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Effect of PANDER in βTC6-cell lipoapoptosis and the protective role of exendin-4

Jing-Nan Xiang, Dan-Ling Chen, Li-Yong Yang *

Department of Endocrinology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian 350005, China

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ABSTRACT

Chronic exposure to high concentrations of saturated fatty acids, such as palmitic acid (PA), leads to apoptosis of pancreatic β-cells through the activation of the c-Jun N-terminal kinase (JNK) signaling pathway. This study of β-cell lipoapoptosis was designed to investigate the roles of pancreatic-derived factor (PANDER), a pro-apoptosis cytokine-like peptide, and exendin-4, a long-acting agonist of the hormone glucagon-like peptide-1 (GLP-1) receptor and anti-apoptosis factor. The glucose-sensitive mouse β-pancreatic cell line, βTC6, was used to investigate the mechanisms of PA-induced apoptosis. Twenty-four hours of PA exposure led to increased PANDER expression in a dose- and time-dependent manner, and significantly increased phosphorylation of JNK. Treatment with the JNK-specific inhibitor SP600125 reduced the PA-induced PANDER expression. After the 24 h of PA exposure, cells also underwent marked apoptosis and showed increased activation of the apoptosis protease, caspase-3. The small interfering (si)RNA-mediated silencing of PANDER gene expression significantly reduced both of these effects. When PA-treated βTC6 cells were exposed to exogenous exendin-4, JNK activation was inhibited, PANDER expression was decreased, and the numbers of apoptotic cells were reduced. Collectively, these results demonstrated that the JNK-mediated signaling mechanism of PA-induced β-cell apoptosis involves up-regulated expression of PANDER and activation of caspase-3. Exendin-4 may protect against lipoapoptosis by interfering with the JNK-PANDER pathway.

1. Introduction

The detrimental effects of free fatty acids (FFAs) on pancreatic β-cells have long been recognized. Chronic exposure of pancreatic β-cells to high concentrations of saturated fatty acids, such as palmitic acid (PA), leads to apoptosis [1–3], a process known as lipoapoptosis. However, the underlying molecular mechanisms remain to be fully elucidated, and may represent feasible therapeutic targets to protect against loss of the insulin-producing pancreatic β-cells and consequent hyperglycemia.

Recently, a novel cytokine-like peptide was identified as a possible regulator of β-cell apoptosis. The pancreatic-derived factor (PANDER) is not only highly expressed in pancreatic islet β-cells but also in the islet α-cells, which produce the insulin-counteracting hormone glucagon [4]. Since both of these cell types are the key mediators of glucose metabolism, PANDER has been hypothesized to play an important pathophysiological role in diabetes mellitus [5–7].

It is well known that gut-secreted factors influence glucose metabolism. One such factor is the hormone glucagon-like peptide-1 (GLP-1), which is secreted by the intestinal L-cells in response to nutrient ingestion [8]. GLP-1 binding to its cognate receptor GLP1R, a glucagon-receptor family member expressed on pancreatic β-cells, activates an adenylyl cyclase signaling pathway that stimulates insulin synthesis and secretion. A GLP-1 receptor agonist, exendin-4, was identified from the Gila monster in 1992 and found to share 53% homology with GLP-1. While the two hormones exert the same antidiabetic actions, exendin-4 has a significantly longer duration of action [9,10]. Both GLP-1 and exendin-4 have been developed as glucose control therapeutic agents and successfully applied in clinic. Exogenous administration of these hormones has been shown to enhance glucose-dependent insulin secretion, decrease glucagon release, stimulate β-cell proliferation and islet neogenesis, and inhibit β-cell apoptosis [11–14].

In order to gain a better understanding of the lipoapoptotic process in β-cells, this study was designed to investigate the putative role of PANDER and the possible protective effect of exendin-4.

2. Methods

2.1. βTC6 cell culture

The mouse βTC6 insulinoma cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were grown at 37 °C in a humidified, 5% CO_2/95% air (v/v) atmosphere with low glucose (5.6 mM) Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal

* Corresponding author. Fax: +86 591 83318716.
E-mail address: yly_lm@sina.com (L.-Y. Yang).

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bovine serum (FBS; Gibco). The medium was changed twice a week, and the cells were trypsinized once a week for passaging. Cells from passages 30 to 70 were used for all experiments.

