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Increased expression of ERp57/GRP58 is protective against pancreatic beta cell death caused by autophagic failure

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ABSTRACT

Autophagy is a tightly regulated self-digestion system. As in other cell types, autophagy plays an essential role in the homeostasis of pancreatic beta cells. However, the mechanisms involved in the deterioration of beta cell function caused by autophagic failure have not yet been fully elucidated. To gain insight into its mechanisms, we compared the protein expression of islets from beta cell-specific Atg7-deficient mice ($Atg7^{\Delta\beta\text{-cell}}$ mice) and their controls ($Atg7^{f/f}$ mice). Liquid chromatography/mass spectrometry after 1-dimensional electrophoresis identified the increased expression of ERp57/GRP58 in islets isolated from $Atg7^{\Delta\beta\text{-cell}}$ mice compared with those from $Atg7^{f/f}$ mice. The expression level of ERp57 was also elevated in rat insulinoma INS-1 cells by inducible knock-down of the *atg7*-gene. In Atg7 knock-down INS-1 cells, the suppression of ERp57 expression by siRNA resulted in an increase in the level of cleaved Caspase-3 protein and a decrease in the number of live cells. Furthermore, cell cycle analyses demonstrated that the suppressed expression of ERp57 increased the sub-G1 population. These data reveal that increased expression of ERp57 may contribute to the protection from beta cell death caused by autophagic failure.

1. Introduction

Type 2 diabetes mellitus is characterized by insulin resistance and pancreatic beta cell dysfunction. During the development of type 2 diabetes, pancreatic beta cells initially compensate for the insulin resistance, however, beta cells gradually become unable to compensate for this insulin resistance, because of the inadequate expansion of beta cell mass and inadequate insulin secretion [1–3]. Therefore, to gain insight into the pathogenesis of type 2 diabetes, elucidating the mechanisms of beta cell failure is one of the most important research themes.

Autophagy is an intracellular process that plays crucial roles in cellular homeostasis through the degradation and recycling of organelles. Since this process is essential for protein quality control, impairment of autophagy causes structural and functional abnormalities in a variety of cells including pancreatic beta cells [4–9]. Beta-cell-specific Atg7-deficient mice ($Atg7^{\Delta\beta\text{-cell}}$ mice) showed increased apoptosis and decreased proliferation of beta cells, thus resulting in the reduction of beta-cell mass, hypoinsulinemia and hyperglycemia [8,9]. These results clearly demonstrated that autophagy is indispensable for maintaining the structure, mass and function of beta cells. In addition, we recently reported that expression of p62, a specific substrate of autophagy, is increased in the islets of *db*/*db* mice and patients with type 2 diabetes mellitus [10]. These data suggest that autophagic failure in beta cells is involved in the pathogenesis of beta cells in type 2 diabetes mellitus.

Abbreviations: $Atg7^{\Delta\beta\text{-cell}}$ mice, beta-cell-specific Atg7-deficient (RIP-Cre+:Atg7-flox/flox) mice; $Atg7^{f/f}$ mice, Atg7flox/flox mice; LC3, microtubule-associated protein-1 light chain 3; LC–MS, liquid chromatography/mass spectrometry; PDI, protein disulfide isomerase; RIP-Cre, Rat Insulin Promoter-driven Cre recombinase; UPR, unfolded protein response.

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Beta cells show a high biosynthesis rate of proinsulin, which forms a complex tertiary structure containing three disulfide bonds [11,12]. The correct folding of proinsulin is easily disturbed by inflammatory reactions and oxidative stress in beta cells [13]. Thus, it has been conceived that beta cells are easily exposed to ER stress, and an increase in ER stress is associated with beta cell dysfunction in type 2 diabetes [13,14]. As a protective system against ER stress, autophagy is induced by ER stress and contributes to the improvement of cell survival by eliminating damaged organelles [15]. According to a recent study, treatment with rapamycin, an autophagy enhancer prevented beta-cell apoptosis and improved hyperglycemia in Akita mice, in which beta cells are genetically exposed to enhanced ER stress [16]. In addition, the islets isolated from $Atg7^{\Delta\beta\text{-cell}}$ mice show enhanced thapsigargin-induced cell death and an impaired unfolded protein response (UPR) [8,9]. These data suggest that autophagic failure in beta cells renders them susceptible to cell death caused by ER stress.

ERp57/GRP58 is a member of the protein disulfide isomerase (PDI) family. Whereas ERp57 is present in many subcellular locations and potentially has diverse functions, it is mainly localized in the ER and supports the proper folding of glycoproteins, by forming complexes with calreticulin and calnexin. However, regulation of the expression of ERp57 and its role in islets associated with autophagic failure have not yet been investigated. In this study, we found that the expression of ERp57 was enhanced in the islets isolated from $Atg7^{\Delta\beta\text{-cell}}$ mice. Accordingly, we investigated the role of ERp57 in the survival of beta cells with autophagic failure.

