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Regulation of store-operated Ca^{2+} entry activity by cell cycle dependent up-regulation of Orai2 in brain capillary endothelial cells

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ABSTRACT

Store-operated Ca^{2+} entry (SOCE) via Orai1 and STIM1 complex is supposed to have obligatory roles in the regulation of cellular functions of vascular endothelial cells, while little is known about the contribution of Orai2. Quantitative PCR and Western blot analyses indicated the expression of Orai2 and STIM2, in addition to Orai1 and STIM1 in bovine brain capillary endothelial cell line, t-BBEC117. During the exponential growth of t-BBEC117, the knockdown of Orai1 and STIM1 significantly reduced the SOCE activity, whereas Orai2 and STIM2 siRNAs had no effect. To examine whether endogenous SOCE activity contributes to the regulation of cell cycle progression, t-BBEC117 were synchronized using double thymidine blockage. At the G2/M phase, Ca^{2+} influx via SOCE was decreased and Orai2 expression was increased compared to the G0/G1 phase. When Orai2 was knocked down at the G2/M phase, the decrease in SOCE was removed, and cell proliferation was partly attenuated. Taken together, Orai1 significantly contributes to cell proliferation via the functional expression, which is presumably independent of the cell cycle phases. In construct, Orai2 is specifically up-regulated during the G2/M phase, negatively modulates the SOCE activity, and may contribute to the regulation of cell cycle progression in brain capillary endothelial cells.

1. Introduction

Brain capillary endothelial cells (BCECs) form the blood brain barrier (BBB) that regulates the movement of essential metabolites and paracrine factors from the circulation and blocks the invasion of noxious materials into the brain to maintain the homeostasis of central nervous system. Cellular turnover of BCECs is mediated by the delicate balance of cell proliferation and death, and that the balance is essential for the integrity of the BBB [1].

Cytosolic Ca^{2+} signals are crucial for regulating a variety of cellular functions, including contraction, gene expression, proliferation and apoptosis in various types of cells. Ca^{2+} influx in non-excitable cells such as endothelial cells is mediated mainly by store-operated Ca^{2+} entry (SOCE) through Ca^{2+} release-activated Ca^{2+}(CRAC) channels [2]. SOCE is activated by the depletion of intracellular Ca^{2+} stores. When stromal interaction molecules (STIM) on the endoplasmic reticulum membrane sense store depletion, they translocate to regions close to the plasma membrane and then form functional complexes with Orai molecules, the pore-forming subunits of CRAC channels on the cell membrane. Orai channels and STIM proteins are both required for CRAC channel activity [3]. Three genes for Orai (Orai1, Orai2 and Orai3) and two for STIM (STIM1 and STIM2) have been identified in a number of different tissues. Among these subunits, Orai1 and STIM1 predominantly combine to form CRAC channels that play a critical role in the regulation of essential physiological functions. (e.g., migration, cell proliferation, gene expression, and differentiation).

Cytosolic Ca^{2+} signals are involved in various aspects of the cell cycle. The cell cycle consists of four distinct phases, namely two gap

Abbreviations: BCEC, brain capillary endothelial cell; CRAC, calcium release-activated calcium; SOCE, store-operated calcium entry; TRPC, transient receptor potential canonical.

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phases (G1 and G2), the DNA synthesis phase (S), and the mitosis phase (M). The transitions among these phases are strictly regulated, and four major checkpoints have been identified as the G1/S transition, the late S, the G2/M transition, and the metaphase to anaphase transition. Ca^{2+} signals are thought to be involved in all of these checkpoints. A transient change in $[Ca^{2+}]_i$ occurs at the exit from the G0 phase, at the G1/S transition, during the S phase and at the exit from the M phase [4].