2.2. PANDER small interfering (si)RNA construct and transient transfection

An siRNA targeting mouse PANDER mRNA (GenBank Accession Number: 52793; CCAGCACUCUCUACAACAUTT) and a negative control (UUCUCCGAACGUGUCACGUTT) were designed and synthesized by GenePharma Co., Ltd. (Shanghai, China). The siRNAs were transfected, respectively, into βTC6 cells (1.5×10^6 cells/well in 6-well plates) using Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After 4–6 h of incubation, the transfection medium was replaced with DMEM (5.6 mM glucose, 10% FBS) and allowed to incubate for an additional 72 h. PANDER mRNA and protein expression was analyzed by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) and Western blotting.

2.3. RNA extraction and qRT-PCR

Total RNA was extracted from βTC6 cells by using the TRIzol reagent (Invitrogen). RNase-free DNase (Invitrogen) was used according to the manufacturer's instructions to remove any contaminating genomic DNA. cDNA was synthesized from the total RNA by using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, Ontario, Canada) and following the manufacturer's protocol. The qRT-PCR reactions were carried out in a Thermal Cycler Dice Real Time System (TaKaRa, Shiota, Japan) with SYBR® Premix Ex*Taq* (TaKaRa) under the following conditions: 95 °C for 3 min (for initial denaturation) followed by 40 cycles of 95 °C for 30 s (denaturation)/62 °C for 40 s (annealing)/72 °C for 30 s (extension). Primers for mouse PANDER were: forward, 5′-CC AAACTGAAGGCTCAAGCAAAG-3′; reverse, 5′-TTTCTCTCTCGATTTCT GAAGGGAG-3′. Primers for the normalization gene, mouse β-actin, were: forward, 5′-AGTGTGACGTTGACATCCGTA-3′; reverse, 5′-GCC AGAGCAGTAATCTCCTTCT-3′ (Invitrogen).

2.4. Protein extraction and Western blotting

Cells were lysed with ice-cold radioimmune precipitation assay (RIPA) buffer and 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma–Aldrich, St. Louis, MO, USA). Lysates were centrifuged at 12,000*g* at 4 °C for 10 min, and the supernatant containing the cellular protein was collected. The total protein of each sample was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Invitrogen). The membranes were incubated overnight with primary antibodies on a platform rocker, washed, and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h. Immunoreactive protein bands were detected with the WesternBreeze kit (Invitrogen). Primary antibodies were used at the following working dilutions: rabbit anti-human PANDER polyclonal antibody (1:200 dilution, sc-83250; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse anti-human JNK polyclonal antibody (1:500, sc-7345; Santa Cruz Biotechnology), mouse anti-human phosphorylated (p)-JNK polyclonal antibody (1:500, sc-6254; Santa Cruz Biotechnology), rabbit anti-human cleaved caspase-3 polyclonal antibody (1:200, Cat. No. 9664; Cell Signaling Technology, Beverly, MA, USA), and mouse monoclonal antibody β-actin (1:1000, sc-47778; Santa Cruz Biotechnology). For PANDER and cleaved caspase-3, the secondary antibodies were goat anti-rabbit IgG-HRP (1:5000, sc-2004; Santa Cruz Biotechnology). For β-actin, JNK and p-JNK, the secondary antibodies were goat anti-mouse IgG-HRP (1:5000, sc-2005; Santa Cruz Biotechnology).

2.5. Flow cytometric analysis

Apoptotic cells were quantified by the Annexin V and propidium iodide (PI) double-staining technique using an Annexin V–FITC and PI apoptosis detection kit (Becton–Dickinson Bioscience, Franklin Lakes, NJ, USA). βTC6 cells were harvested by centrifugation, and labeling was carried out by serial addition of Annexin V and PI in the dark, according to the manufacturer's instructions. Cell apoptosis was analyzed on a Cytomics™ FC500 Flow Cytometer (Beckman Coulter, Brea, CA, USA). Annexin V-FITC(+)/PI(−) cells were scored as apoptotic, and Annexin V-FITC(+)/PI(+) (double-stained) cells were considered either necrotic or late apoptotic.

2.6. PA, SP600125 and exendin-4 intervention

βTC6 cells were seeded in 6-well plates (1.5×10^6 cells/well). When ~70–80% confluency was reached, the medium was replaced with fresh DMEM medium with the final concentration of BSA adjusted to 0.5% (w/v). To measure PA-induced PANDER expression, cells were treated with PA (dissolved in ethanol; Sigma–Aldrich) at various concentrations (0, 0.125, 0.25, 0.5 and 1.0 mmol/L) for different times (0, 6, 12, 24 and 48 h). Cells treated with 0.5% BSA/ethanol in DMEM without PA (uninduced) served as the control.

