Biochemical and Biophysical Research Communications 422 (2012) 615–620

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Surf4 modulates STIM1-dependent calcium entry

Yoko Fujii [a,b], Masayuki Shiota [c], Yasuyuki Ohkawa [d], Akemi Baba [e], Hideki Wanibuchi [f], Tatsuo Kinashi [b], Tomohiro Kurosaki [a,b,e,*], Yoshihiro Baba [a,e,*]

[a] Laboratory for Lymphocyte Differentiation, RIKEN Research Center for Allergy and Immunology, Yokohama, Kanagawa 230-0045, Japan
[b] Department of Molecular Genetics, Institute of Biomedical Science, Kansai Medical University, Moriguchi, Osaka 570-8506, Japan
[c] Department of Pharmacology, Osaka City University Medical School, Osaka 545-8585, Japan
[d] Faculty of Medicine Div. Epigenetics, Kyushu University, Fukuoka 812-8582, Japan
[e] Laboratory for Lymphocyte Differentiation, WPI Immunology Frontier Research Center, Osaka University, Suita, Osaka 565-0871, Japan
[f] Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan

ARTICLE INFO

Article history:
Received 6 May 2012
Available online 15 May 2012

Keywords:
Store-operated calcium entry
STIM1
Surf4

ABSTRACT

Store-operated Ca^{2+} entry (SOCE) is crucial for various physiological responses in immune cells. Although it is known that STIM1 relocates into discrete puncta juxtaposed to the plasma membrane to initiate SOCE, the machinery modulating the function of STIM1 remains unclear. We explored to find its modulators using affinity purification for STIM1-binding proteins and identified surfeit locus protein 4 (Surf4). Surf4 associated with STIM1 in the endoplasmic reticulum. Deletion of Surf4 in DT40 B cells resulted in marked increase of SOCE and facilitation of STIM1 clustering upon store-depletion. These findings suggest the modulatory function of Surf4 for STIM1-mediated SOCE.

1. Introduction

Store-operated Ca^{2+} entry (SOCE) through Ca^{2+} release-activated Ca^{2+} (CRAC) channel in immune cells is essential for gene regulation, cell proliferation, cytokine production and a variety of physiological responses [1–5]. Previous studies have identified STIM1, a Ca^{2+}-binding membrane protein localized in the endoplasmic reticulum (ER), as the ER Ca^{2+} sensor and critical CRAC activator [6,7]. STIM1 contains a Ca^{2+}-binding EF-hand motif and a sterile alpha-motif (SAM) domain in the luminal side of the ER, and beyond the single transmembrane (TM) segment, it has two coiled-coil (CC) domains and carboxy-terminal cytoplasmic region including multiple serine and proline residues (S/P rich) [8]. At resting state, STIM1 distributes widely in the ER while the reduction of Ca^{2+} concentration in the ER induces dissociation of Ca^{2+} from EF-hand motif in STIM1 and subsequent oligomerization of STIM1 through EF-SAM domain [6]. This oligomerization leads to the relocalization of STIM1 into puncta at the ER–plasma membrane (PM) junction region [5,9], which is absolutely required for the activation of SOCE [5,8]. Subsequent studies have identified Orai1 (also known as CRACM1) as a pore subunit of the CRAC channels [10,11]. The interaction of STIM1 with Orai1 is essential for gating of Orai1 to induce SOCE [5,8,12,13]. In addition to Orai1, STIM1 has been reported to interact with several proteins such as STIM2 [14], sarco-endoplasmic reticulum calcium ATPase 2 (SERCA2) [15], end binding protein (EB1) [16], CRAC regulator 2A (CRACR2A) [17], voltage-gated Ca^{2+} channel $Ca_V1.2$ [18,19], calnexin, exportin1, transportin1 [20], and ERp57 [21]. However, the molecular machinery modulating STIM1 activity remains poorly understood.

Here, we report that surfeit locus protein 4 (Surf4), the mammalian ortholog of the yeast cargo receptor Erv29p, is identified as a binding partner of STIM1, which modulates STIM1-mediated SOCE. Surf4 interacted with STIM1 in the lumina of the ER. Surf4-deficeint DT40 B cells showed a significant increase of SOCE in response to ER store deletion induced by the stimulation with B cell receptor (BCR) or thagsigargin (TG). Furthermore, in the absence of Surf4, clustering of STIM1 at the ER–PM junctions was markedly enhanced after BCR stimulation, suggesting that Surf4 regulates the clustering and/or relocation of STIM1 at the ER–PM junction area through their physical interaction. These observations provide the evidence for the modulatory function of Surf4 in STIM1-mediated SOCE.

2. Materials and methods

2.1. Cells, Abs, and reagents

Wild-type and various mutant DT40 B cells were maintained in RPMI 1640 medium (Wako) supplemented with 10% FCS, 1%

Abbreviations: ER, endoplasmic reticulum; SOCE, store-operated calcium entry; PM, plasma membrane; TG, thapsigargin; BCR, B cell receptor; Ab, antibody.

* Corresponding authors at: Laboratory for Lymphocyte Differentiation, WPI Immunology Frontier Research Center, Osaka University, Suita, Osaka 565-0871, Japan. Fax: +81 66 879 4460.

