, 46]. This impairment was ameliorated after knocking down ATX expression. Meanwhile, we did not observe any difference in fasting blood glucose, body weight, or food intake among these mice. The lower fasting glucagon level in Sirt3 KO mice with HFD feeding was consistent with our previous study [14]. In other cellular experiments, MAPKs appeared to be the downstream effectors of LPA pathways [47, 48]. In type 1 diabetes, activation of JNK1/2 and JNK could mediate the effects of pro-inflammatory cytokines to cause β cell apoptosis [49]. There was also evidence of JNK activation in diabetic Zucker fatty rats, INS-1 cells under hyperglycemia and human islets from patients with type 2 diabetes [50, 51]. Other experimental studies also indicated that JNK1/2 could cause cytoplasmic translocation of PDX1 to suppress insulin gene expression under hyperglycemic and oxidative stress conditions [52]. Our previous study also demonstrated that Sirt3 deficiency induced ATX overexpression in islets [14, 46]. In conclusion, we demonstrated that Sirt3 played a critical role in maintaining functional β cells in HFD-fed mice. In conclusion, Sirt3 deficiency induced ATX overexpression in pancreatic β cells ameliorated HFD-induced β cell dedifferentiation and glucose intolerance found in Sirt3 KO mice. Similarly, knockdown of ATX in pancreatic β cells relieved the HFD suppression on insulin production in Sirt3 KO mice. These results supported the notion that ATX was the mediator of Sirt3 deficiency-induced β cell dysfunction.

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activation to impair β cell function, making the ATX-LPA pathway a potential therapeutic target.

**DUALITY OF INTEREST**

GR has received grant funding from and is a consultant for Sun Pharmaceuticals Inc.

**AUTHOR CONTRIBUTIONS**

J.C.N.C., A.C.K.C., A.P.S.K., H.C., and X.Y.T. contributed to the study concept and design. A.P.S.K., H.C., G.A.R., and X.Y.T. contributed to the data analysis and the writing of the manuscript. H.C., X.M., D.M., H.M.L. and X.C. conducted the in vitro and in vivo studies. All authors contributed to the critical review of the manuscript and approved the final version of the manuscript. H.C., X.Y.T., and A.P.S.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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**CONFLICT OF INTEREST STATEMENT**

JNC has received research grants and/or honorarium for consultancy or giving lectures, from AstraZeneca, Bayer, Bristol-Myers Squibb, Boehringer Ingelheim, Daiichi-Sankyo, Eli-Lilly, GlaxoSmithKline, Merck Serono, Merck Sharp & Dohme, Novo-Nordisk, Pfizer and Sanofi.

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**APPENDIX A. SUPPLEMENTARY DATA**

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**REFERENCES**

[1] Defronzo, R.A., 2009. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58(4):773–795.

[2] Kassem, S.A., Ariel, I., Thornton, P.S., Scheinberg, I., Glaser, B., 2000. Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. Diabetes 49(8):1325–1333.

[3] Accili, D., Talchai, S.C., Kim- Muller, J.Y., Cinti, F., Ishida, E., Oredelheide, A.M., et al., 2016. When beta-cells fail: lessons from dedifferentiation. Diabetes, Obesity and Metabolism 18(Suppl 1):117–122.

[4] Talchai, C., Xuan, S., Lin, H.V., Susse1, L., Accili, D., 2012. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. Cell 150(6):1223–1234.

[5] Rutter, G.A., Georgiadou, E., Martinez-Sanchez, A., Pullen, T.J., 2020. Metabolic and functional specialisations of the pancreatic beta cell: gene disallows, mitochondrial metabolism and intercellular connectivity. Diabetologia 63(10):1990–1998.

[6] Yin, Q., Ni, Q., Wang, Y., Zhang, H., Li, W., Nie, A., et al., 2020. Raptor determines beta-cell identity and plasticity independent of hyperglycemia in mice. Nature Communications 11(1):2538.

[7] Pullen, T.J., Huisjes, M.O., Rutter, G.A., 2017. Analysis of purified pancreatic islet beta and alpha cell transcriptomes reveals 11beta-hydroxysteroid dehydrogenase (Hsd11b2) as a novel disallowed gene. Frontiers in Genetics 8:41.