We have previously shown that the regulation of Ca^{2+} influx due to membrane hyperpolarization via the activation of K^+ channels (SK2 and/or Kir2.1) induced cell proliferation or cell death in t-BBEC117, respectively [5–7]. However, the expression of Ca^{2+}-permeable channels and their functions in BCECs remain to be elucidated. The present study was undertaken to examine the following hypothesis; CRAC channels are responsible for the SOCE and regulates cell cycle progression and proliferation in t-BBEC117. The results denote that Orai2 is specifically up-regulated during the G2/M phase and negatively regulates the SOCE activity to modulate cell cycle progression in BCECs.

2. Materials and methods

2.1. Cell culture

The immortalized endothelial cell line t-BBEC117 was established from primary-cultured BBECs by the transfection of SV40 large T-antigen-expressing vector and cultured as described previously [8].

2.2. Real-time PCR and western blotting

Real-time PCR and Western blotting analyses were performed as described previously [7]. PCR primers and antibodies used in this study were listed in the online supplement.

2.3. Transfection of plasmid constructs and siRNA

Bovine Orai2 (NM_001191348) was subcloned into pEYFP-C1 (Clontech Laboratories, Mountein View, USA). t-BBEC117 cells were transiently transfected with cDNA using Lipofectamine2000 (Invitrogen, Carlsbad, USA), or with siRNA (Invitrogen) using an Amaxa Basic Nucleofector kit V (Lonza, Basel, Switzerland). Details of siRNA utilized are provided in the online supplement.

2.4. Measurement of intracellular Ca^{2+}concentrations

Cells loaded with Fura2-AM were superfused with HEPES-buffered solution [in mM: 137 NaCl, 5.9 KCl, 2.2 $CaCl_2$, 1.2 $MgCl_2$, 14 glucose, 10 HEPES (pH 7.4 adjusted by NaOH)] as previously described [7]. Ca^{2+}-free solution was prepared by replacing 2.2 mM $CaCl_2$ by 5 mM EGTA.

2.5. MTT assay

Cell viability was measured by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma–Aldrich, St Louis, USA) assay, as described previously [7]. MTT assay was performed against 2,000 cells/well.

2.6. Cell cycle analysis

Propidium iodide (PI) staining was performed using an established protocol. The synchronization of the cell cycle phases was performed with thymidine (Sigma–Aldrich), as described previously [9]. Cells were incubated: (i) in 2.5 mM thymidine-containing medium for 24 h, then (ii) in standard medium for 15 h, and finally (iii) in 2.5 mM thymidine-containing medium for 24 h. After this thymidine blockage, t-BBEC117 cells were cultured in standard medium and collected at 0, 6, 12, and 24 h after subculture.

2.7. Drugs

The following compounds were used in this study: thapsigargin (Wako, Osaka, Japan), lanthanum chloride (Sigma-Aldrich), gadolinium chloride (Sigma-Aldrich), SK&F96365 (Sigma-Aldrich), and 2-aminoethyldiphemyl borate (2-APB; Tocris Bioscience, Bristol, UK).

2.8. Statistical analysis

Pooled data are expressed as means ± SE. Statistical significance was examined using the Student's *t*-test for two groups and Tukey's test for more than three groups, after one-way analysis of variance. *P* values <0.05 were considered as statistical significance. The total number of cells used and the number of trials performed in each series of experiments were represented as “n” and “N”, respectively.

3. Results

3.1. Effects of Orai and STIM knockdown by siRNA in t-BBEC117

The functional expression and contribution of Orai1 and STIM1 to SOCE have been reported in endothelial cells from several types of vasculatures, such as human umbilical vein and coronary artery [10,11]. However, the contribution of TRP channels to SOCE has been also demonstrated in several reports, including our previous study [12,13]. The component susceptible to 10 μM SK&F96365, which shows some selectivity to receptor operated Ca^{2+} entry mainly due to TRP channel activation, was approximately 40%. In addition, real-time PCR revealed the abundant transcript expression of Orai1, Orai2, and STIM2, while Orai3 and STIM1 transcripts were also detected (Supplementary Fig. 3A). Moreover, it has been shown that the subtypes of Orai channels (Orai1, Orai2, and Orai3) have different sensitivities to 2-aminoethoxydiphenyl borate (2-APB) [3]. Thus, using 2-APB as a pharmacological tool, we examined the subtypes of Orai family and found that Orai1 and Orai2 but not Orai3 may contribute to SOCE in t-BBEC117 (Supplementary Fig. 2). Western blot analysis revealed that Orai2 and STIM1 proteins were abundantly expressed in t-BBEC117 (Supplementary Fig. 3B,C,D,E). Unfortunately, the anti-Orai1 antibody and anti-STIM2 antibody, which we could obtained (see Supplementary Materials), appeared to be not available to bovine Orai1 and STIM2, while these proteins in Jurkat T cells were clearly detected, respectively.