To investigate the effect of JNK on PA-induced PANDER expression, cells were pre-treated with 25 μmol/L of the JNK-specific inhibitor SP600125 (dissolved in 0.1% DMSO (v/v); Sigma–Aldrich) for 1 h, then exposed to 0.5 mmol/L of PA plus 25 μmol/L of SP600125 for 24 h. PA-induced cells without SP600125 and uninduced cells exposed to SP600125 only served as controls.

To investigate the effects of exendin-4 on PA-induced lipoapoptosis, cells were pre-treated with 50 nmol/L of exendin-4 (dissolved in sterile H_2O and diluted in DMEM; Sigma–Aldrich) for 24 h, then exposed to 0.5 mmol/L of PA plus 50 nmol/L of exendin-4 for 24 h. PA-induced cells without exendin-4 and uninduced cells exposed to exendin-4 only served as controls.

2.7. Statistical analysis

Statistical analysis was performed using the Student's *t*-test for unpaired data when two samples were compared. Data are expressed as means ± standard deviation (SD). A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Effects of PA on PANDER gene expression in βTC6 cells

As detected by qRT-PCR, uninduced βTC6 cells expressed PANDER mRNA, which was used as the baseline level of expression. PA exposure induced PANDER expression in a dose- and time-dependent manner (Fig. 1A and B, respectively). The peak value of PA-induced PANDER mRNA expression occurred with the 0.5 mmol/L concentration at 12 h of incubation. The PANDER protein expression pattern detected by Western blotting was consistent with the mRNA expression pattern. PANDER protein levels increased in conjunction with increases in PA concentrations and exposure times (Fig. 1C and D, respectively). The maximal enhancement of PANDER protein expression was also induced by 0.5 mmol/L PA but occurred at 24 h of incubation.

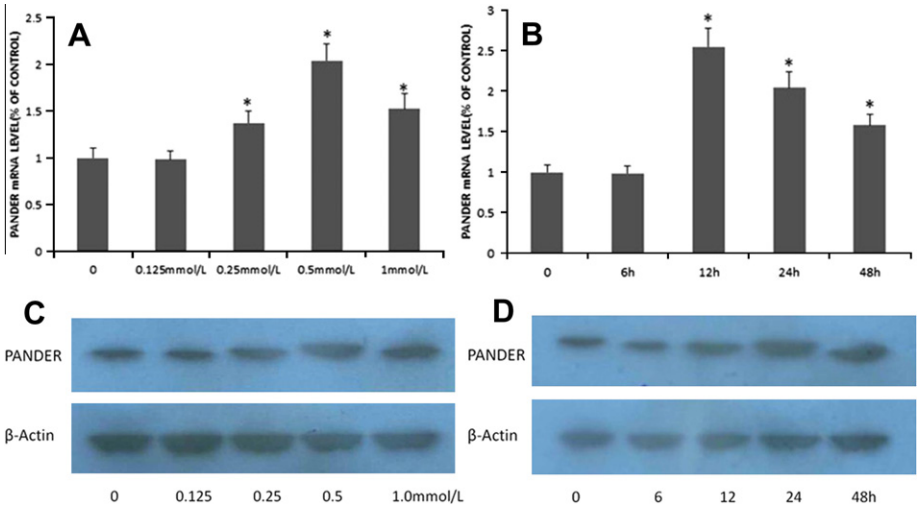

Fig. 1. PA induces PANDER mRNA and protein expression in βTC6 cells, as detected by qRT-PCR (A and B) and Western blotting (C and D), respectively. (A and C) βTC6 cells were stimulated with 0, 0.125, 0.25, 0.5 or 1.0 mmol/L of PA for 24 h. (B and D). βTC6 cells were stimulated with 0.5 mmol/L of PA for 0, 6, 12, 24 or 48 h. All qRT-PCR data are presented as mean ± SD of three independent experiments. β-actin was detected in Western blotting as the loading control. A representative Western blot of three independent experiments is shown.

3.2. Role of JNK in PA-induced PANDER expression in βTC6 cells

JNK activation in response to PA exposure and in conjunction with stimulated PANDER expression was assessed by detecting the level of phosphorylated JNK using Western blotting. As shown in Fig. 2, 24 h exposure to PA significantly increased the levels of p-JNK. Treatment of βTC6 cells with the JNK-specific inhibitor SP600125 reduced the PA-induced PANDER expression.

