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ORIGINAL ARTICLE

SNARE-binding protein synaptosomal-associated protein of 29 kDa (SNAP29) regulates the intracellular sequestration of glucose transporter 4 (GLUT4) vesicles in adipocytes

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Keywords
Glucose transporter type 4 (GLUT4), Insulin, Synaptosomal-associated protein of 29 kDa (SNAP29)

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ABSTRACT

Aims/Introduction: Insulin stimulates translocation of glucose transporter 4 (GLUT4) from the perinuclear location to the plasma membrane. In the unstimulated state, intracellular vesicles containing GLUT4 are sequestered into specialized storage vesicles that have come to be known as the insulin-responsive compartment (IRC). The IRC is a functional compartment in the perinuclear region that is a target of the insulin signaling cascade, although its precise nature is unclear. Here, we report a novel molecular mechanism facilitating formation of the IRC.

Materials and Methods: We determined synaptosomal-associated protein of 29 kDa (SNAP29) by mass spectrometry to be an EH domain-containing protein 1 (EHD1)-binding protein. Then, its expression was confirmed by western blotting. Subcellular localization of SNAP29 was determined by immunofluorescent microscopy. Interactions between SNAP29 and syntaxins were determined by immunoprecipitation. We measured glucose uptake and GLUT4 translocation in 3T3-L1 adipocyte expressing SNAP29 or silencing SNAP29.

Results: We found SNAP29 to be localized in the perinuclear region and to show partial co-localization with GLUT4 under basal conditions. We also found that SNAP29 binds to syntaxin6, a Qc-SNARE, in adipocytes. In SNAP29-expressing cells, vesicles containing GLUT4 were observed to aggregate around the perinuclear region. In contrast, when SNAP29 was silenced, perinuclear GLUT4 vesicles were dispersed throughout the cytosol. Insulin-stimulated glucose uptake was inhibited in both SNAP29-expressing and SNAP29-silenced cells.

Conclusions: These data suggest that SNAP29 sequesters and anchors GLUT4-containing vesicles in the perinuclear region, and might have a role in the biogenesis of the perinuclear IRC.

INTRODUCTION

Glucose transporter 4 (GLUT4) is the major insulin responsive transporter of glucose, expressed in skeletal muscle and adipose tissue. Insulin enhances the translocation of vesicles containing GLUT4 to the plasma membrane. This redistribution of glucose transporter GLUT4 is thought to be the rate-limiting step for insulin action in humans[1,2]. In the basal state, most GLUT4 vesicles are in the perinuclear compartment (similar to the microtubule-organizing center), and the residual vesicles are within endosomal compartments (in the cytosol). When the cells are stimulated by insulin, perinuclear GLUT4 vesicles are translocated to the plasma membrane in a few minutes, and

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externalized GLUT4 protein facilitates glucose transport across the plasma membrane. After GLUT4 takes up glucose in response to insulin, GLUT4 is recycled back into the intracellular compartments. This intracellular sequestration of GLUT4 vesicles is important for another round of recycling and sustainable glucose uptake in response to insulin stimulation[3]. Despite numerous investigations, the precise mechanism responsible for GLUT4 vesicle recycling remains unclear.

In the past decade, numerous investigations have been carried out in efforts to show the molecular mechanisms of GLUT4 vesicle trafficking in response to insulin. Most were studies designed to identify the mechanisms of GLUT4-vesicle exocytosis in response to insulin stimulation; that is, GLUT4 translocation. The molecular basis of GLUT4 translocation (exocytotic processes of GLUT4 vesicles), which includes insulin signaling molecules, molecular motors[4–7], cytoskeletons[8–10], and SNARE and SNARE-related proteins[11–14], has already been extensively shown.

In contrast, only a few leading researchers have reported on the endocytotic trafficking of GLUT4 vesicles[15,16]. We and other groups have investigated the function of the GLUT4 compartment in the perinuclear region[8,17,18]. This region overlaps with the microtubule organizing center, and is thought to be important for rapid translocation of GLUT4 vesicles to the plasma membrane[4,5,8,19]. In other words, the perinuclear compartment is thought to serve as an important insulin responsive compartment (IRC)[8,19]. However, very little is known about the molecular mechanism of IRC formation.

Eps15 homology (EH)-domain containing 1 (EHD1) was first cloned by Mintz *et al.*[20] as a family of proteins encoding eps15 homology (EH) domain . The EH domain has been shown to be an important motif involved in protein–protein interactions, endocytosis, vesicle transport and signal transduction[21–23].

