SIDT2 is involved in the NAADP-mediated release of calcium from insulin secretory granules

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Abstract

The Sidt2 global knockout mouse (Sidt2−/−) has impaired insulin secretion. The aim of this study was to assess the role of SIDT2 protein in glucose-induced insulin secretion in primary cultured mouse β-cells. The major metabolic and electrophysiological steps of glucose-induced insulin secretion of primary cultured β-cells from Sidt2−/− mice were investigated. The β-cells from Sidt2−/− mice had normal NAD(P)H responses and KATP and KV currents. However, they exhibited a lower [Ca2+]i peak height when stimulated with 20 mM glucose compared with those from WT mice. Furthermore, it took a longer time for the [Ca2+]i of β-cell from Sidt2−/− mice to reach the peak. Pretreatment with ryanodine or 2-aminoethoxydiphenyl borate (2-APB) did not change [Ca2+]i the response pattern to glucose in Sidt2−/− cells. Extraordinarily, pretreatment with bafilomycin A1 (Baf-A1) led to a comparable [Ca2+]i increase pattern between these two groups, suggesting that calcium traffic from the intracellular acidic compartment is defective in Sidt2−−/−β-cells. Bath-mediated application of 50 nM nicotinic acid adenine dinucleotide phosphate (NAADP) normalized the [Ca2+]i response of Sidt2−/−β-cells. Finally, glucose-induced CD38 expression increased to a comparable level between Sidt2−/− and WT islets, suggesting that Sidt2−/− islets generated NAADP normally. We conclude that Sidt2 is involved in NAADP-mediated release of calcium from insulin secretory granules and thus regulates insulin secretion.

Key Words
- insulin secretory granule
- Sidt2
- Ca2+
- NAADP
- CD38

Introduction

Lysosomal proteomic analyses suggest that, in addition to the well-known lysosomal membrane proteins, e.g. LAMP1, LAMP2, some less-abundant proteins without clear physiological function reside on lysosomal membranes, including SIDT2 (Bagshaw et al. 2005, Chapel et al. 2013, Zhang et al. 2007). As a member of the SID1 transmembrane family (Elhassan et al. 2012), SIDT2 was presumed to be a small RNA membrane transporter. However its exact function has not been explored experimentally.

Currently, no known human diseases are caused by SIDT2 gene mutations. To explore its pathophysiological function, we generated a global knockout Sidt2−/− mouse model, which exhibited age-dependent increased plasma glucose levels and impaired glucose tolerance (Gao et al. 2011). We hypothesized that SIDT2 participates in insulin secretion. In this study, we investigated the role of SIDT2 protein in glucose-induced insulin secretion in primary cultured mouse β-cells.
Islets from Sidt2\(^{-/-}\) mice cultured \textit{in vitro} produced less insulin when stimulated with a high concentration of glucose or a depolarizing concentration of KCl.

Under physiological conditions, glucose-induced insulin release depends on rapid glucose uptake and metabolism in the pancreatic \(\beta\)-cells, which results in increased cytosolic ATP/ADP, sequential closure of ATP-sensitive potassium channels (K\(_{\text{ATP}}\)) and depolarization of the cell membrane (Bratanova-Tochkova \textit{et al.} 2002, Eliasson, \textit{et al.} 1997, Gyfle \textit{et al.} 2000). Such depolarization activates membrane voltage-gated Ca\(^{2+}\) channels and allows extracellular Ca\(^{2+}\) to enter the cytoplasm, which in turn induces Ca\(^{2+}\) release from intracellular stores. The resulting rise in [Ca\(^{2+}\)], then triggers insulin secretory granule (ISG) exocytosis. Abnormalities in intracellular Ca\(^{2+}\) signals have been associated with impaired insulin secretion in both rodent and human pancreatic \(\beta\)-cells (Islam 2010).

Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) from intracellular stores is a critical step during insulin secretion in both INS cell line and mouse pancreatic \(\beta\)-cells (Kang \textit{et al.} 2005, Kang and Holz 2003, Roderick \textit{et al.} 2003, Varadi and Rutter 2004). Acidic organelles such as lysosomes and ISGs are the physiological sources of intracellular free Ca\(^{2+}\) (Christensen \textit{et al.} 2002, Suckale and Solimena 2010), which are mobilized in response to the second messenger nicotinic acid adenine dinucleotide phosphate (NAADP) (Galione \textit{et al.} 2009, Kinnear \textit{et al.} 2004, Yamasaki \textit{et al.} 2004). Local Ca\(^{2+}\) release from the acidic stores is then amplified by ER Ca\(^{2+}\) release via a CICR-dependent manner (Patel \textit{et al.} 2001). However, the NAADP receptor remains elusive (Calcraft \textit{et al.} 2009, Morgan and Galione 2014, Wang \textit{et al.} 2012). Two-pore channels (TPCs) were proposed as the long sought-after NAADP receptor in the intracellular acidic compartment (Calcraft \textit{et al.} 2009, Ruas \textit{et al.} 2015). However, study by other groups has suggested that TPCs are NAADP-independent highly selective Na\(^{+}\) channels (Cang \textit{et al.} 2013, Wang \textit{et al.} 2012). More study on this pathway is imperative.

The aim of this study was to investigate the role of SIDT2 during glucose-induced insulin secretion. We show that SIDT2 is localized in ISGs, where it participates in NAADP-mediated ISG calcium release, playing a critical role in regulating glucose homeostasis.

### Materials and methods

#### Animals

Generation of the Sidt2\(^{-/-}\) mice was described previously (Gao \textit{et al.} 2013). All mice were housed in Animal Laboratory Center at Xinhua hospital, Shanghai, China. The Institutional Review Ethics Board of Xinhua Hospital approved all procedures involving mice. Male mice aged 6 months age were used in this study.

#### Islet isolation and primary cell culture

The islets were isolated as described previously (Lacy and Kostianovsky 1967). Briefly, islets were incubated in 0.05 mg/ml trypsin without phenol red for 3 min at 37°C. The cells were then centrifuged, and re-suspended in RPMI 1640 supplemented with 10% fetal calf serum (Gibco), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Single cells were then spread onto coverslips pre-treated with poly-\(\gamma\)-lysine (15–30kDa; Sigma). The cells began to form monolayers after 48 h of culture.

#### Sidt2 RNA analysis

Total RNA was extracted from the pancreatic islets. The forward and reverse primers of pair 1 were located within exons 17 and 18, respectively. Qualitative real-time RT-PCR (qPCR) using pair 1 was performed to analyze the residual RNA content. The forward and reverse primers of pair 2 were designed against regions in exons 2 and 5, respectively, whereas those of pair 3 were in exons 1 and 6. To confirm the deletion of exon 2 of Sidt2 gene at the mRNA level, RT-PCR was performed using primer pairs 2 and 3. Amplicons from primer pair 3 were directly sequenced. The sequences of three primer pairs were shown in Table 1.

#### Western blotting analysis

A total of about 200 isolated islets were lysed in 100\(\mu\)l 1 \times RIPA buffer supplemented with protease inhibitors.

### Table 1

<table>
<thead>
<tr>
<th>Oligonucleotide primers used to amplify Sidt2 cDNA</th>
<th>Reverse primer</th>
<th>Length</th>
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<tr>
<td>Primer1</td>
<td>AGCGAGCTGGTCAACATCTAC</td>
<td>161 bp</td>
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<tr>
<td>Primer2</td>
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</tr>
<tr>
<td>Primer3</td>
<td>GGTACATGCCAATGAGGCTA</td>
<td>535 bp</td>
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(Beyotime, Shanghai, China). Total proteins were extracted and separated using a 10% SDS–PAGE gel followed by wet transfer onto PVDFs. The primary antibodies used were goat polyclonal anti-SIDT2 (1:300 dilution; Santa-Cruz Biotechnology), rabbit anti-CD38 (1:500 dilution; Novus Biologicals, Littleton, CO, USA), and mouse monoclonal anti-tubulin (1:1000 dilution; Beyotime). Signals were detected using HRP-conjugated secondary antibodies and ECL Detection Kit (Beyotime).