E-mail addresses: kurosaki@ifrec.osaka-u.ac.jp (T. Kurosaki), babay@ifrec.osaka-u.ac.jp (Y. Baba).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.05.037

chicken serum, 50 mM 2-mercaptoethanol, 4 mM L-glutamate, and antibiotics. HeLa and 293T cells were cultured in DMEM (Wako) supplemented with 10% FCS and antibiotics. Anti-Flag mAb (M2) and anti-V5 Ab were purchased from Sigma or Invitrogen, respectively.

2.2. Crosslinking and affinity protein purification

Wild-type or Flag-STIM1 expressing STIM1-deficient DT40 B cells [22] were crosslinked with 1% w/v paraformaldehyde (PFA) for 5 min at room temperature. To stop the cross-linking reaction, glycine was added to a final concentration of 15 mM for 5 min. For affinity purification of Flag-STIM1 complexes, cells were solubilized in 1% Nonidet P-40 and 0.05% SDS lysis buffer supplemented with protease and phosphatase inhibitors as described previously [22] and precleared lysates were incubated with anti-Flag mAb-conjugated agarose (Sigma). Immunoprecipitates were eluted by 3× Flag peptides (Sigma) and incubated in SDS sample buffer at 65 °C for 20 min. To reverse cross-linking, the eluted samples were heated at 95 °C for 20 min.

2.3. Multidimensional protein identification technology (MudPIT)

Proteome analysis was performed on a DiNa-AI nano LC System (KYA Technologies) coupled to a QSTAR Elite hybrid mass spectrometer (AB Sciex) through a NanoSpray ion source (AB Sciex). Precipitated Flag-STIM1- or Flag-tag binding protein preparations were digested with trypsin, and analyzed by QSTAR Elite LC–MS/MS described previously [23]. The obtained MS/MS spectra were mapped with Protein Pilot (AB Sciex) to amino acid sequence defined as refseq protein (*Gallus gallus*). The validated peptides with a statistically significant p value ($p < 0.01$) were further aligned to amino acid sequences defined as refseq protein (*G. gallus*: NCBI) for calculating the coverage [24]. Proteins with more than two peptides and not less than 5% of sequence coverage were considered real hit candidates.

2.4. Plasmids and transfection

cDNAs of V5-Surf4, mCherry-STIM1, and mutants of Flag-STIM1 were generated by PCR. mCherry was inserted immediately downstream of the predicted signal sequence of the STIM1 gene. Flag-STIM1 and GFP-STIM1 were described previously [22]. The mouse Surf4 cDNA was obtained from Open Biosystem. V5-Surf4 and Flag- or mCherry-STIM1 mutants were cloned into pcDNA3.1 or pApuro expression vector. The deletion mutant of the SAM domain (ΔSAM) and coiled-coil domain (ΔCC), and EF hand mutant (mEF; D76A/D78A/N80A/E87A) were previously described [22]. The deletion mutant of the Ser/Pro-rich C-terminal domain (ΔS/P: 391 to stop codon), cytoplasmic fragment (cyto: 235 to stop codon) and ER luminal region with TM (ER–TM; start codon to 234) were generated by PCR. Nucleotide sequences of these constructs were verified by sequencing. Stable transfection into DT40 B cells was performed by electroporation as described [22]. Transient transfection using HeLa or 293T cells was performed by FuGENE HD (Promega) according to manufacturer's protocol.

2.5. Generation of Surf4-deficient DT40 B cells

We searched chicken homolog of surf4 using the expressed sequence tag database and obtained the genomic clone by PCR using oligonucleotides, and DT40 genomic DNA was used as a template. The targeting vectors, pSurf4-Neo or pSurf4-HisD, were constructed by replacing the genomic fragment containing exon 3–6, which represents amino acids from a first transmembrane segment to stop codon, with neo or hisD cassette. Targeting vectors were sequentially transfected by electroporation, and homologous recombination was identified by PCR using following primers a; 5′-gaagctggctgccttgactctagtc-3′, b (neo); 5′-caagctcttcagcaatatcacg-3′, b (hisD); 5′-ccgaagccaaacgtcaggtcagccag-3′, c; 5′-atgaattccagaacactttgcagagc-3′. For RT-PCR, total RNA was purified with the TRIzol reagent (Invitrogen) and subjected to cDNA synthesis using SuperScript first-strand synthesis system (Invitrogen) according to the manufacturer's instructions. The following primer pairs were used: sense primer 5′-accgctgaggacttcgcggatcag-3′ and antisense primer 5′-ccaccaagataatcagggctgtgc-3′ (surf4); sense primer 5′-caacatccacaagcagatgg-3′ and antisense primer 5′-gcattgttcaccgctaacct-3′ (stim1).

2.6. Ca^{2+} measurement

Cytosolic Ca^{2+} concentrations were measured as described previously [22]. Briefly, cells were loaded with indo-1 acetoxylmethylester (Indo-1 AM) and Pluronic F-127 (Invitrogen) and stimulated with 10 μg/ml anti-chicken IgM mAb (clone M4) or 2 μM thapsigargin (Calbiochem). Changes in fluorescence intensity were monitored on a LSRII flow cytometer (BD Biosciences). Data were analyzed with FlowJo software (TreeStar).