2. Materials and methods

2.1. Animals and isolation of islets

The study protocol was reviewed and approved by the Animal Care and Use Committee of Juntendo University. All mice were housed in specific pathogen-free barrier facilities, maintained under a 12 h light/dark cycle, and fed standard rodent food (Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. We used the Rat Insulin Promoter-driven Cre recombinase (RIP-Cre) to delete the *ATG7* gene in a pancreatic beta cell-specific manner. The generation of Atg7flox/+ mice was described previously [5]. We crossed RIP-Cre+:Atg7flox/flox mice with Atg7flox/flox ($Atg7^{f/f}$ mice) mice to generate $Atg7^{f/f}$ mice and RIP-Cre+:Atg7flox/flox mice ($Atg7^{\Delta\beta\text{-cell}}$ mice). Pancreatic islets were isolated from $Atg7^{\Delta\beta\text{-cell}}$ and $Atg7^{f/f}$ mice at the age of 11–12 weeks, by the collagenase digestion method as described previously [17].

2.2. Electrophoresis and silver stain

The isolated islets were lysed in lysis buffer (20 mM Tris–HCl [pH 7.5], 1 mM ethylenediaminetetraacetic acid [EDTA], 140 mM NaCl, 1% Nonidet P-40 [NP-40], 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride [PMSF], 50 mM NaF, and 10 μl/ml proteinase cocktail [Sigma, St. Louis, MO]). Ten micrograms of the cell lysates were separated by 1-dimensional SDS–PAGE with 5–15% gradient gels. Then, the gels were stained with a PlusOne Silver Staining Kit (GE Healthcare Life Sciences, Uppsala, Sweden) and soaked in the fixing solution (30% ethanol/10% glacial acetic acid) for 30 min, in the sensitizing solution (30% ethanol/4% sodium thiosulphate [5% w/v]/sodium acetate 17 g) for 30 min, in the silver solution (10% silver nitrate solution [2.5% w/v]) for 20 min and in the developing solution (sodium carbonate 6.25 g/0.4% formaldehyde [37% w/v]) for 2–5 min, and finally in the stop solution (1.46% EDTA-Na) for 10 min. The gels were preserved in a solution containing 30% ethanol/4.6% glycerol (87% w/w).

2.3. In-gel digestion of protein samples

The proteins separated on 1-dimensional SDS–PAGE were subjected to gel digestion as described previously [18,19]. Briefly, bands were excised manually using a razor blade, placed in microtubes, washed with H_2O (10 min, 37 °C, 5 times), and destained in 100 μl of a working solution (30 mM potassium ferricyanide:100 mM sodium thiosulfate = 1:1) for 10 min at 37 °C until colorless. Gels were dehydrated in 100 ml CH_3CN in a microtube for 10 min at 37 °C and in a MicroVac MV-100 (Tomy, Tokyo, Japan) for 5 min. The dried residue was rehydrated by adding 50 μl of 0.001% trypsin (Promega, Madison, WI) in 100 mM ammonium bicarbonate (pH 8.5) and incubated overnight at 37 °C. The incubation mixture in the microtube was centrifuged, and the residue was extracted with 50% CH_3CN and 0.1% trifluoroacetic acid, and centrifuged again. The residue was further extracted with 15% isopropyl alcohol, 20% formic acid, 25% CH_3CN, 40% H_2O, and finally with 80% CH_3CN. The supernatant and all extracts were dried in a single microtube, and the residue was dissolved in 5–10 μl of 0.1% formic acid. Aliquots were used for peptide identification by mass spectrometry.

2.4. Liquid chromatography/mass spectrometry (LC–MS) analysis and protein identification

The digested peptides were analyzed using a nano-flow LC–MS system with a direct nano-flow LC system (DiNa; KYA Technologies, Hachioji, Japan) and an LTQ Orbitrap XL ETD mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). The samples were injected into a nano LC Column C18 RP chromatograph (0.1 mm, inner diameter × 100 mm, KYA Technologies) and eluted with 0.1% formic acid (solvent A) and 0.1% formic acid in 90% CH_3CN (solvent B) using a program of 0% solvent B for 30 min, a gradient of 1.45%/min for 55 min, 100% solvent B for 10 min, and a flow rate of 300 nl/min. The eluted peptides were introduced online into the mass spectrometer. Data were searched against the UniPortKB/SwissProt (ver. 2014. 4) database using the Mascot search engine (Matrix Science Inc, Boston, MA).

