Tissue and Cell 49 (2017) 141–149

Contents lists available at ScienceDirect

Tissue and Cell

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

SPCA2 couples Ca^{2+} influx via Orai1 to Ca^{2+} uptake into the Golgi/secretory pathway

Susanne Smaardijk, Jialin Chen, Frank Wuytack, Peter Vangheluwe*

Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium

ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form 29 July 2016
Accepted 2 September 2016
Available online 17 September 2016

Keywords:
Orai1
Store independent Ca^{2+} entry
Store dependent Ca^{2+} entry
Ca^{2+} Transport
Lactation
Breast cancer
Golgi
Secretory pathway

ABSTRACT

Dysregulation of the Golgi/Secretory Pathway Ca^{2+} transport ATPase SPCA2 is implicated in breast cancer. During lactation and in luminal breast cancer types, SPCA2 interacts with the plasma membrane Ca^{2+} channel Orai1, promoting constitutive Ca^{2+} influx, which is termed store independent Ca^{2+} entry (SICE). The mechanism of SPCA2/Orai1 interaction depends on the N- and C-termini of SPCA2. These extensions may play a dual role in activating not only Orai1, but also Ca^{2+} transport into the Golgi/secretory pathway, which we tested by investigating the impact of various SPCA2 N- and/or C-terminal truncations on SICE and Ca^{2+} transport activity of SPCA2. C-terminal truncations impair SICE and SPCA2 activity, but also affect targeting, whereas N-terminal truncations affect targeting and inactivate SPCA2, but remarkably, SICE activation remains unaffected. Importantly, overexpression of SPCA2 increases the Ca^{2+} content of non-ER stores, which depends on Orai1 and SPCA2 activity. Thus, Orai1-mediated Ca^{2+}-influx and SPCA2-mediated Ca^{2+} uptake activity into the Golgi/secretory pathway might be coupled possibly in a microdomain. This channel/pump complex may efficiently transfer Ca^{2+} into the secretory pathway, which might play a role in SPCA2-expressing secretory cells, such as mammary gland during lactation.

1. Introduction

Ca^{2+} is an important second messenger that is implicated in a variety of cellular processes, including secretion, muscle contraction, cell division and apoptosis (Berridge et al., 2003). The Golgi Apparatus participates in shaping the spatiotemporal regulation of Ca^{2+} storage and cytosolic Ca^{2+} signaling in the cell (Pizzo et al., 2011; Rizzuto and Pozzan, 2006; Vanoevelen et al., 2005b). The concentration of Ca^{2+} in the Golgi Apparatus is heterogeneous, ranging between 250 μM in the *cis*-Golgi to 130 μM in the *trans*-Golgi (Pizzo et al., 2010). The high luminal Ca^{2+} concentration promotes protein folding and is needed for enzymatic activities such as glycosylation (Durr et al., 1998; Vangheluwe et al., 2009). The Golgi Apparatus also contributes to Ca^{2+} signaling by releasing Ca^{2+} via inositol-1,4,5-triphosphate receptors or ryanodine receptors in the Golgi (Wong et al., 2013). Finally, local Ca^{2+} uptake in the *trans*-Golgi network organizes luminal protein sorting possibly leading to recruitment of Ca^{2+} binding proteins into budding vesicles for transport (Kienzle et al., 2014; von Blume et al., 2011).

Two Ca^{2+} pumps control Ca^{2+} uptake into the Golgi: the sarco/endoplasmic reticulum Ca^{2+} transport ATPase SERCA2b, which is mainly found in the endoplasmic reticulum (ER), but also in the *cis*-Golgi, and the more abundant secretory pathway Ca^{2+}/Mn^{2+} transport ATPase SPCA, which is detected throughout the Golgi Apparatus and in secretory vesicles. These closely related Ca^{2+} transport ATPases undergo a reaction cycle that involves two major conformational states: E1 with high Ca^{2+} affinity binding sites facing the cytosol, and E2 with low Ca^{2+} affinity binding sites facing the lumen. During transport, the pumps undergo transient autophosphorylation at a conserved aspartate residue, which controls access to the Ca^{2+} binding sites (Moller et al., 2005).