2.7. Immunoprecipitation and Immunoblotting

For immunoprecipitation, cells were solubilized in 0.2% Nonidet P-40 lysis buffer supplemented with protease and phosphatase inhibitors as described previously [22] and precleared lysates were incubated with anti-Flag mAb-resin (Sigma). Immunoprecipitates or whole cell lysates were resolved on SDS/PAGE, transferred to a polyvinyldifluoride membrane (Millipore), and incubated with appropriate Abs and secondary horseradish peroxidase (GE Healthcare) or alkaline phosphatase-labeled (Santa Cruz Biotechnology) Abs.

2.8. Immunofluorescence and TIRF microscopy

GFP images in living DT40 B cells before and after stimulation with anti-IgM were collected using the total internal reflection fluorescence (TIRF) microscopy system (Nikon). To quantity changes in evanescent field fluorescence, the average intensities of several regions of interest (ROIs) were measured and the ratios (F/Fo) of average intensity at indicated times (F) and at resting (Fo) were calculated. The images of GFP-Surf4 and mCherry-STIM1 in HeLa cells were obtained by confocal fluorescence microscope (Olympus, FV10i-DOC).

2.9. Statistical analysis

Statistical analyses were performed with the two-tailed unpaired Student's t test.

3. Results and discussion

3.1. Identification of STIM1 binding partners

To identify novel regulators of SOCE, affinity purification for STIM1 was conducted by using DT40 B cells stably expressing Flag-STIM1. We used *in vivo* PFA cross-linking of cells to preserve STIM1 protein complexes and to validate possible STIM1-interacting proteins. Formaldehyde is a highly specific cross-linker that is reactive with primary amines to one another, is easily reversible, and is used to preserve protein–protein complexes [25]. To identify the binding proteins of Flag-STIM1, the STIM1 complexes in cross-linked cells were immunoprecipitated with anti-FLAG mAb and

eluted with the FLAG peptide. In cross-linked cells expressing Flag-STIM1, the apparent molecular size of Flag-STIM1 which binds with other proteins were increased as analyzed by immunoblotting while after reverse cross-linking, the appropriate molecular size of Flag-STIM1 (approximately 90 kDa) was observed (Fig. 1A). These bands were not detected in cross-linked wild-type DT40 B cells immunoprecipitated with anti-Flag mAb. Thus, these results strongly suggest that STIM1 stably associates with other proteins *in vivo*.

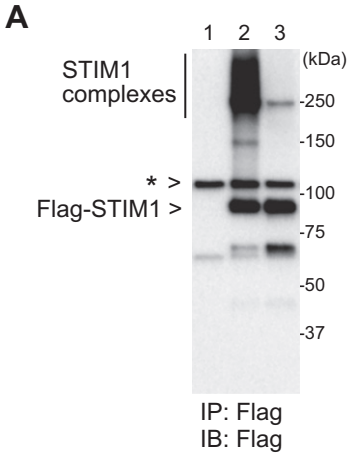
To identify proteins containing STIM1 complexes, multidimensional protein identification technology (MudPIT) analysis was performed on final elutes from wild-type or Flag-STIM1 expressing DT40 B cells. This analysis identified 37 high-confidence STIM1-associated proteins, but not in the control wild-type cells, with statistically significant ($p < 0.01$) values (Fig. 1B). Highly abundant proteins (e.g., ribosomal proteins, tubulins, histones and keratins) that were also directly associated with anti-Flag mAb in wild-type DT40 cells were considered as background and excluded. Protein identifications were sorted according to the amino acid sequence coverage of their detection, which represents the percentage of the protein covered by matching tryptic peptides. The previously reported STIM1-binding proteins such as STIM2 and SERCA2 were identified in this study (Fig. 1B). In addition to these proteins, Surf4, the mammalian ortholog of the yeast cargo receptor Erv29p [26], was identified as a binding partner of STIM1 by searching ER-resident proteins that have relatively high score. Surf4 is a multispanning membrane protein with its N and C terminus predicted to face the cytosol and has di-lysine ER retention signal in the C terminus. A published study has shown that Surf4 localized in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) and ER, suggesting that Surf4 is a cycling protein of the early secretory pathway [27]. Although Surf4 has a high sequence homology with yeast cargo receptor Erv29p [26], its function as a cargo receptor has not been reported. In addition, Surf4 together with ERGIC-53 may participate maintenance of the architecture of ERGIC and Golgi, however, silencing Surf4 alone by siRNA knockdown has no effect on them [28]. Thus, the physiological and molecular function of Surf4 remains elusive.

3.2. Surf4 associates with STIM1 in the ER

To further investigate the interaction between Surf4 and STIM1, immunoprecipitation was conducted in 293T cells transiently transfected with plasmids encoding Surf4 and STIM1. As shown in Fig. 2A, Surf4 was co-immunoprecipitated with STIM1 and its association was not affected when stimulated with TG, an ER Ca^{2+} pump inhibitor. However, STIM1 with EF-hand mutations, which fail to bind Ca^{2+}, showed decreased interaction with Surf4, indicating that Ca^{2+}-bound form of STIM1 effectively interacts with Surf4 rather than Ca^{2+}-unbound one. It is noted that TG stimulation did not appear to alter the binding of Surf4 and STIM1 even as this treatment can induce an intense reduction of the concentration of Ca^{2+} in the ER. A possible reason for the discrepancy is that all STIM1 EF-hand mutants exhibit the active structure whereas a part of STIM1 proteins may detach Ca^{2+} when cells are stimulated with TG. Another possibility is that Ca^{2+}-unbound STIM1 may relocate at the ER–PM junction regions where Surf4 may not exist.