Immunofluorescence staining on pancreas sections and isolated primary β-cells

Five-micrometer paraffin-embedded pancreas sections were stained as described previously (Gao et al. 2013). Isolated primary β-cells were fixed with 4% paraformaldehyde for 20min, permeabilized for 20min in 0.2% Triton X-100 buffer, blocked with PBS containing 5% BSA, and subjected to double immunofluorescence staining. They were first incubated with anti-SIDT2 (1:200) and anti-insulin antibodies (1:100 dilution, Cell Signaling Technology). Then they were incubated with Alexa Fluor 488 donkey-anti-rabbit IgG and Alexa Fluor 594 chicken-anti-goat IgG (1:200 dilution, Molecular Probes). Images were visualized by confocal microscopy (Leica 710, Mannheim, Germany). The co-localization of SIDT2 with insulin was analyzed by Image Pro Plus 6.0 and expressed as Pearson’s correlation coefficient, where P values >0.5 indicated a significant correlation.

Assessment of NAD(P)H using a fluorimetric assay and an enzymatic recycling method

Suspension of dispersed islet cells (10⁶ cells/ml) was in 96-well plates in a temperature controlled chamber in Krebs–Ringer Bicarbonate Buffer (KRBB) supplemented with 3 mM glucose. NAD(P)H fluorescence (340nm excitation, 420nm emission) was measured using a Hitachi F-2000 fluorescence spectrophotometer. The fluorescence intensity was represented as arbitrary units (AU).

Batches of 100 islets were incubated in 20mM glucose for 10min, and then extracted using RIPA buffer. The aliquots were heated at 60°C for 10min to eliminate both NAD and NADP. NADPH levels were then determined by the enzymatic recycling method using NAD(P)H Assay Kit (AAT Bioquest, Sunnyvale, CA, USA). The mean changes in NADPH were expressed as a percentage of unstimulated batches.

Electrophysiological measurements of Kᴀᴛ, and Kᴠ currents in single islet β-cells

Whole-cell patch-clamp recordings in dispersed β-cells were conducted using a patch-clamp amplifier EPC10 (HEKA Electronik, Lambrecht, Germany) as reported previously with some modification (Deng et al. 2014). The β-cells were identified by a cell capacitance >5 pF (Barg et al. 2000). Stimulation and recordings were controlled by PULSE software. The patch electrodes were fabricated from borosilicate glass and had a resistance of 2.5–5 MΩ while filled with internal recording solution. Capacitive transients and series resistance errors were minimized before recording. All experiments were carried out at room temperature (22–25°C).

Kᴀᴛ currents were recorded by applying 10mV hyperpolarizing and depolarizing voltage pulses (duration, 200ms; pulse interval, 2 s) from a holding potential of −70mV using the perforated patch whole-cell configuration. The extracellular solution consisted of (in mM) 138NaCl, 5.6KCl, 2.6CaCl₂, 1.2MgCl₂, and 5 HEPES (pH 7.4 with NaOH) and was supplemented with glucose as indicated. The pipette solution consisted of (in mM) 76K₂SO₄, 10KCl, 1MgCl₂, 5 HEPES (pH 7.35 with KOH), and 0.24 mg/ml of the pore-forming antifungal agent amphotericin B. The voltage clamp was considered satisfactory when the series conductance was stable and greater than 35 nS.

Kᵥ currents were recorded by a series of 10mV voltage steps to the potentials between −40 and +60mV from the holding potential −70mV using standard whole-cell patch-clamp recording mode. The extracellular solution consisted of (in mM) 135 NaCl, 5.4 KCl, 1 CaCl₂, 1.2MgCl₂, 10 HEPES, and 3 glucose (pH 7.4 with NaOH). The pipette solution consisted of (in mM) 140 KCl, 1 CaCl₂, 1MgCl₂, 10 HEPES, 10 EGTA, and 3MgATP (pH 7.3 with KOH).