[8] Boland, B.B., Brown Jr., C., Boland, M.L., Cunn, J., Sullikowski, M., Hansen, G., et al., 2019. Pancreatic beta-cell rest replenishes insulin secretory capacity and attenuates diabetes in an extreme model of obese type 2 diabetes. Diabetes 68(1):131–140.

[9] Gomez-Baroy, N., Guseh, J.S., Li, G., Rubio-Navarro, A., Chen, T., Porier, B., et al., 2019. Adipin preserves beta cells in diabetic mice and associates with protection from type 2 diabetes in humans. Nature Medicine 25(11):1739–1747.

[10] Caton, P.W., Richardson, S.J., Kieswich, J., Bugliani, M., Holland, M.L., Marchetti, P., et al., 2013. Sirtuin 3 regulates mouse pancreatic beta cell function and is suppressed in pancreatic islets isolated from human type 2 diabetic patients. Diabetologia 56(5):1068–1077.

[11] Zhang, Y., Zhou, F., Bai, M., Liu, Y., Zhang, L., Zhu, Q., et al., 2019. The pivotal role of protein acetylation in linking glucose and fatty acid metabolism to beta-cell function. Cell Death & Disease 10(2):66.

[12] Kim, M., Lee, J.S., Oh, J.E., Nan, J., Lee, H., Jung, H.S., et al., 2015. SIRT3 overexpression attenuates palmitate-induced pancreatic beta-cell dysfunction. PLoS One 10(4):e0124744.

[13] Zhou, Y., Chung, A.C.K., Fan, R., Lee, H.M., Xu, G., Tomlinson, B., et al., 2017. Sirt3 deficiency increased the vulnerability of pancreatic beta cells to oxidative stress-induced dysfunction. Antioxidants and Redox Signaling 27(13):962–976.

[14] Ming, X., Chung, A.C.K., Mao, D., Cao, H., Fan, B., Wong, W.K.K., et al., 2021. Pancreatic Sirtuin 3 deficiency promotes hepatic steatosis by enhancing 5-hydroxytryptamine synthesis in mice with diet-induced obesity. Diabetes 70(1):119–131.

[15] Stefan, C., Jansen, S., Bollen, M., 2005. NPY-type ectophosphodiesterases: unity in diversity. Trends in Biochemical Sciences 30(10):542–550.

[16] Barbayanni, E., Kaffe, E., Aidinis, V., Kokotos, G., 2015. Autotaxin, a secreted lysophospholipase D, as a promising therapeutic target in chronic inflammation and cancer. Progress in Lipid Research 54:76–96.

[17] Ninou, I., Magkioti, C., Aidinis, V., 2018. Autotaxin in pathophysiology and pulmonary fibrosis. Frontiers of Medicine 5:180.

[18] Valdes-Rivas, S.A., Gonzalez-Arenas, A., 2017. Autotaxin-lysophosphatic acid: from inflammation to cancer development. Mediators of Inflammation 2017:9173090.

[19] Kaffe, E., Katsifla, A., Xylousigidis, N., Ninou, I., Zannikou, M., Harokopos, V., et al., 2017. Hepatocyte autotaxin expression promotes liver fibrosis and cancer. Hepatology 65(4):1369–1383.

[20] Oklonomou, N., Mouratis, M.A., Tzouvelakis, A., Kaffe, E., Valavanis, C., Vilaras, G., et al., 2012. Pulmonary autotaxin expression contributes to the pathogenesis of pulmonary fibrosis. American Journal of Respiratory Cell and Molecular Biology 47(5):566–574.

[21] Nikolopoulou, I., Oklonomou, N., Karouzakis, E., Sevastou, I., Nikolaidou-Katsaridou, N., Zhao, Z., et al., 2012. Autotaxin expression from synovial fibroblasts attenuates collagen-induced arthritis in mice: a potential therapeutic target.
fibroblasts is essential for the pathogenesis of modeled arthritis. Journal of Experimental Medicine 209(5):925–933.

[22] Balood, M., Zahednasab, H., Siroos, B., Mesbah-Namin, S.A., Torbati, S., Harirchian, M.H., 2014. Elevated serum levels of lysophosphatidic acid in patients with multiple sclerosis. Human Immunology 75(5):411–413.

[23] Rancoule, C., Attane, C., Gres, S., Fourmel, A., Dusaulcy, R., Bertrand, C., et al., 2013. Lysophosphatidic acid impairs glucose homeostasis and inhibits insulin secretion in high-fat diet obese mice. Diabetologia 56(6):1394–1402.

