Sorting nexin 19 regulates the number of dense core vesicles in pancreatic β-cells

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ABSTRACT

Aims/Introduction: Insulinoma-associated protein 2 (IA-2) regulates insulin secretion and the number of dense core vesicles (DCV). However, the mechanism of regulation of DCV number by IA-2 is unknown. We examined the effect of sorting nexin 19 (SNX19), an IA-2 interacting protein, on insulin secretion and the number of dense core vesicles (DCV).

Materials and Methods: Stable SNX19 knockdown (SNX19KD) MIN6, a mouse pancreatic β-cell line, and stable SNX19-reintroduced SNX19KD MIN6 were established. Quantification of DCV, and lysosomes was carried out using electron micrographs. The half-life of DCV was detected by pulse-chase experiment.

Results: Insulin secretion and content were decreased in stable SNX19KD MIN6 cells compared with those in control MIN6 cells. Electron micrographs showed that DCV number in SNX19KD cells was decreased by approximately 75% and that DCV size was decreased by approximately 40% compared with those in control cells, respectively. Furthermore, when SNX19 was reintroduced in SNX19KD cells, insulin content, insulin secretion and DCV number were increased. The half-life of DCV was decreased in SNX19KD cells, but was increased in SNX19KD cells in which SNX19 was reintroduced. The number of lysosomes and the activity of lysosome enzyme cathepsin D were increased by approximately threefold in SNX19KD cells compared with those in control cells. In contrast, they were decreased to approximately half to one-third in SNX19-reintroduced SNX19KD cells.

Conclusions: SNX19 regulates the number of DCV and insulin content by stabilizing DCV in β-cells. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00138.x, 2012)

KEY WORDS: Sorting nexin 19, Insulinoma-associated protein 2, Dense core vesicles

INTRODUCTION

The sorting nexins (SNX) belong to a large family involved in protein sorting and intracellular trafficking[1,2]. SNX19 is a 992 amino acid member of this family that has a phox (PX) domain (a binding motif to phosphatidylinositol) at the COOH-terminus and a PX-associated (PXA) domain at the NH2-terminus[3,4]. The function of SNX19 is not known, but it binds to the dense core vesicle (DCV) transmembrane protein insulinoma-associated protein 2 (IA-2)[5].

IA-2 is a major autoantigen in type 1 diabetes[6,7], and autoantibodies to it are found in 70–80% of newly diagnosed patients. These autoantibodies appear years before the onset of clinical disease, and individuals with autoantibodies to both IA-2 and GAD65 have approximately a 50% risk of developing type 1 diabetes within 5 years. Based on sequence, IA-2 is a member of the protein tyrosine phosphate (PTP) family, but because of two amino acid substitutions in the PTP domain, it is enzymatically inactive with conventional PTP substrates[8]. IA-2 is present in neuroendocrine cells throughout the body and knockout of IA-2 in mice results in impaired secretion of hormones and neurotransmitters, and a variety of phenotypes characterized by impaired insulin secretion, glucose intolerance[9,10], female infertility[11], abnormalities in learning and behavior[12], and loss of circadian rhythm[13]. Overexpression of IA-2 in MIN6 cells and rat pheochromocytoma cell line PC12 cells increased insulin secretion[14] and dopamine release[15], respectively.

Because SNX19 binds to IA-2, the present experiments were initiated to study the effects of knockdown and reconstitution of SNX19 in MIN6 cells on the biology and physiology of DCV, including their half-life, and number and the cellular content and secretion of insulin. We show here that SNX19 regulates the DCV number and insulin content by modulating the half-life of DCV in pancreatic β-cells.

MATERIALS AND METHODS

Reagents

pCMV-Tag3 mammalian expression vectors with G418 resistance gene were purchased from Agilent technologies (Santa Clara, CA, USA), pSilencer3.1-CMV hygro mammalian siRNA expression vector from Applied Biosystem (Austin, TX, USA), Effectene transfection reagent from Qiagen (Santa Clarita, CA, USA), mouse IA-2 antibody from LAD (Berlin, Germany), mouse anti-α-tubulin antibody from Sigma (St. Louis, MO,

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USA), anti-SNX19 antibody from Santa Cruz biotechnology (Santa Cruz, CA, USA) and mouse insulin ELISA kit from Shibayagi (Shibukawa, Japan).

Plasmids

Two SNX19 siRNA were synthesized by Takara (Otsu, Japan); the sequences were 5′-AATTGCACCTGGAACGATTCA-3′ and 5′-AAAGGCAGCTGGAACAGGAGA-3′, and were inserted into pSilencer 3.1-CMV hygro vector. Primers for complementary SNX19 were synthesized by Takara. The forward and reverse primer sequences for SNX19 were 5′-CCGCTCGAGATGAAGACAGAAACAGTG-3′ and 5′-CCGCTCGAGCTAAGAGGAGACACCCAT-3′. SNX19 polymerase chain reaction (PCR) product was inserted into pCMV-Tag3 at *Xho*I sites. All plasmids were sequenced and no mutations were found.

Establishment of Stable Cell Lines

MIN6 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 25 mmol/L D-glucose (high glucose), supplemented with 15% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in 95% air and 5% CO_2. SNX19 siRNA inserted into pSilence3.1-CMV hygro vector were introduced into MIN6 cells with Effectene transfection reagent, and stably transfected cells were selected by 200 μg/mL of hygromycin and by limiting dilution. Full length IA-2 and full length SNX19 were inserted into pCMV-Tag3 vectors and introduced into SNX19 knockdown MIN6 cells using Effectene transfection reagent, and stably transfected cells were selected by 300 μg/mL of G418 and 200 μg/mL of hygromycin, and by limiting dilution. SNX19 and IA-2 expression were confirmed by western blot.