Two SPCA isoforms exist in humans: the ubiquitous Ca^{2+} pump SPCA1, and SPCA2, which displays a more restricted expression pattern limited mainly to secretory cells in mammary epithelia, the gastrointestinal tract and testes (Vanoevelen et al., 2005a; Xiang et al., 2005). SPCA1 primarily fulfills a housekeeping function to provide the Golgi with Ca^{2+} and Mn^{2+}, although SPCA1 is also involved in Mn^{2+} detoxification in the liver (Leitch et al., 2011). Heterozygous mutations in human SPCA1 give rise to Hailey–Hailey disease, an autosomal dominant skin disease that is characterized by acantholysis and dyskeratosis (Sudbrak et al., 2000), whereas mouse *Atp2c1* null mutants are embryonically lethal by failure of neural tube closure (Okunade et al., 2007). The predominant expression of SPCA2 in secretory cell types suggest a role of SPCA2 in secretion (Vanoevelen et al., 2005a; Xiang et al., 2005).

* Corresponding author at: Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, KU Leuven Campus Gasthuisberg, Herestraat 49, Box 802, B3000 Leuven, Belgium.
E-mail address: peter.vangheluwe@kuleuven.be (P. Vangheluwe).

http://dx.doi.org/10.1016/j.tice.2016.09.004
0040-8166/

In lactating mammary gland, SPCA1 and SPCA2 are highly upregulated and promote Ca^{2+} into the Golgi/secretory pathway supporting *trans*-epithelial Ca^{2+} transport, which accounts for 40% of the Ca^{2+} release into the milk (Faddy et al., 2008; Reinhardt et al., 2000; Reinhardt et al., 2004). Of interest, SPCA2, but not SPCA1, directly interacts with and activates the Ca^{2+} channel Orai1, the plasma membrane (PM) constituent of Ca^{2+}-release activated Ca^{2+} (CRAC) channels, which in mammary gland may potentiate cellular Ca^{2+} uptake for release into the milk (Feng et al., 2010). Within the PM, the N-terminus of SPCA2 first binds to Orai1, thereby enabling the much shorter C-terminus to come close enough to interact and subsequently activate the CRAC channel. This process is termed Store-Independent Ca^{2+} Entry (SICE), since it occurs independent of ER Ca^{2+} depletion and relocalization of STIM1 (Feng et al., 2010). This is different from Store-Operated Ca^{2+} entry (SOCE), the 'classical' mechanism of activation of CRAC channels by STIM1 in conditions of reduced ER Ca^{2+} levels (Park et al., 2009; Zhang et al., 2005).

Orai1, SPCA1 and SPCA2 are implicated in breast cancer. Orai1 expression is elevated in basal-type tumor samples and in mammary tumor cell lines, including MCF-7 and MDA-MB-231 (McAndrew et al., 2011). In basal-like breast cancer cell lines, including MDA-MB-231 cells, SPCA1 is elevated (Dang and Rao, 2016; Feng et al., 2010; Grice et al., 2010) and inhibition of SPCA1 may be considered as a therapeutic strategy, since in MDA-MB-231 cells this impairs the processing of the insulin-like growth factor receptor, which is involved in tumor progression (Grice et al., 2010). SPCA2 expression is upregulated in luminal-type breast cancer types (Faddy et al., 2008). In MCF-7 cells, SPCA2 physically interacts with Orai1 leading to a constitutive Ca^{2+} influx that stimulates proliferation (Feng et al., 2010). Indeed, knockdown of either SPCA2 or Orai1 in MCF-7 cells attenuates cell proliferation, colony formation in soft agar and tumor formation in nude mice, whereas SPCA2 overexpression in non-tumorigenic MCF-10A cells promotes colony formation in soft agar (Feng et al., 2010). Thus, the SPCA2/Orai1 dysregulation leads to a pathological Ca^{2+} influx that contributes to the proliferative nature and tumorigenic potential of these breast cancer cell types.

In this paper, we confirm and further extend insights into the interaction between SPCA2 and Orai1 by the N- and C-termini of SPCA2. Since isoform specific N- and C-terminal extensions of Ca^{2+} transport ATPases often exert signaling/scaffolding functions, which are coupled to the regulation of Ca^{2+} transport (Falchetto et al., 1992; Gorski et al., 2012), we hypothesized that N- and C-terminal stretches of SPCA2 might exert a dual function stimulating Ca^{2+} influx via Orai1, while promoting Ca^{2+} uptake into the Golgi/secretory pathway. To explore this, we generated a series of N- and C-terminally truncated constructs of SPCA2, which were tested for their subcellular localization, auto-phosphorylation activity, intracellular Ca^{2+} uptake and stimulation of SICE.