[Ca²⁺]i, recording in single islet β-cells

[Ca²⁺]i was measured by placing the coverslip on the stage of IX71 inverted microscope (Olympus) (Deng et al. 2014). The single islet cells were loaded with 4μM Fura 2-AM (Sigma) for 30min at 37°C in KRBB containing 3mM glucose, then they were washed with KRBB, and stimulated with 20mM glucose. The single cells were illuminated by excitation at 340nm (F340) and 380nm (F380) using a monochromator (Till Photonics, Munich, Germany), and the emission signals were detected at 510nm using an image-intensifying CCD camera (SensiCam, PCO, Kelheim, Germany). The images were
collected at 10 s intervals. The [Ca^{2+}]_i was expressed as the ratio of F340/F380 (AU). The [Ca^{2+}]_i peak height upon stimulation was calculated as the ratio of difference between the highest [Ca^{2+}]_i value and the baseline. The time to the peak [Ca^{2+}]_i (T_peak) was defined as a period from the point when the stimulation was applied to the point when the highest [Ca^{2+}]_i value was obtained. The maximal rate of Ca^{2+} rise was defined as the max velocity increase in the [Ca^{2+}]_i curves (max.d[Ca^{2+}]_i/dt). Calcium channel blockers, including ryanodine and 2-APB, and the vacuolar ATPase inhibitor Baf-A1 were purchased from Gene Operation (Ann Arbor, MI, USA). NAADP was obtained from Sigma. Calcium-free KRBB was prepared by omitting CaCl_2 from its composition. All the drugs were applied through a superfusion system.

### Measuring the pH in ISGs and fibroblast lysosomes

The islet β-cells were stained with 2µM LysoSensor Green DND-189 (Molecular Probes) in 1ml KRBB for 30 min at 37°C. Green fluorescence that accumulated in acidic organelles was imaged by Leica 710 confocal microscopy (excitation 443 nm, emission 505 nm). The fluorescence intensity was also represented as AU.

Primary cultured fibroblasts isolated from mouse tail tip were loaded with the same pH indicator to measure the pH of lysosomes. The signals were observed by Leica 710 confocal microscopy and were detected using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). Data were analyzed using Flojo version 7.6.1 (Treestar, Ashland, OR, USA).

### Statistical analysis

Results are expressed as means±s.d. An independent-samples t test was used to evaluate differences between groups. Differences were statistically significant when P≤0.05.

### Results

#### Expression and localization of SIDT2 in pancreatic β-cells

SIDT2 expression was observed in WT islets using pair 1, and RNA expression was reduced by 70% in Sidt2−/−
islets (Fig. 1A). The Sidt2 gene-specific product obtained using pair 2 was amplified from the WT islets (Fig. 1B, lane 2), but not from Sidt2−/− islets (Fig. 1B, lane 3). The amplicon from Sidt2−/− islets using pair 3 (Fig. 1B, lane 5) was shorter than that obtained from WT islets (Fig. 1B, lane 4, WT, 535 bp; Sidt2−/−, 413 bp). Direct sequencing of the primer pair 3 amplicon confirmed the deletion of exon 2 in the Sidt2−/− islets, which was presumed to cause a premature stop codon at amino acid 83 of SIDT2 protein (Fig. 1C). Western blotting analysis revealed that SIDT2 was expressed endogenously in INS-1 cells and WT islets, whereas its expression was almost completely lost in Sidt2−/− islets (Fig. 1B). The SIDT2 signals colocalized with the insulin granules of β-cells in WT islets (Fig. 1E), as well as in single β-cells (Fig. 1F). The calculated Pearson’s value for SIDT2 with insulin was 0.86 in isolated primary β-cells.

**Normal NAD(P)H responses to glucose in Sidt2−/− β-cells**

To assess whether SIDT2 deficiency affected glucose metabolism in β-cells, the NAD(P)H autofluorescence response to 20mM glucose was measured. The patterns of NAD(P)H responses to 20mM glucose in WT and Sidt2−/− β-cells were similar (Fig. 2A). Moreover, the autofluorescence levels under 3mM or 20mM glucose were comparable between these two groups (n=10 islets for each group, P>0.05, Fig. 2B). NAD(P)H increase to 20mM glucose in Sidt2−/− β-cells was not different from WT (50.1±7.6 AU vs 49.8±6.3 AU, WT vs Sidt2−/−, P>0.05, cells from 6 mice per group).