Western Blot

Cells were washed twice with PBS, detached from plates with trypsin-EDTA, collected, washed two more times with PBS and then sonicated in lysis buffer. Equivalent amounts of protein were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 4–12% acrylamide gels (Invitrogen, Carlsbad, CA, USA) and transferred to polyvinylidene fluoride membranes (Invitrogen), followed by immunoblotting with antibodies to detect respective proteins.

Cell Proliferation Assay

A total of 1.0×10^4 cells/mL were seeded into a 96-well culture late and incubated for 10 days in 25 mmol/L glucose DMEM media. Cell proliferation was measured at indicated times by a bromodeoxyuridine (BrdU) cell proliferation assay kit (Calbilchem, Damstadt, Germany) as previously reported[16].

Insulin Secretion Test

MIN6 cells were seeded in 96-well culture plates at a density of 3.0×10^4 cells per well and cultured for 3 days. The attached cells were washed twice with 3 mmol/L glucose Krebs–Ringer bicarbonate HEPES (KRBH) buffer (124 mmol/L NaCl, 5.6 mmol/L KCl, 2.5 mmol/L $CaCl_2$ and 20 mmol/L HEPES at pH 7.4). The cells were then incubated at 37°C for 60 min in KRBH buffer, washed and incubated again for 60 min in KRBH at 3 mmol/L glucose. Supernatant was collected and insulin release measured by ELISA kit (Shibayagi). The cells then were incubated at 25 mmol/L glucose in KRBH for 60 min and the amount of insulin released measured again.

Insulin Content

Cells were seeded in 24-well culture plates at a density of 1.0×10^5 cells per well and cultured for 3 days at 25 mmol/L glucose. Media then were removed and replaced again with 25 mmol/L glucose. The cells were incubated for 16 h and the insulin content was determined by ELISA.

Electron Microscopy

Cells were cultured in 25 mmol/L glucose for 3 days. The culture media then were replaced with 25 mmol/L glucose containing fresh DMEM media for 16 h. Cells were washed with PBS three times and fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.4, and used for electron microscopy study.

Quantification of DCV and Lysosomes Per Cytoplasmic Area

Quantification of DCV was carried out as previously reported[14]. Briefly, 15 cells were selected at random and the images were taken at 8 k magnification. The number of DCV/cytoplasmic area or lysosome/cytoplasmic area was quantified by two operators blind to their status using national Institutes of Health images. Approximately 20 cytoplasmic areas taken by electron microscopy were estimated.

Half-life of DCV

Insulin half-life was determined as previously described[14]. Cells were seeded in 6-well culture plates and incubated for 2 days in 25 mmol/L glucose to obtain a steady state. The cells were then washed with KRBH buffer and incubated in 25 mmol/L glucose luecine-free media with [^{3}H]leucine (Amersham Biosciences, Piscataway, NJ, USA) for 24 h. The media then was changed to 3 mmol/L low glucose without [^{3}H]leucine for a 48 h chase. The cells and supernatant were collected at different times and the cells were lysed by repeated freezing and thawing. The cell lysates and supernatants were incubated at 4°C for 2 h in the presence of anti-insulin or anti-proinsulin antibodies. Antibody-antigen complexes then were precipitated by adding 5 mg of protein A-Sepharose in 100 μL of glycine/BSA/NP-40 buffer. After mixing at 4°C for 2 h, the immunoreactive material bound to the protein A-Sepharose was separated from unbound material in the supernatant by centrifugation (8000 *g*, 30 s). After washing the precipitates twice with 250 μL of glycine/BSA/NP-40, the precipitants were suspended in 250 μL of 1 mol/L acetic acid and 2.5 mg/mL of BSA. The suspended precipitates were added to liquid scintillation vials, the activity ratios measured and the insulin half-life determined. Incorporation of

[^{3}H]leucine into total protein under high glucose over 24 h was determined by precipitation with trichloroacetic acid (TCA). The data is expressed as the ratio of radiolabeled (pro)insulin/TCA precipitated protein.

Cathepsin D Activity

To measure cathepsin D activity, 1.0×10^4 cells were thoroughly washed in glucose-free Hank's solution and dissolved by sonication in 200 mL acetate-EDTA buffer (1.1 mmol/L