3.3. Role of PANDER in PA-induced apoptosis in βTC6 cells

3.3.1. Effects of siRNA-mediated PANDER silencing

Transfection of siRNA targeting endogenous PANDER mRNA in βTC6 cells caused a 78% decrease PANDER expression, compared to the expression detected in cells transfected with the negative control (NC) siRNA (Fig. 3A). The NC siRNA had no significant effect on PANDER expression, compared with βTC6 cells exposed to transfection reagent alone (MOCK) or no treatment (BC) (Fig. 3A and B, respectively).

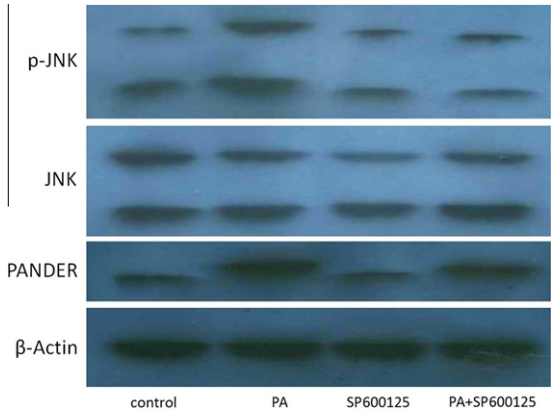

Fig. 2. JNK contributes to PA-induced PANDER expression in βTC6 cells, as detected by Western blotting. activated JNK (p-JNK), JNK and PANDER protein expression was detected in PA-induced (0.5 mmol/L PA for 24 h) βTC6 cells with or without SP600125 (25 μmol/L), and compared with levels detected in the uninduced (control) and JNK-inhibited (SP600125) βTC6 cells. β-actin was detected as the loading control. A representative Western blot of three independent experiments is shown.

3.3.2. Role of PANDER in PA-induced apoptosis

Detection of the apoptotic marker Annexin V/PI ratio indicated that 24 h exposure of βTC6 cells to PA led to a significant increase in the percentage of apoptotic cells. When PANDER expression was silenced by siRNA, the PA-induced apoptosis rate decreased by ~10%, compared with that observed in NC-transfected PA-induced cells (Fig. 3C).

3.3.3. Effects of PANDER in PA-induced activation of caspase-3

Detection of cleaved caspase-3 by Western blotting indicated that 24 h exposure of βTC6 cells to PA led to increase the levels of activated caspase-3. When PANDER expression was silenced by siRNA, the PA-induced level of cleaved caspase-3 was decreased, compared with that observed in NC-transfected PA-induced cells (Fig. 3D).

3.4. Effects of exendin-4 on PA-induced apoptosis in βTC6 cells

3.4.1. Exendin-4 inhibits PA-induced apoptosis

To determine whether exendin-4 was able to exert its anti-apoptotic effects on PA-induced apoptosis, βTC6 cells were exposed to PA or a combination of PA and exendin-4. As shown above, PA exposure for 24 h induced a statistically significant increase in apoptosis. This effect was partially reduced by exendin-4 (Fig. 4A).

3.4.2. Effects of exendin-4 on JNK activation, PANDER expression, and caspase-3 activation in PA-treated βTC6 cells

Western blotting was used to analyze whether exendin-4 exerted its anti-apoptotic effects by modulating p-JNK, PANDER, and cleaved caspase-3 protein levels in PA-induced cells. Exendin-4 treatment alone had no effect on the protein levels, compared with those in untreated/uninduced βTC6 cells. After 24 h of exposure to PA, however, βTC6 cells showed significantly increased levels of p-JNK, PANDER, and cleaved caspase-3. When cells were induced by PA in the presence of exendin-4, the PA-induced increases in p-JNK, PANDER, and cleaved caspase-3 were less extensive (Fig. 4B).