2.5. Cell culture and immunoblot analyses

Tetracycline-inducible Atg7 knock-down INS-1 (Atg7KD INS-1) cells that we recently established [10] were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. To delete the Atg7 gene, INS-1 cells were cultured with 2 μg/ml tetracycline for 72 h. To analyze the accumulation of microtubule-associated protein-1 light chain 3 (LC3)-II, 10 μg/ml pepstatin A (Nacalai tesque, Tokyo, Japan) and 10 μg/ml E64d (Peptide Institute Inc., Minoh, Japan) were added to the culture medium at 2 h before harvesting of the cells. Immunoblot analyses using islet cell extracts and cell extracts from Atg7KD INS-1 cells were performed as described previously [17]. An anti-LC3 antibody from Sigma Aldrich Japan (Tokyo, Japan), an anti-p62 antibody from PROGEN (Heidelberg, Germany), an anti-Atg7 antibody prepared as described previously [20], an anti-ERp57 antibody from Cell Signaling Technology Japan (Tokyo, Japan), an anti-Caspase-3 from Cell Signaling Technology Japan, an anti-cleaved Caspase-3 antibody from Cell Signaling Technology Japan, and an anti-GAPDH antibody from Cell Signaling Technology Japan were used and the amount of the proteins were quantified with a Fuji LAS 3000 system (Fuji Film, Tokyo, Japan).

2.6. Silencing of ERp57 using siRNA

Gene expression of ERp57 was suppressed by the siRNA method using Stealth RNAi® (Life Technology Japan, Tokyo, Japan). The siR-

NA sequence used were as follows; siERp57-1: CCUAGGACUG CCGAUGGAAUUGUCA siERp57-2: CCUUCUCCAUAUGAAGUCAAG GGUU, siERp57-3: GAUUCCUGUUGUGGCUAU-CAGAACU. Two nanograms of siRNA was introduced into 2.0×10^5 Atg7KD INS-1 cells via nucleofection with a Nucleofector II (Lonza Japan, Tokyo, Japan) using Solution V/program T-20 as described in the instruction manual. As a negative control, transfection with an irrelevant siRNA (Invitrogen USA, Cat. No. 12935-300) was performed.

2.7. Live-cell count and sub-G1 analyses

After treatment with or without tetracycline, Atg7KD INS-1 cells were seeded at 2.0×10^5 cells/well in a 24-well plate. After culturing for 72 h, cells were transfected with siRNA for ERp57 or the control siRNA. After the Atg7KD INS-1 cells were cultured for another 3 days, they were washed with PBS and treated with Trypsin–EDTA for a few minutes. Then, after the addition of culture medium containing fetal bovine serum, the cells were harvested, centrifuged and then re-suspended in 350 μl PBS to count the number of live cells, or in 350 μl propidium iodine solution (50 μl/ml) with 4 mM Na-citrate, 450 μl/ml RNase and 0.1% Triton X and incubated for 10 min for sub-G1 analysis. Regarding the live-cell count, after trypan blue staining, the number of cells negative for trypan blue were counted automatically using a Countess automatic cell counter (Life Technologies). Regarding the sub-G1 analyses, 50 μl NaCl (1.5 M) was added to the mixtures and then incubated for 10 min. These samples were subjected to DNA content analyses using a FACSort flow cytometer (Becton Dickinson, Franklin Lakes, NJ). The number of cells belonging to sub-G1 was counted automatically. Sub-G1 populations were calculated as the number of cells belonging to sub-G1 divided by the total number of cells.

2.8. Data analyses

Results are presented as means ± SEM. Statistical differences between the 2 groups were calculated by the unpaired Student's *t*-test or Mann–Whitney *U*-test as appropriate, and between more than 3 groups by non-repeated ANOVA with the Bonferroni's post hoc test. A p value less than 0.05 was considered to indicate a statistically significant difference between the groups.

3. Results

3.1. ERp57 expression is increased in Atg7$^{\Delta\beta\text{-cell}}$ islets

To search for molecules that play roles in the deterioration of beta cells upon autophagic failure, we compared the protein expression patterns of islets isolated from Atg7$^{\Delta\beta\text{-cell}}$ mice with those from the control Atg7$^{f/f}$ mice by 1-dimensional electrophoresis with silver staining (Fig. 1A). We identified 6 bands that are more intense in the lanes of Atg7$^{\Delta\beta\text{-cell}}$ islets compared with Atg7$^{f/f}$ islets. We isolated these 6 bands and subjected them to LC-MS, and identified several peptides derived from several proteins (Supplemental Table 1). Among them, we focused on ERp57 (GRP58/PDIA3), ERp59, carboxypeptidase E and protein disulfide-isomerase A4, and investigated their protein expression in the islets of both Atg7$^{\Delta\beta\text{-cell}}$ mice and Atg7$^{f/f}$ mice. Whereas the expression of ERp59, carboxypeptidase E and protein disulfide-isomerase A4 were comparable between Atg7$^{\Delta\beta\text{-cell}}$ islets and Atg7$^{f/f}$ islets (data not shown), the expression of ERp57 was significantly increased in the Atg7$^{\Delta\beta\text{-cell}}$ islets (Fig. 1B).