[24] Scher, M.B., Vaquero, A., Reinberg, D., 2007. SirT3 is a nuclear NAD+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. Genes & Development 21(8):920–928.

[25] Gao, P., Jiang, Y., Wu, H., Sun, F., Li, Y., He, H., et al., 2020. Inhibition of mitochondrial calcium overload by SIRT3 prevents obesity- or age-related whitening of brown adipose tissue. Diabetes 69(2):165–180.

[26] Diedisheim, M., Oshima, M., Albagli, O., Huldt, C.W., Ahlstedt, I., Clausen, M., et al., 2018. Modeling human pancreatic beta cell dedifferentiation. Molecular Metabolism 10:74–86.

[27] Yung, Y.C., Stoddard, N.C., Chun, J., 2014. LPA receptor signaling: pharmacology, physiology, and pathophysiology. The Journal of Lipid Research 55(7):1192–1214.

[28] Palomer, X., Roman-Azcona, M.S., Pizarro-Delgado, J., Planavila, A., Yung, Y.C., Stoddard, N.C., Chun, J., 2014. Lysophosphatidic acid impairs glucose homeostasis and inhibits insulin secretion in high-fat diet obese mice. Diabetologia 56(6):1394–1402.

[29] Wang, Z., Zhu, T., Rehman, K.K., Bertera, S., Zhang, J., Chen, C., et al., 2006. Potential in pancreatic beta-cells. Biochemical Pharmacology 80(6):874–883.

[30] Accili, D., 2018. Insulin action research and the future of diabetes treatment: the 2017 banting medal for scientific achievement lecture. Diabetes 67(9):1701–1709.

[31] Palomer, X., Roman-Azcona, M.S., Pizarro-Delgado, J., Planavila, A., Villarroya, F., Valenzuela-Alcaraz, B., et al., 2020. SIRT3-mediated inhibition of FOS through histone H3 deacetylation prevents cardiac fibrosis and inflammation. Signal Transduction and Targeted Therapy 5:14.

[32] Argaud, D., Boulanger, M.C., Chignon, A., Mkannez, G., Mathieu, P., 2019. Enhancer-mediated enrichment of interacting JMJD3-DDX21 to ENPP2 locus c regulation of autotaxin with obesity in human adipose tissue. The Journal of Lipid Research 59(10):1815–1807.

[33] Merchant, P., Bugiani, M., De Tata, V., Suleiman, M., Marselli, L., 2017. Pancreatic beta cell identity in humans and the role of type 2 diabetes. Frontiers in Cell and Developmental Biology 5:55.

[34] Dhawan, S., Tschen, S.I., Zeng, C., Guo, T., Hebrok, M., Matveyenko, A., et al., 2015. DNA methylation directs functional maturation of pancreatic beta cells. Journal of Clinical Investigation 125(7):2851–2860.

[35] Rutter, G.A., Pullen, T.J., Hudson, D.J., Martinez-Sanchez, A., 2015. Pancreatic beta-cell identity, glucose sensing and the control of insulin secretion. Biochemical Journal 462(2):203–218.

[36] Ishida, E., Kim-Muller, J.Y., Accili, D., 2017. Pair feeding, but not insulin, phlorizin, or risoglinide treatment, curtails markers of beta-cell dedifferentiation in db/db mice. Diabetes 66(8):2092–2101.

[37] White, M.G., Shaw, J.A., Taylor, R., 2016. Type 2 diabetes: the pathogenic basis of reversible beta-cell dysfunction. Diabetes Care 39(11):2080–2088.

[38] Zhyshnevskaya, S.V., Al-Mabhe, A., Peters, C., Barnes, A., Aribalba, B., Hollingsworth, K.G., et al., 2020. Time course of normalization of functional beta-cell capacity in the diabetes remission clinical trial after weight loss in type 2 diabetes. Diabetes Care 43(4):813–820.

[39] Dusaulcy, R., Rancoule, C., Gres, S., Warnecc, E., Colom, A., Guigne, C., et al., 2011. Adipose-specific disruption of autotaxin enhances nutritional fattening and reduces plasma lysophosphatic acid. The Journal of Lipid Research 52(6):1247–1255.