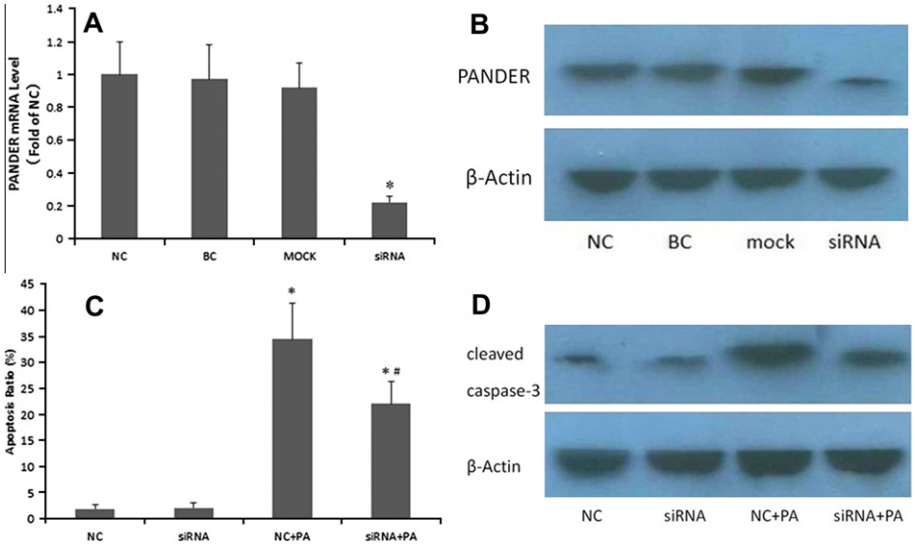

Fig. 3. PANDER contributes to PA-induced apoptosis in βTC6 cells. (A) Quantification of PANDER mRNA expression by qRT-PCR in no-treatment control βTC6 cells (BC), transfection reagent-exposed βTC6 cells (MOCK), and cells transfected with negative control siRNA (NC) or PANDER siRNA. Data are presented as the percent of PANDER expression in cells relative to that in NC cells. (B) PANDER protein expression levels detected by Western blotting in NC, BC, MOCK and PANDER siRNA-transfected βTC6 cells. (C) Flow cytometric analysis of apoptotic cells stained with Annexin V and PI. NC-transfected cells and PANDER siRNA-transfected cells were cultured without PA or with 0.5 mmol/L of PA for 24 h. (D) Cleaved caspase-3 protein expression levels detected by Western blotting in NC- or PANDER siRNA-transfected cells cultured without PA or with 0.5 mmol/L of PA for 24 h. For qRT-PCR in (A) and flow cytometry (C), the data are presented as mean ± SD of three independent experiments; $^{*}p < 0.05$ vs. NC and $^{\#}p < 0.05$ vs. NC + PA. For Western blots in (B and D), β-actin was detected as the loading control and a representative blot of three independent experiments is shown .

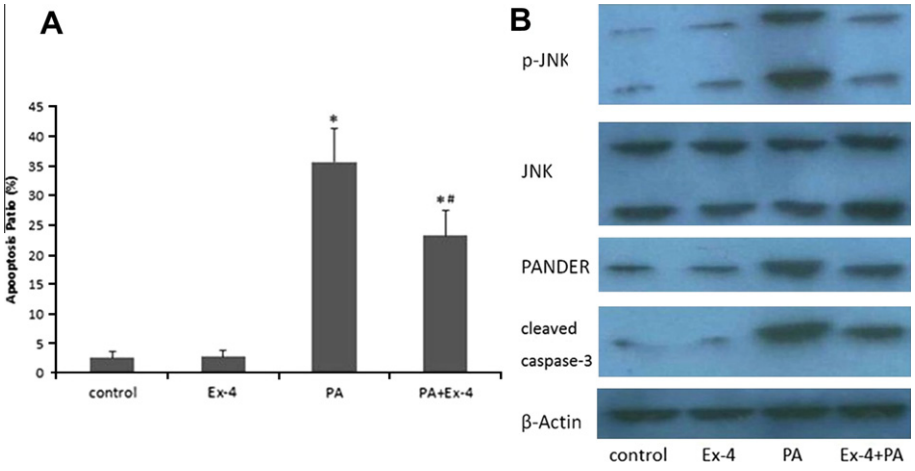

Fig. 4. Exendin-4 inhibits PA-induced apoptosis in βTC6 cells. Uninduced or PA-induced βTC6 cells were cultured in the presence or absence of exendin-4 (50 nmol/L) for 24 h. (A) Flow cytometric analysis of apoptotic cells stained with Annexin V and PI. The data are shown as mean ± SD of three independent experiments; $^{*}p < 0.05$ vs. control and $^{\#}p < 0.05$ vs. PA. (B) PANDER, p-JNK, JNK and cleaved caspase-3 protein expression levels detected by Western blotting. A representative Western blot from three independent experiments is shown.

4. Discussion

The PANDER cytokine-like peptide was recently cloned and characterized. In addition to its high expression levels in pancreatic islet β-cells, PANDER appears to colocalize with the insulin-containing secretory granules. However, when PANDER was over-expressed in β-cells through infection with adenovirus-PANDER, the β-cells became apoptotic [7]. Studies to elucidate the regulatory mechanisms of gene expression of PANDER demonstrated that glucose and some cytokines (such as interferon (IFN)-γ, monocyte chemoattractant protein-1 (MCP-1) or a combination of interleukin (IL)-1β, tumor necrosis factor (TNF)-α and IFN-γ [15–17]) act as positive regulators, suggesting that PANDER may participate in the glucose- or cytokine-mediated β-cell apoptosis.