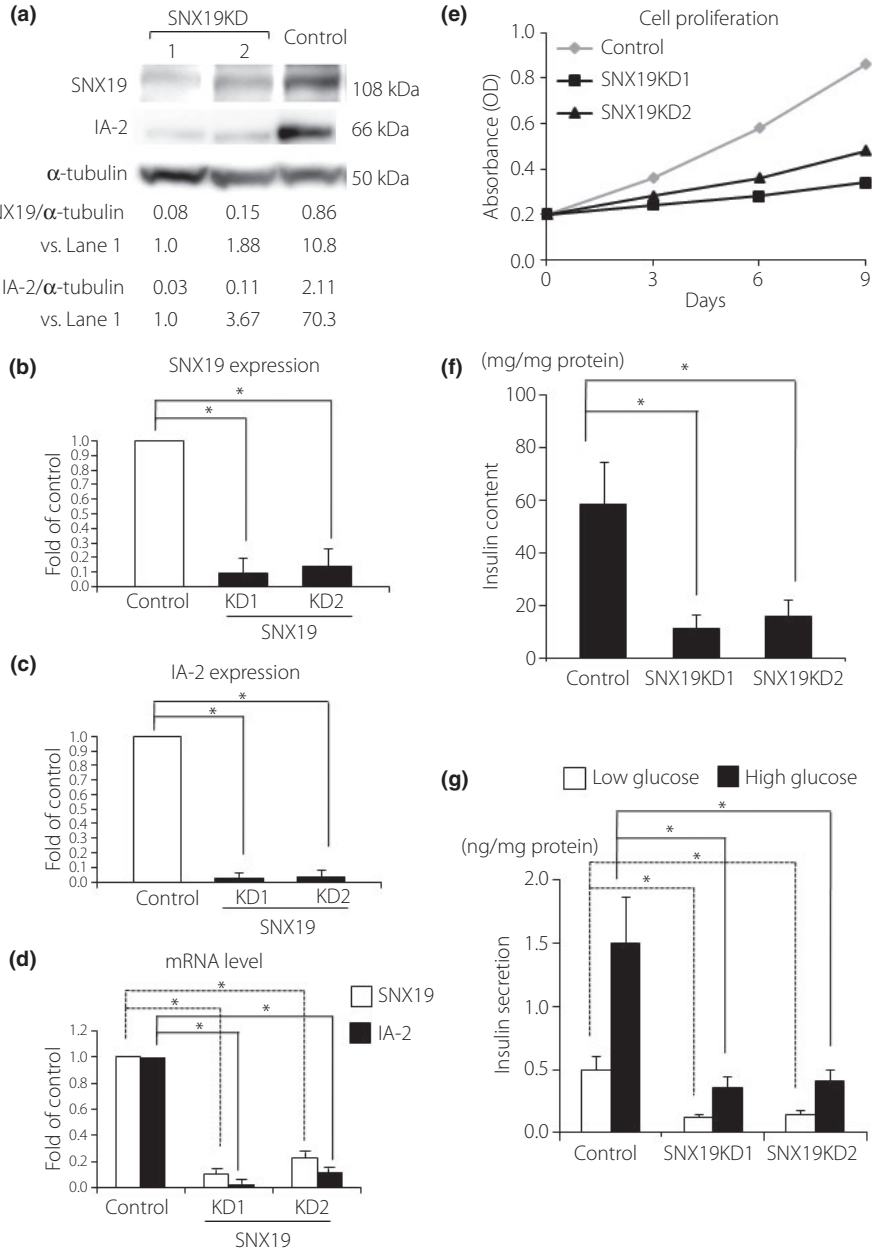

Figure 1 | Decrease in sorting nexin 19 (SNX19) expression slowed cell proliferation and reduced insulin content and insulin secretion. (a) Establishment of SNX19 knockdown MIN6 cells. Western blot analysis of SNX19 and insulinoma-associated protein 2 (IA-2) expression in sorting nexin 19 knockdown (SNX19KD) and control MIN6 cells. (b) Relative ratio of SNX19 expression in SNX19KD MIN6 cells compared with that in control MIN6 cells. (c) Relative ratio of IA-2 expression in SNX19KD MIN6 cells compared with that in control MIN6 cells. (d) Relative ratio of mRNA level of SNX19 or IA-2 quantified by real time polymerase chain reaction in SNX19KD MIN6 cells compared with that in control MIN6 cells. (e) Cell proliferation measured by a bromodexyuridine cell proliferation assay in control, SNX19KD MIN6 cells. (f) Insulin content in control and SNX19KD MIN6 cells. (g) Low (3 mmol/L) and high (25 mmol/L) glucose-stimulated insulin secretion in control and SNX19KD MIN6 cells. Images are representative of three independent experiments. Data are means ± SE of four independent experiments. *$P < 0.01$.

EDTA, 5 mmol/L acetate, pH 5.0). Aliquots were used to measure lysosomal activity determined by cathepsin D activity kit (Sigma).

Statistical Analysis

All data are expressed as mean ± standard error. Student's *t*-test was used to determine statistical significance.

RESULTS

Knockdown of SNX19 Decreases Insulin Content and Insulin Secretion in MIN6 cells

We established two permanent SNX19 knockdown (SNX19KD) MIN6 cell lines (SNX19KD1 and SN19KD2). Western blot showed that SNX19 expression was decreased to approximately one-tenth and one-fifth in SNX19KD1 and SNX19KD2 MIN6 cells, respectively, compared with that in scrambled siRNA-expressing MIN6 cells (control; Figure 1a,b). IA-2 expression was decreased to less than one-thirtieth in SNX19KD1 and SNX19KD2 MIN6 cells compared with that in control MIN6 cells (Figure 1a,c). Quantitative real-time PCR showed that messenger RNA level of SNX19 was decreased by approximately one-tenth and one-fifth in SNX19KD1 and SNX19KD2 MIN6 cells, respectively (Figure 1d). Messenger RNA level of IA-2 also was decreased by one-thirtieth and one-tenth in SNX19KD1 and SNX19KD2 MIN6 cells, respectively, as observed in western blot (Figure 1d). Cell proliferation of the SNX19KD cells were decreased by approximately one-third to one-quarter compared with that of control cells (Figure 1e). As a reduction in IA-2 expression and cell proliferation in pancreatic β-cells is known to decrease insulin content and secretion[9,14], we examined insulin content and glucose-stimulated insulin secretion in SNX19KD MIN6 cells. Insulin content was