To determine which domains of STIM1 is important for interaction with Surf4, several STIM1 mutants as indicated in Fig. 2B were tested. We found that the luminal side of STIM1, not cytosolic domains, associated with Surf4 (Fig. 2B). Because SAM domain was not required for the interaction (Fig. 2B), these data suggests that the intraluminal region in the ER except for SAM domain is involved in the interaction between STIM1 and Surf4.

To assess the spatial relationship between STIM1 and Surf4, the distribution of these proteins was investigated in HeLa cells transiently expressed with GFP-Surf4 and mCherry-STIM1. At steady state, a large portion of GFP-Surf4 colocalized with mCherry-STIM1 in the ER (Fig. 2C). Store depletion induced by histamine and TG resulted in redistribution of mCherry-STIM1 into puncta in the



B

Gene name	Descriptive name	Sequence coverage (%)
STIM1	STIM1	30.3
UBIQ	Ubiquitin	27.3
LOC769670	uncharacterized protein	24.3
ENPL	Endoplasmin	23.1
Q0KKP4	Lanosterol 14alpha-demethylase	22.7
HS3ST6	Heparan sulfate glucosamine 3-O-sulfotransferase 6	21.0
Q5ZIF2	DHCR24	19.6
ENSGALP00000015657	POTE ankyrin domain family, member J	17.6
PPIB	Peptidyl-prolyl cis-trans isomerase B	17.0
Q9DEH4	Nebulin	15.7
Q5ZM80	ribophorin II	15.5
PPIC	Peptidyl-prolyl cis-trans isomerase C	13.7
ENOA	Enolase	12.6
PGK	Phosphoglycerate kinase	12.2
SURF4	SURF4	10.8
STIM2	STIM2	10.4
Q98940	Cardiac muscle factor 1	9.7
SLC25A6	ADP/ATP translocase 3	8.4
SQLE	Squalene monooxygenase	8.3
LOC415922	guanylate-binding protein 4-like	7.9
AT2A2	SERCA2	7.8
RTN4RL1	Reticulon-4 receptor-like 1	7.7
Q76CF0	3-hydroxy-3-methylglutaryl-CoA reductase	7.4
SPON1	Spondin-1	6.9
OSBPL6	Oxysterol-binding protein-related protein 6	6.7
Q9I8D6	T-complex protein 1 subunit delta	6.7
NDE1	Nuclear distribution protein nudE homolog 1	6.7
FKBP8	FKBP8	6.6
IF4A2	Eukaryotic initiation factor 4A-II	6.4
NME3	Nucleoside diphosphate kinase 3	6.3
Q5ZJA5	Hypothetical protein	6.2
HYOU1	Hypoxia up-regulated protein 1	6.1
LRRC42	Leucine-rich repeat-containing protein 42	6.1
AT2A3	SERCA3	5.9
BTD	Biotinidase	5.4
OST48	DDOST 48 kDa subunit	5.1
TBC1D14	TBC1 domain family member 14	5.1

Fig. 1. Identification of Surf4 as a STIM1-binding protein. (A) Affinity purification of STIM1 protein complexes. Immunoprecipitation was conducted with anti-FLAG resin in cross-linked wild-type DT40 B cells (lane 1) or STIM1-deficient DT40 B cells expressing Flag-STIM1 (lane 2), eluted with the FLAG peptide and detected by immunoblotting by anti-Flag mAb. Elute sample from lane 2 was heated to 95 °C to reverse cross-linking (lane 3). Asterisk indicates a non-specific band. Molecular mass standards are indicated in kilodaltons (kDa). (B) The list of STIM1-associated proteins identified by MudPIT analysis. Proteins identified only in DT40 B cells expressing Flag-STIM1, but not in the control wild-type cells, with the validated peptides ($p < 0.01$), more than two peptides and relatively high sequence coverage (>5%) are presented. Sequence coverage (in%) represents the percentage of the protein covered by the matching tryptic peptides.