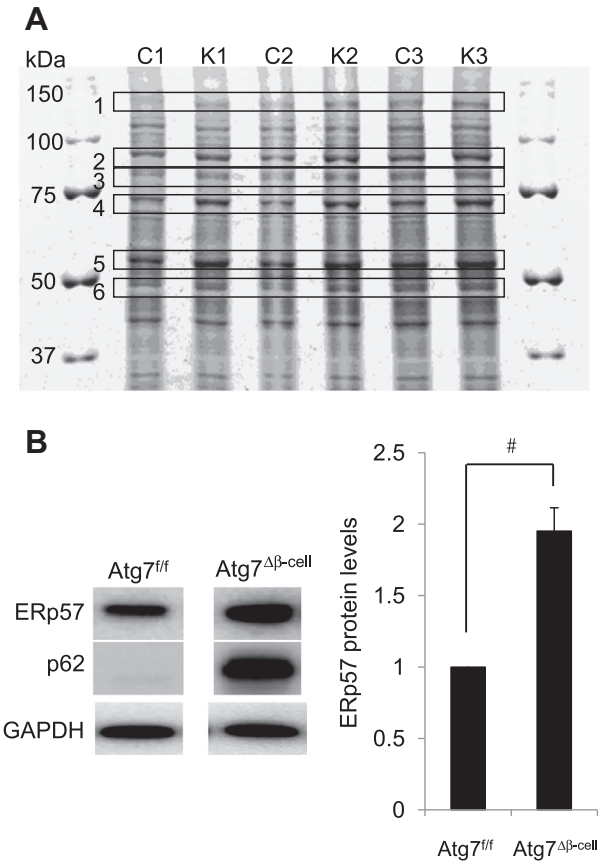

Fig. 1. Identification of ERp57 as a protein with increased expression in Atg7$^{\Delta\beta\text{-cell}}$ islets. (A) One-dimensional electrophoresis pattern of whole-cell lysates from Atg7$^{f/f}$ (C1–3) and Atg7$^{\Delta\beta\text{-cell}}$ (K1–3) islets. The bands excised from the gel and analyzed for LC–MS are shown as numbers. (B) Protein expression levels of ERp57 in Atg7$^{f/f}$ and Atg7$^{\Delta\beta\text{-cell}}$ islets. Representative results (left panel) and quantification of protein expression (right panel). Data are means ± SEM of 6 independent experiments. $^{\#}p < 0.01$.

3.2. ERp57 expression is increased in Atg7 knock-down INS-1 cells

In Atg7$^{\Delta\beta\text{-cell}}$ islets, autophagic failure is theoretically present from before birth. Thus, the increased expression of ERp57 could be a result of systemic metabolic changes elicited by autophagic failure in the Atg7$^{\Delta\beta\text{-cell}}$ islets. To investigate whether these changes are cell-autonomous effects, we assessed the expression of ERp57 in the tetracycline-inducible Atg7KD INS-1 cells. In this cell line, the expression of Atg7 was decreased by approximately 90% after treatment with tetracycline for 72 h [10]. We found that the expression of ERp57 was increased in the tetracycline-treated Atg7KD INS-1 cells compared with tetracycline non-treated Atg7KD INS-1 cells (Fig. 2). These data suggest that the increased expression of ERp57 in the islets of Atg7$^{\Delta\beta\text{-cell}}$ mice might be due to cell-autonomous effects.

3.3. ERp57 demonstrates protective effects against apoptotic cell death in beta cells with autophagic failure

To investigate the role of ERp57 in beta cells with autophagic failure, we treated Atg7KD INS-1 cells with or without tetracycline and then transfected them with 3 kinds of ERp57-targeted siRNAs to reduce the expression of ERp57. The transfection of all siRNAs for ERp57 (siERp57-1,2,3) reduced the expression of ERp57 effectively in both tetracycline-treated and non-treated Atg7KD INS-1 cells compared with non-targeting (NT) siRNA transfected cells

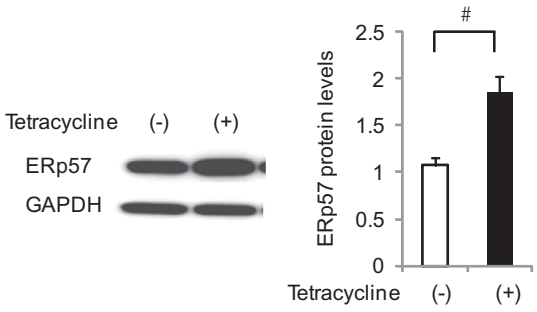

Fig. 2. Deletion of Atg7 increases the expression of ERp57 in Atg7KD INS-1 cells. Protein levels of ERp57 in Atg7KD INS-1 cells treated with or without tetracycline. Representative results (left panel) and quantification of protein expression (right panel). Data are means ± SEM of 6 independent experiments. $^{\#}p < 0.01$ vs. tetracycline-treated Atg7KD INS-1 cells.

(Fig. 3A). Live-cell count analyses showed that the reduced expression of both Atg7 and ERp57 significantly reduced the cell viability of these cells. ERp57 suppression at least by siER57-2 and siERp57-3 on reduced expression of Atg7 in INS-1 cells further reduced cell viability (Fig. 3B).

