Biochemical and Biophysical Research Communications xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

SAD-A, a downstream mediator of GLP-1 signaling, promotes the phosphorylation of Bad S155 to regulate in vitro β-cell functions

Kai Wang [a, 1], Dechen Liu [a, 1], Yaqin Zhang [a, 1], Xiaoai Chang [a], Rufeng Xu [a], Jing Pang [a], Kai Li [a], Peng Sun [a], Yunxia Zhu [a, b], Xiao Han [a, b, *]

[a] Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, Nanjing, 211166, China
[b] Collaborative Innovation Center for Cardiovascular Disease Translational Medicine, Nanjing Medical University, Nanjing, 211166, China

ARTICLE INFO

Article history:
Received 1 December 2018
Accepted 7 December 2018
Available online xxx

Keywords:
SAD-A
GLP-1
Phosphorylation
Bad
β cells

ABSTRACT

The incretin hormone GLP-1 reduces β-cell failure in patients with type 2 diabetes. Previous studies demonstrated that GLP-1 activates SAD-A, a member of the AMPK family, to regulate glucose-stimulated secretion (GSIS), but the underlying mechanisms of SAD-A regulation of β-cell functions remain poorly understood. Here, we propose that activation of SAD-A by GLP-1 promotes the phosphorylation of Bad S155, which in turn positively affects GSIS and β-cell survival. Bad therefore appears to be a downstream molecule of a SAD-A pathway that mediates the GLP-1-triggered reduction in β-cell failure. Knockdown of endogenous SAD-A expression significantly exacerbated in vitro β-cell dysfunction under lipotoxic conditions and promoted lipotoxicity-induced apoptosis, whereas overexpression of SAD-A inhibited β-cell apoptosis. SAD-A silencing increased ER stress and inhibited the autophagic flux, which contributed to β-cell apoptosis. Thus, SAD-A appears to function as a downstream molecule of GLP-1 signaling that results in Bad S155 phosphorylation. This phosphorylation might therefore be involved in the GLP-1-linked protection against β-cell dysfunction and apoptosis.

1. Introduction

The failure of pancreatic β-cell functions contributes to the onset and development of type 2 diabetes (T2DM). Although the underlying causes are still unknown, lipotoxicity is known to play an important role in β-cell failure in T2DM [1], as prolonged exposure to high levels of free fatty acids induces ER stress and leads to impairment of β-cell [2]. The survival of β-cell can be improved by the incretin hormone GLP-1, which has glucose-lowering properties that make it useful clinically in the treatment of T2DM [3]. GLP-1 treatment improve glucose-stimulated insulin secretion (GSIS) by β-cell from patients with T2DM to normalize blood glucose in these patients [4]. The GLP-1 receptor agonist liraglutide also protects β-cells from lipotoxicity-induced apoptosis [5]. However, the molecular mechanisms by which GLP-1reduces β-cell failure are unclear.

One mediator of the GLP-1 signaling pathway is SAD-A, a serine/threonine protein kinase of the AMPK subfamily that regulates GSIS under physiological conditions [6,7]. SAD-A is abundantly expressed in the brain and pancreas and is involved in a variety of neuron functions ranging from neuronal polarity to neurotransmitter release [8,9]. In β-cells, SAD-A is dephosphorylated by PKA to regulate GSIS partially through its action on F-actin remodeling [6,10]. SAD-A functions as a mediator of mTORC1 signaling to control islet β-cell size [11], indicating important roles of SAD-A in pancreatic β-cell physiology. However, the mechanisms by which SAD-A regulates GLP-1 signaling and β-cell functions and the nature of downstream molecules of SAD-A signaling are not yet established.

In mice, glucose metabolism and apoptosis are regulated by phosphorylation of Ser-112,136 and 155 of Bad, a proapoptotic member of the BCL-2 family. In particular, the phosphorylation at Ser 155, within the BH3 domain, promotes the β-cells GSIS and survival in diabetes [12,13]. Biochemical and structural analyses have shown that the phospho-BAD S155 directly binding to activate the glucokinase (GK) [12,13]. This then promotes insulin secretion by increasing the cytoplasmic ATP/ADP ratio, which causes the closure of ATP-sensitive potassium channels (K^+ATP channels), resulting in membrane depolarization, opening of voltage-gated calcium channels, and then Ca^{2+} influx. This glucokinase

* Corresponding author. Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, Nanjing, 211166, China.
E-mail address: hanxiao@njmu.edu.cn (X. Han).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.bbrc.2018.12.063

activation can also suppress the expressions of CHOP and Bax to reduce the ER stress-induced apoptosis of β-cells [14]. The phosphorylation of S155 also causes a conformational change in Bad that disrupts the interaction with the prosurvival Bcl-2 family members such as BCL-2, BCL-XL, and BCL-W proteins on the outer mitochondrial membrane, thereby blocking the apoptotic activity associated with Bad [15,16]. Therefore, the phosphorylation of Bad S155 has dual functions: it contributes to the insulin secretion and it promotes the β-cell survival.

In the present study, we found that SAD-A was activated by GLP-1 to phosphorylate the Bad S155. We hypothesized that the phosphorylation of Bad S155 might be involved in the process whereby SAD-A, as a downstream molecule of GLP-1, mediated lipotoxicity-induced pancreatic β-cells dysfunction and apoptosis.