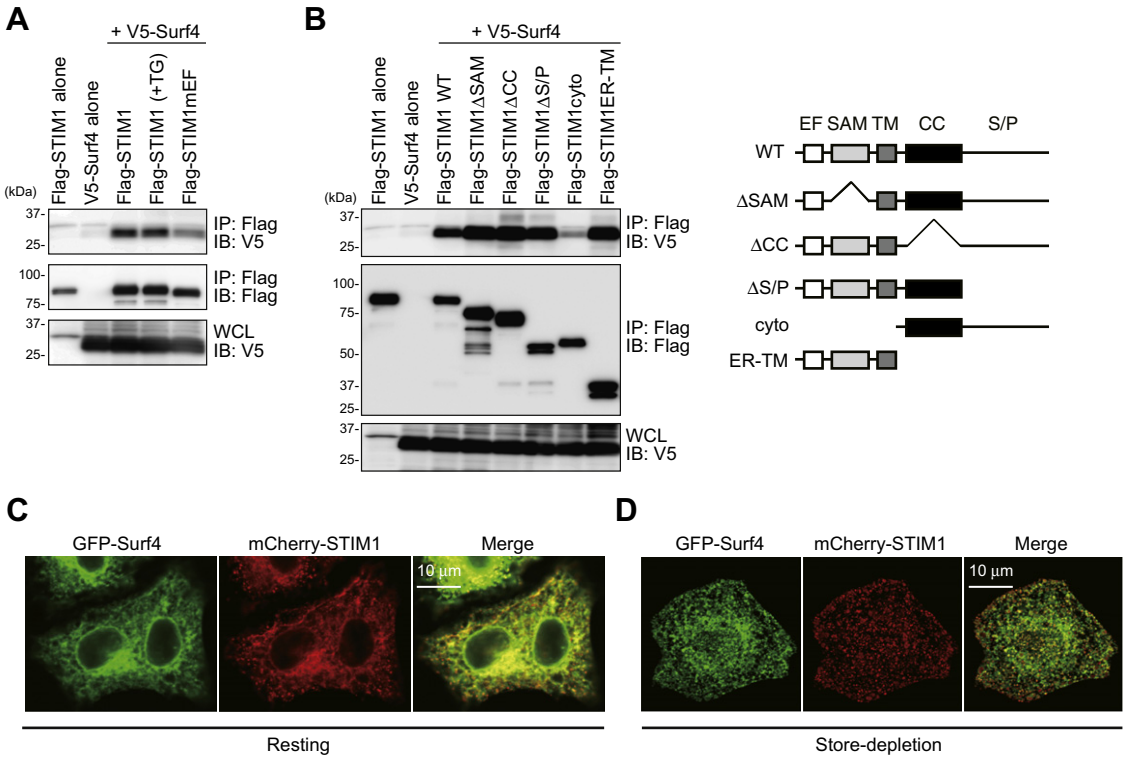


Fig. 2. Association and subcellular distribution of STIM1 and Surf4. (A) Co-immunoprecipitation of V5-Surf4 and Flag-STIM1 or STIM1 EF-hand mutant (mEF) in 293T cells without or with 2 μM TG treatment (+TG). WCL; whole cell lysate (B) schematic representation of STIM1 mutants and the functional domains, including EF-hand motif (EF), a single transmembrane (TM), SAM, coiled-coil (CC), and Ser/Pro-rich C-terminal (S/P) domains. cyto; cytoplasmic region, ER–TM; ER luminal region and TM (right). Co-immunoprecipitation of V5-Surf4 and Flag-STIM1 mutants in 293T cells (left). (C and D) Confocal microscopy analysis of HeLa cells expressing GFP-Surf4 and mCherry-STIM1 in the absence of stimuli (resting) (C) or upon store-depletion by stimulation with 100 μM histamine and 2 μM TG for 15 min (D).

immediate vicinity of the ER near the PM (Fig. 2D). In contrast, a part of GFP-Surf4 still showed colocalization with STIM1, however, GFP-Surf4 poorly formed clusters, suggesting that Surf4 is distributed differently from aggregated STIM1 at the ER–PM junctions. At rest, STIM1 has been shown to accumulate in comet-like shape at polymerizing microtubule ends where associate with the ER [16,22]. In accordance with these studies, mCherry-STIM1 showed comet-like accumulations, but GFP-Surf4 did not (data not shown). These results suggest that Surf4 binds with STIM1 that distributes widely in the ER, not with oligomerized STIM1.

3.3. Surf4 functions as a modulator for SOCE

To examine the molecular function of Surf4, we established Surf4-deficient DT40 B cells by a gene-targeting method (Fig. 3A). Deletion of Surf4 was verified by genomic PCR and RT-PCR (Fig. 3B). To determine the effects of Surf4 on SOCE, we monitored cytosolic Ca^{2+} in wild-type and Surf4-deficient DT40 B cells after Ca^{2+} depletion from ER stores in response to anti-IgM (BCR stimulation) or TG. Surf4-deficient DT40 B cells showed a significant increase of SOCE after BCR or TG stimulation compared with control wild-type DT40 B cells (Fig. 3C and D). Moreover, we transduced Surf4 in Surf4-deficient DT40 B cells to confirm the specificity of the effect of Surf4 on SOCE. An introduction of Surf4 into Surf4-deficient DT40 B cells resulted in a reduction of SOCE to a similar extent to that seen in wild-type cells (Fig. 3C and D). Because internal Ca^{2+} elevation was comparable between wild-type and Surf4-deficient DT40 B cells (Fig. 3D), the absence of Surf4 has no effect on Ca^{2+} pool size in the ER and the release of Ca^{2+} from stores after stimulation. These results collectively indicate that Surf4 negatively modulate STIM1-mediated SOCE.

3.4. Deletion of Surf4 facilitates STIM1 puncta formation

SOCE activation is dependent on oligomerization of STIM1 and subsequent formation of discrete clusters in close proximity to the PM. To assess whether enhanced SOCE in the absence of Surf4 was due to the alternation of STIM1 puncta formation, we established Surf4-deficient DT40 B cells expressing GFP-STIM1 and examined the BCR-induced clustering of GFP-STIM1. The similar expression level of transfected proteins compared with control cells was confirmed by flow cytometry (Fig. 4A). As previously shown [22], GFP-STIM1 accumulated near the PM in DT40 B cells following BCR stimulation (Fig. 4B and C). In DT40 B cells lacking Surf4, the BCR-mediated clustering of GFP-STIM1 was much intensely accelerated (Fig. 4B and C), suggesting that Surf4 modulate STIM1 puncta formation and/or relocation to the ER–PM junctions.