In beta cells, autophagic failure enhances apoptosis [10]. Therefore, we investigated the effect of ERp57 depletion on apoptosis in beta cells. Tetracycline-treated Atg7KD INS-1 cells induced cleaved Caspase-3 protein that represents the early state of apoptosis more than tetracycline non-treated cells. The suppression of ERp57 further increased the levels of cleaved Caspase-3 (Fig. 3C and D). Furthermore, sub-G1 analyses to detect cells in the late stage of the apoptosis process showed that suppression of ERp57 did not alter the sub-G1 population in non-treated Atg7KD INS-1 cells, and that the sub-G1 population was comparable between tetracycline-treated and non-treated Atg7KD INS-1 cells. However, suppression of ERp57 increased the sub-G1 population only in tetracycline-treated Atg7KD INS-1 cells (Fig. 4A and B).

4. Discussion

Autophagy is regarded as an essential mechanism to maintain cell homeostasis by degrading and recycling old organelles and unfolded proteins. Indeed, the accumulation of disorganized mitochondria and ubiquitinated proteins was observed together with impaired beta cell function in the islets of Atg7$^{\Delta\beta\text{-cell}}$ mice [8]. As a first step towards elucidating the mechanism of beta cell dysfunction upon autophagic failure, we searched for proteins that play roles in beta cells with autophagic failure, and identified ERp57 as a protein with higher expression levels in Atg7$^{\Delta\beta\text{-cell}}$ islets compared with control islets. The increased expression of ERp57 was also observed in Atg7 knock-down INS-1 cells, suggesting that