Syntaxin6 and EHD1 protein have also been reported to facilitate retrograde trafficking of GLUT4 vesicles from early endosomes to recycling endosomes or perinuclear compartments[24,25]. However, the molecular mechanisms underlying the functions of EHD1 and syntaxin6 in intracellular sequestration of GLUT4 are poorly understood. Thus, to identify the novel proteins that regulate retrograde traffic of GLUT4 vesicles, we first screened for proteins that can bind to EHD1, and found synaptosomal-associated protein of 29 kDa (SNAP29) in 3T3-L1 adipocytes. SNAP29 is a cytosolic SNARE protein involved in multiple membrane trafficking steps[26,27]. This protein can also bind syntaxins, such as syntaxin6 and other intracellular syntaxins[28,29]. Recently, EHD1 was reported to bind with SNAP29, and was suggested to play a role in insulin-like growth factor-1 receptor endocytosis[30]. Based on prior observations, we hypothesized that SNAP29 might have essential roles in GLUT4 recycling and the formation of IRC.

In the present study, we focused on SNAP29 and explored its role in GLUT4 vesicle recycling, perinuclear GLUT4 compartment formation and insulin stimulated glucose uptake in adipocytes. We found that SNAP29 co-localized with GLUT4 vesicles, and that manipulating SNAP29 expression upward and downward disturbed insulin stimulated glucose uptake. SNAP29 might have an essential role in formation of the IRC, as well as in GLUT4 vesicle recycling.

MATERIALS AND METHODS

Plasmids and antibodies

Mouse full-length EHD1 and SNAP29 cDNAs were cloned by reverse transcription polymerase chain reaction amplification with total messenger ribonucleic acid (mRNA) from 3T3-L1 adipocytes, and then subcloned into green fluorescent protein (peGFP)-C2 (Clontech, Mountain View, CA, USA) and pcDNA3-3xHA in-house modified vectors. We also constructed the APA-mutant of SNAP29 corresponding to the NPF motif (amino acids 9–11) using a QuikChange Site-Directed Mutagenesis Kit (Stratagene, San Diego, CA, USA). All plasmids were verified by deoxyribonucleic acid sequencing.

The following antibodies were used: anti-GLUT4, anti-GFP, anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-HA (HA-7; Sigma Aldrich, St. Louis, MO, USA); anti-SNAP29, anti-syntaxin4, anti-syntaxin6 (Synaptic Systems, Gottingen, Germany); anti-phospho-Akt (Ser473), anti-Akt (pan; Cell Signaling Technology, Danvers, MA, USA); and secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA).

SNAP29 short hairpin-RNA lentivirus

Short hairpin RNA (shRNA) and corresponding control-RNA were purchased from Thermo Scientific (Waltham, MA, USA). All transfections were carried out according to the manufacturer's instructions (GIPZ Lentiviral shRNA system; Thermo Scientific).

Cell culture

3T3-L1 fibroblasts were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were differentiated into adipocytes and used for the experiments, as previously described[4,14].

Preparation of recombinant adenovirus vectors

eGFP and eGFP-tagged SNAP29 genes were inserted into adenovirus vectors by using an AdEasy adenovirus vector system (Stratagene). All adenovirus particles were purified using cesium chloride gradient centrifugation and stored at −80°C.

Immunoprecipitation and immunoblotting

Cells were lysed in lysis buffer (20 mmol/L HEPES [pH 7.2], 100 mmol/L NaCl, 1 nmol/L ethylenediaminetetraacetic acid, 25 mmol/L NaF, 1 mmol/L sodium vanadate, 1 mmol/L benzamidine, 5 μg/mL leupeptin, 5 μg/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L dithiothreitol, 0.5% NP-40) and adjusted its protein concentration using bicinchoninic acid (BCA) protein assay reagent (Pierce, Waltham,

MA, USA). Immunoprecipitations were carried out using pre-cleared cell lysates with protein-G/A-Sepharose. Then, the cleared lysates were incubated with primary antibody at 4°C overnight followed by incubation with protein-G/A-Sepharose. The immunoprecipitated proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Amersham, Uppsala, Sweden). Quantitative image analyses of western blots were processed and analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA)[31].

Identification of EHD1-binding proteins

3T3-L1 adipocytes were serum-starved for 2 h, and treated with or without 100 nmol/L of insulin for 10 min at 37°C. Then, the cells were lysed in the aforementioned lysis buffer. The samples were then immunoprecipitated with anti-EHD1 antibody as described in the "Immunoprecipitation and immunoblotting" section. Binding proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and stained with silver. The bands were excised and subjected to in-gel digestion according to the method described by Shevchenko *et al.*[32] with minor modifications. Mass spectra were acquired using a time-of-flight mass spectrometer (AXIMA-QIT-TOF MS; Shimadzu, Kyoto, Japan). The peptide mass fingerprint was analyzed by the Mascot engine (Matrix Science, Columbus, OH, USA). Details of in-gel digestion were described in our prior report[33].