Here, we have identified Surf4 as a STIM1 binding partner in the ER. In the absence of Surf4, STIM1 clustering induced by store-depletion is dramatically accelerated and consequently, SOCE is significantly enhanced. Therefore, our findings suggest that the interaction of Surf4 with STIM1 attenuates STIM1 clustering and SOCE.

Surf4 is the mammalian ortholog of yeast cargo receptor Erv29p [26], packaging a soluble cargo protein such as glycosylated α-factor pheromone precursor into COPII vesicles budding from the ER [29,30]. Given the extent of sequence similarity with Erv29p, Surf4 may function as a mammalian cargo receptor for the transport of soluble secretory proteins. Although STIM1 is not soluble protein, future investigation is needed to elucidate whether Surf4 may determine the distribution of STIM1, leading to affect STIM1 function for SOCE.

Although the topology of Surf4 has not been proved, Surf4 may contain four transmembrane spanning segments based on the

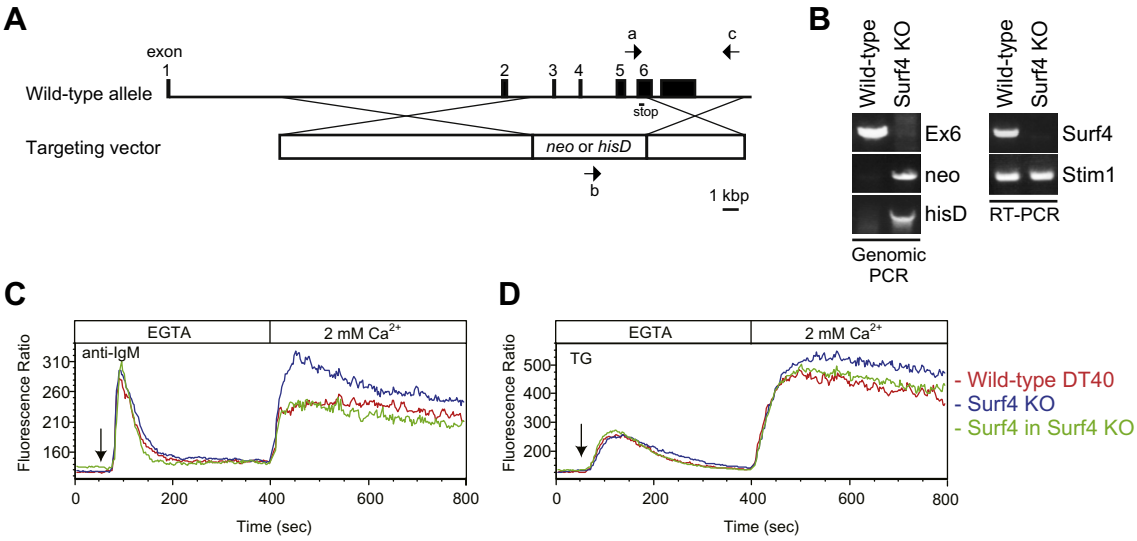


Fig. 3. Generation and SOCE analyses of Surf4-deficeint DT40 B cells. (A) Schematic representation of the targeting strategy. Exons 3–6 of chicken surf4 were replaced with drug resistant cassettes (neo and hisD). (B) Analysis of genomic PCR and RT-PCR. Surf4 genotype was confirmed by genomic PCR using primer pairs a and c (Ex6) for wild-type allele or pairs b and c (neo or hisD) for targeted alleles as indicated in A (left). RT-PCR of mRNA encoding surf4 and stim1 in wild-type and Surf4-deficient (Surf4 KO) DT40 B cells (right). (C) Ca^{2+}-mobilization profiles, monitored by Indo-1 AM imaging. Ca^{2+} release was elicited in wild-type, Surf4 KO and Surf4 expressing Surf4 KO (Surf4 in Surf4 KO) DT40 B cells. Cells by stimulation with anti-IgM or TG in Ca^{2+}-free conditions (0.5 mM EGTA), and Ca^{2+} influx was induced by restoration of the extracellular Ca^{2+} concentration to 2 mM. All values are plotted as the FL5/FL4 fluorescence ratio (FL4 = 500–520 nm; FL5 = 400–420 nm). Data are representative of at least three independent experiments.

