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ORIGINAL ARTICLE

Functional interactions among STIM1, Orai1 and TRPC1 on the activation of SOCs in HL-7702 cells

Zhen-Ya Zhang · Li-Jie Pan · Zong-Ming Zhang

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Abstract STIM1, Orai1 and TRPC1 are all reported to be important for store-operated Ca^{2+} entry (SOCE) in diverse cells. However, there is no evidence for the functional interaction of the three proteins in SOCE in human liver cells. The objective of this study is to determine whether they are involved in SOCE in normal human liver cells. Liposomal transfection method was used to increase expression levels of the three proteins in HL-7702 cells, a normal human liver cell line. Western blot and single cell RT–PCR were applied to evaluate transfection effectiveness. Changes in store-operated current (I_{SOC}) and SOCE were investigated using whole-cell patch-clamp recording and calcium imaging. I_{SOC} is detected in HL-7702 cells and it is inhibited either by 2-Aminoethoxydiphenyl borate (2-APB) or La^{3+}. Overexpression of STIM1 or Orai1 alone did not induce any change in I_{SOC}. TRPC1-transfection, however, caused approximate 2.5-fold increase in I_{SOC}. A large increase (>10-fold) in I_{SOC} emerged when both STIM1 and Orai1 were co-transfected into HL-7702 cells. Co-overexpression of STIM1 + TRPC1 also caused >10-fold increase in I_{SOC}, and addition of Orai1 did not cause any further increase. In HL-7702 cells, TRPC1 and Orai1 take part in SOCE independently of each other. Functional interactions of STIM1 and Orai1 or TRPC1 contribute to I_{SOC} activation.

Keywords Store-operated channels · STIM1 · Orai1 · TRPC1 · Human liver cells

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Abbreviations

2-APB	2-Aminoethoxydiphenyl borate
EDTA	Ethylene diamine tetraacetic acid
EGFP	Enhanced green fluorescence protein
EGTA	Ethylene glycol tetraacetic acid
ER	Endoplasmic reticulum
FBS	Fetal bovine serum
I_{CRAC}	Ca^{2+} release-activated Ca^{2+} current
I_{SOC}	Store-operated current
PBS	Phosphate-buffered saline
ROCs	Receptor-operated channels
RT-PCR	Reverse transcription-polymerase chain reaction
SCID	Severe combined immunodeficiency
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOCE	Store-operated Ca^{2+} entry
SOCs	Store-operated channels
SR	Sarcoplasmic reticulum
STIM1	Stromal interacting molecular 1
Tg	Thapsigargin
TRPC1	Transient receptor potential canonical 1
VOCCs	Voltage-operated Ca^{2+} channels

Introduction

Ca^{2+} has long been recognized as an important second messenger, and intracellular Ca^{2+} has been shown to play an important role in regulating a variety of physiological processes in both excitable and non-excitable cells. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) may be increased through various mechanisms (1) the release of Ca^{2+} from the endoplasmic reticulum (ER) or sarcoplasmic reticulum

(SR), (2) Ca^{2+} entry from extracellular space through voltage-operated Ca^{2+} channels (VOCCs) or receptor-operated channels (ROCs) or store-operated channels (SOCs) (Barritt 1999; Parekh and Putney 2005). The functional control of SOCs is dependent upon the Ca^{2+} concentration in the ER/SR lumen: when intracellular Ca^{2+} stores are full, Ca^{2+} influx does not occur; as the stores deplete, however, Ca^{2+} entry develops. The concept of SOCE or I_{SOC} was proposed in 1986 (Putney 1986) and developed more fully during the past 20 years. With the introduction of electrophysiological techniques, several I_{SOC} types have been characterized including the Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}), typically found in T lymphocytes and mast cells (Roos et al. 2005). The molecular compositions of SOCs, and the exact signaling pathways that activate these channels remain debated. In recent years, several independent groups have identified STIM1 and Orai1 to be essential components of SOCs, especially the CRAC channels (Parekh 2006; Huang et al. 2006; Prakriya et al. 2006).