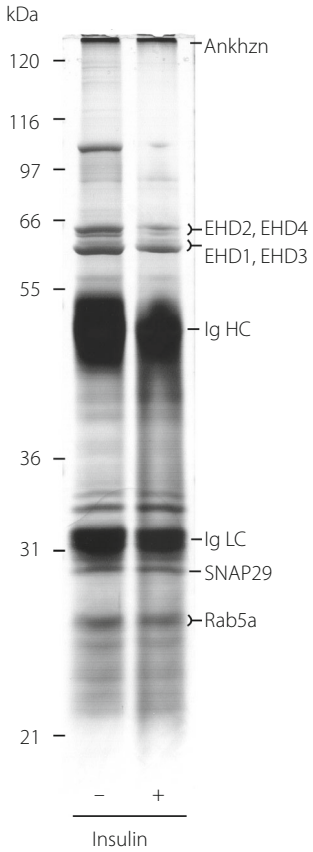

Figure 1 | Identification of Eps15 homology-domain containing 1 (EHD1)-binding protein. After 2-h serum starvation, 3T3-L1 adipocytes were treated with or without 100 nmol/L insulin for 15 min. Clear cell lysates were prepared and immunoprecipitated with anti-EHD1 antibody. The precipitates were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and the proteins were visualized by silver staining. Gel-bands were excised, and in-gel digested and analyzed by mass spectrometry. Synaptosomal-associated protein of 29 kDa (SNAP29) and other proteins were identified.

Immunofluorescence microscopy and digital image analysis

Differentiated 3T3-L1 adipocytes on coverslips were serum-starved for 4 h, followed by incubation with or without insulin for 20 min at 37°C. Then, the cells were fixed and immunostained by the methods described before[14]. Cell imaging was carried out by a LSM 510 META laser scanning confocal microscope and its processing software (Carl Zeiss, Oberkochen, Germany) using a 63x/1.4 numerical aperture oil immersion lens. The co-localization of endogenous SNAP29 with perinuclear GLUT4 was measured using Intensity Correlation Analysis function from the Colocalization plugin (Coloc2) in ImageJ software.

2-Deoxy-glucose uptake

Differentiated 3T3-L1 adipocytes were infected with the recombinant adenoviruses or lentiviruses. After 2 h of serum starvation in Krebs–Ringer–HEPES buffer, the adipocytes were treated with or without 100 nmol/L of insulin for 10 min.

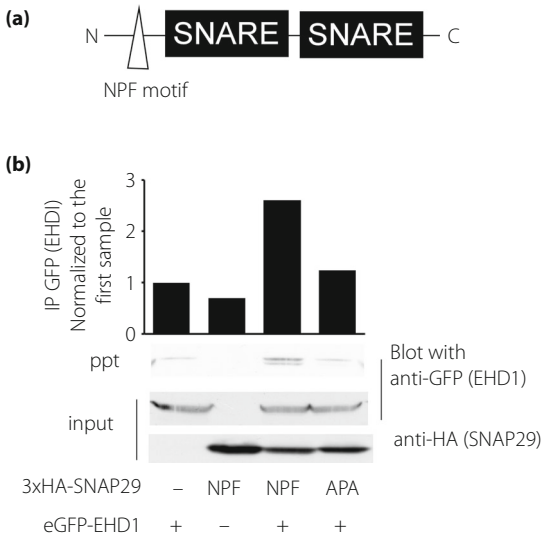

Figure 2 | Synaptosomal-associated protein of 29 kDa (SNAP29) binds to Eps15 homology-domain containing 1 (EHD1) through its NPF motif. (a) Domain structure of SNAP29. (b) 3xHA-tagged SNAP29 wildtype (NPF) and 3xHA-tagged SNAP29 APA mutants were co-transfected with green fluorescent protein (eGFP)-EHD1 in COS cells. SNAP29–EHD1 interactions were confirmed by immunoprecipitation experiments using anti-HA antibody. The graph shows the results of quantitative analysis for each band by Image J software.