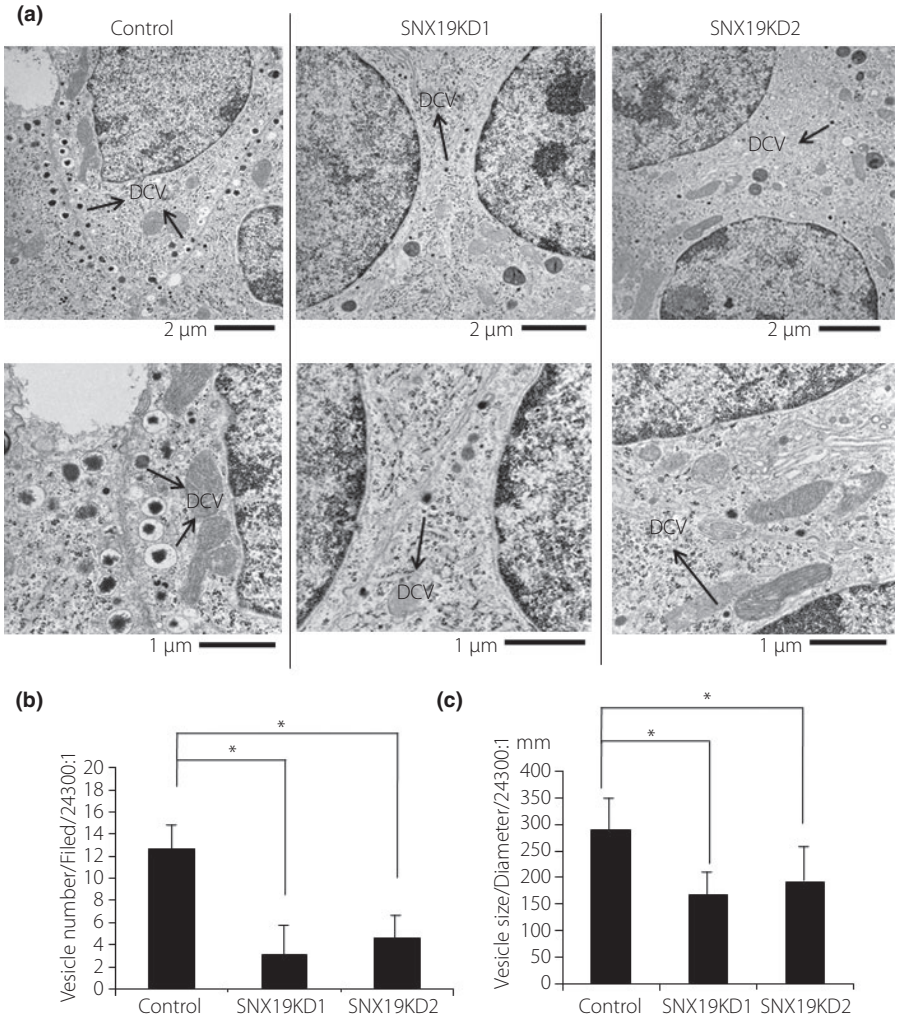

Figure 2 | Knockdown of sorting nexin 19 (SNX19) decreased the number of dense core vesicles (DCV). (a) Representative electron micrographs of 15 images in control, sorting nexin 19 knockdown (SNX19KD)1 and SNX19KD2 MIN6 cells. Black arrows indicate DCV. (b) Average number of DCV in control, SNX19KD1 and SNX19KD2 MIN6 cells. (c) Average size of DCV in control, SNX19KD1 and SXN19KD2 MIN6 cells. Data are means ± SE of four independent experiments. *$P < 0.01$.

decreased to one-seventh and one-quarter in SNX19KD1 and SNX19KD2 MIN6 cells, respectively, compared with that in control MIN6 cells (Figure 1f). The amounts of constitutive and glucose-stimulated insulin secretion also were decreased to approximately one-quarter and one-third in SNX19KD1 and SNX19KD2 cells, respectively, compared with that in control MIN6 cells (Figure 1g). These results suggest that SNX19 regulates insulin content and insulin secretion with a decrease in IA-2 expression.

Knockdown of SNX19 Decreases the Number and the Size of DCV in MIN6 Cells

We then examined the number of DCV in SNX19KD MIN6 cells. Electron micrographs showed that the number of DCV