STIM1 protein was reported to contain several important domains: an EF-hand domain, and a sterile-alpha motif (SAM) in the N-terminus, and a coiled coil region, and a lysine-rich region in the C-terminus. It is thought that STIM1 is the Ca^{2+} store-sensing component of SOCs and mutations in acidic residues within the Ca^{2+}-binding pocket of the EF-hand domain of STIM1, which presumably lower the affinity for Ca^{2+}, produce constitutive Ca^{2+} entry that is independent of store depletion (Smyth et al. 2006; Liou et al. 2005; Zhang et al. 2005).

Orai1 was first discovered from genetic linkage analysis performed to identify mutations contributing to a rare form of clinical severe combined immunodeficiency (SCID), and it was predicted to be a membrane-spanning protein with four transmembrane domains (Feske et al. 2006; Vig et al. 2006). The SCID patients with the mutation for Orai1 were reported to have a decreased I_{CRAC} activity in T cells. This was confirmed by the failure of the mutant Orai1 to rescue the I_{CRAC} activity when transfected into T cells and fibroblasts, whereas the wild-type Orai1 did (Feske et al. 2006).

There is also increasing evidence that members of the canonical subgroup of transient receptor potential channels constitute SOCs (Parekh and Putney 2005; Pedersen et al. 2005; Albert et al. 2007). Currently, TPRC1 is thought to be the most likely candidate for the formation of some SOCs. Several recent studies had shown that SOCs were impaired in different mammalian cells when TRPC1 expression was inhibited using antibodies, antisense or siRNA methods (Saleh et al. 2006, 2008; Takahashi et al. 2007).

As a type of non-excited cells, hepatocytes (liver cells) are functionally regulated by intracellular Ca^{2+}. Despite the role as a second message (Barritt et al. 1981), changes in $[Ca^{2+}]_i$ in hepatocytes constitute an essential intracellular signaling event which functions in unison with numerous other signaling pathways, including the extensive protein kinase network, in regulating hepatocyte and liver function under normal and pathological conditions (Gaspers and Thomas 2005; Teoh and Farrell 2003; Nieuwenhuijs et al. 2006). Furthermore, the functions of SOCs have been shown in hepatocytes and liver cell lines (Graf and Häussinger 1996; Auld et al. 2000). Also, in accord with investigations in other cell types, the results of recent experiments with liver cells indicate that STIM1, Orai1 and TRPC1 exist in rat liver cells and play essential roles in the formation and regulation of SOCs (Aromataris et al. 2008; Barritt et al. 2009; Chen and Barritt 2003; Brereton et al. 2001).

Although the characteristics of SOCs in animal hepatocytes have been reported (Graf and Häussinger 1996; Barritt et al. 2009; Chen and Barritt 2003), the study on SOCs in human liver cells is still lacking. Since STIM1, Orai1 and TRPC1 are all necessary for SOCs, the interactions among these proteins provide the mechanism for activating SOCs in different mammalian cells (Li et al. 2007; Jardin et al. 2008; Ong et al. 2007). However, there is no evidence for functional interaction among the three proteins in the activation of SOCs in human liver cells. The aim of the present study was to investigate the roles of STIM1, Orai1 and TRPC1 on the activation of SOCs in human liver cells.

Materials and methods

Reagents

Cell culture supplies: RPMI-1640, trypsin-EDTA, and antibiotics were purchased from Gibco. Fetal bovine serum (FBS) was bought from Hyclone. Thapsigargin (Tg), Fast Red TR and Naphthol AS-MX phosphate were products of Sigma-Aldrich. Fluo-4/AM, TRIZOL and Lipofectamine 2000 were purchased from Invitrogen. Antibodies for STIM1 and Orai1 were acquired from Proteintech, TRPC1 antibodies were bought from Abnova. Peroxidase-conjugated secondary antibodies were from Beijing Zhongshan Goldenbridge Company. 2-APB was from Calbiochem. All other reagents were of reagent grade.