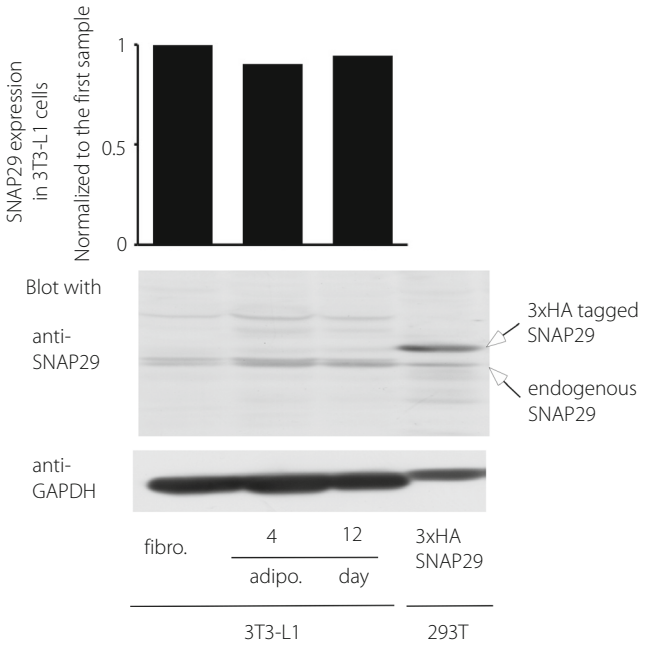

Figure 3 | Synaptosomal-associated protein of 29 kDa (SNAP29) expression profiles. The endogenous expressions of SNAP29 were determined by western blotting in 3T3-L1 fibroblasts, differentiated adipocytes (days 4 and 12), and 3xHA-transfected 293 T cells, serving as a positive control. The graph shows the results of quantitative analysis for each band by Image J software.

Then, glucose transport activity was determined using 2-deoxy-D-[2,6 ^{3}H] glucose, as previously described[14].

Statistical analysis

Data are presented as the mean ± standard deviation. Multiple comparisons between more than two experimental groups were carried out using one-way ANOVA. *P*-values <0.05 were considered significant.

RESULTS

Identification of SNAP29 as an EHD1-binding protein

As EHD1 protein was reported to facilitate retrograde trafficking of GLUT4 vesicles from early endosomes to recycling endosomes or perinuclear compartments[25,34], we first screened for proteins capable of binding EHD1 using immunoprecipitation with anti-EHD1 antibody and mass-spectrometric analysis. We thereby identified SNAP29, which is a cytosolic SNARE protein involved in multiple membrane trafficking steps (Figure 1).

SNAP29 contains two SNARE domains and an asparagine-proline-phenylalanine (NPF) motif at its N terminus (Figure 2a). EH domains are protein interaction modules that recognize NPF motifs, thereby mediating critical events during endocytosis[35]. Therefore, SNAP29 can bind EHD1 through the NPF motif-EH domain structure. Based on this information, we created mutations in the NPF motif of SNAP29; that is, an APA (N9A, F11A) mutant, and examined their interactions with EHD1. As shown in Figure 2b, EHD1 was capable of

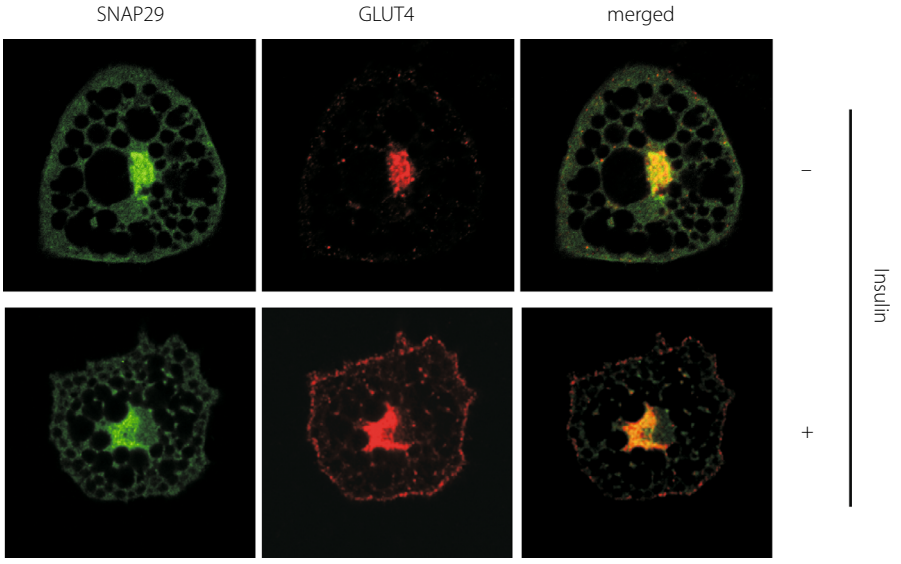

Figure 4 | Intracellular localization of synaptosomal-associated protein of 29 kDa (SNAP29) and glucose transporter 4 (GLUT4) vesicles. 3T3-L1 adipocytes were serum-starved for 2 h and treated with or without 100 nmol/L insulin for 15 min. Then, the cells were fixed and stained with anti-SNAP29 and anti-GLUT4 antibody followed by fluorescein isothiocyanate and Cy3-labeled secondary antibodies, and then observed using confocal microscopy. Quantitative co-localization analyses of endogenous SNAP29 and GLUT4 in perinuclear areas were carried out using the Intensity Correlation Analysis function from the co-localization plugin (Coloc2) in ImageJ software. Pearson's correlation coefficients were 0.85 and 0.78 in cells with and without insulin, respectively, suggesting partial co-localization between SNAP29 and GLUT4 vesicles.

binding to the SNAP29 wild type, but not the APA mutant of SNAP29. Based on the results presented here, we hypothesized that SNAP29, in co-operation with EHD1, might regulate endocytic recycling of GLUT4 vesicles in adipocytes.