[40] Nishimura, S., Nagasaki, M., Okudaira, S., Aoki, J., Ohmori, T., Ohkawa, R., et al., 2014. ENPP2 contributes to adipose tissue expansion and insulin resistance in diet-induced obesity. Diabetes 63(12):4154–4164.

[41] Giese, J., Thorarensen, A., Beltey, K., Bradshaw-Pierce, E., Cortes-Burgos, L., Hall, T., et al., 2010. A novel autotaxin inhibitor reduces lysophosphatic acid levels in plasma and the site of inflammation. Journal of Pharmacology and Experimental Therapeutics 334(1):310–317.

[42] Rancoule, C., Dusaulcy, R., Treguer, K., Gres, S., Attane, C., Saulnier-Blache, J.S., 2014. Involvement of autotaxin/lysophosphatic acid signaling in obesity and impaired glucose homeostasis. Biochimie 96:140–143.

[43] Rancoule, C., Dusaulcy, R., Treguer, K., Gres, S., Attane, C., Saulnier-Blache, J.S., et al., 2012. Depot-specific regulation of autotaxin with obesity in human adipose tissue. Journal of Physiology & Biochemistry 68(4):635–644.

[44] Reeves, V.L., Trybula, J.S., Wills, R.C., Goodpaster, B.H., Dube, J.J., Kienesberger, P.C., et al., 2015. Serum Autotaxin/ENPP2 correlates with insulin resistance in older humans with obesity. Obesity 23(12):2371–2376.

[45] D‘oussa, K., Nizirorera, C., Cowie, A.M., Varghese, G.P., Eichmann, T.O., et al., 2018. Autotaxin-LPA signaling contributes to obesity-induced insulin resistance in muscle and impairs mitochondrial metabolism. The Journal of Lipid Research 59(10):1805–1817.

[46] Peterson, B.S., Campbell, J.E., Ilkayeva, O., Grimsrud, P.A., Hirschey, M.D., Newgard, C.B., 2018. Remodeling of the acetyltpeptone by SirT3 manipulation fails to affect insulin secretion or beta cell metabolism in the absence of overnutrition. Cell Reports 24(1):209–223 e6.

[47] Zhang, J., Li, Y., Wang, C., Wang, Y., Zhang, Y., Huang, L., et al., 2020. Lysophosphatidic acid induces apoptosis of PC12 cells through LPA1 receptor/ LPA2 receptor/MAPK signaling pathway. Frontiers in Molecular Neuroscience 13:16.

[48] Budnik, L.T., Brunswig-Spichenheier, B., Mukhopadhyay, A.K., 2003. Lysophosphatidic acid signals through mitogen-activated protein kinase-extracellular signal regulated kinase regulated signal kinase in ovarian theca cells expressing the LPA1/edg2-receptor: involement of a nonclassical pathway? Molecular Endocrinology 17(8):1593–1606.

[49] Bonny, C., Oberon, A., Negri, S., Saeurer, C., Schorderet, D.F., 2001. Cell-permeable peptide inhibitors of JNK: novel blockers of beta-cell death. Diabetes 50(1):77–82.

[50] Syed, I., Jayaram, B., Subasinghe, W., Kowluw, A., 2010. Tiam1/Rac1 signaling pathway mediates palmitate-induced, ceramide-sensitive generation of superoxides and lipid peroxides and the loss of mitochondrial membrane potential in pancreatic beta-cells. Biochemical Pharmacology 80(6):874–883.

[51] Syed, I., Kysthanahalli, C.N., Jayaram, B., Govind, S., Rhodes, C., Kowluru, R.A., et al., 2011. Increased phagocyte-like NADPH oxidase and ROS generation in type 2 diabetic ZDF rat and human islets: role of Rac1-JNK1/2 signaling pathway in mitochondrial dysregulation in the diabetic islet. Diabetes 60(11):2843–2852.

[52] Kawamori, D., Kajimoto, Y., Kaneto, H., Umayahara, Y., Fujitani, Y., Miyatsuka, T., et al., 2003. Oxidative stress induces nuclear-cytoplasmic translation of pancreatic transcription factor PDX-1 through activation of c-Jun NH(2)-terminal kinase. Diabetes 52(12):2896–2904.

[53] Sidarala, V., Veluthakal, R., Syeda, K., Vlaar, C., Newsholme, P., Kowluru, A., et al., 2018. Modeling human pancreatic beta cell dedifferentiation. Molecular Medicine 209(5):925.