Cell culture, plasmid construction and transfection

Human liver cell line, HL-7702, purchased from Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, was cultured at 37°C in 5% (v/v) CO_2 in air in RPMI-1640 medium and 10% (v/v) heat-inactivated FBS. pBS-T-TRPC1 plasmid was constructed and verified as

previously described (Rao et al. 2006). STIM1-mORANGE and Orai1-EGFP were gifts from Prof. Tao Xu (Institute of Biophysics, Chinese Academy of Sciences, China). HL-7702 cells were allowed to grow to ~80% confluence and transfected with required DNA at concentration of 1 μg/ml, using Lipofectamine 2000 and protocols supplied by the manufacturer.

RNA isolations, first strain cDNA synthesis, and semiquantitative RT-PCR analysis

Total RNA was extracted from HL-7702 cells using TRIZOL reagent according to the instructions offered by the manufacturer. The primer sequence used for TRPC1, STIM1 and Orai1 are given in Table 1. First strand cDNA synthesis and RT-PCR were performed as described previously (Rao et al. 2006). After PCR, 10 μl of the PCR product were analyzed on a 1% agarose gel. Band recognition and semiquantitative calculation were all performed by the software, Gelpro Analyzer 4.0 (Media Cybernetics, USA).

Western blotting

HL-7702 cells were cultured and transfected with the indicated plasmids as described earlier. The cells were harvested, lysed and stored at −80°C. An aliquot of 20 μg proteins was diluted to 45 μl in the loading buffer (44.6 mM Tris at pH 6.8, 1.6 mM EDTA, 44.5 mM boric acid, 10% glycerol, 2% SDS, 5% β-mercaptoethanol and 0.1% bromphenol blue), separated by 3 and 9% SDS–PAGE gel, transferred to polyvinylidene difluoride membranes, and probed with anti-TRPC1, anti-STIM1 and anti-Orai1 antibodies. Peroxidase-conjugated secondary antibodies were used to label the proteins. The proteins on the membranes were stained by a staining solution containing Fast Red TR and Naphthol AS-MX phosphate. Finally, the red protein bands were cut and placed in 200 μl of dimethyl sulfoxide (DMSO) for discolouration, and the absorbance of the resulting DMSO solution was measured in a micro plate reader (Thermo, USA) at 492 nm.

Electrophysiology

Whole-cell patch clamping was performed at room temperature (22–25°C) using a computer-based patch-clamp amplifier (EPC-10, HEKA Electronics, Lambrecht/Pfalz, Germany) and PatchMaster software (HEKA Electronics). HL-7702 cells grown in 35-mm dishes were perfused with a standard external solution containing the following (in mM): 140 NaCl, 4 CsCl, 2 $MgCl_2$, 10 $CaCl_2$, 10 D-glucose and 10 Hepes (pH 7.4, adjusted with NaOH). The patch pipettes were pulled from borosilicate glass and fire polished. Pipette resistance ranged between 4 and 8 MΩ after filling with the standard intracellular solution containing (in mM): 15 CsCl, 135 Cs-glutamate, 10 Hepes and 10 EGTA (pH 7.2, adjusted with CsOH). In all whole cell experiments, the recording started when the series resistance dropped to below 20 MΩ. After establishment of the whole cell configuration, voltage ramps of 100 ms duration, spanning a range of −160 to +100 mV, were delivered from a holding potential of 0 mV every 2 s over a period of 300–800 s. All voltages were corrected for a liquid-junction potential of 17 mV (estimated by JPCalc). Capacitative currents were determined and compensated for automatically by the EPC-10 amplifier. The maximal currents at −100 mV were used for statistical analysis.