FFAs contribute to β-cell dysfunction and apoptosis [18–21]. Our previous study demonstrated that FFAs exert pleiotropic effects in pancreatic β-cells, as evidenced by a glucose-stimulated insulin assay [22]. The study presented herein was designed to further investigate the observed lipotoxicity in β-cells. By using the glucose-sensitive, insulin secreting βTC6 cell line, we found that the saturated fatty acid, PA, stimulated PANDER expression in a dose- and time-dependent manner and induced apoptosis. Furthermore, the underlying mechanism of PA-induced apoptosis in βTC6 cells involved PANDER, as evidenced by the siRNA-mediated knock-down of PANDER expression.

The cysteine-containing aspartate-specific protease (CASPASE) family is known to function as important mediators of FFA-induced apoptosis of β-cells [18]. The caspase-3 family member has been characterized as a major effector caspase of the apoptosis signaling cascade [23]. A recent study of PANDER-induced β-cell apoptosis revealed that caspase-3 is critically involved in this process [5]. In our current study, PA-induced activation of caspase-3 was

partially blocked in PANDER siRNA-transfected βTC6 cells, illustrating the role of PANDER in PA stimulation of caspase-3 activation.

JNK is widely recognized as a principal signaling node in the regulation of cell survival in response to stress-activated apoptosis. A number of studies have demonstrated that saturated FFAs are a major source of metabolic stress and activators of JNK [18,24–27]. Intriguingly, the promoter region of PANDER harbors a binding site for one of the key JNK downstream transcription factors, activating protein-1 (AP-1) [16]. Furthermore, the protein kinase C (PKC)–JNK–AP-1 signaling pathway was implicated in the MCP-1-induced expression of the PANDER gene [17]. We found that when JNK was specifically inhibited by a pharmacologic agent in βTC6 cells, the PA-induced increase in PANDER expression was attenuated. This finding indicated that JNK-mediated signaling is involved in PA stimulation of PANDER expression.

GLP-1 and its long-acting homologue exendin-4 are known to reduce apoptosis in rodent and human isolated β-cells and intact islets exposed to FFAs, cytokines, or toxic agents [28–32]. It has recently been revealed that the anti-apoptotic effects of exendin-4 are related to JNK activation [13,33,34]. The results in the present study showed that exendin-4 inhibits the activation of JNK, decreases the expression of PANDER, and protects βTC6 cells from lipoapoptosis. These findings led us to hypothesize that exendin-4 inhibits activation of JNK, leading to down-regulation of PANDER expression and blocking the lipoapoptosis-promoting signaling events.

In summary, the results of this *in vitro* study demonstrated that PANDER participates in the process of PA-induced β-cell apoptosis through the activation of caspase-3 in β-cells. The protective effects of exendin-4 on lipoapoptotic β-cells may involve interference of the JNK-PANDER signaling pathway.

5. Disclosures

The authors have no conflicts to disclose.

Acknowledgments

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References

[1] N. Omae, M. Ito, S. Hase, M. Nagasawa, J. Ishiyama, T. Ide, K. Murakami, Suppression of FoxO1/cell death-inducing DNA fragmentation factor alpha-like effector A (Cidea) axis protects mouse beta-cells against palmitic acid-induced apoptosis, Mol. Cell Endocrinol. 348 (2012) 297–304.

[2] V. Nemcova-Furstova, R.F. James, J. Kovar, Inhibitory effect of unsaturated fatty acids on saturated fatty acid-induced apoptosis in human pancreatic beta-cells: activation of caspases and ER stress induction, Cell Physiol. Biochem. 27 (2011) 525–538.

[3] A.M. Engelbrecht, J.L. Toit-Kohn, B. Ellis, M. Thomas, T. Nell, R. Smith, Differential induction of apoptosis and inhibition of the PI3-kinase pathway by saturated, monounsaturated and polyunsaturated fatty acids in a colon cancer cell model, Apoptosis 13 (2008) 1368–1377.

[4] Y. Zhu, G. Xu, A. Patel, M.M. McLaughlin, C. Silverman, K. Knecht, S. Sweitzer, X. Li, P. McDonnell, R. Mirabile, D. Zimmerman, R. Boyce, L.A. Tierney, E. Hu, G.P. Livi, B. Wolf, S.S. Abdel-Meguid, G.D. Rose, R. Aurora, P. Hensley, M. Briggs, P.R. Young, Cloning, expression, and initial characterization of a novel cytokine-like gene family, Genomics 80 (2002) 144–150.