Based on these results, we hypothesized that Orai1, 2 and STIM1, 2 are functionally expressed and significantly contribute to SOCE in t-BBEC117, and carried our further experiments to detect their molecular functions, particularly in cell cycle progression and proliferation.

To obtain direct evidence for the involvement of Orai and STIM in SOCE in t-BBEC117, siRNA was applied to knockdown Orai and STIM expression. The specific siRNA knockdown of Orai1, 2 and STIM1, 2 was confirmed by real-time PCR (Fig. 1A,C,E,F). Fig. 1B and D demonstrate that the knockdown of Orai1 and STIM1 markedly reduced the SOCE, respectively. Summarized data indicate the significant SOCE inhibition by specific siRNA (si-Orai1, 0.23 ± 0.02, n = 90, $p < 0.01$ vs. sc-Orai1, 0.43 ± 0.02, n = 77; si-STIM1, 0.55 ± 0.03, n = 88, $p < 0.01$ vs. sc-STIM1, 0.81 ± 0.04, n = 64; N = 4–6; Supplementary Fig. 4A,B). On the contrary, the knockdown of Orai2 and STIM2 had no effect on the SOCE (si-Orai2,

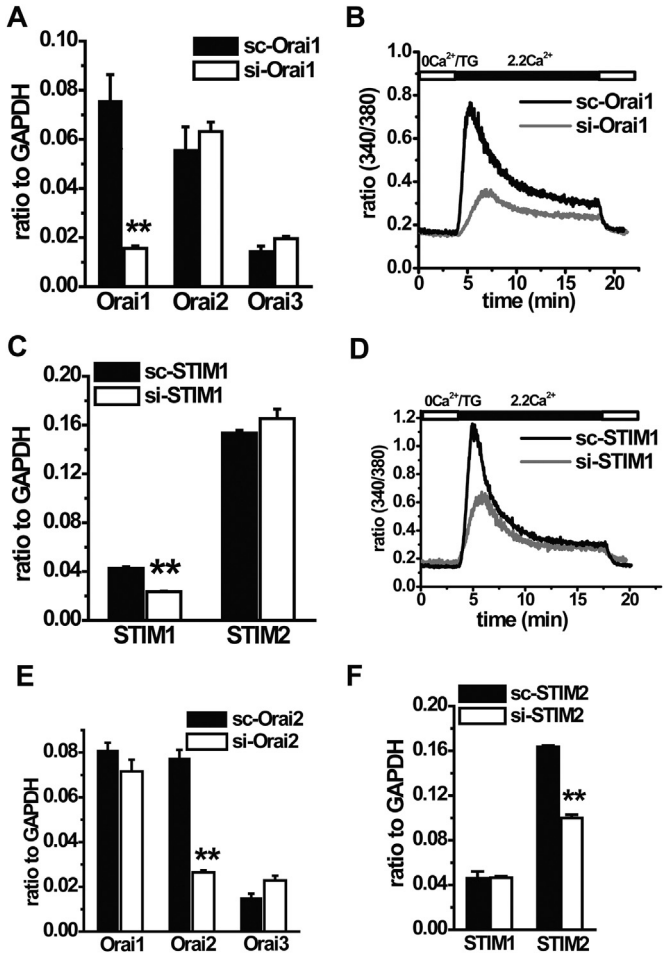

Fig. 1. Effects of knockdown of Orai or STIM on SOCE in t-BBEC117. A, C, E, and F, The specificity of each siRNA was confirmed by real-time PCR analyses. Scrambled siRNA was used as a negative control (N = 4 for each group, **p < 0.01 vs. scrambled siRNA). B and D, Representative records of $[Ca^{2+}]_i$ changes due to SOCE activation in t-BBEC117 transfected with Orai1 or STIM1 siRNA. sc-Orai1, n = 77; si-Orai1, n = 90; sc-STIM1, n = 64; si-STIM1, n = 88; N = 4–6; **p < 0.01 vs. scrambled siRNA.

0.64 ± 0.02, n = 91, p > 0.05 vs. sc-Orai2, 0.71 ± 0.03, n = 112; si-STIM2, 0.68 ± 0.03, n = 85, p > 0.05 vs. sc-STIM2, 0.71 ± 0.04, n = 51; N = 4–6; Supplementary Fig. 4C,D). Western blotting analysis revealed that the treatment with si-STIM1 or si-Orai2 markedly reduced protein expression of STIM1 or Ora2, respectively (not shown).

3.2. Dependence of the SOCE activity on the cell cycle phase in t-BBEC117

It has been reported that SOCE is strongly suppressed during mitosis in several cell lines [14,15]. To determine whether endogenous SOCE activity plays a role in regulating cell cycle progression, t-BBEC117 were synchronized using double thymidine blockage in the G1/S boundary followed by removal of thymidine (i.e., the release) for 24 h. The G0/G1, S and G2/M phase were defined as a function of time after the release from thymidine blockage (Fig. 2A). Double thymidine block caused a marked increase in the fraction of cells (74 ± 2%, N = 5) in the G0/G1 phase compared to the control (66 ± 4%, N = 6, not shown). 