2. Materials and methods

2.1. Plasmids, antibodies, and reagents

Lipofectamine-2000 was purchased from Invitrogen. Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium and Fetal bovine serum (FBS) were purchased from GIBCO. Antibodies against SAD-A, SAD-B, cleaved-PARP1, cleaved-Caspase-3, p-eIF2α, eIF2α, Bad, LC3, and p62 were purchased from Cell Signaling Technology. Actin and p-Bad S155 antibodies were separately purchased from Sigma-Aldrich and Ablcam. The TUNEL assay kit was purchased from Vazyme Biotech. Nifedipine and Bay K 8664 were purchased from Selleck. The Flag-SAD-A adenovirus was constructed by the Vigene Biosciences. The SAD-A siRNA sequence was: 5′-GAGAGGAACAACAUCCGUATT-3′ and siRNA with sequence of 5′- UUCUCCGAACGUGUCACGUTT-3′was selected as the negative control siRNA (NC-siRNA). The siRNA was constructed by GenePharma. Palmitic acid was purchased from Sigma-Aldrich. The SAD-A, K48M and T443A plasmids were used as described previously [10].

2.2. Cell culture and transient transfection

The mouse MIN6 pancreatic β-cell line was cultured in DMEM medium with 25 mmol/L D-glucose supplemented with 15% FBS, 100 μg/mL streptomycin, 10 mmol/L HEPES, and 50 μmol/L β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO) and 100 U/mL penicillin. The rat INS-1 pancreatic β cell line was cultured in RPMI-1640 medium with 11.1 mmol/L D-glucose supplemented with 8% FBS, 100 μg/mL streptomycin, 10 mmol/L HEPES, 50 μmol/L β-mercaptoethanol, and 100 U/mL penicillin. The protocols for transient transfection of SAD-A plasmids and siRNA into the MIN6 and INS-1 cells were as described previously [6,10] and the treatment with palmitic acid was as described previously [17].

2.3. Islet isolation

Mouse islets were isolated as described previously [17]. Isolated islets were transferred to sterile 6-well plates and cultured in RPMI-1640 containing 11.1 mM glucose supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin for 24 h. Islets were then picked into 48-well plates (8 islets per well) and used for GSIS assays.

2.4. GSIS assays

MIN6 cells or isolated islets were plated in 48-well plates and transfected with siRNA or infected with SAD-A adenovirus for 48 h for GSIS assays. The MIN6 cells or isolated islets were pre-incubated for 1 h in HEPES-balanced Krebs-Ringer bicarbonate buffer (KRBH) containing 2 mM glucose and 1 g/L bovine serum albumin (BSA). The MIN6 cells or isolated islets were then incubated for 1 h in KRBH containing basal glucose (2.8 mM), stimulatory glucose (16.7 mM). After this static incubation period, the culture media were collected and frozen at −80 °C for subsequent determination of insulin concentration. The insulin levels were measured using a radioimmunoassay (RIA), as described previously [6].

2.5. TUNEL assays

The TUNEL assay kit was purchased from Vazyme Biotech. TUNEL staining was performed according to the manufacturer's protocol [18] and the stained cells were scanned with a laser scanning confocal microscope (FV1200, Olympus, Japan).

2.6. Western blot assays

MIN6 cells, INS-1 cells, and isolated mouse islets were cultured and treated with forskolin, exendin-4, palmitate, Tg and siRNA or infected with SAD-A adenovirus as described above. The cells and islets were lysed in a buffer containing 50 mmol/L Tris-HCl, 1% NP-40, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulphonyl fluoride and protease inhibitor cocktail. Western blot assays were performed as previously reported [18].

2.7. Statistical analysis

All data were expressed as mean ± SEM. Prism 7.0 software (GraphPad, La Jolla, CA) were used for statistical analysis of all data using the Student *t*-test or ANOVA, where appropriate. In all cases, $P < 0.05$ was considered statistically significant.

3. Results

3.1. SAD-A is activated by GLP-1 signaling to phosphorylate Bad S155 in β-cells

Evaluation of the activation of SAD-A in response to GSIS by immunoblotting of MIN6 cell proteins confirmed that SAD-A have highly abundant and less abundant forms, which represent the activation of SAD-A in the neuron [19]. The highly abundant form shifted to the less abundant form in response to treatment with 25 mM glucose for 5 min or 15 min but not for 30 min (Fig. 1A). GLP-1 regulates GSIS partially through cAMP and Ca^{2+} signaling pathways, so treatment with Forskolin (an adenylyl cyclase agonist) and exendin-4 (a GLP-1 analogue) also caused a shift from the highly abundant to the less abundant form in MIN6 cells (Fig. 1B and C) and in the isolated mouse islets (Fig. 1E). Conversely, the levels of the abundant form did not change in the αTC-6 cells treated with glucose and Forskolin (Fig. 1D). The levels of abundant forms of SAD-B, which are analogues of SAD-A, did not change in the isolated mouse islets following treatment with forskolin (Fig. 1E). Ca^{2+} influxes also contribute to the onset of GSIS in pancreatic β cells. In the present study, Ca^{2+} influxes also induced the switch to the less abundant form of SAD-A (Fig. 1F). As shown in Fig. 1F, the inductor of Ca^{2+} influx KCl treatment of INS-1 cells and MIN6 cells significantly promoted the shift from the SAD-A abundant form to the less abundant form, whereas L-type calcium channel blocker nifedipine inhibited the shift. L-type calcium channel agonist Bay k8664 also promoted the shift from the abundant form. These results confirmed that SAD-A was activated by cAMP signaling and Ca^{2+} influxes in pancreatic β-cells in response to GSIS.