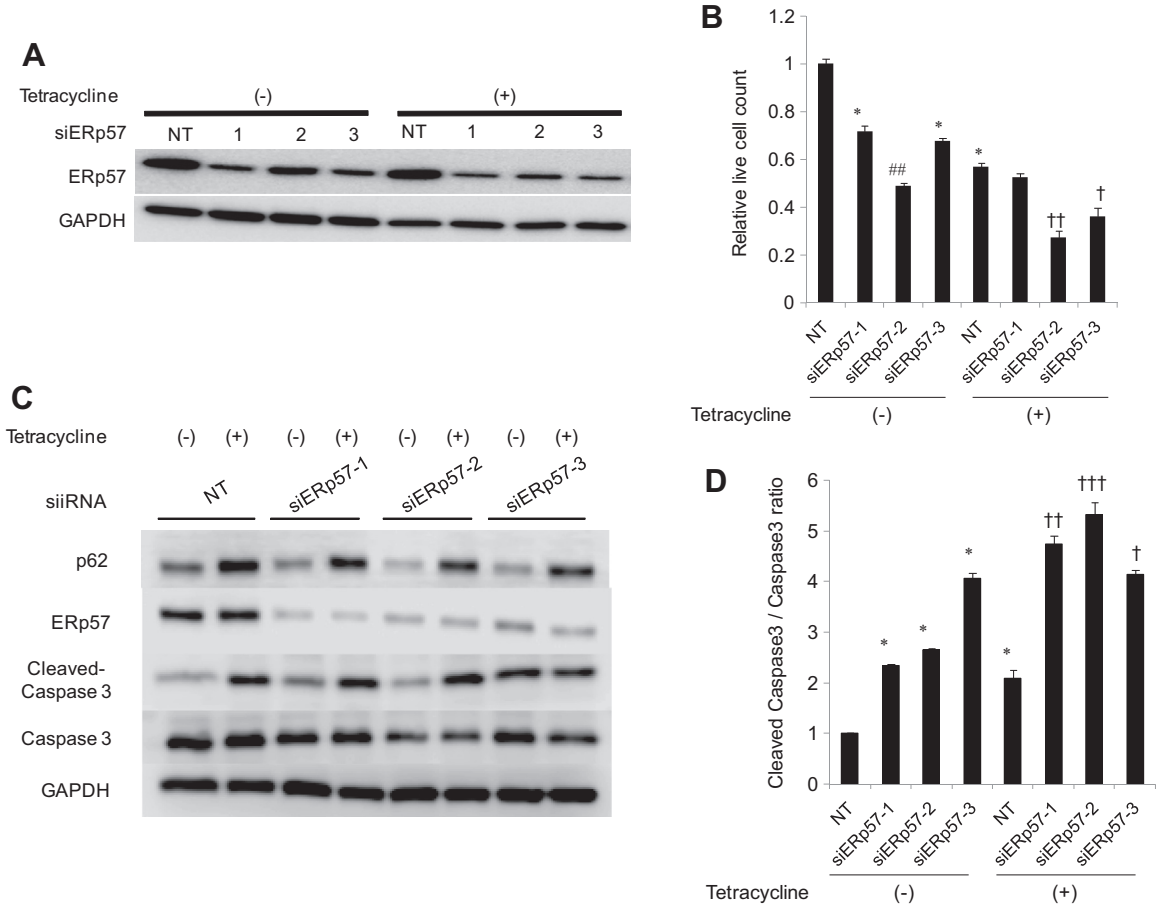

Fig. 3. Reduction of ERp57 further enhances cleaved Caspase-3 in tetracycline-treated Atg7KD INS-1 cells. (A) Representative results of the expression of ERp57 in tetracycline-treated and non-treated Atg7KD INS-1 cells transfected with a non-targeting siRNA (NT) or 3 kinds of ERp57-targeting siRNA (siERp57-1,2,3). (B) Relative number of live cells in tetracycline non-treated and treated Atg7KD INS-1 cells treated with NT-siRNA or siERp57s (siERp57-1,2,3). (C) Representative results of immunoblotting using an anti-cleaved Caspase-3 antibody. (D) Quantification of the protein expression of cleaved Caspase-3. Data are means ± SEM of 12 independent experiments. $^{*}p < 0.001$ vs. tetracycline non-treated Atg7KD INS-1 cells with NT, $^{\dagger}p < 0.001$ vs. tetracycline-treated Atg7KD INS-1 cells with NT, $^{\dagger\dagger}p < 0.001$ vs. tetracycline-treated Atg7KD INS-1 cells with NT and siERp57-1, $^{\dagger\dagger\dagger}p < 0.001$ vs. tetracycline-treated Atg7KD INS-1 cells with NT and siERp57-2.

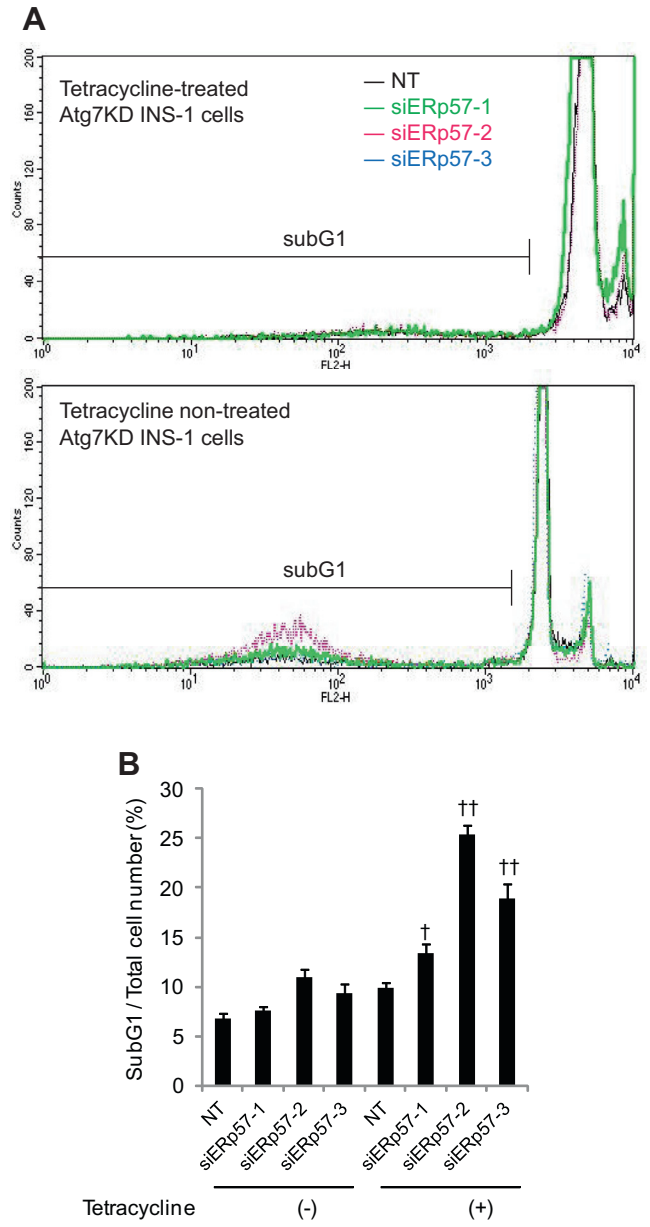

Fig. 4. Reduction of ERp57 further enhances sub-G1 cells in tetracycline-treated Atg7KD INS-1 cells. (A) Representative results of sub-G1 analyses (B) and quantification of the area under the curve (AUC) of the sub-G1 population. Data are means ± SEM of 10 independent experiments. $^{\dagger}p < 0.001$ vs. tetracycline-treated Atg7KD INS-1 cells with NT, $^{\dagger\dagger}p < 0.001$ vs. tetracycline-treated Atg7KD INS-1 cells with NT and siERp57-1, $^{\dagger\dagger\dagger}p < 0.001$ vs. tetracycline-treated Atg7KD INS-1 cells with NT and siERp57-2.

the increased ERp57 expression by autophagic failure is regulated in a cell-autonomous manner. Furthermore, suppression of ERp57 in Atg7 knock-down INS-1 cells elicits an increase in the expression of cleaved Caspase 3, an increase in the sub-G1 population, and a decrease in live cells. These data suggest that ERp57 plays protective roles against apoptosis in autophagy-deficient beta cells.