Identification of intracellular localization of SNAP29

To test the aforementioned hypothesis, we first confirmed the protein expression of SNAP29 in differentiated 3T3-L1 adipocytes. As shown in Figure 3, SNAP29 was expressed in both 3T3-L1 fibroblasts and 3T3-L1 mature adipocytes. Next, we determined the intracellular localization of SNAP29 in adipocytes. As shown in Figure 4, immunofluorescent microscopic analysis revealed the most SNAP29 to be localized at the perinuclear region and unchanged in response to insulin stimulation. A few SNAP29 resulted in a fine punctate or granular appearance throughout the cytoplasm. These observations are consistent with our aforementioned hypothesis that SNAP29 might have a role in the sequestration of GLUT4 vesicles in the perinuclear compartment.

Identification of SNAP29-binding partners

As membrane fusion of GLUT4 vesicles is thought to be mediated by SNARE and SNARE-related proteins, we can reasonably speculate that SNAP29 also interacts with other SNARE proteins. Therefore, we attempted to identify SNAP29-binding partners by carrying out an immunoprecipitation experiment. As shown in Figure 5a, SNAP29 was capable of binding with syntaxin6, but not with syntaxin4. Syntaxin6 belongs to the Qbc subfamily of SNARE proteins and regulates GLUT4 sorting from endosomes into the perinuclear compartment[24]. We also found that insulin enhanced the SNAP29–syntaxin6 interaction, as shown in Figure 5b. We do not know why synatxin4, an abundant syntaxin form, did not bind to SNAP29. Distinct intracellular localizations of syntaxins might influence this interaction.

These results, presented in Figures 2 and 5, are consistent with our aforementioned hypothesis that SNAP29 might sequester GLUT4 vesicles into the perinuclear compartment. To confirm this possibility, we next carried out a series of experiments using adipocytes expressing SNAP29, as well as cells in which SNAP29 was silenced.

Role of SNAP29 in glucose transport into adipocytes

To clarify the role of SNAP29 in glucose transport, we next introduced shRNA (shRNA-SNAP29) into cultured adipocytes using the recombinant lentiviruses described above to induce specific degradation of the SNAP29 messenger RNA. SNAP29