6 Hours after the release, the cell population in the G2/M phase reached its maximum (57 ± 2%, N = 5). 6–24H after the release, cells progressed to the G0/G1 phase (Fig. 2B).

Based on the results in Fig. 2A and B, the cells at 0 and 6 h after the release from the thymidine block were used as those in the G0/G1 and G2/M phases, respectively. The SOCE was significantly decreased in cells at the G2/M phase compared to the G0/G1 phase (0.26 ± 0.02, n = 25 and 0.36 ± 0.02, n = 49, N = 4 for each, p < 0.01) (Fig. 2C and D). Thus, our results obtained in t-BBEC117 well fit to those reported in other type of cells. Interestingly, this suppression of Ca^{2+} influx via SOCE recovered in accordance with the cell cycle progression from the G2/M phase to the G0/G1 phase. In addition, the SOCE activity was markedly increased in exponentially growing cells at 12 h after the release compared to the G2/M phase (0.38 ± 0.03, n = 28, N = 4, p < 0.01 vs. 6 h) (Fig. 2C and D).

3.3. Suppression of SOCE at G2/M phase due to the up-regulation of Orai2

Changes in the mRNA expression of Orai and STIM subfamilies were examined in cell-cycle synchronized t-BBEC117. Real-time PCR analysis revealed the up-regulation of Orai2 transcript, 6 h after the release (0.08 ± 0.04 at 0 h, N = 4; 0.12 ± 0.01 at 6 h, N = 4, p < 0.05) (Fig. 3A). The increase in Orai2 mRNA disappeared at 12 h. However, there was no significant changes in the mRNA expressions of Orai1, Orai3, STIM1, and STIM2 (Supplementary Fig. 5). We previously showed that mRNAs of TRPC1, TRPC3, and TRPC5 are expressed in t-BBEC117 [7]. Any or all of these channels could be potential molecular candidates for SOC channels [16,17]. However, no change in these transcripts was detected (Supplementary Fig. 5). Western blot analysis showed the up-regulation of Orai2 protein in whole cell fraction of t-BBEC117 at 6 h after the release compared to that at 0 h (N = 4, p < 0.05) (Fig. 3B). This finding supports the results of the real-time PCR shown in Fig. 3A.