Although the regulation of ERp57 expression is not fully elucidated, we found that the expression of ERp57 is increased by autophagic failure in beta cells. Whereas the expression of ERp57 was reportedly increased by different stress-inducing agents, Murray et al. reported that this response was robust following the UPR [21]. With regard to the UPR in beta cells with autophagic failure, we as well as Quan et al. identified a compromised UPR response in beta cells with autophagic failure [10,22]. Thus, it is possible that the compromised UPR induced by autophagic failure may be involved in the mechanism of increased ERp57 expression.

Here, we found that ERp57 may have protective roles against apoptosis in autophagy-deficient beta cells. Previous data also demonstrated that ERp57 plays a protective role against apoptosis in cancer cells treated with agents that increase ER stress [23,24]. In addition, it has been shown that prion replication is accompanied by the increased expression of ERp57, and the damage caused by prions is inversely related to the amount of ERp57 [25]. Furthermore, ERp57 plays a protective role by preventing the self-association of the amyloid-β peptide that is involved in Alzheimer's disease as the main component of the amyloid plaques found in the brains of Alzheimer's disease patients [26]. Although the mechanisms of cell protection against apoptosis by the increased expression of ERp57 are not clear, accumulated data support that ERp57 plays a protective role against apoptotic cell death.

In conclusion, we found the increased expression of ERp57 in autophagy-deficient beta cells and identified its protective role against beta cell apoptosis due to autophagic failure. Considering that the levels of autophagy are insufficient in islets with type 2 diabetes mellitus, ERp57 could be a new target for the development of new drugs for the treatment of type 2 diabetes mellitus.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.040.

References

[1] J.L. Leahy, Natural history of beta-cell dysfunction in NIDDM, Diabetes Care 13 (1990) 992–1010.
[2] C. Weyer, C. Bogardus, D.M. Mott, R.E. Pratley, The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus, J. Clin. Invest. 104 (1999) 787–794.
[3] C.J. Rhodes, Type 2 diabetes-a matter of β?-cell life and death?, Science 307 (2005) 380–384
[4] M. Komatsu, S. Waguri, T. Chiba, S. Murata, J. Iwata, I. Tanida, T. Ueno, M. Koike, Y. Uchiyama, E. Kominami, K. Tanaka, Loss of autophagy in the central nervous system causes neurodegeneration in mice, Nature 441 (2006) 880–884.
[5] M. Komatsu, S. Waguri, T. Ueno, J. Iwata, S. Murata, I. Tanida, J. Ezaki, N. Mizushima, Y. Ohsumi, Y. Uchiyama, E. Kominami, K. Tanaka, T. Chiba, Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice, J. Cell Biol. 169 (2005) 425–434.
[6] A. Nakai, O. Yamaguchi, T. Takeda, Y. Higuchi, S. Hikoso, M. Taniike, S. Omiya, I. Mizote, Y. Matsumura, M. Asahi, K. Nishida, M. Hori, N. Mizushima, K. Otsu, The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress, Nat. Med. 13 (2007) 619–624.