The exact mechanisms of SAD-A regulation of the GSIS have not been established; however, the phosphorylation of Bad S155 is known to promote GSIS [12,13]. In the present study, we transfected

Please cite this article as: K. Wang et al., SAD-A, a downstream mediator of GLP-1 signaling, promotes the phosphorylation of Bad S155 to regulate in vitro β-cell functions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2018.12.063

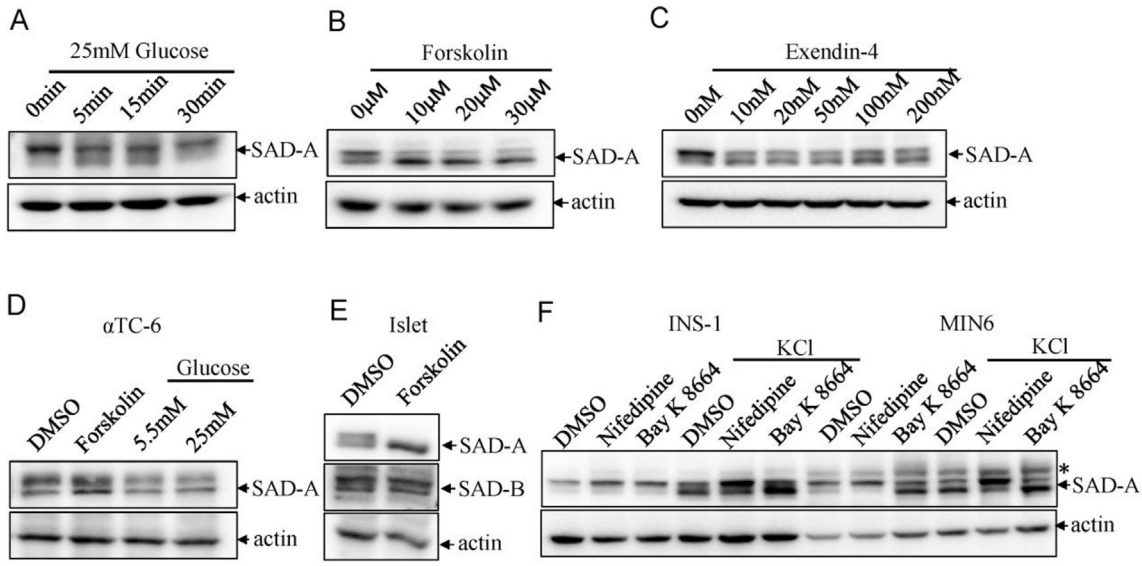

Fig. 1. SAD-A is activated by the cAMP signaling and Ca^{2+} signaling pathway in islet β-cells. (A) MIN6 cells were treated with 25 mM high-glucose for 0, 5, 15, and 30 min and then the SAD-A expression was detected by western blotting. (B) MIN6 cells were treated with 0, 10, 20, and 30 μM forskolin for 30 min and then the SAD-A expression was detected by western blotting. (C) MIN6 cells were treated with 0, 10, 20, 50, 100, and 200 nM exendin-4 for 30 min and then the SAD-A expression was detected by western blotting. (D) The αTC-6 cells were treated with DMSO (control) or 30 μM forskolin and either, 5.5 mM or 25 mM glucose for 30 min and then the SAD-A expression was detected by western blotting. (E) Isolated mouse islets were treated with DMSO or 30 μM forskolin for 30 min and then the SAD-A and SAD-B expressions were detected by western blotting. (F) INS-1 cells and MIN6 cells were treated by 40 mM KCl alone or combination with 10 μM nifedipine and Bay K 8664 for 1 h and then the SAD-A expression was detected by western blotting. * indicates a non-specific band.

INS-1 cells with plasmids containing SAD-A, a kinase-dead SAD-A mutant (K48M), or a constitutively activated SAD-A mutant (T443A) and examined Bad expression by western blotting. As showed in Fig. 2A, overexpression of SAD-A significantly decreased Bad expression, whereas expression of K48M kinase mutant partially restored SAD-A expression, and T443A expression inhibited this restoration. Taken together, these results indicated that SAD-A kinase promoted the phosphorylation of Bad, and this was confirmed by the finding that SAD-A kinase expression significantly enhanced the phosphorylation level of Bad S155 (Fig. 2B). Conversely, knockdown of SAD-A expression significantly decreased the forskolin-induced phosphorylation of Bad S155 in INS-1 cells (Fig. 2C). Knockdown of SAD-A expression also significantly decreased the forskolin-induced phosphorylation of Bad S155 in isolation mouse islets (Fig. 2D). These findings suggested that Bad might be functioning as a downstream molecule of SAD-A in response to GSIS. Therefore, SAD-A might be activated by GLP-1 signaling to phosphorylate Bad in response to GSIS.