[5] B.R. Burkhardt, S.R. Greene, P. White, R.K. Wong, J.E. Brestelli, J. Yang, C.E. Robert, T.M. Brusko, C.H. Wasserfall, J. Wu, M.A. Atkinson, Z. Gao, K.H. Kaestner, B.A. Wolf, PANDER-induced cell-death genetic networks in islets reveal central role for caspase-3 and cyclin-dependent kinase inhibitor 1A (P21), Gene 369 (2006) 134–141.

[6] X. Cao, Z. Gao, C.E. Robert, S. Greene, G. Xu, W. Xu, E. Bell, D. Campbell, Y. Zhu, R. Young, M. Trucco, J.F. Markmann, A. Naji, B.A. Wolf, Pancreatic-derived factor (FAM3B), a novel islet cytokine, induces apoptosis of insulin-secreting beta-cells, Diabetes 52 (2003) 2296–2303.

[7] X. Cao, J. Yang, B.R. Burkhardt, Z. Gao, R.K. Wong, S.R. Greene, J. Wu, B.A. Wolf, Effects of overexpression of pancreatic derived factor (FAM3B) in isolated mouse islets and insulin-secreting betaTC3 cells, Am. J. Physiol. Endocrinol. Metab. 289 (2005) E543–E550.

[8] P.J. Larsen, M. Tang-Christensen, J.J. Holst, C. Orskov, Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem, Neuroscience 77 (1997) 257–270.

[9] J. Eng, W.A. Kleinman, L. Singh, G. Singh, J.P. Raufman, Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas, J. Biol. Chem. 267 (1992) 7402–7405.

[10] Y.E. Chen, D.J. Drucker, Tissue-specific expression of unique mRNAs that encode proglucagon-derived peptides or exendin 4 in the lizard, J. Biol. Chem. 272 (1997) 4108–4115.

[11] A. De Silva, S.R. Bloom, Gut hormones and appetite control: a focus on PYY and GLP-1 as therapeutic targets in obesity, Gut Liver 6 (2012) 10–20.

[12] Z. Yu, T. Jin, New insights into the role of cAMP in the production and function of the incretin hormone glucagon-like peptide-1. GLP-1, Cell Signal 22 (2010) 1–8.

[13] M. Ferdaoussi, S. Abdelli, J.Y. Yang, M. Cornu, G. Niederhauser, D. Favre, C. Widmann, R. Regazzi, B. Thorens, G. Waeber, A. Abderrahmani, Exendin-4 protects beta-cells from interleukin-1 beta-induced apoptosis by interfering with the c-Jun NH2-terminal kinase pathway, Diabetes 57 (2008) 1205–1215.

[14] S. Klinger, C. Poussin, M.B. Debril, W. Dolci, P.A. Halban, B. Thorens, Increasing GLP-1-induced beta-cell proliferation by silencing the negative regulators of signaling cAMP response element modulator-alpha and DUSP14, Diabetes 57 (2008) 584–593.

[15] W. Xu, Z. Gao, J. Wu, B.A. Wolf, Interferon-gamma-induced regulation of the pancreatic derived cytokine FAM3B in islets and insulin-secreting betaTC3 cells, Mol. Cell Endocrinol. 240 (2005) 74–81.

[16] O. Wang, K. Cai, S. Pang, T. Wang, D. Qi, Q. Zhu, Z. Ni, Y. Le, Mechanisms of glucose-induced expression of pancreatic-derived factor in pancreatic beta-cells, Endocrinology 149 (2008) 672–680.

[17] X. Hou, O. Wang, Z. Li, H. Mou, J. Chen, B. Deng, L. Qian, X. Liu, Y. Le, Upregulation of pancreatic derived factor (FAM3B) expression in pancreatic beta-cells by MCP-1 (CCL2), Mol. Cell Endocrinol. 343 (2011) 18–24.

[18] R. Lupi, F. Dotta, L. Marselli, S. Del Guerra, M. Masini, C. Santangelo, G. Patane, U. Boggi, S. Piro, M. Anello, E. Bergamini, F. Mosca, U. Di Mario, S. Del Prato, P. Marchetti, Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated, Diabetes 51 (2002) 1437–1442.

[19] I. Kharroubi, L. Ladriere, A.K. Cardozo, Z. Dogusan, M. Cnop, D.L. Eizirik, Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress, Endocrinology 145 (2004) 5087–5096.