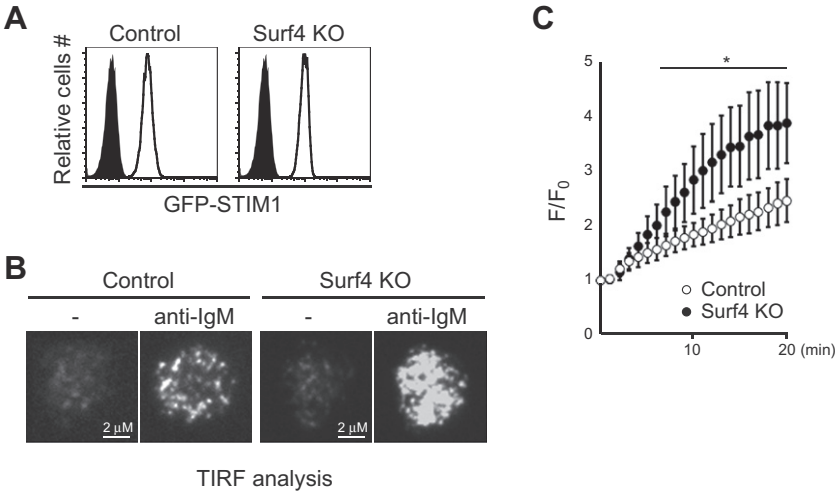


Fig. 4. STIM1 puncta formation in the absence of Surf4. (A) The expression of GFP-STIM1 in STIM1-deficient (control) and Surf4 KO DT40 B cells was detected by flow cytometry. Filled and open histograms represent non-transfected and GFP-STIM1-transfected cells, respectively. (B) The relocation of GFP-STIM1 was monitored before (-) and after BCR stimulation (anti-IgM, 20 min) in the absence of extracellular Ca^{2+}. Representative stills from the time-lapse TIRF images are shown. (C) Kinetic analysis of the average region of interest intensities of GFP-STIM1 in control (open circle; $n = 27$) and Surf4 KO DT40 B cells (closed circle; $n = 21$). Cells were stimulated with anti-IgM mAb after 1 min in the absence of extracellular Ca^{2+}. F/Fo, the ratios of average intensities of regions of interest at indicated times after stimulation (F) and at steady state (Fo). Regions of interest are for whole cells. Error bars represent standard deviations of mean. $^{*}p < 0.001$ versus control.

sequence similarity of Erv29p [31]. Therefore, Surf4 can interact with the ER luminal side of STIM1 probably through the luminal loop of Surf4. The induction of SOCE is required for STIM1 oligomerization and ultimate translocation at the ER–PM junctions. Whether the binding of Surf4 with STIM1 interfere the oligomerization, puncta formation or relocation of STIM1 awaits further studies.

In summary, we have identified a novel STIM1-binding protein, Surf4 that plays the modulatory role for STIM1-mediated SOCE.

Acknowledgments

We thank R. Tsien (University of California at San Diego) for providing the pRSETB-mCherry plasmid. We are thankful to M. Matsumoto for critical reading of the manuscript. This work was supported by Grant-in-Aids for the Ministry of Education, Culture, Sports, Science and Technology, Japan (Y.B. and T.K.) and for Japan Science and Technology Agency and Core Research for Evolutional Science and Technology (T.K.) and "HISHO" The Top Thirty Young Researchers of Osaka University (Y.B.).

References

[1] Y. Baba, K. Nishida, Y. Fujii, T. Hirano, M. Hikida, T. Kurosaki, Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses, Nat. Immunol. 9 (2008) 81–88.

[2] M. Matsumoto, Y. Fujii, A. Baba, M. Hikida, T. Kurosaki, Y. Baba, The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production, Immunity 34 (2011) 703–714.