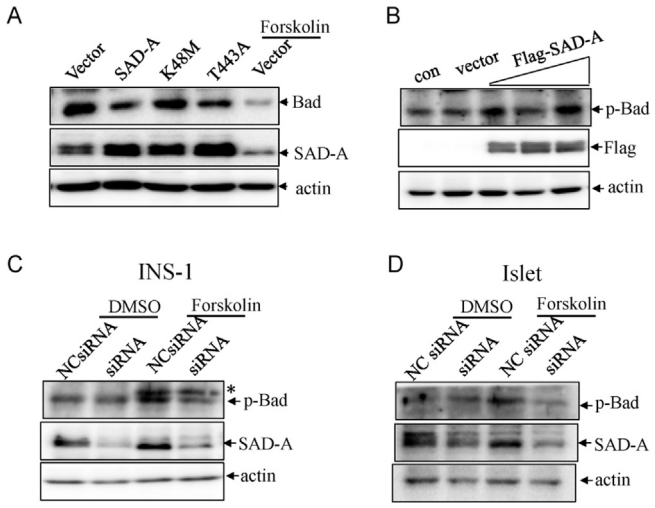

Fig. 2. SAD-A kinase promotes the phosphorylation of Bad S155 in response to glucose-stimulated insulin secretion (GSIS). (A) INS-1 cells were transfected with vector, SAD-A, K48M, or T443A plasmids for 48 h, followed by treatment with 30 μM forskolin for 30 min as a positive control. Bad, SAD-A, and actin expressions were detected by western blotting. (B) INS-1 cells were infected with different doses of the Flag-SAD-A adenovirus for 24 h or infected with the vector adenovirus as a negative control. P-Bad S155, Flag-SAD-A, and actin expressions were detected by western blotting. (C) The INS-1 cells were transfected with the SAD-A siRNA for 48 h and then treatment with DMSO or 30 μM forskolin for 30 min. P-Bad S155, SAD-A, and actin expressions were detected by western blotting. (D) The isolated islets were transfected with the SAD-A siRNA for 48 h and then treated with DMSO or 30 μM forskolin for 30 min. P-Bad S155, SAD-A, and actin expressions were detected by western blotting. * indicates a non-specific band.

3.2. SAD-A mediates the palmitate-induced impairment of GSIS

The phosphorylation of Bad S155 contributes to GSIS [12,13], and SAD-A promotes GSIS under both physiological and lipotoxic conditions. Overexpression of SAD-A significantly increased GSIS in isolated mouse islets (Fig. 3A), whereas downregulation of SAD-A expression by SAD-A siRNA notably decreased GSIS (Fig. 3B). Similarly, knockdown of SAD-A with siRNA in MIN6 cells significantly decreased insulin secretion in response to glucose and Forskolin (Fig. 3C).

Long-term exposure of pancreatic β-cells to high levels of free fatty acids (FFAs) contributes to pancreatic β-cell dysfunction, including the inhibition of GSIS. Long-term (24 h) treatment of INS-1 cells with palmitate significantly decreased the endogenous SAD-A expression in INS-1 cells (Fig. 3D). Long-term exposure to palmitate significantly inhibited GSIS, and SAD-A knockdown enhanced this inhibition (Fig. 3E).

3.3. SAD-A mediates the protection of GLP-1 against the palmitate-induced β-cell apoptosis

The phosphorylation level of Bad S155 controls apoptosis of β

Please cite this article as: K. Wang et al., SAD-A, a downstream mediator of GLP-1 signaling, promotes the phosphorylation of Bad S155 to regulate in vitro β-cell functions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2018.12.063