In addition, an enzymatic recycling method was used to measure the changes in NADPH in islet extracts. In WT islets, NADPH levels rose by 59±9.3% after the addition of 20mM glucose. Similarly, a 62±13.6% increase was observed in Sidt2−/− mouse islets (WT vs Sidt2−/−, P>0.05, n=300 islets from 6 mice per group, Fig 2C and Fig. 2D).

**Attenuated and delayed glucose-stimulated Ca2+ response of Sidt2−/− β-cells**

Upon stimulation with 20mM glucose, the [Ca2+]i in WT β-cells increased rapidly to plateau levels. In contrast, the [Ca2+]i rise was impaired in Sidt2−/− β-cells (Fig. 4A). The peak height of [Ca2+]i in Sidt2−/− β-cells was decreased by 45.3% compared with WT (92.9±9.5 AU vs 169.8±16.4 AU, P<0.01, n=20 cells, Fig. 4B). Moreover, The time to the peak of [Ca2+]i (Tpeak) was longer in Sidt2−/− β-cells compared with WT (4.8±0.9 min vs 1.1±0.25 min.

These data indicate that mitochondrial glycolytic function was normal in Sidt2−/− mouse islets.

**Normal K_{ATP} and K_{o} currents in single β-cells from Sidt2−/− mice**

The K_{ATP} current amplitudes were stable in 3mM glucose, which were inhibited markedly by 20mM glucose in both groups (Fig. 3A and Fig. 3B). The maximum steady-state outward currents were reduced by 65.1±4.13% in WT and 59.3±7.9% in Sidt2−/− β-cells on perfusion with 20mM glucose (P>0.05, n=15 cells).

K_{o} channels in pancreatic β-cells negatively regulate Ca^{2+} entry and insulin secretion (Su et al. 2001). The outward voltage-dependent K^{+} currents in Sidt2−/− β-cells were similar to those in WT β-cells, and could be antagonized by 20mM glucose (Figs 3C and Fig. 3E). The peak outward K_{o} currents were reduced from 1127.4±97.6 pA/pF (3mM glucose) to 459.2±60.6 pA/pF (20mM glucose) in WT β-cells (P<0.01, n=20 cells), and from 1159.7±95.8 to 544.4±51.1 pA/pF in Sidt2−/− β-cells (P<0.01, n=20 cells). The reduction in K^{+} current by 20mM glucose was statistically significant in both groups when the membrane potential was clamped at 0mV (n=20 cells, Figs 3D and Figs 3F).

**Figure 2**

Glucose-induced NAD(P)H changes in Sidt2−/− β-cells. (A) The representative curves for NAD(P)H autofluorescence. (B) Quantification of NAD(P)H fluorescence (β-cells from 6 mice per group). (C) NADPH responses to 20mM glucose compared with the basal levels at 3mM glucose with the enzymatic method (n=300 islets from 6 mice per group). (D) Quantification of the NADPH responses to 20mM glucose compared with the basal levels at 3mM glucose (n=300 islets from 6 mice per group). A P>0.05, **P<0.01.
Sidt2 is involved in the NAADP-mediated release of ER ryanodine receptors (RyRs) and 50μM 2-APB (an inhibitor of ER inositol trisphosphate receptors (IP3Rs)) dramatically reduced the glucose-stimulated Ca^{2+} response in WT and Sidt2−/− β-cells (Fig. 4E and Fig. 4F). There were no differences in the extent of [Ca^{2+}]_{i} reduction by the ER blockers in both groups (ryanodine, 54.9 ± 7.6% vs 49.3 ± 5.2%, P > 0.05; 2-APB, 53.8 ± 6.3% vs 47.5 ± 5.4%, P > 0.05, n = 20, WT vs Sidt2−/−, Fig. 4G). These data indicate that there was no defect in Ca^{2+} trafficking from ER stores via RyRs or IP3R during glucose-induced CICR in Sidt2−/− β-cells. Therefore, the release of Ca^{2+} from intracellular stores other than the ER might be impaired in Sidt2−/− β-cells.