[3] M. Oh-Hora, M. Yamashita, P.G. Hogan, S. Sharma, E. Lamperti, W. Chung, M. Prakriya, S. Feske, A. Rao, Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance, Nat. Immunol. 9 (2008) 432–443.
[4] D. Varga-Szabo, A. Braun, C. Kleinschnitz, M. Bender, I. Pleines, M. Pham, T. Renne, G. Stoll, B. Nieswandt, The calcium sensor STIM1 is an essential mediator of arterial thrombosis and ischemic brain infarction, J. Exp. Med. 205 (2008) 1583–1591.
[5] P.G. Hogan, R.S. Lewis, A. Rao, Molecular basis of calcium signaling in lymphocytes: STIM and ORAI, Annu. Rev. Immunol. 28 (2010) 491–533.
[6] J. Liou, M.L. Kim, W.D. Heo, J.T. Jones, J.W. Myers, J.E. Ferrell Jr., T. Meyer, STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx, Curr. Biol. 15 (2005) 1235–1241.
[7] J. Roos, P.J. DiGregorio, A.V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J.A. Kozak, S.L. Wagner, M.D. Cahalan, G. Velicelebi, K.A. Stauderman, STIM1, an essential and conserved component of store-operated Ca^{2+} channel function, J. Cell Biol. 169 (2005) 435–445.
[8] Y. Baba, T. Kurosaki, Physiological function and molecular basis of STIM1-mediated calcium entry in immune cells, Immunol. Rev. 231 (2009) 174–188.
[9] J. Liou, M. Fivaz, T. Inoue, T. Meyer, Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca^{2+} store depletion, Proc. Natl. Acad. Sci. USA 104 (2007) 9301–9306.
[10] S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S.H. Puppel, B. Tanasa, P.G. Hogan, R.S. Lewis, M. Daly, A. Rao, A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function, Nature 441 (2006) 179–185.
[11] M. Vig, C. Peinelt, A. Beck, D.L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner, J.P. Kinet, CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry, Science 312 (2006) 1220–1223.
[12] J.C. Mercer, W.I. Dehaven, J.T. Smyth, B. Wedel, R.R. Boyles, G.S. Bird, J.W. Putney Jr., Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1, J. Biol. Chem. 281 (2006) 24979–24990.
[13] C. Peinelt, M. Vig, D.L. Koomoa, A. Beck, M.J. Nadler, M. Koblan-Huberson, A. Lis, A. Fleig, R. Penner, J.P. Kinet, Amplification of CRAC current by STIM1 and CRACM1 (Orai1), Nat. Cell Biol. 8 (2006) 771–773.
[14] J. Soboloff, M.A. Spassova, T. Hewavitharana, L.P. He, W. Xu, L.S. Johnstone, M.A. Dziadek, D.L. Gill, STIM2 is an inhibitor of STIM1-mediated store-operated Ca^{2+} entry, Curr. Biol. 16 (2006) 1465–1470.
[15] A. Sampieri, A. Zepeda, A. Asanov, L. Vaca, Visualizing the store-operated channel complex assembly in real time: identification of SERCA2 as a new member, Cell Calcium 45 (2009) 439–446.
[16] I. Grigoriev, S.M. Gouveia, B. Van der Vaart, J. Demmers, J.T. Smyth, S. Honnappa, D. Splinter, M.O. Steinmetz, J.W. Putney, C.C. Hoogenraad, A. Akhmanova, STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER, Curr. Biol. 18 (2008) 177–182.
[17] S. Srikanth, H.J. Jung, K.D. Kim, P. Souda, J. Whitelegge, Y. Gwack, A novel EF-hand protein, CRACR2A, is a cytosolic Ca^{2+} sensor that stabilizes CRAC channels in T cells, Nat. Cell Biol. 12 (2010) 436–446.
[18] C.Y. Park, A. Shcheglovitov, R. Dolmetsch, The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels, Science 330 (2010) 101–105.
[19] Y. Wang, X. Deng, S. Mancarella, E. Hendron, S. Eguchi, J. Soboloff, X.D. Tang, D.L. Gill, The calcium store sensor, STIM1, reciprocally controls Orai and CaV1.2 channels, Science 330 (2010) 105–109.
[20] N. Saitoh, K. Oritani, K. Saito, T. Yokota, M. Ichii, T. Sudo, N. Fujita, K. Nakajima, M. Okada, Y. Kanakura, Identification of functional domains and novel binding partners of STIM proteins, J. Cell Biochem. 112 (2011) 147–156.
[21] D. Prins, J. Groenendyk, N. Touret, M. Michalak, Modulation of STIM1 and capacitative Ca^{2+} entry by the endoplasmic reticulum luminal oxidoreductase ERp57, EMBO Rep. 12 (2011) 1182–1188.
[22] Y. Baba, K. Hayashi, Y. Fujii, A. Mizushima, H. Watarai, M. Wakamori, T. Numaga, Y. Mori, M. Iino, M. Hikida, T. Kurosaki, Coupling of STIM1 to store-operated Ca^{2+} entry through its constitutive and inducible movement in the endoplasmic reticulum, Proc. Natl. Acad. Sci. USA 103 (2006) 16704–16709.
[23] A. Kakehashi, N. Ishii, T. Shibata, M. Wei, E. Okazaki, T. Tachibana, S. Fukushima, H. Wanibuchi, Mitochondrial prohibitins and septin 9 are implicated in the onset of rat hepatocarcinogenesis, Toxicol. Sci. 119 (2011) 61–72.
[24] S. Sato, C. Tomomori-Sato, T.J. Parmely, L. Florens, B. Zybailov, S.K. Swanson, C.A. Banks, J. Jin, Y. Cai, M.P. Washburn, J.W. Conaway, R.C. Conaway, A set of consensus mammalian mediator subunits identified by multidimensional protein identification technology, Mol. Cell 14 (2004) 685–691.
[25] V. Orlando, H. Strutt, R. Paro, Analysis of chromatin structure by in vivo formaldehyde cross-linking, Methods 11 (1997) 205–214.
[26] J.E. Reeves, M. Fried, The surf-4 gene encodes a novel 30 kDa integral membrane protein, Mol. Membr. Biol. 12 (1995) 201–208.
[27] J. Dancourt, C. Barlowe, Protein sorting receptors in the early secretory pathway, Annu. Rev. Biochem. 79 (2010) 777–802.
[28] S. Mitrovic, H. Ben-Tekaya, E. Koegler, J. Gruenberg, H.P. Hauri, The cargo receptors Surf4, endoplasmic reticulum–Golgi intermediate compartment (ERGIC)-53, and p25 are required to maintain the architecture of ERGIC and Golgi, Mol. Biol. Cell 19 (2008) 1976–1990.
[29] W.J. Belden, C. Barlowe, Role of Erv29p in collecting soluble secretory proteins into ER-derived transport vesicles, Science 294 (2001) 1528–1531.
[30] S. Otte, C. Barlowe, Sorting signals can direct receptor-mediated export of soluble proteins into COPII vesicles, Nat. Cell Biol. 6 (2004) 1189–1194.
[31] D.A. Foley, H.J. Sharpe, S. Otte, Membrane topology of the endoplasmic reticulum to Golgi transport factor Erv29p, Mol. Membr. Biol. 24 (2007) 259–268.