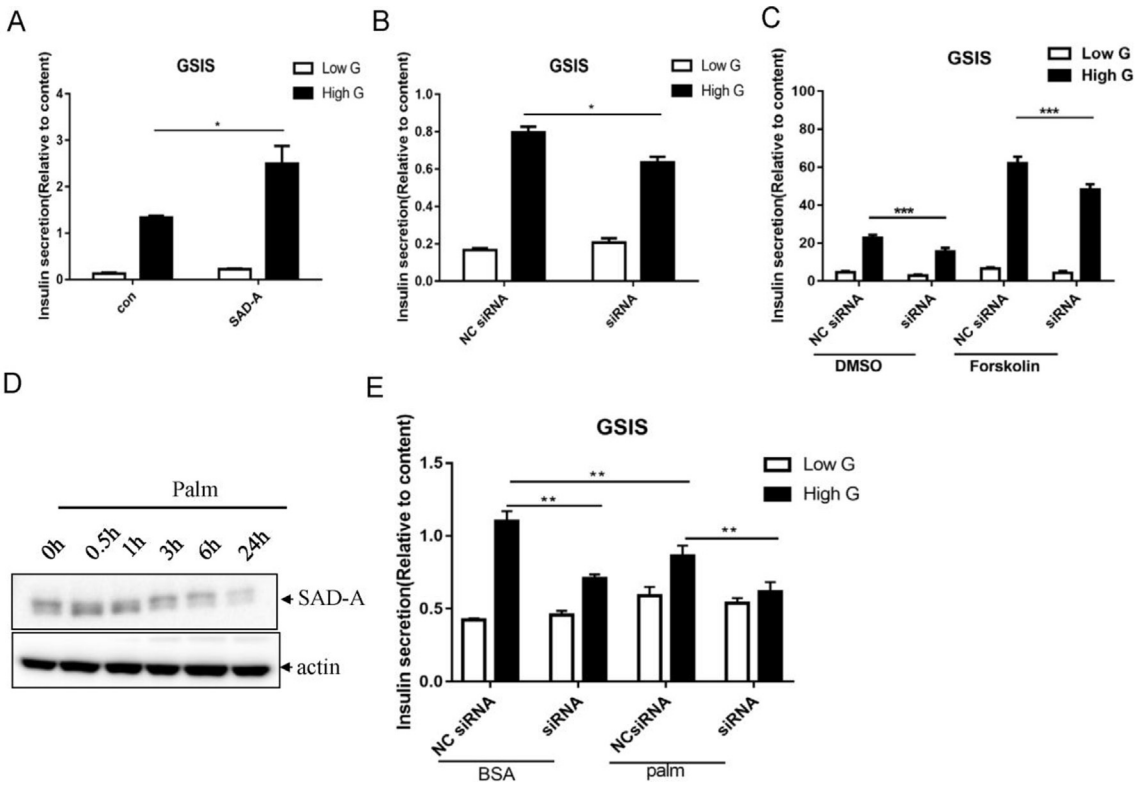

Fig. 3. SAD-A mediates the palmitate-induced impairment of glucose-stimulated insulin secretion (GSIS). (A) The mouse isolated islets were infected by the SAD-A adenovirus for 24 h and then the insulin secretion were assessed by GSIS assays. (B) The mouse isolated islets were transfected with the SAD-A siRNA for 48 h and then the insulin secretion were assessed by GSIS assays. (C) MIN6 cells were transfected with SAD-A siRNA for 48 h, followed by treatment with 30 μM forskolin for 30 min. Insulin secretion were then assessed by GSIS assays. (D) INS-1 cells were treated with the 0.4 mM palmitate for 0, 0.5, 1, 3, 6, and 24 h and SAD-A and actin expressions were detected by western blotting. (E) MIN6 cells were transfected with SAD-A siRNA for 48 h and then treated with BSA (control) or 0.4 mM palmitate for a long-term 24 h exposure. Insulin secretion was detected by GSIS assays. Means ± SEM were shown. White bar: 2.8 mmol/L glucose, black bar: 16.7 mmol/L glucose. *P < 0.05, **P < 0.01, ***P < 0.001.

cells [12,13,16,20]. Long-term exposure of islet β-cells to high levels of FFAs also causes apoptosis at least in part through ER stress [21]. Suppression of SAD-A expression with siRNA further increased INS-1 cells apoptosis in response to palmitate (Fig. 4A) or to thapsigargin (Tg), an ER stress inducer (Fig. 4B). TUNEL assays showed that Tg induced significant apoptosis in MIN6 cells and that overexpression of SAD-A suppressed this Tg-induced apoptosis (Fig. 4C and D). Knockdown of SAD-A expression by siRNA and treatment with 0.3 mM palmitate for 48 h (Fig. 4A) or 1 μg/mL Tg for 24 h (Fig. 4B) indicated that SAD-A knockdown significantly promoted the phosphorylation of eIF2α, which representing the activation of ER stress. Inhibition of autophagic flux also promotes palmitate-induced β-cell apoptosis [22]. SAD-A knockdown significantly inhibited the expression of the autophagic marker, LC3-II, and degradation of the autophagic substrate p62, implying that SAD-A knockdown inhibited the autophagic fluxes during lipotoxicity-induced β cell apoptosis. SAD-A knockdown therefore promoted ER stress during β-cell apoptosis.

GLP-1 protects against palmitate-induced apoptosis [23,24]. Treatment of INS-1 cells with the palmitate promoted the expression of cleaved-PARP1, and this expression was promoted by SAD-A knockdown and attenuated by treatment with exendin-4. SAD-A knockdown attenuated the inhibition of palmitate-induced INS-1 cells apoptosis by exendin-4 (Fig. 4E and F), indicating that SAD-A mediated the protective role of GLP-1 against the palmitate-induced β cell apoptosis.

4. Discussion

GLP-1 is used to reduce β-cell failure as a treatment for type 2 diabetes. Previous studies demonstrated that GLP-1 signaling is mediated by SAD-A to regulate GSIS [6], but the underlying mechanisms have not been identified. In the present study, we found that SAD-A was activated by GLP-1 through cAMP and Ca^{2+} signaling. This activation then triggered phosphorylation of Bad S155, suggesting that this phosphorylation might mediate the protective action of GLP-1 against β-cell dysfunction and apoptosis.

The SAD-A kinase, also referred to as BRSK2, is abundantly expressed in the brain and pancreas. Previous studies on neurons have indicated that SAD-A regulates neuronal development, the neuronal polarity, and neurotransmitter release [9,25,26]. In the neuron, SAD-A kinase can be activated by the NT-3/TrkC signals by promoting the dephosphorylation of the C-terminal domain; this appears, as the less abundant form of SAD-A in the protein bands on SDS-PAGE [19]. We found an induction of this less abundant form of SAD-A in islet β-cell by glucose, cAMP signaling, and even Ca^{2+} influx. This finding further confirmed that SAD-A kinase is activated by the cAMP and Ca^{2+} signaling pathways, which are both confirmed to exist in pancreatic β-cells [27]. Activation of SAD-A by cAMP and Ca^{2+} signaling in β-cells is consistent with positive regulation of GSIS. Interestingly, the levels of the abundant form of SAD-B in mouse islets did not change in response to forskolin treatment, implying that SAD-A and SAD-B have different functions in the β-cells. SAD-A shows highly structural conservation with SAD-B, but they differ at key sites, such as Thr443, which is an autoinhibitory phosphorylation site in the PRD domain of SAD-A that is absent in SAD-B [10].