The functional roles of Orai2 in the cell cycle-dependent SOCE activity were examined by Orai2 knockdown in t-BBEC117 at both G0/G1 and G2/M phase. At 0 h, there was no significant difference in Ca^{2+} influx via SOCE between the scrambled and Orai2 siRNA (sc-Orai2, 0.40 ± 0.02, n = 25; si-Orai2, 0.36 ± 0.03, n = 36, p > 0.05). However, at 6 h, Ca^{2+} influx was markedly increased in t-BBEC117 transfected with si-Orai2 compared to control cells (sc-Orai2, 0.32 ± 0.02, n = 41; si-Orai2, 0.43 ± 0.03, n = 31; N = 4 for each; p < 0.01) (Fig. 3C).

Effects of Orai2 overexpression on the SOCE-induced $[Ca^{2+}]_i$ increase were also examined in t-BBEC117 transiently transfected with the YFP-labeled bovine Orai2. YFP-Orai2 was overexpressed in non-synchronized t-BBEC117. Overexpression of YFP-Orai2 in t-BBEC117 significantly decreased the SOCE in comparison to mock control cells (mock, 0.24 ± 0.02, n = 24; Orai2, 0.12 ± 0.02, n = 22; N = 3 for each; p < 0.01) (Fig. 3D).Taken together, results in Fig. 3 strongly suggest that the up-regulation of Orai2 expression may cause the suppression of SOCE activity at the G2/M phase.

3.4. Decrease in the cell cycle distribution at G0/G1 phase and attenuation of cell proliferation due to knockdown of Orai2

We have also examined whether the regulation of $[Ca^{2+}]_i$ signaling by Orai2 significantly affects the cell cycle progression (Fig. 3E) and cell proliferation (Fig. 3F) in non-synchronized t-BBEC117. In t-BBEC117 transfected with Orai2 siRNA, G0/G1 population was significantly decreased compared to control cells (si-Orai2, G0/G1 0.57 ± 0.01, S 0.14 ± 0.01, G2/M 0.30 ± 0.01, N = 4; sc-Orai2, G0/G1 0.62 ± 0.01, S 0.12 ± 0.01, G2/M 0.27 ± 0.01, N = 4) (Fig. 3E). This result suggests that gene silencing of Orai2 induces the cell cycle arrest at the G2/M phase or promote the G1-S phase transition.

Next, the effect of si-Orai2 on cell proliferation was measured using MTT cell viability assay. After these cells were transfected