[7] T. Hara, K. Nakamura, M. Matsui, A. Yamamoto, Y. Nakahara, R. Suzuki-Migishima, M. Yokoyama, K. Mishima, I. Saito, H. Okano, N. Mizushima, Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice, Nature 441 (2006) 885–889.
[8] C. Ebato, T. Uchida, M. Arakawa, M. Komatsu, T. Ueno, K. Komiya, K. Azuma, T. Hirose, K. Tanaka, E. Kominami, R. Kawamori, Y. Fujitani, H. Watada, Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet, Cell Metab. 8 (2008) 325–332.
[9] H.S. Jung, K.W. Chung, J. Won Kim, J. Kim, M. Komatsu, K. Tanaka, Y.H. Nguyen, T.M. Kang, K.H. Yoon, J.W. Kim, Y.T. Jeong, M.S. Han, M.K. Lee, K.W. Kim, J. Shin, M.S. Lee, Loss of autophagy diminishes pancreatic β? Cell mass and function with resultant hyperglycemia, Cell Metab. 8 (2008) 318–324.
[10] H. Abe, T. Uchida, A. Hara, H. Mizukami, K. Komiya, M. Koike, N. Shigihara, Y. Toyofuku, T. Ogihara, Y. Uchiyama, S. Yagihashi, Y. Fujitani, H. Watada, Exendin-4 improves β?-cell function in autophagy-deficient β?-cells, Endocrinology 154 (2013) 4512–4524.
[11] L. Van Lommel, K. Janssens, R. Quintens, K. Tsukamoto, D. Vander Mierde, K. Lemaire, C. Denef, J.C. Jonas, G. Martens, D. Pipeleers, F.C. Schuit, Probe-independent and direct quantification of insulin mRNA and growth hormone mRNA in enriched cell preparations, Diabetes 55 (2006) 3214–3220.
[12] F.C. Schuit, P.A. In't Veld, D.G. Pipeleers, Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic beta cells, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 3865–3869.
[13] D.L. Eizirik, A.K. Cardozo, M. Cnop, The role for endoplasmic reticulum stress in diabetes mellitus, Endocr. Rev. 29 (2008) 42–61.
[14] D. Scheuner, R.J. Kaufman, The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes, Endocr. Rev. 29 (2008) 317–333.
[15] M. Ogata, S. Hino, A. Saito, K. Morikawa, S. Kondo, S. Kanemoto, T. Murakami, M. Taniguchi, I. Tanii, K. Yoshinaga, S. Shiosaka, J.A. Hammarback, F. Urano, K. Imaizumi, Autophagy is activated for cell survival after endoplasmic reticulum stress, Mol. Cell. Biol. 26 (2006) 9220–9231.
[16] E. Bachar-Wikstrom, J.D. Wikstrom, Y. Ariav, B. Tirosh, N. Kaiser, E. Cerasi, G. Leibowitz, Stimulation of autophagy improves endoplasmic reticulum stress-induced diabetes, Diabetes 62 (2013) 1227–1237.
[17] T. Uchida, N. Iwashita, M. Ohara-Imaizumi, T. Ogihara, S. Nagai, J.B. Choi, Y. Tamura, N. Tada, R. Kawamori, K.I. Nakayama, S. Nagamatsu, H. Watada, Protein kinase Cdelta plays a non-redundant role in insulin secretion in pancreatic beta cells, J. Biol. Chem. 282 (2007) 2707–2716.
[18] T. Fujimura, Y. Shinohara, B. Tissot, P.C. Pang, M. Kurogochi, S. Saito, Y. Arai, M. Sadilek, K. Murayama, A. Dell, S. Nishimura, S.I. Hakomori, Glycosylation status of haptoglobin in sera of patients with prostate cancer vs. benign prostate disease or normal subjects, Int. J. Cancer 122 (2008) 39–49.
[19] T. Nara, M. Hashimoto, H. Hirawake, C.W. Liao, Y. Fukai, S. Suzuki, A. Tsubouchi, J. Morales, S. Takamiya, T. Fujimura, H. Taka, R. Mineki, C.K. Fan, D.K. Inaoka, M. Inoue, A. Tanaka, S. Harada, K. Kita, T. Aoki, Molecular interaction of the first 3 enzymes of the de novo pyrimidine biosynthetic pathway of *Trypanosoma cruzi*, Biochem. Biophys. Res. Commun. 418 (2012) 140–143.
[20] I. Tanida, E. Tanida-Miyake, T. Ueno, E. Kominami, The human homolog of *Saccharomyces cerevisiae* Apg7p is a protein-activating enzyme for multiple substrates including human Apg12p, GATE-16, GABARAP, and MAP-LC3, J. Biol. Chem. 276 (2001) 1701–1706.
[21] J.I. Murray, M.L. Whitfield, N.D. Trinklein, R.M. Myers, P.O. Brown, D. Botstein, Diverse and specific gene expression responses to stresses in cultured human cells, Mol. Biol. Cell 15 (2004) 2361–2374.
[22] W. Quan, K.Y. Hur, Y. Lim, S.H. Oh, J.C. Lee, K.H. Kim, G.H. Kim, S.W. Kim, H.L. Kim, M.K. Lee, K.W. Kim, J. Kim, M. Komatsu, M.S. Lee, Autophagy deficiency in beta cells leads to compromised unfolded protein response and progression from obesity to diabetes in mice, Diabetologia 55 (2012) 392–403.
[23] P.E. Lovat, M. Corazzari, J.L. Armstrong, S. Martin, V. Pagliarini, D. Hill, A.M. Brown, M. Piacentini, M.A. Birch-Machin, C.P. Redfern, Increasing melanoma cell death using inhibitors of protein disulfide isomerases to abrogate survival responses to endoplasmic reticulum stress, Cancer Res. 68 (2008) 5363–5369.
[24] M. Corazzari, P.E. Lovat, J.L. Armstrong, G.M. Fimia, D.S. Hill, M. Birch-Machin, C.P. Redfern, M. Piacentini, Targeting homeostatic mechanisms of endoplasmic reticulum stress to increase susceptibility of cancer cells to fenretinide-induced apoptosis: the role of stress proteins ERdj5 and ERp57, Br. J. Cancer 96 (2007) 1062–1071.
[25] C. Hetz, M. Russelakis-Carneiro, S. Walchli, S. Carboni, E. Vial-Knecht, K. Maundrell, J. Castilla, C. Soto, The disulfide isomerase Grp58 is a protective factor against prion neurotoxicity, J. Neurosci. 25 (2005) 2793–2802.
[26] R.R. Erickson, L.M. Dunning, D.A. Olson, S.J. Cohen, A.T. Davis, W.G. Wood, R.A. Kratzke, J.L. Holtzman, In cerebrospinal fluid ER chaperones ERp57 and calreticulin bind beta-amyloid, Biochem. Biophys. Res. Commun. 332 (2005) 50–57.