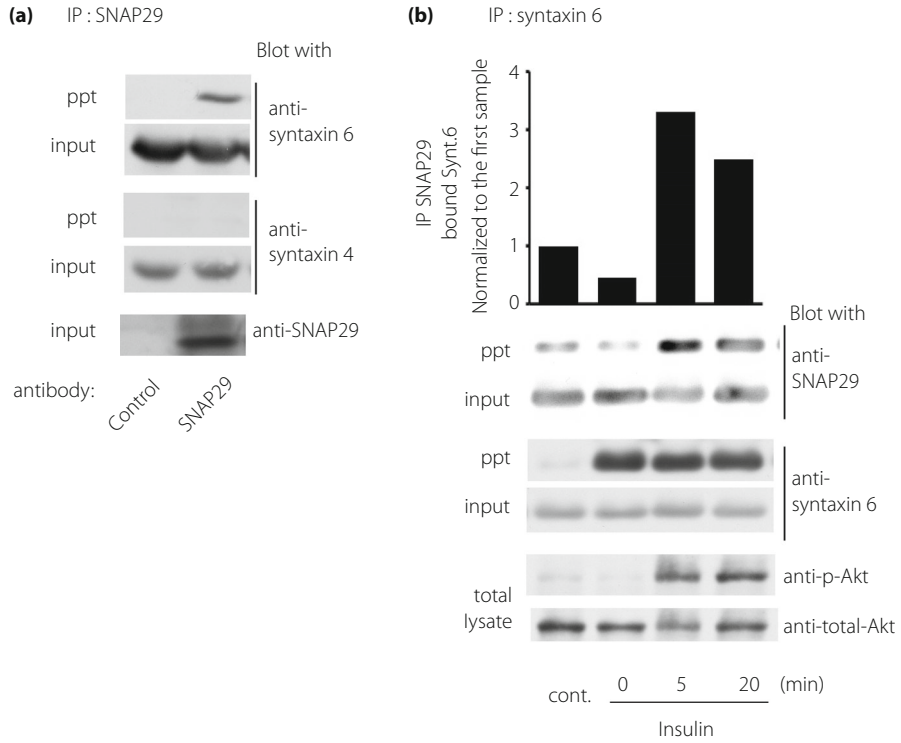

Figure 5 | Synaptosomal-associated protein of 29 kDa (SNAP29)–syntaxins interaction. (a) 3T3-L1 adipocytes were not serum-starved. The SNAP29–syntaxins interactions were determined by immunoprecipitation using anti-SANP29, anti-syntaxin4, anti-syntaxin6 or non-immune control rabbit antibodies. (b) 3T3-L1 adipocytes were serum-starved for 2 h and treated with 100 nmol/L insulin for 0, 5 or 20 min. The SNAP29–syntaxin6 interaction was determined by immunoprecipitation using anti-synatxin6 antibody, and the precipitates and start lysates were immunoblotted with anti-SNAP29, anti-syntaxin6, antiphospho-Ser473 protein kinase B (Akt) and anti-Akt antibodies. The graph shows the results of quantitative analysis for each band by Image J software.

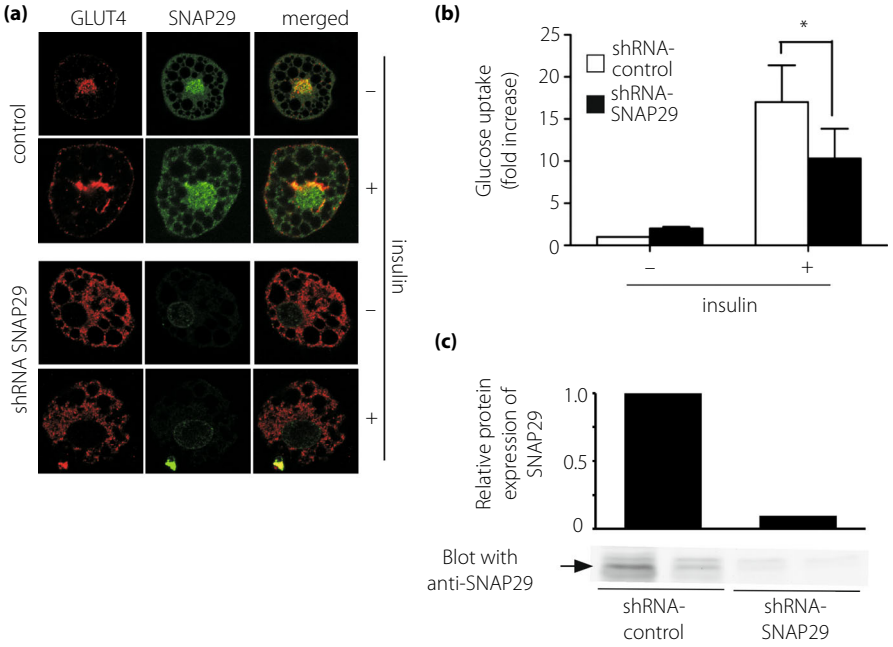

Figure 6 | Effects of synaptosomal-associated protein of 29 kDa (SNAP29) silencing on intracellular localization of glucose transporter 4 (GLUT4) and insulin stimulated glucose-uptake in 3T3-L1 adipocytes. (a) 3T3-L1 pre-adipocytes were infected with short hairpin ribonucleic acid (shRNA) lentivirus or its control virus at a multiplicity of infection of 100. Two days thereafter, the cells were re-seeded onto glass plates. After being serum-starved for 3–4 h, the cells were treated with or without 100 nmol/L insulin for 15 min, then the cells were fixed and immunostained with anti-SNAP29 and anti-GLUT4 antibodies. Intracellular localizations of GLUT4 and SNAP29 were then observed using confocal microscopy. (b) The cells were serum-starved for 2 h in Krebs–Ringer–HEPES buffer and treated with or without 100 nmol/L of insulin for 10 min. Glucose uptake measurements were carried out. Each bar represents the mean ± standard deviation of three independent experiments. (c) Determination of the efficacy of SNAP29 knockdown. Approximately 80–90% of SNAP29 protein was silenced in 3T3-L1 adipocytes. The graph shows the results of quantitative analysis for each band by ImageJ software.

protein expression was decreased to 10–20% of the control level (Figure 6c). We also confirmed that SNAP29 knockdown did not affect insulin signaling to Akt (Ser 473) phosphorylation (data not shown). Interestingly, GLUT4 vesicles were dispersed in the cytosol in SNAP29-silenced cells (Figure 6a). As expected, inhibition of insulin stimulated glucose uptake was observed in SNAP29 knockdown cells, as compared with the control cells (Figure 6b).