The phosphorylated form of Bad S155 could be binding to GK to regulate GSIS [12,13]. In the present study, SAD-A was activated by GLP-1 signaling to phosphorylate Bad S155. Ca^{2+}-cAMP is known to

Please cite this article as: K. Wang et al., SAD-A, a downstream mediator of GLP-1 signaling, promotes the phosphorylation of Bad S155 to regulate in vitro β-cell functions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2018.12.063

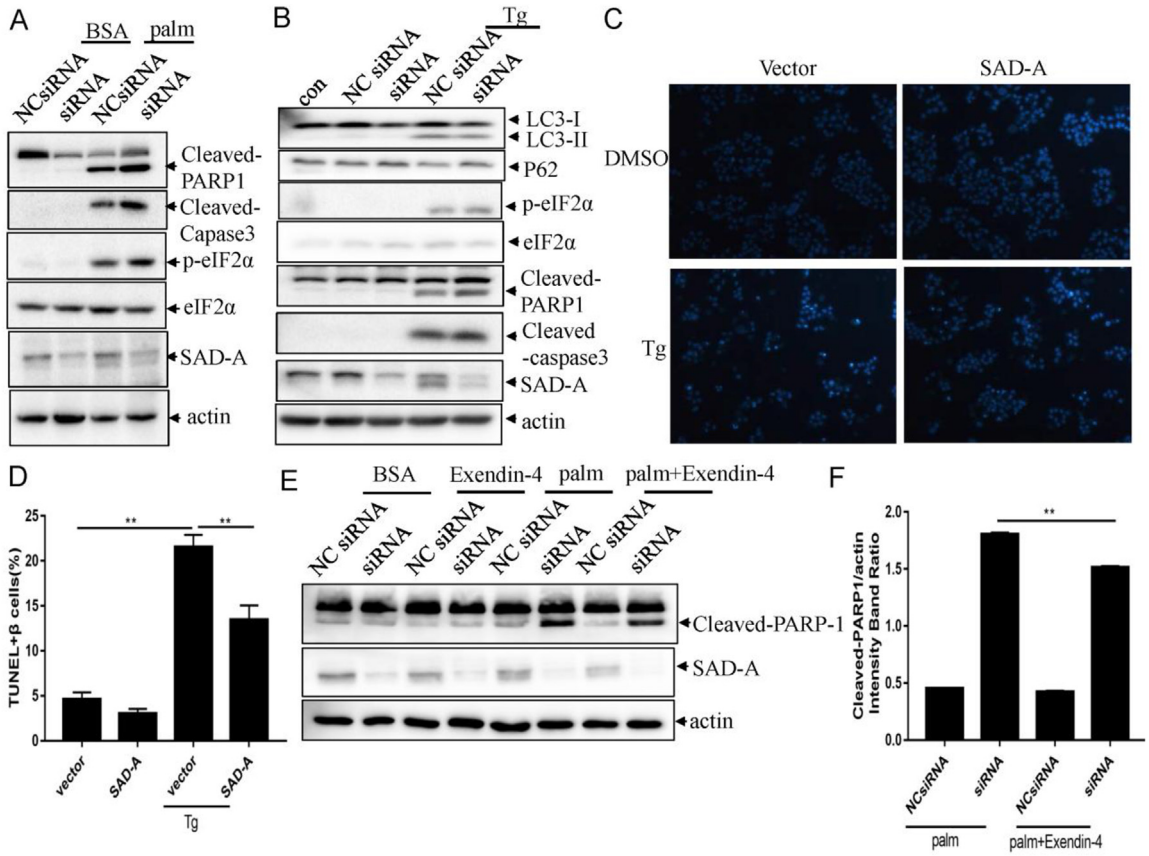

Fig. 4. SAD-A mediates the protective effect of GLP-1 against the pancreatic β-cell apoptosis induced by long-term exposure to palmitate. (A) INS-1 cells were transfected with SAD-A siRNA for 48 h and then treated with BSA or 0.4 mM palmitate for 24 h. The expressions of cleaved-PARP1, SAD-A, cleaved-caspase 3, p-eIF2α, eIF2α and actin were detected by western blotting. (B) INS-1 cells were transfected with SAD-A siRNA for 48 h and then treated with 1 μg/mL thapsigargin (Tg) for 24 h. The expressions of cleaved-PARP1, SAD-A, cleaved-caspase 3, p-eIF2α, eIF2α, LC3, P62 and actin were detected by western blotting. (C) MIN6 cells were transfected with SAD-A plasmids for 48 h and then treated with 1 μg/mL Tg for 24 h. Apoptosis was detected by TUNEL assays. (D) TUNEL-positive β cells were counted. (E) INS-1 cells were transfected with SAD-A siRNA for 24 h and then treated with BSA (control) or 0.4 mM palmitate for 24 h, alone or combination with 10 nM exendin-4 for 12 h. Expressions of cleaved-PARP1, SAD-A, and actin were detected by western blotting. (F) The optical density of the cleaved-PARP1 band in Fig. 4E was measured by densitometric analysis using ImageJ software and the ratio of cleaved-PARP1/actin was calculated. Means ± SEM were shown. $^{**}P < 0.01$.

signal the phosphorylation of Bad S155 [28–30] and the Ca^{2+}-cAMP oscillatory circuit is present in pancreatic β-cells [27], indicating that SAD-A may be activated by GLP-1 signaling and then phosphorylates Bad S155 to regulate the β-cell functions. SAD-A regulated GSIS under both physiological and lipotoxic-conditions. Saturated FFAs such as palmitate, one of the most abundant FFAs in plasma, are highly toxic to β-cells [22]. SAD-A expression decreased in INS-1 cells following long-term exposure to palmitate, and knockdown of SAD-A expression further inhibited the GSIS-caused by long-term exposure to palmitate.