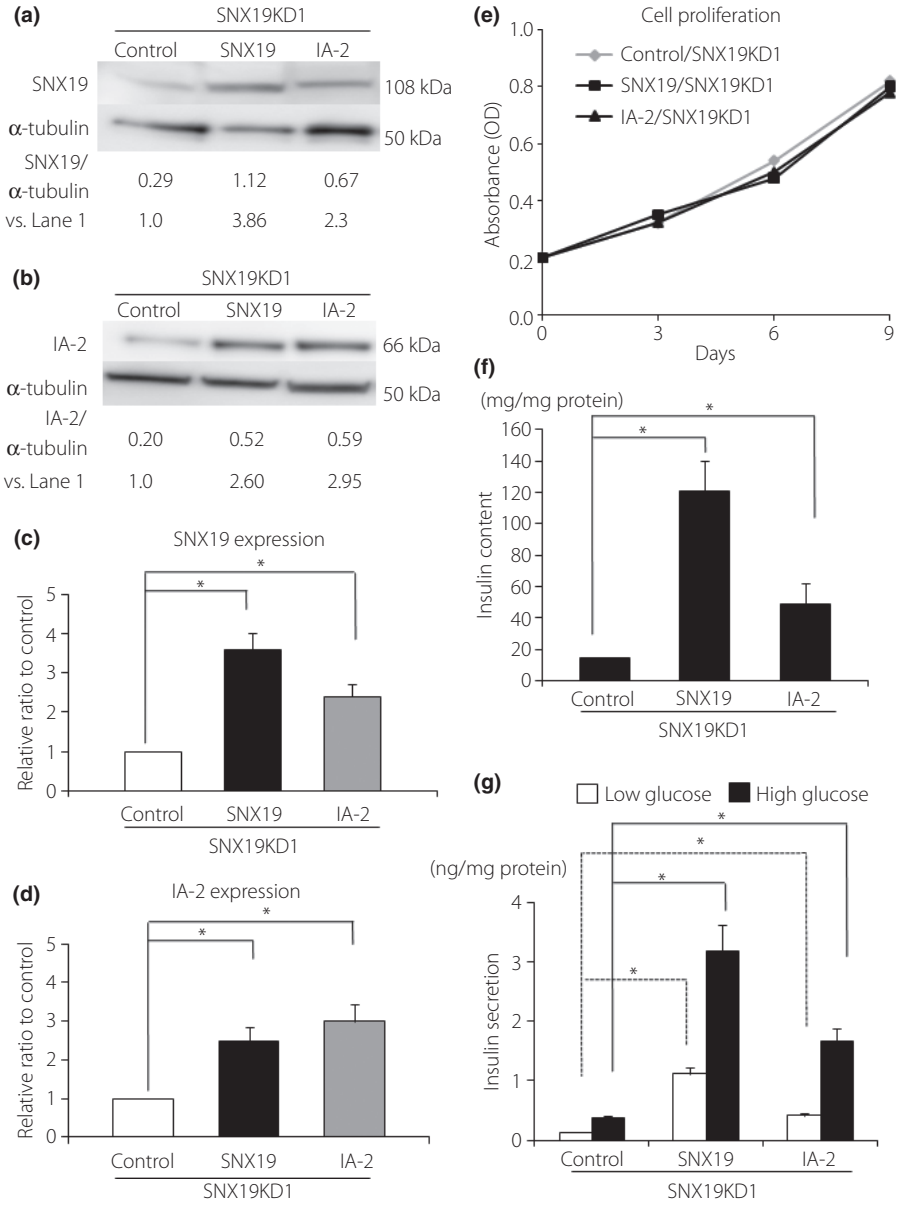

Figure 3 | Reintroduction of sorting nexin 19 (SNX19) or insulinoma-associated protein 2 (IA-2) in sorting nexin 19 knockdown (SNX19KD) MIN6 cells restored cell proliferation rate, insulin content and insulin secretion. (a) Western blot analysis of SNX19 expression in control, SNX19/ and IA-2/SNX19KD1 MIN6 cells. (b) Western blot analysis of IA-2 expression in control, SNX19/ and IA-2/SNX19KD1 MIN6 cells. (c) Relative ratio of SNX19 expression in SNX19/ and IA-2/SNX19KD1 MIN6 cells compared with that in control/SNX19KD1 MIN6 cells. (d) Relative ratio of IA-2 expression in SNX19/ and IA-2/SNX19KD1 MIN6 cells compared with that in control/SNX19KD1 MIN6 cells. (e) Cell proliferation measured by a bromodexyuridine cell proliferation assay in control/, SNX19/ and IA-2/SNX19KD1 MIN6 cells. (f) Insulin content in control/, SNX19/ and IA-2/SNX19KD1 MIN6 cells. (g) Low (3 mmol/L) and high (25 mmol/L) glucose-stimulated insulin secretion in control/, SNX19/ and IA-2/SNX19KD1 MIN6 cells. Images are representative of three independent experiments. Data are means ± SE of four independent experiments. *$P < 0.01$.

was dramatically decreased in SNX19KD1 and SNX19KD2 MIN6 cells compared with that in control cells (Figure 2a). In addition, the size of DCV was smaller in SNX19KD1 and SNX19KD2 MIN6 cells compared with that in control cells (Figure 2a). The average number of DCV was decreased to approximately one-sixth and one-quarter in SNX19KD1 and SNX19KD2 MIN6 cells, respectively, compared with that in control cells (Figure 2b). The size of DCV in SNX19KD1 and SNX19KD2 MIN6 cell lines were also decreased by approximately 40 and 35%, respectively, compared with that in control cells (Figure 2c).

Reintroduction of SNX19 and IA-2 in SNX19KD MIN6 Cells Restores Insulin Content and Insulin Secretion

To confirm the effect of SNX19 on the number of DCV, we used established permanent human SNX19-reintroduced SNX19KD1 (SNX19/SNX19KD1) MIN6 cells and human IA-2-reintroduced SNX19KD1 (IA-2/SNX19D1) MIN6 cells. Western blot analysis showed that SNX19 expression was increased by fourfold in SNX19/SNX19KD1 cells and by approximately twofold in IA-2/SNX19KD1 cells, respectively, compared with that in control vector-transfected SNX19KD1 cells (control/SNX19KD1; Figure 3a,c). In addition, IA-2 expression was