Finally, we examined the effects of SNAP29 overexpression on glucose uptake and intracellular localization of GLUT4 vesicles. As shown in Figure 7, overexpression of eGFP-SNAP29 decreased insulin stimulated-glucose uptake to 75% of the control level without affecting insulin signaling. Interestingly, the cells expressing SNAP29 showed accumulation of GLUT4 vesicles in the perinuclear region, and this was observed even in cells treated with insulin. Collectively, these results suggest that SNAP29 mediates the accumulation of GLUT4 vesicles in the perinuclear area, but has little effect on GLUT4 vesicle exocytotic processes.

DISCUSSION

Insulin stimulates translocation of the GLUT4 glucose transporter from the intracellular membrane (intracellular compartments) to the cell surface. After glucose is taken up at the cell surface, GLUT4 returns to intracellular compartments, such as the endosomal and perinuclear membranes. Although several trafficking models have been proposed, the actual function of the perinuclear compartment is still unclear. In the basal state, most GLUT4 vesicles are in the perinuclear region, which overlaps with the microtubule organizing center. Insulin stimulates the translocation of GLUT4 vesicles along with microtubules and other cytoskeletal systems[8,10,11]. Therefore, formation of this perinuclear compartment is extremely important for rapid glucose uptake and insulin action. However, the precise mechanism by which endocytosed GLUT4 vesicles are sequestered into the perinuclear region remains largely unknown.

A key finding of the present study was identification of SNAP29, a molecule that promotes the accumulation of GLUT4 vesicles in the perinuclear region. As SNAP29 has no membrane spanning domains, this SNARE is basically a cytosolic protein, and regulates membrane fusion processes through interaction with vesicle-associated membrane proteins and syntaxins. A recent proteomic analysis of GLUT4 storage vesicles showed SNAP29 to be present in GLUT4 vesicles[36], suggesting a role of membrane recycling processes through SNAP29 and

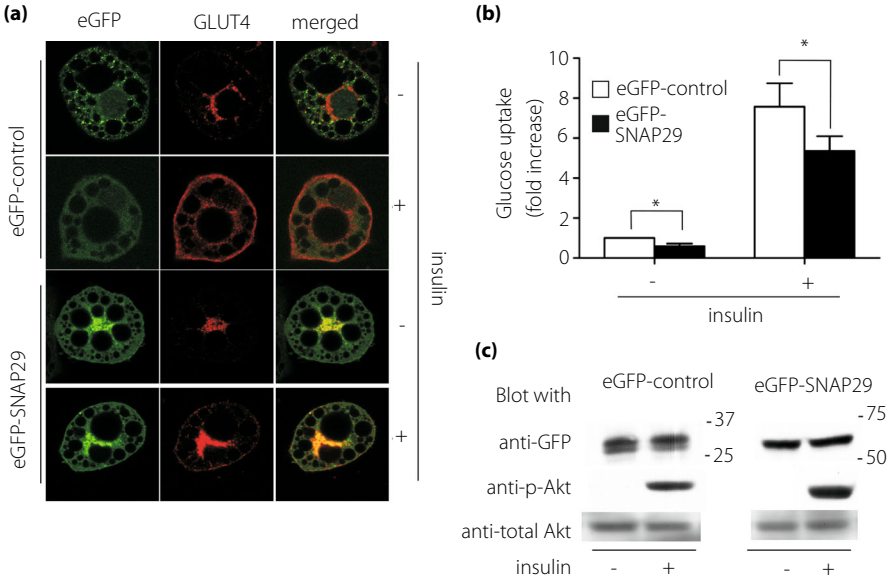

Figure 7 | Effects of synaptosomal-associated protein of 29 kDa (SNAP29) expression on intracellular localization of glucose transporter 4 (GLUT4) and insulin stimulated glucose-uptake in 3T3-L1 adipocytes. (a) 3T3-L1 adipocytes were infected with recombinant adenovirus encoding wild-type SNAP29 and green fluorescent protein (eGFP) vector alone (as a control) at an multiplicity of infection of 50. Two days thereafter, the cells were re-seeded onto glass plates. After being serum-starved for 3–4 h, the cells were treated with or without 100 nmol/L of insulin for 15 min. Then, the cells were fixed and immunostained with anti-GLUT4 antibodies. Intracellular localization of GLUT4 was then observed using confocal microscopy. (b) The cells were serum-starved for 2 h in Krebs–Ringer–HEPES buffer and treated with or without 100 nmol/L of insulin for 10 min. Glucose uptake measurements were carried out. Each bar represents the mean ± standard deviation of three independent experiments. (c) Expression levels of recombinant proteins and protein kinase B (Akt) phosphorylation were determined by western blotting using anti-eGFP, anti-phospho-Akt (Ser473) and anti-total-Akt antibodies.