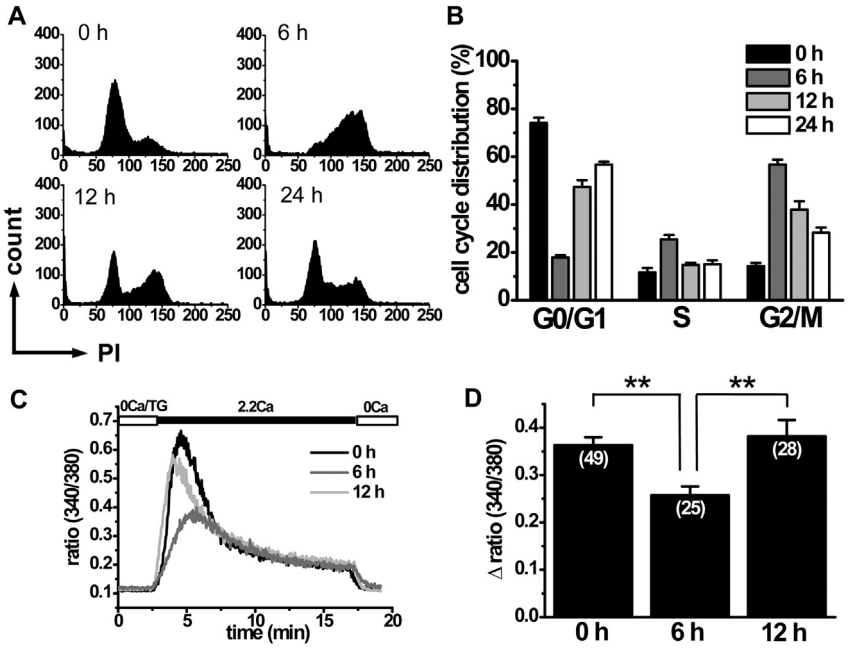

Fig. 2. Changes in SOCE activity according to the cell cycle phase. A, Time course of the cell cycle phases in t-BBEC117 after the release from double thymidine blockage. DNA contents are shown as function of PI fluorescence at different times after the release. B, Cell cycle distribution at each time point (0, 6, 12, 24 h, N = 5 for each). C, Representative records of $[Ca^{2+}]_i$ changes due to SOCE activity in t-BBEC117 at 0, 6, and 12 h after the removal. D, Summarized data from experiments shown in C (0 h, n = 49; 6 h, n = 25; 12 h, n = 28; N = 4 for each; **p < 0.01).

with scrambled or Orai2 siRNA, they were incubated for 0, 24, 48, 72, and 96 h. The relative viability at each time point was calculated (Fig. 3F). The cell proliferation at 48 h and 72 h was significantly attenuated by the silencing of Orai2 (scramble control, 1.56 ± 0.02 at 24 h, 2.13 ± 0.04 at 48 h, 2.74 ± 0.06 at 72 h, 3.38 ± 0.12 at 96 h, N = 4; Orai2 siRNA, 1.49 ± 0.08 at 24 h, 1.87 ± 0.01 at 48 h, 2.39 ± 0.09 at 72 h, 3.07 ± 0.13 at 96 h, N = 5). Effects of Orai1 or STIM1 knockdown on cell proliferation were also examined in non-synchronized t-BBEC117 (Supplementary Fig. 6A,B). The suppression of cell proliferation by si-Orai1 appears to be the most significant among the knockdown of Orai1, Orai2 and STIM1.

4. Discussion

The present study clearly demonstrates that Orai2 in addition to Orai1 plays a substantial role in the activation of SOCE in t-BBEC117, a model of BCECs. We found that Orai2 is up-regulated at the G2/M phase of cell cycle and consequently decreases Ca^{2+} influx via SOCE. To our knowledge, this is the first report, regardless of cell types, describing a specific change in the expression and functional contribution of Orai2 to SOCE during cell cycle progression. Orai2 as well as Orai1 may be a potential regulator of cell proliferation in BCECs.

4.1. Molecular components responsible for SOCE activity in BCECs

CRAC channels consisting of Orai and STIM have been identified as one set of molecular components responsible for SOCE in a variety of cell types. It is a matter of importance that SOCE signaling is involved in migration and cell cycle progression of various cancer cells [18,19]. Moreover, Abdullaev et al. have demonstrated that the activation of CRAC channels composed of Orai1 facilitates cell proliferation in HUVECs [10].

The SOCE in t-BBEC117 showed the typical pharmacological properties of CRAC channel: (i) SOCE was blocked by lanthanides (La^{3+} and Gd^{3+}) and partly by SK&F96365 and (ii) the difference in the sensitivity to 2-APB at lower and higher concentrations demonstrated the relevance of Orai1 and/or Orai2 to SOCE (Supplementary Fig. 1,2). Real-time PCR and Western blotting analyses also suggest the expression of Orai1 and Orai2 in t-BBEC117 (Supplementary Fig. 3). SOCE activity was markedly decreased by siOrai1 or siSTIM1 but not affected significantly by either siOrai2 or siSTIM2, in spite of supposed expression of Orai2 and STIM2 at relatively high levels (Fig. 1 and Supplementary Fig. 3). In addition, it has been demonstrated that STIM2 is a weaker activator of Orai channels than STIM1 and that the overexpression of STIM2 has a strong negative effect on endogenous SOCE [20].