Table 1 Primer sequences for RT-PCR

Human	Forward	Reverse
STIM1	5′-GTTTGCCTATATCCAGAACCGTTA-3′	5′-TACCATGAGCTGTGAGATTCTAGC-3′
Orai1	5′-AATCTCAACTCGGTCAAGGAGTC-3′	5′-ACTGTCGGTCAGTCTTATGGCTA-3′
TRPC1	5′-GCAATGATACCTTCCATTCGTTC-3′	5′-CGATGCACTAGGCAGCAGATC-3′

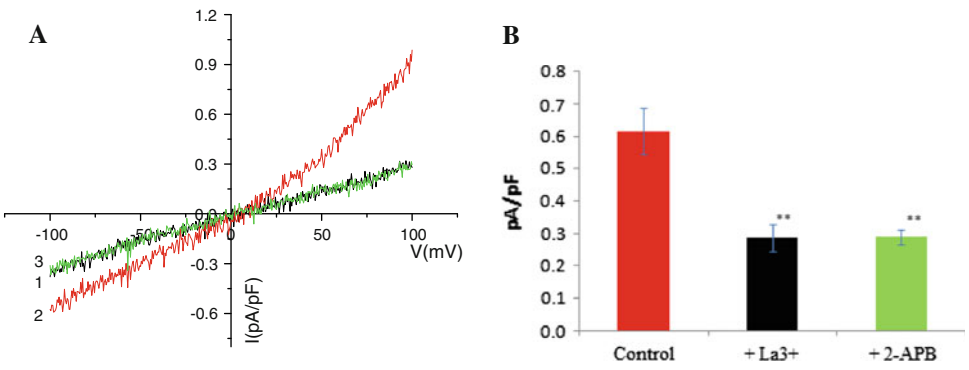

Fig. 1 Native store-operated currents in HL-7702 cells A. I–V curves of Tg-induced currents in normal HL-7702 cells in the absence (*red trace*) or presence of 100 μM La^{3+} (*black trace*) or 50 μM 2-APB (*green trace*). B. Bar graph of mean peak current density at −100 mV ($n = 6$)