Treatment of cells with 3μM Baf-A1, an inhibitor of vacuolar H^{+}-ATPase, induced a transient increase in [Ca^{2+}]_{i} in both groups (peak height, 74.1 ± 2.1 AU in WT and 71 ± 8.3 AU in Sidt2−/−, P > 0.05, n = 15, Figs 4H and Fig. 4I). Subsequent stimulation with 20mM glucose induced a similar [Ca^{2+}]_{i} increase in both groups (peak height, 94.8 ± 8.5 AU vs 87.7 ± 7.2 AU, WT vs Sidt2−/−, P > 0.05, n = 15) (Fig. 4H and Fig. 4I). When Baf-A1
Sidt2 is involved in the NAADP-mediated release

depleted the calcium stores in the acidic compartments, the glucose-induced Ca^{2+} release from the ER and other stores was comparable between Sidt2^{−/−} and WT β-cells. This suggests that the attenuated [Ca^{2+}]_{i} increase in Sidt2^{−/−} β-cells was due to impaired Ca^{2+} release from acidic stores.

The pH of ISGs and lysosomes

The intracellular acidic organelles of β-cell, including ISGs and lysosomes, contain high levels of calcium, and their calcium contents are affected by pH (Christensen et al. 2002, Suckale and Solimena 2010). In 3mM glucose, the granular pH-dependent fluorescence of
β-cells was stable for 3 min. The mean fluorescence intensity of WT and Sidt2−/− ISGs was 103.85 ± 15.75 AU and 100.67 ± 13.43 AU, respectively (n = 20 cells, P > 0.05). Stimulation with 20 mM glucose caused a rapid and monophasic increase of fluorescence intensity in both WT and Sidt2−/− β-cells (52.2 ± 6.7 AU vs 54.2 ± 9.1 AU, respectively, P > 0.05, n = 20, Figs 5A and Figs 5B). These data indicate that SIDT2 deficiency had no effect on the acidification of ISGs.

Similarly, no difference was obtained in the lysosomal pH of Sidt2−/− fibroblasts compared with WT, which was observed by confocal microscopy (Fig. 5C) and assessed by flow cytometer (785 ± 72.1 AU vs 798 ± 31.8 AU, WT vs Sidt2−/−, P > 0.05, Fig. 5D).

Efficacy of NAADP treatment in Sidt2−/− β-cells

It was reported that NAADP-induced calcium signals were not observed in extracellular calcium-free conditions (Kim et al. 2008), which is similar to our observation that the rise in [Ca2+]i in response to glucose was nearly abolished in Sidt2−/− β-cells without extracellular calcium. Therefore, we assessed the effect of NAADP on Sidt2−/− β-cells. The peak height of [Ca2+]i in Sidt2−/− β-cells in the presence of 50 nM NAADP was comparable with WT β-cells without NAADP to the stimulus of 20 mM glucose (156.9 ± 12.1 AU vs 169.8 ± 16.4 AU, Sidt2−/− vs WT, n = 10 cells, P > 0.05); the respective Tpeak was also comparable (1.0 ± 0.3 min vs 1.1 ± 0.3 min, n = 10 cells, P > 0.05). Thus,
bath-mediated application of NAADP rescued $[\text{Ca}^{2+}]_{i}$ response of Sidt2$^{-/-}$-β-cells to glucose (Fig. 6A).

In the presence of 12mM glucose, stimulation with 50nM NAADP induced a comparable $[\text{Ca}^{2+}]_{i}$ response in both the WT and Sidt2$^{-/-}$ groups (peak height, 112.9±6.5 AU vs 111.5±8.6 AU, WT vs Sidt2$^{-/-}$, $P>0.05$, $n=10$) (Fig. 6B).