Taken together, the results in this study about the molecular components responsible for SOCE activity are consistent with those previously reported and suggest the large contribution of Orai1 and STIM1 to SOCE in BCECs. Even though, partial contribution of TRP channels to SOCE activity in this type of cells cannot be ruled out. Further study is required to elucidate the whole molecular features underlying SOCE activity in BCECs.

4.2. Cell cycle dependent SOCE activity via up-regulation of Orai2 at G2/M phase

It is well-known that cell cycle progression involves various types of $[Ca^{2+}]_i$ signaling events, ranging from a single Ca^{2+} spike to continuous Ca^{2+} oscillations that are caused by Ca^{2+} release and Ca^{2+} influx [21]. It has been reported that Ca^{2+} influx due to SOCE following histamine receptor activation in HeLa cells is diminished during mitosis [15]. Recently, the regulation of I_{CRAC} has been shown to be dependent on the cell cycle progression in the mast cell line RBL-2H3. I_{CRAC} is substantially suppressed in mitosis, and significantly increased during G1/S phase [14]. During meiosis in *Xenopus* oocytes, SOCE is inactivated via the inhibition of STIM1 clustering into puncta, as well as Orai1 internalization [22]. Moreover, the phosphorylation of STIM1 causes an attenuation of STIM1 function. This phosphorylation is mediated by the activation of