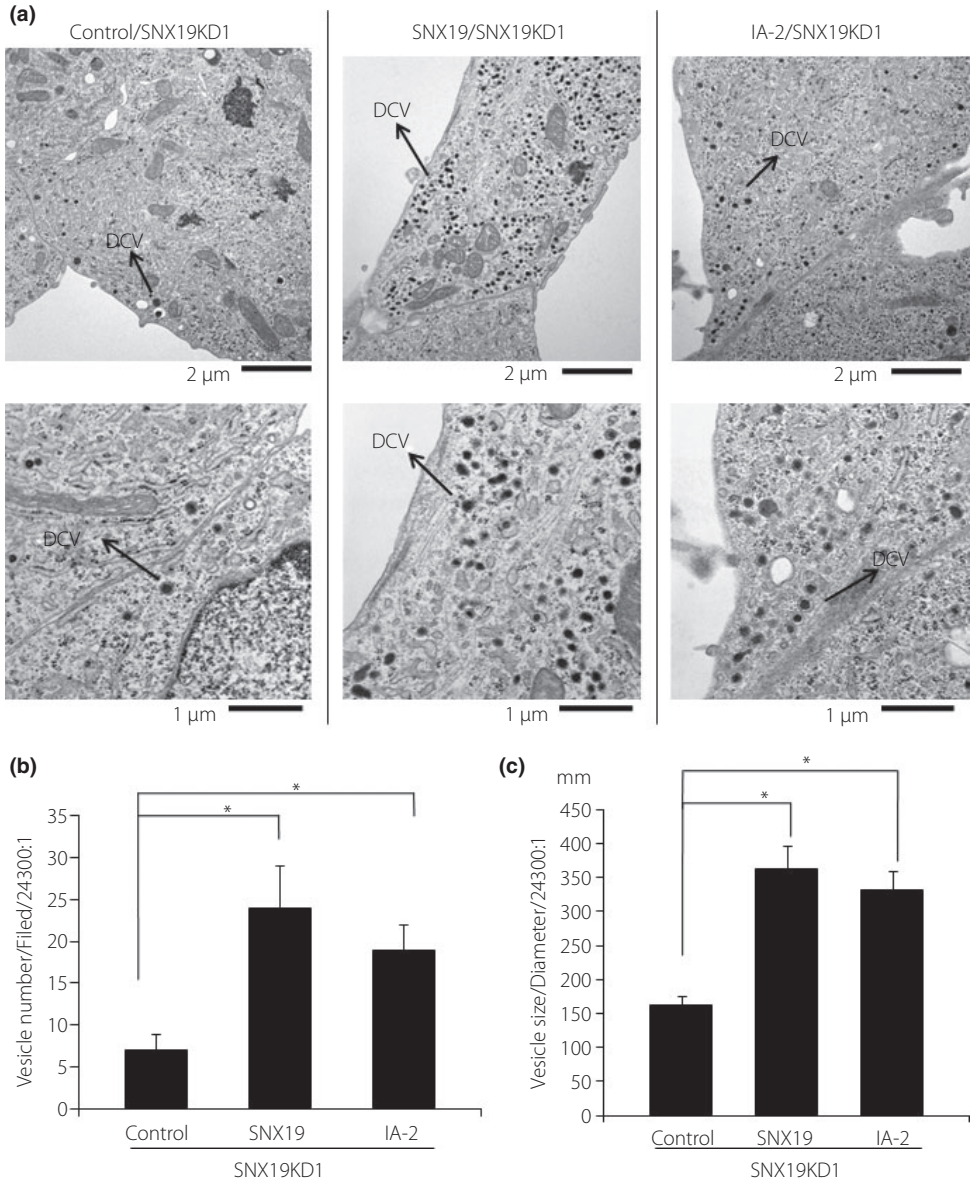

Figure 4 | Reintroduction of sorting nexin 19 (SNX19) or insulinoma-associated protein 2 (IA-2) restored the number of dense core vesicles (DCV). (a) Representative electron micrographs of 15 images in control, SNX19/ sorting nexin 19 knockdown (SNX19KD)1 and IA-2/SNX19KD1 MIN6 cells. Black arrows indicate DCV. (b) Average number of DCV in control, SNX19/SNX19KD1 and IA-2/SNX19KD1 MIN6 cells. (c) Average size of DCV in control, SNX19/SNX19KD1 and IA-2/SXN19KD1 MIN6 cells. Data are means ± SE of four independent experiments. *$P < 0.01$.

increased by approximately 2.5-fold in SNX19/SNX19KD1 MIN6 cells and approximately threefold in IA-2/SNX19KD1 MIN6 cells, respectively (Figure 3b,d). Cell proliferation of SNX19/SNX19KD1 and IA-2/SNX19KD1 MIN6 cells were almost the same as that of control/SNX19KD1 MIN6 cells (Figure 3e). Accordingly, insulin content was increased by approximately sevenfold and threefold in SNX19/SNX19KD1 and IA-2/SNX19KD1 cells, respectively, compared with that in control/SNX19KD1 cells (Figure 3f). Constitutive and glucose-stimulated insulin secretion also were increased by approximately sixfold and threefold in SNX19/SNX19KD1 and IA-2/SNX19KD1 cells, respectively, compared with those in control/SNX19KD1 cells (Figure 3g).

Reintroduction of SNX19 and IA-2 Increases the Number and the Size of DCV in SNX19KD MIN6 Cells

Electron micrographs showed that the number of DCV was increased in both SNX19/SNX19KD1 and IA-2/SNX19KD1 MIN6 cells compared with that in control/SNX19KD1 cells (Figure 4a). The number of DCV was increased by approximately fourfold in SNX19/SNX19KD1 cells and approximately threefold in IA-2/SNX19KD1 cells, respectively, compared with that in control/SNX19KD1 cells (Figure 4b). The size of DCV was increased by approximately twofold in both SNX19/SNX19KD1 and IA-2/SNX19KD1 cells compared with that in control/SNX19KD1 MIN6 cells (Figure 4c).

SNX19 Affects the Half-life of DCV

To investigate the involvement of SNX19 in DCV stability, we measured the half-life of DCV in SNX19KD1 and SNX19/SNX19KD1 cells. A pulse-chase experiment showed that the half-life of DCV in SNX19KD1 cells was 11.6 h, approximately half of that in control cells (Figure 5a). In contrast, the half-life of DCV in SNX19/SNX19KD1 was 30.4 h, approximately threefold of that in control/SNX19KD1 cells (Figure 5b). To ascertain that the decreased half-life of DCV in SNX19KD1 cells was not the result of a decrease in biosynthesis of proinsulin/insulin, cells were pulsed with [^{3}H]leucine, and newly synthesized proinsulin/insulin was measured. At the end of a 24-h pulse, the amount of proinsulin/insulin in SNX19KD1 cells was almost equal to or slightly lower than that in control cells (Figure 5a). Similarly, the amount of newly synthesized proinsulin/insulin in SNX19/SNX19KD1 cells was almost equal to or slightly greater than that in control/SNX19KD1 cells (Figure 5b). These results show that SNX19 stabilizes DCV.