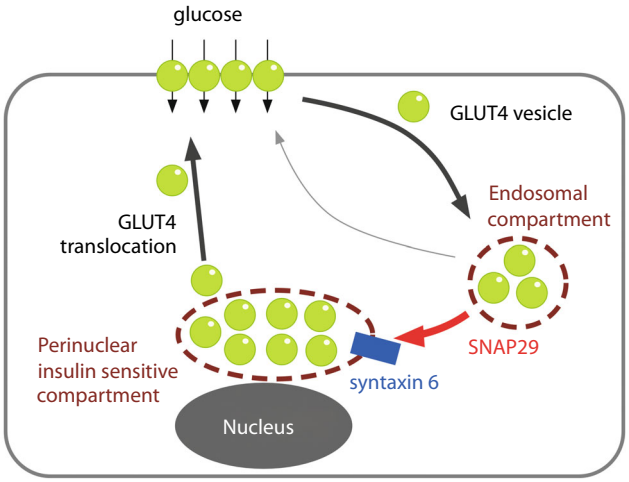

Figure 8 | Schematic model of synaptosomal-associated protein of 29 kDa (SNAP29)-mediated glucose transporter 4 (GLUT4) intracellular sequestration. SNAP29 traps endocytosed GLUT4 and tethers it within the insulin-responsive compartment (IRC). Another mechanism might be necessary to release GLUT4-vesicles from the IRC.

related SNARE proteins. As shown in Figures 2 and 5, SNAP29 can interact with EHD1 and syntaxin6 through their NPF motifs and SNARE domains, respectively. These binding partners have already been reported to facilitate endocytic recycling of GLUT4 vesicles[24,25]. These observations are consistent with our hypothesis that SNAP29 regulates the endocytic recycling of GLUT4 vesicles. Furthermore, immunofluorescent microscopy showed GLUT4 vesicles to be consolidated around the perinuclear region in SNAP29 overexpressing cells, whereas GLUT4 vesicles were dispersed in SNAP29-silenced cells. These findings are consistent with our aforementioned hypothesis that SNAP29 sequesters GLUT4 vesicles into the perinuclear compartment, and might have an essential role in maintaining the actions of insulin in adipocytes.

If this hypothesis is valid, glucose uptake might be increased in cells expressing SNAP29. However, as previously shown (Figure 7), insulin-stimulated glucose uptake was decreased in the cells expressing SNAP29, but this had no effect on insulin signaling. Interestingly, basal glucose uptake was decreased by approximately 30% in cells expressing SNAP29 (Figure 7b). These observations support the idea that SNAP29 anchors and sequesters GLUT4 vesicles in the perinuclear region. These data are also consistent with the microscopic observation that GLUT4 vesicles accumulated in the perinuclear region in cells expressing SNAP29. These results suggested the complexity of IRC regulation. Similar observations were reported previously. In brief, overexpression of syntaxin6 enhanced GLUT4 vesicle endocytosis, but affected neither GLUT4 translocation to the plasma membrane nor glucose uptake[24]. One explanation of

these findings is that SNAP29 only traps endocytosed GLUT4 and tethers it within the IRC, and that another mechanism is necessary to release GLUT4-vesicles from the IRC. A mechanism by which SNAP29 might trap GLUT4 vesicles in the IRC is schematically illustrated in Figure 8.

The data and considerations described here also raise the possibility of the GLUT4-perinuclear compartment being a major target of insulin action, and that this compartment might constitute a departure station for GLUT4 translocation to the plasma membrane. Many investigators have suggested the existence of two distinct GLUT4-recycling pathways[37]. One is a short pathway between the endosomal recycling compartment and the plasma membrane. The other is a pathway between the perinuclear compartment and the plasma membrane. As shown in Figure 6, GLUT4 vesicles were dispersed around the cytosol, and insulin-stimulated glucose uptake was disturbed in the SNAP29-silenced cells. These results suggest that the SNAP29-sensitive endosomal compartment is a major insulin sensitive compartment in adipocytes. The present data strongly support the importance of IRC formation in the insulin-dependent rapid-translocation of GLUT4 vesicles. Although there is still room for discussion on the detailed intracellular localization of IRC, our results and proposal for SNAP29-dependent GLUT4 dynamics are consistent with the recycling models proposed by other groups, such as McGraw, James and their colleagues[38,39].

In conclusion, we identified SNAP29, functioning as a SNARE protein against GLUT4 vesicles in the perinuclear region, and this SNARE protein sequesters and anchors GLUT4-vesicles within the insulin-sensitive compartment. We intend to design future experiments allowing the identification of a molecule causing GLUT4-containing vesicles to bud and release their vesicles from the IRC.

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DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: N/A.
Informed consent: N/A.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.

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