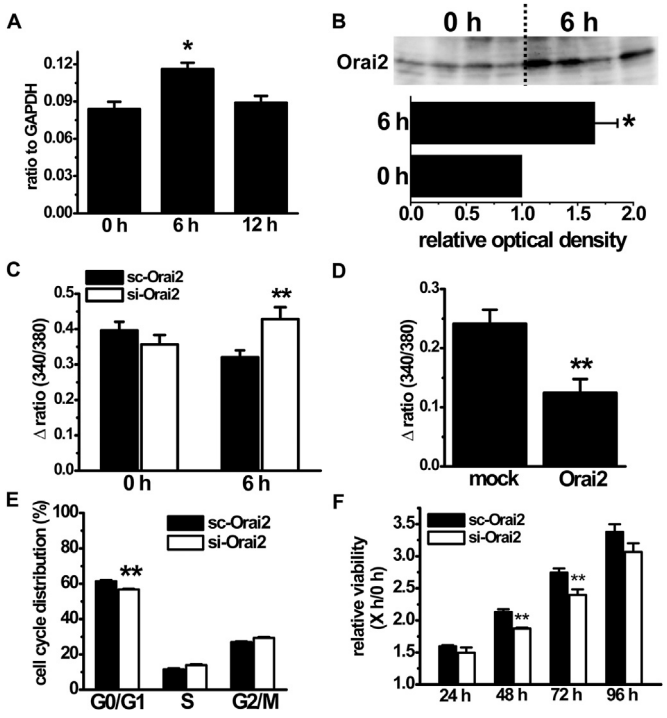

Fig. 3. Suppression of SOCE at the G2/M phase due to the up-regulation of Orai2. A, Real-time PCR analyses of Orai2 in t-BBEC117 at 0, 6, and 12 h after the release of thymidine blockage. Expression levels of Orai2 mRNA have been normalized to GAPDH level (N = 4 for each). *p < 0.05 vs. 0 h. B, Western blotting analysis for Orai2 in t-BBEC117 at 0 and 6 h after the release (N = 4 for each). Protein expression is shown as relative optical density, normalized to the value at 0 h. *p < 0.05 vs. 0 h. C, The effects of Orai2 knockdown on SOCE activity at the G2/M phase. Synchronization of t-BBEC117 transfected with Orai2 siRNA was performed to examine the roles of Orai2 on the SOCE activity at the G2/M phase. SOCE was measured at 0 and 6 h after the release (0 h: si-Orai2, n = 36; sc-Orai2, n = 25; 6 h: si-Orai2, n = 31; sc-Orai2, n = 41; N = 4 for each). **p < 0.01 vs. sc-Orai2. D, Effects of Orai2 overexpression on SOCE was examined in t-BBEC117 transfected with an empty vector (mock, n = 24, N = 3) and YFP-Orai2 in non-synchronized t-BBEC117 (Orai2, n = 22, N = 3). **p < 0.01 vs. mock. E, Effects of Orai2 knockdown on the cell population were compared between cell cycle phases. Cell cycle progression was monitored by the detection of DNA contents using FCM analysis. DNA was labeled with PI. Data describing cell cycle phase distribution in t-BBEC117 cells, that were transfected with scramble siRNA (N = 5) or Orai2 siRNA (N = 4), are summarized in terms of the percentage of the G0/G1, S, G2/M phases. **p < 0.01 vs. sc-Orai2. F, The influence of si-Orai2 on cell proliferation was measured in non-synchronized t-BBEC117 using the MTT assay. Data about viability of t-BBEC117 transfected with scrambled or Orai2 siRNA after the incubation in normal medium for 24, 48, 72, and 96 h (N = 4 and 5, respectively) were normalized at 0 h (1.0) and summarized. **p < 0.01 vs. sc-Orai2.

maturation-promoting factor (MPF) composed of cyclin B and cyclin-dependent kinase1 (CDK1) that stimulate the mitotic phase of the cell cycle [23].

In t-BBEC117, SOCE activity decreased at the G2/M phases compared to the G0/G1 phases, as has been reported in several types of cell lines. Our analyses using double thymidine blockade revealed that only the expression of Orai2 transcripts and protein was significantly increased at G2/M phase. The mRNA expression of other SOCE related factors including Orai1, Orai3, STIM1, STIM2 and some TRP channels did not change during cell cycle phases (Supplementary Fig. 5). Knockdown of Orai2 enhanced SOCE in cells synchronized at the G2/M phase, facilitated the cell arrest, and reduced cell proliferation (Fig. 3C,E and F). On the contrary, the overexpression of YFP-tagged Orai2 reduced SOCE in t-BBEC117 (Fig. 3D). These results strongly suggest that the suppression of the SOCE activity at G2/M phase can be, at least in part, attributed to the specific up-regulation of Orai2 in t-BBEC117.

It has been reported that Orai2 may play a dominant-negative role in the activation of CRAC channel, presumably composed of Orai1 and STIM1 [24]. Moreover, Orai2 can make molecular complex with Orai1 to form a CRAC channel presumably as a hetero-multimer [25]. Alternatively, the ability of Orai2 as a molecular component of CRAC channel may be simply lower than that of Orai1 [3]. In this study, the mechanism underlying the up-regulation of Orai2 expression at G2/M phase could not be determined. Nevertheless, the newly-found up-regulation of Orai2 expression is apparently an important mechanism for the cell cycle-dependent SOCE activity, in addition to the regulatory factors and mechanisms mentioned in the previous paragraph.

The t-BBEC117 proliferation was suppressed not only by siOrai1 but also by siOrai2 in different extents (Fig. 3F and Supplementary Fig. 6). The suppression of cell proliferation by siOrai1 developed time-dependently and was significant at any time from 24 to 96 h (Supplementary Fig. 6). In contrast, the suppression by siOrai2 was almost maximal at 48 h and was significant only at 48 and 72 h. These results suggest that Orai1 is one of essential factors for the regulation of cell proliferation. Further study is required to elucidate the relation between the recovery of SOCE activity at G2/M phase by siOrai2 and the slight but significant suppression of cell proliferation by siOrai2 at 48 and 72 h.

Here, we have shown that Orai1 plays a central role in SOCE activity independently of the cell cycle phases, whereas Orai2 is up-regulated specifically at the G2/M phase and can reduce the SOCE activity in a cell cycle-dependent manner. The present study provides a new insight into the molecular functions of Orai subfamily in the regulation of cell cycle progression and proliferation of BCEC and, thus, in the maintenance of BBB homeostasis.

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the author(s).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.127.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.127.

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