The phosphorylated form of Bad S155, in addition to activating GK, could also be interacting with the Bcl-2 family to regulate apoptosis [14,16,20], thereby providing a mechanism by which SAD-A could mediate β-cell apoptosis. SAD-A silencing promoted β-cell apoptosis, whereas overexpression of SAD-A inhibited palmitate-induced β-cell apoptosis, indicating that the protective effect of GLP-1 against the palmitate-induced β-cell apoptosis is probably mediated by SAD-A. Palmitate induced β-cell apoptosis occurs as a consequence of ER stress [31], and SAD-A silencing enhanced the phosphorylation of eIF2α, a hallmark of ER stress, during lipid-induced β-cell apoptosis. Autophagy also plays a protective role in ER stress-mediated β-cell apoptosis [31], and GLP-1 protects β-cells from apoptosis by increasing autophagic flux [24]. Knockdown of SAD-A expression inhibited the expression of LC3-II and degradation of the autophagic substrate p62 in the β-cells, suggesting that SAD-A mediated this protective effect of GLP-1.

In summary, we propose here that SAD-A, is a downstream molecule of GLP-1 signaling that promotes the phosphorylation of Bad S155 to regulate β-cell functions, and that SAD-A is a key component of the mechanism controlling lipotoxicity-induced β-cell apoptosis. Further studies will be needed to elucidate the precise function of SAD-A in regulating GSIS and pancreatic β-cell apoptosis, as well as its exact roles in pancreatic β-cells.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This work was supported by NSFC Major International (Regional) Joint Research Program (81420108007), National Key Research and Development Program of China (2016YFC 1304804 to X.H.), grants from the National Natural Science Foundation (81600598, 81603169, 81702882).

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.12.063.

References

[1] S.E. Kahn, R.L. Hull, K.M. Utzschneider, Mechanisms linking obesity to insulin resistance and type 2 diabetes, Nature 444 (2006) 840–846.

[2] T.J. Biden, E. Boslem, K.Y. Chu, N. Sue, Lipotoxic endoplasmic reticulum stress, beta cell failure, and type 2 diabetes mellitus, Trends Endocrinol. Metabol. 25 (2014) 389–398.

[3] L. Prasad-Reddy, D. Isaacs, A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond, Drugs Context 4 (2015) 212283.

[4] Y.S. Lee, H.S. Jun, Anti-diabetic actions of glucagon-like peptide-1 on pancreatic beta-cells, Metabolism 63 (2014) 9–19.

[5] X.Y. Miao, Z.Y. Gu, P. Liu, Y. Hu, L. Li, Y.P. Gong, H. Shu, Y. Liu, C.L. Li, The human glucagon-like peptide-1 analogue liraglutide regulates pancreatic beta-cell proliferation and apoptosis via an AMPK/mTOR/P70S6K signaling pathway, Peptides 39 (2013) 71–79.

[6] J. Nie, B.N. Lilley, Y.A. Pan, O. Faruque, X. Liu, W. Zhang, J.R. Sanes, X. Han, Y. Shi, SAD-A potentiates glucose-stimulated insulin secretion as a mediator of glucagon-like peptide 1 response in pancreatic beta cells, Mol. Cell Biol. 33 (2013) 2527–2534.

[7] J. Nie, C. Sun, Z. Chang, N. Musi, Y. Shi, SAD-A promotes glucose-stimulated insulin secretion through phosphorylation and inhibition of GDIalpha in male islet beta-cells, Endocrinology (2018) 3036–3047.

[8] A.P. Barnes, B.N. Lilley, Y.A. Pan, L.J. Plummer, A.W. Powell, A.N. Raines, J.R. Sanes, F. Polleux, LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons, Cell 129 (2007) 549–563.

[9] E. Inoue, S. Mochida, H. Takagi, S. Higa, M. Deguchi-Tawarada, E. Takao-Rikitsu, M. Inoue, I. Yao, K. Takeuchi, I. Kitajima, M. Setou, T. Ohtsuka, Y. Takai, SAD: a presynaptic kinase associated with synaptic vesicles and the active zone cytomatrix that regulates neurotransmitter release, Neuron 50 (2006) 261–275.

[10] J. Nie, C. Sun, O. Faruque, G. Ye, J. Li, Q. Liang, Z. Chang, W. Yang, X. Han, Y. Shi, Synapses of amphids defective (SAD-A) kinase promotes glucose-stimulated insulin secretion through activation of p21-activated kinase (PAK1) in pancreatic beta-Cells, J. Biol. Chem. 287 (2012) 26435–26444.

[11] J. Nie, X. Liu, B.N. Lilley, H. Zhang, Y.A. Pan, S.R. Kimball, J. Zhang, W. Zhang, L. Wang, L.S. Jefferson, J.R. Sanes, X. Han, Y. Shi, SAD-A kinase controls islet beta-cell size and function as a mediator of mTORC1 signaling, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 13857–13862.

[12] N.N. Danial, L.D. Walensky, C.Y. Zhang, C.S. Choi, J.K. Fisher, A.J. Molina, S.R. Datta, K.L. Pitter, G.H. Bird, J.D. Wikstrom, J.T. Deeney, K. Robertson, J. Morash, A. Kulkarni, S. Neschen, S. Kim, M.E. Greenberg, B.E. Corkey, O.S. Shirihai, G.I. Shulman, B.B. Lowell, S.J. Korsmeyer, Dual role of proapoptotic BAD in insulin secretion and beta cell survival, Nat. Med. 14 (2008) 144–153.