[20] N.M. Doliba, W. Qin, S.A. Vinogradov, D.F. Wilson, F.M. Matschinsky, Palmitic acid acutely inhibits acetylcholine- but not GLP-1-stimulated insulin secretion in mouse pancreatic islets, Am. J. Physiol. Endocrinol. Metab. 299 (2010) E475–E485.

[21] V.C. Tuei, J.S. Ha, C.E. Ha, Effects of human serum albumin complexed with free fatty acids on cell viability and insulin secretion in the hamster pancreatic beta-cell line HIT-T15, Life Sci. 88 (2011) 810–818.

[22] P. Wu, L. Yang, X. Shen, The relationship between GPR40 and lipotoxicity of the pancreatic beta-cells as well as the effect of pioglitazone, Biochem. Biophys. Res. Commun. 403 (2010) 36–39.

[23] J.G. Walsh, S.P. Cullen, C. Sheridan, A.U. Luthi, C. Gerner, S.J. Martin, Executioner caspase-3 and caspase-7 are functionally distinct proteases, Proc. Natl. Acad. Sci USA 105 (2008) 12815–12819.

[24] K. Komiya, T. Uchida, T. Ueno, M. Koike, H. Abe, T. Hirose, R. Kawamori, Y. Uchiyama, E. Kominami, Y. Fujitani, H. Watada, Free fatty acids stimulate autophagy in pancreatic beta-cells via JNK pathway, Biochem. Biophys. Res. Commun. 401 (2010) 561–567.

[25] A. Jaeschke, R.J. Davis, Metabolic stress signaling mediated by mixed-lineage kinases, Mol. Cell. 27 (2007) 498–508.

[26] E. Bachar, Y. Ariav, M. Ketzinel-Gilad, E. Cerasi, N. Kaiser, G. Leibowitz, Glucose amplifies fatty acid-induced endoplasmic reticulum stress in pancreatic beta-cells via activation of mTORC1, PLoS One 4 (2009) e4954.

[27] S.C. Martinez, K. Tanabe, C. Cras-Meneur, N.A. Abumrad, E. Bernal-Mizrachi, M.A. Permutt, Inhibition of Foxo1 protects pancreatic islet beta-cells against fatty acid and endoplasmic reticulum stress-induced apoptosis, Diabetes 57 (2008) 846–859.

[28] S. Bregenholt, A. Moldrup, N. Blume, A.E. Karlsen, B. Nissen Friedrichsen, D. Tornhave, L.B. Knudsen, J.S. Petersen, The long-acting glucagon-like peptide-1 analogue, liraglutide, inhibits beta-cell apoptosis in vitro, Biochem. Biophys. Res. Commun. 330 (2005) 577–584.

[29] J. Buteau, W. El-Assaad, C.J. Rhodes, L. Rosenberg, E. Joly, M. Prentki, Glucagon-like peptide-1 prevents beta cell glucolipotoxicity, Diabetologia 47 (2004) 806–815.

[30] H. Hui, A. Nourparvar, X. Zhao, R. Perfetti, Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5'-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway, Endocrinology 144 (2003) 1444–1455.
[31] L. Farilla, A. Bulotta, B. Hirshberg, S. Li Calzi, N. Khoury, H. Noushmehr, C. Bertolotto, U. Di Mario, D.M. Harlan, R. Perfetti, Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets, Endocrinology 144 (2003) 5149–5158.
[32] L. Farilla, H. Hui, C. Bertolotto, E. Kang, A. Bulotta, U. Di Mario, R. Perfetti, Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats, Endocrinology 143 (2002) 4397–4408.
[33] Y. Kawasaki, S. Harashima, M. Sasaki, E. Mukai, Y. Nakamura, N. Harada, K. Toyoda, A. Hamasaki, S. Yamane, C. Yamada, Y. Yamada, Y. Seino, N. Inagaki, Exendin-4 protects pancreatic beta cells from the cytotoxic effect of rapamycin by inhibiting JNK and p38 phosphorylation, Horm. Metab. Res. 42 (2010) 311–317.
[34] A. Natalicchio, F. De Stefano, M.R. Orlando, M. Melchiorre, A. Leonardini, A. Cignarelli, R. Labarbuta, P. Marchetti, S. Perrini, L. Laviola, F. Giorgino, Exendin-4 prevents c-Jun N-terminal protein kinase activation by tumor necrosis factor-alpha (TNFalpha) and inhibits TNFalpha-induced apoptosis in insulin-secreting cells, Endocrinology 151 (2010) 2019–2029.