**CD38 expression in WT and Sidt2$^{-/-}$ islets**

Since the above data implied reduced $[\text{Ca}^{2+}]_{i}$ increase in Sidt2$^{-/-}$-β-cells was related with NAADP signaling, we investigated whether NAADP generation was deficient in Sidt2$^{-/-}$-β-cells. CD38 is located in endosomes, secretory granules, and lysosomes. It is an ADP-ribosyl cyclase responsible for endogenous NAADP generation in β-cells (Kim et al. 2008). As shown in Figs 6C and Figs 6D, glucose-induced CD38 expression increased by 27±9.1% in WT and 32.3±10.8% in Sidt2$^{-/-}$-β-cells ($P>0.05$), suggesting that NAADP production was normal in Sidt2$^{-/-}$-β-cells upon glucose stimulation.

**Discussion**

Previous proteomic studies identified some lysosomal-associated membrane proteins such as LAMP2, Syntaxin7, VAMP8, and Cathepsins L1, D, and F are expressed in ISGs (Brunner et al. 2007, Schwartz et al. 2012). The current study demonstrated that Sidt2, which was identified previously in mouse/rat liver lysosomes, was also endogenously expressed in mouse islet β-cells, and that it co-localized with ISGs.

The β-cells in rodent models have been estimated to contain ~10,000 ISGs, which corresponds to 12–20% of the total cell volume (PM 1973). ISGs contain the highest calcium concentrations among all intracellular compartments. Compared with the total calcium concentrations in the cytoplasm of β-cells, ISGs contain two-fold higher levels of calcium (Nolund et al. 1987). Only a small number of lysosomes are present in the islet β-cells (Barg et al. 2001), and the lysosomal volume of mouse islet β-cells was only 3–5% of that of ISGs (Schnell Landstrom et al. 1991). Therefore, the impaired $[\text{Ca}^{2+}]_{i}$ response in Sidt2$^{-/-}$-β-cells likely results mainly from defects in calcium release from the ISG stores.

NAADP is the second messenger that mobilizes $[\text{Ca}^{2+}]_{i}$ from acidic organelles. NAADP-mediated $[\text{Ca}^{2+}]_{i}$ release plays a key role in triggering glucose-induced $[\text{Ca}^{2+}]_{i}$ signaling events (Arredouani et al. 2010). However, the identity of the NAADP receptor channel in acidic organelles remains elusive. Recent data have indicated that neither TPCs nor the transient receptor potential channel mucolipin 1 (TRP-ML1) is the NAADP receptor (Lin-Moshier et al. 2012, Zhang et al. 2011). Bath-application of extracellular NAADP has been shown to induce intracellular calcium increase, at least partially through increase in intracellular NAADP (Djerada and Millart 2013, Singaravelu and Deitmer 2006). In the current study, the bath-application of NAADP corrected the impaired $[\text{Ca}^{2+}]_{i}$ increase in Sidt2$^{-/-}$-β-cells, suggesting that SIDT2 exerts its function on the upstream of NAADP-mediated calcium release from acidic store. In other words, SIDT2 may regulate NAADP production in ISGs or function as a modulator to the NAADP-regulated $[\text{Ca}^{2+}]_{i}$ channel.

In this study, the equal glucose-induced CD38 expression in WT and Sidt2$^{-/-}$-islets indicates that the generation of NAADP was not different between the two groups. Based on the localization of SIDT2 on the membranes of intracellular acidic compartments and its nine transmembrane domain structures (Jialin et al. 2010), and taken together the results of this study, we propose that SIDT2 may be required to assemble with TPCs to form a functional $[\text{Ca}^{2+}]_{i}$ release channel, or function as a modulator to the NAADP-regulated $[\text{Ca}^{2+}]_{i}$ channel in ISGs.

In conclusion, this study demonstrated that SIDT2 was expressed in mouse pancreatic β-cells, and co-localized...
predominantly with ISGs. It plays an important role in regulating NAADP-mediated ISG calcium release and glucose-induced insulin secretion.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author contribution statement
All authors contributed to the study conception and design. G C and R Y researched the data. Y C and A N helped the electrophysiological recording of cells. G C and H Z wrote the paper. H Z and X G designed the project.

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