SNX19 Knockdown Increases the Activity of Lysosomes

The finding that SNX19 affected the half-life of DCV suggested that the reduction of DCV number in the SNX19KD cells might be the result of accelerated DCV degradation. The number of lysosomes in SNX19KD1 cells was increased by approximately fourfold compared with that in control MIN6 cells (Figure 6a,b). In contrast, the number of lysosomes in SNX19/SNX19KD1

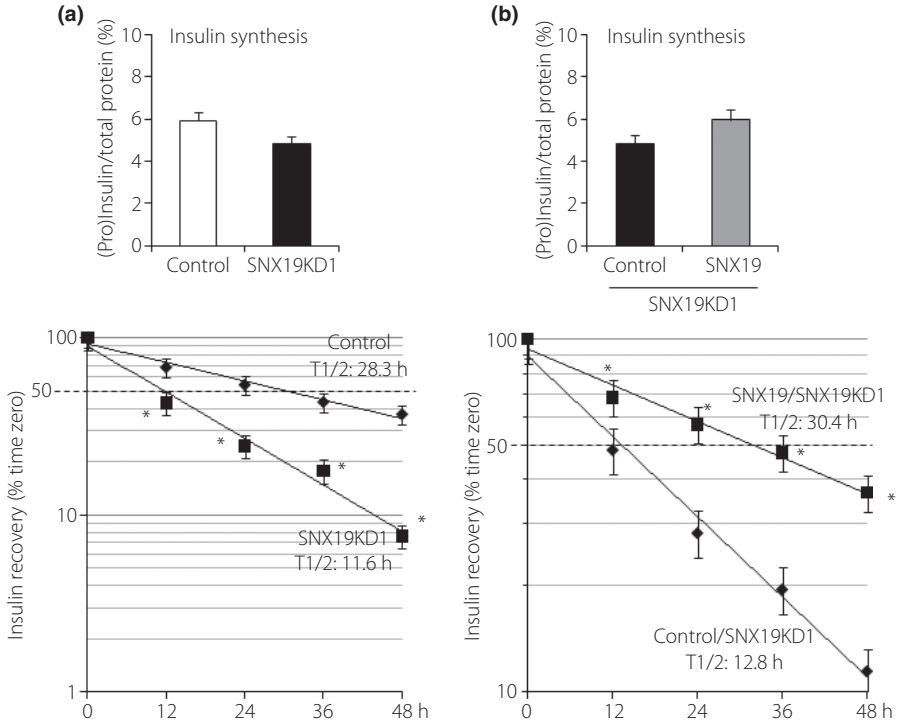

Figure 5 | Sorting nexin 19 (SNX19) affected the half-life of dense core vesicles (DCV). (a) Half-life of DCV and insulin and proinsulin biosynthesis in control and sorting nexin 19 knockdown (SNX19KD)1 MIN6 cells; (b) in control/ and SNX19/SNX19KD1 MIN6 cells. Data are means ± SE of four independent experiments. *$P < 0.01$.

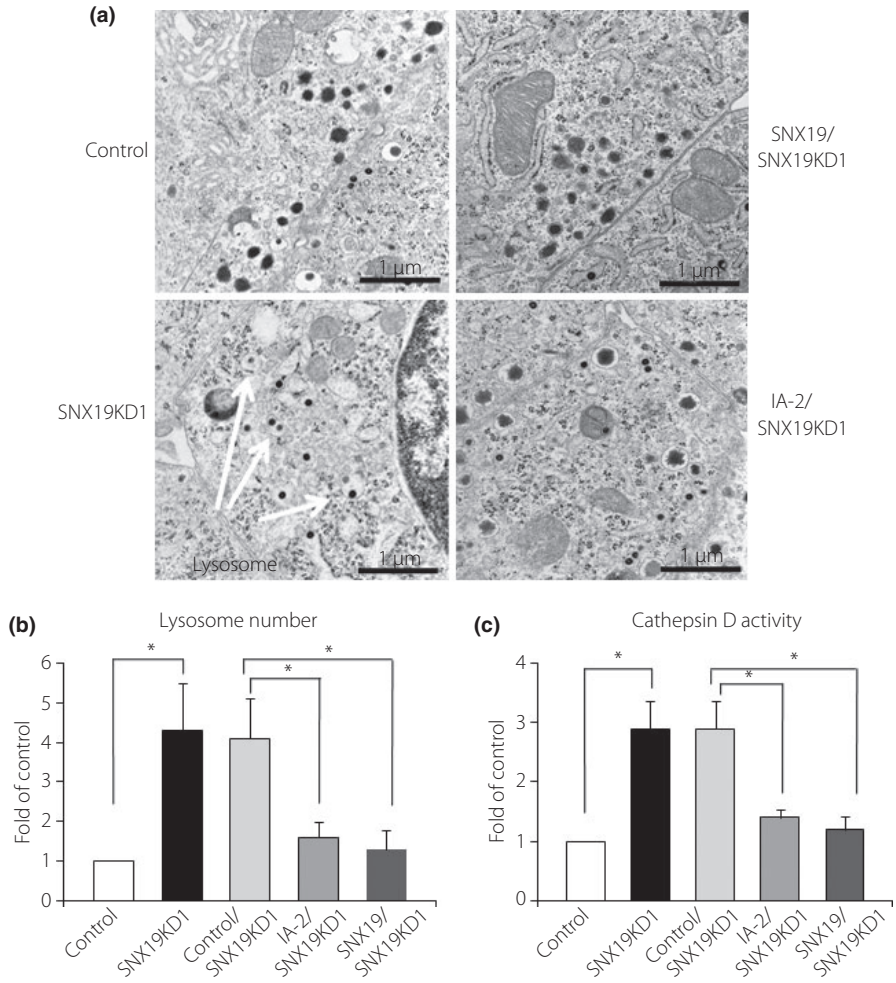

Figure 6 | Decreased expression of sorting nexin 19 (SNX19) increased activity of lysosomes and autophagy. (a) Representative electron micrographs of 15 images in control, sorting nexin 19 knockdown (SNX19KD)1, IA-2/SNX19KD1 and SNX19/SNX19KD1 MIN6 cells. White arrows indicate lysosomes. (b) Average lysosome number per image of 20 images. (c) Cathepsin D activity. Data are means ± SE of four independent experiments. *$P < 0.01$.