[13] S. Ljubicic, K. Polak, A. Fu, J. Wiwczar, B. Szlyk, Y. Chang, J.C. Alvarez-Perez, G.H. Bird, L.D. Walensky, A. Garcia-Ocana, N.N. Danial, Phospho-BAD BH3 mimicry protects beta cells and restores functional beta cell mass in diabetes, Cell Rep. 10 (2015) 497–504.

[14] Y. T, J. Shirakawa, E. Sakamoto, M. Kaji, K. Tajima, K. Orime, H. Inoue, N. Kubota, T. Kadowaki, Y. Terauchi, Glucokinase activation ameliorates ER stress-induced apoptosis in pancreatic beta-cells, Diabetes 62 (2013) 11.

[15] A. K, S.R. Datta, L. Hu, A. Petros, S.W. Fesik, M.B. Yaffe, M.E. Greenberg, 14-3-3 proteins and survival kinases cooperate to inactivate BAD by BH3 domain phosphorylation, Mol. Cell 6 (2000) 11.

[16] Y. Tan, M.R. Demeter, H. Ruan, M.J. Comb, BAD Ser-155 phosphorylation regulates BAD/Bcl-XL interaction and cell survival, J. Biol. Chem. 275 (2000) 25865–25869.

[17] W. Y, Q. Huang, Y. Li, Y. Sun, Y. Zhou, Y. Zhang, D. Liu, S. Zhan, Y. Zhu, X. Han, Glucolipotoxicity-inhibited miR-299-5p regulates pancreatic beta-cell function and survival, Diabetes 67 (2018) 2280–2292.

[18] Y. Li, T. Zhang, Q. Huang, Y. Sun, X. Chang, H. Zhang, Y. Zhu, X. Han, Inhibition of tumor suppressor p53 preserves glycation-serum induced pancreatic beta-cell demise, Endocrine 54 (2016) 383–395.

[19] B.N. Lilley, Y.A. Pan, J.R. Sanes, SAD kinases sculpt axonal arbors of sensory neurons through long- and short-term responses to neurotrophin signals, Neuron 79 (2013) 39–53.

[20] P. P, K. Virdee, A.M. Tolkovsky, Phosphorylation of the pro-apoptotic protein BAD on serine 155, a novel site contributes to cell survival, Curr. Biol. 10 (2000).

[21] E. D, J.N. Winnay, C.W. Liew, R.N. Kulkarni, C.R. Kahn, p85a deficiency protects beta cells from endoplasmic reticulum stress induced apoptosis, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 1192–1197.

[22] G. Las, S.B. Serada, J.D. Wikstrom, G. Twig, O.S. Shirihai, Fatty acids suppress autophagic turnover in beta-cells, J. Biol. Chem. 286 (2011) 42534–42544.

[23] S. Tsunekawa, N. Yamamoto, K. Tsukamoto, Y. Itoh, Y. Kaneko, T. Kimura, Y. Ariyoshi, Y. Miura, Y. Oiso, I. Niki, Protection of pancreatic beta-cells by exendin-4 may involve the reduction of endoplasmic reticulum stress; in vivo and in vitro studies, J. Endocrinol. 193 (2007) 65–74.

[24] K. C, F.P. Zummo, M. Honkanen-Scott, J.A.M. Shaw, P.E. Lovat, C. Arden, Glucagon-like peptide-1 protects pancreatic beta cells from death by increasing autophagic flux and restoring lysosomal function, Diabetes 66 (2017) 1272–1285.

[25] M. Z, J.G. Crump, Y. Jin, C.I. Bargmann, The SAD-1 kinase regualtes presynaptic vesicle clustering and Axon termination, Neuron 29 (2001) 115–129.

[26] P.Y. Kishi M, J.G. Crump, J.R. Sanes, Mammalian SAD kinases are required for neuronal polarization, Science 307 (2005) 4.

[27] Q. Ni, A. Ganesan, N.N. Aye-Han, X. Gao, M.D. Allen, A. Levchenko, J. Zhang, Signaling diversity of PKA achieved via a Ca2+-cAMP-PKA oscillatory circuit, Nat. Chem. Biol. 7 (2011) 34–40.

[28] N. E, M. Haidar, Y. Ding, E. Kamau, G. Langsley, Transforming growth factor beta 2 promotes transcription of COX2 and EP4 leading to a prostaglandin E2-driven autostimulatory loop that enhances virulence of Theileria annulata-transformed macrophages, Infect. Immun. 83 (2015) 1869–1880.

[29] H.J. Park, K.H. Park, K.S. Shin, M.K. Lee, The roles of cyclic AMP-ERK-Bad signaling pathways on 6-hydroxydopamine-induced cell survival and death in PC12 cells, Toxicol. In Vitro 27 (2013) 2233–2241.

[30] D. Samovski, B. Kalderon, E. Yehuda-Shnaidman, J. Bar-Tana, Gating of the mitochondrial permeability transition pore by long chain fatty acyl analogs in vivo, J. Biol. Chem. 285 (2010) 6879–6890.

[31] A. Bartolome, C. Guillen, M. Benito, Autophagy plays a protective role in endoplasmic reticulum stress-mediated pancreatic beta cell death, Autophagy 8 (2012) 1757–1768.

Please cite this article as: K. Wang et al., SAD-A, a downstream mediator of GLP-1 signaling, promotes the phosphorylation of Bad S155 to regulate in vitro β-cell functions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2018.12.063