2. Materials and methods

2.1. DNA, cell culture and transfection

Human SPCA1a and SPCA2 cDNA was inserted into pcDNA6 destination vectors (Gateway®) with or without an N-terminal mCherry- or GFP-tag. N-terminal mCherry-SERCA2b and mCherry-STIM1 constructs were used (Baron et al., 2010). Mutants were generated using the Quickchange® site-directed mutagenesis kit (Stratagene). HEK293T cells were cultured at 37 °C and 10% CO_2. HeLaT1 and COS cells were cultured at 37 °C and 5% CO_2. Cells were kept in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 mg/ml streptomycin, 2 mM Glutamax (Thermo Fisher Scientific) and non-essential amino acids (Thermo Fisher Scientific, only for HEK293T and COS cells). GeneJuice transfection reagent (Thermo Fisher Scientific) was used for transfection according to the manufacturer's instructions. Cotransfection of plasmid DNA and siRNA oligos (OnTarget Plus SMART pool, Dharmacon) was achieved with Dharmafect Duo (GE Life Science). The siRNA used was a mixture of the following target sequences: non-targeting siRNA: UGGUUUACAUGUCGACUAA, UGGUUUACAUGUUGUGUGA, UGGUUUACAUGUUUUCUGA, UGGUUUACAUGUUUUCCUA; Orai1 siRNA: UCAACGAGCACUCCAUGCA, GGAGUUUGCCCGCUUACAG, GCACCUGUUUGCGCUCAUG, GGCCUGAUCUUUAUCGUCU.

2.2. Fluorescence imaging and NFAT translocation

HEK293T cells or HeLaT1 cells were seeded at 15,000 cells/well in chamber slides (NUNC) and transfected with 300 ng DNA. 72 h after transfection, cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) prior to fluorescence microscopy. For NFAT-GFP translocation, cells were co-transfected with SPCA1a or SPCA2 and NFAT-GFP in a 5:1 ratio. We verified via immunostaining with an SPCA antibody that a similar percentage of cells was transfected with the different SPCA constructs. Per condition, a minimum of 100 cells was compared to determine the percentage of cells with nuclear NFAT translocation. For TGN staining, cells were fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, incubated for 1 h with anti-TGN46 (Thermo Scientific, 1:500) and 1 h with Alexa Fluo 488 anti-rabbit (Fischer Scientific, 1:2000). Visualization was performed on an Olympus IX81 fluorescence microscope with excitation/emission wavelengths 469/510 nm (GFP) or 562/610 nm (mCherry).

2.3. Fura2-AM intracellular Ca^{2+} measurements

For Ca^{2+} imaging, HEK293T cells were transfected with mCherry-tagged SPCA constructs. The procedure was carried out as described in Vervliet et al. (2014). 72 h after transfection, cells were incubated for 30 min in a modified KREBS solution (11.5 mM glucose, 1.5 mM $CaCl_2$, 135 mM NaCl, 6.2 mM KCl, 1.2 mM $MgCl_2$, 12 mM HEPES, pH 7.3) supplemented with 1 μM Fura2-AM (Life Sciences), and subsequently washed for 30 min in the same modified KREBS solution. mCherry-positive cells with comparable fluorescence intensity were selected and visualized at room temperature at excitation wavelengths of 340 and 380 nm and emission wavelength 510 nm on a Zeiss Axio Observer Z1 Inverted Microscope. Extracellular Ca^{2+} was depleted by adding modified KREBS supplemented with 3 mM BAPTA (Alfa Aesar) to the cells. Ca^{2+} release from ER and non-ER stores was assessed by adding respectively 500 nM thapsigargin (Alomone labs) or 2 μM ionomycin (Enzo Life Sciences) in modified KREBS supplemented with 3 mM BAPTA. CRAC channels were inhibited by 10 μM 3,5-bistrifluoromethyl pyrazole (YM-58483/BTP2, Abcam), which was added in modified KREBS (Zitt et al., 2004).

2.4. Total membrane preparation and immunoblotting

4.1 million HEK293T cells were seeded per 15 cm petri dish one day prior to transfection. For MG-132 treatment, cells were incubated for 6 h with 10 μM MG-132 (Sigma-Aldrich) prior to homogenization. Membrane preparation was carried out as described in Sepúlveda et al. (2008). 72 h after transfection, HEK293T cells were homogenized in homogenization buffer (10 mM HEPES pH 7.4, 0.32 M sucrose, 0.5 mM $MgSO_4$, 0.1 mM phenylmethanesulfonyl fluoride, 2 mM β-mercaptoethanol, 1× Sigmafast protease inhibitor (Sigma-Aldrich)) and centrifuged at 1,500*g* for 10 min. The supernatant was centrifuged at 100,000*g* for 45 min to collect the total membrane fraction. The pellet was