and IA-2/SNX19KD1 cells was almost equal to that in control cells and less than one-third of that in control/SNX19KD1 cells (Figure 6a,b). Activity of lysosome enzyme cathepsin D also was increased by approximately threefold in SNX19KD1 cells compared with that in control cells (Figure 6c), whereas activity of cathepsin D in IA-2/ and SNX19/SNX19KD1 cells was decreased to approximately half of that in control/SNX19KD1 cells (Figure 6c).

DISCUSSION

The experiments reported in the present study show that knockdown of SNX19 decreases the number of DCV in MIN6 cells, and also decreases the cellular content and secretion of insulin. Conversely, reintroduction of SNX19 increases the number of DCV in MIN6 cells, and increases the cellular content and secretion of insulin. Thus, SNX19 expression is regulated at the transcriptional level and affects the half-life of DCV, insulin content and secretion. The half-life of DCV in SNX19KD cells was 11.6 h as compared with 28.3 h in control cells and reintroduction of SNX19 increased the half-life of DCV in SNX19KD cells from 12.8 to 30.4 h. The most likely explanation for these findings is that the reduced half-life of the DCV is responsible for their reduced number, which directly underlies the decrease in insulin content and secretion.

Of particular interest, earlier experiments showed that overexpression of IA-2 in MIN6 cells significantly increased the half-life of the DCV, as well as the content and secretion of insulin[14]. Indeed, a very recent experiment found that IA-2 or IA-2β single knockout and IA-2/IA-2β double knockout mice showed a significant decrease in the number of DCV and the content and secretion of insulin (Cai T and Notkins AL, unpublished data, 2011).

IA-2 and IA-2β are transmembrane proteins on the DCV and it is thought that knockout (Cai T and Notkins AL,

unpublished data, 2011) or overexpression of these proteins[14] can decrease or increase, respectively, the stability of the DCV and, in turn, their half-life. Changes in the number of DCV transmembrane proteins can readily affect the stability of these vesicles. SNX19, however, is not a transmembrane protein, but, as determined by the yeast two hybrid system, binds to the cytoplasmic region of IA-2 encompassing amino acids 744–979[5]. Furthermore, SNX19 alone or the IA-2/SNX19 complex binds to several phosphatidylinositols (ptdlins), most strongly to Ptdins(3)P, Ptdlins(4)P and Ptdlins(5)P[16]. In contrast, IA-2 does not bind to the ptdlins. Ptdins(3)P is involved in the recruitment of many different proteins that are important for protein trafficking to membrane[17–19]. PtdIns(4)P is located in the membrane of the Golgi apparatus, and binds to the ADP ribosylation factor (ARF) GTP-binding protein and to four-phosphate-adaptor protein 1 and 2 (FAPP1 and FAPP2) and effector proteins[18,20]. This complex of molecules recruits proteins to the membrane. The function of PtdIns(5)P remains unknown, but it might act in membrane trafficking from late endosomes to the plasma membrane[20,21]. We suggest that binding of the IA-2/SNX19 complex to the ptdlins might be responsible for sorting, trafficking and stabilization of the DVC. In SNX19KD MIN6 cells, lysosomal activities are increased, restored by reintroduction of SNX19 or IA-2. Knockdown of SNX19 decreases IA-2 expression; reintroduction of SNX19 increases IA-2 expression in MIN6 cells. Thus, SNX19 regulates IA-2 expression to allow a complex of SXN19 and IA-2 to stabilize the DCV. Decreased expression of SNX19 reduces IA-2 expression and destabilizes DCV, resulting in increasing lysosomal activities and decreasing DCV half-life. Although the mechanism is not known precisely, based on our findings, the binding of SNX19 to IA-2 might be directly involved in the stabilization of DCV by exposing or protecting IA-2 from degradation or by affecting trafficking or recycling of the DCV through the endosome pathway.

SNX19 also was found to affect cell proliferation. Knockdown of SNX19 inhibited cell proliferation, which was restored by reintroduction of SNX19. We previously reported that overexpression of IA-2 and/or SNX19 induced apoptosis and inhibited cell proliferation together with a decrease in Akt/PKB phosphorylation under high glucose conditions[16]. In contrast, knockdown of IA-2 and/or SNX19 did not induce apoptosis in β-cells (data not shown). However, cell proliferation was inhibited in SNX19 knockdown MIN6 cells with a decrease in insulin content and insulin secretion. On the other hand, reintroduction of SNX19 or IA-2 restored cell proliferation in SNX19KD MIN6 cells with an increase in insulin content and insulin secretion. A possible explanation is that a decrease in insulin secretion, which is important for cell growth in pancreatic β-cells, contributes to inhibition of cell proliferation in SNX19KD MIN6 cells.

In conclusion, the present study shows the importance of SNX19 in DCV physiology. Recent studies have shown that SNX19 can bind not only to IA-2, but also to IA-2β (S.-I. Harashima, unpublished data, 2011). As there are nearly 30 different members of the SNX family, it is an intriguing group of proteins for further study.

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