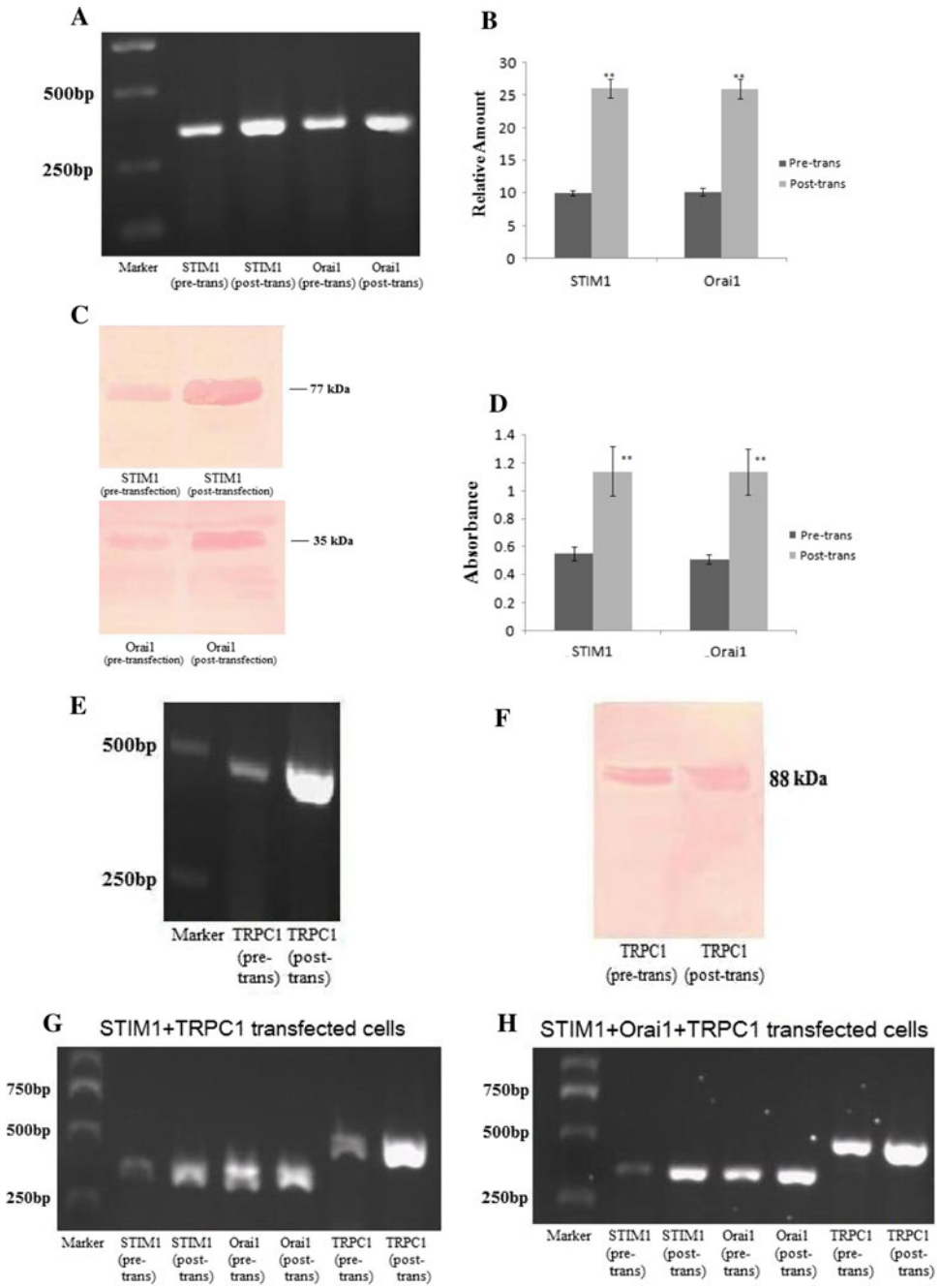

Fig. 2 Expressions of STIM1, Orai1 and TRPC1 in HL-7702 cells. **a** RT-PCR products from cultured HL-7702 cells and STIM1 + Orai1 transfected cells using primers for human STIM1 (342 bps) and Orai1 (343 bps). **b** Semi-quantification of RT-PCR results of STIM1/Orai1 between untransfected and transfected HL-7702 cells, six independent reactions were performed for each condition, ** $p < 0.01$. **c** STIM1 and Orai1 proteins were detected in cultured HL-7702 cells and STIM1 + Orai1 transfected cells using western blotting analysis. **d** Bar graph showing quantification of western blotting results. After transfection, protein expression levels of STIM1 and Orai1 were significantly increased ($n = 6$, ** $p < 0.01$). **e** Single cell RT-PCR products from control cells and TRPC1-transfected cells using primers for human TRPC1 (455 bps). **f** TRPC1 protein was detected in cultured HL-7702 cells and TRPC1-transfected cells using western blotting analysis. It is obvious that the expression level of TRPC1 was increased in the transfected cells. **g** Single cell RT-PCR products from HL-7702 cells and STIM1 + TRPC1 transfected cells. **h** Single cell RT-PCR products from HL-7702 cells and STIM1 + Orai1 + TRPC1 transfected cells. The expressions of STIM1, Orai1 and TRPC1 are all increased after transfection

Calcium imaging

For imaging experiments, HL-7702 cells and transfected cells were seeded in glass-bottomed microwell dishes the day prior to experimentation. Then cells were loaded with 6.7 μmol/L Fluo-4/AM in the standard external solution at 37°C for 30 min, followed by three washes in PBS and 15 min incubation in cultural medium to allow de-esterification of the dye. Changes of $[Ca^{2+}]_i$ in cells were monitored at 5 s intervals with a Leica TCS SP5 confocal laser scanning microscope equipped with an argon laser (Leica, Wetzlar, Germany). The 495 nm laser was used for Fluo-4 excitation, and emitted fluorescence was collected through a 500–530 nm window of the detector. LAS AF was applied for hardware control, image acquisition and image analysis.

Single cell RT-PCR

Single cell RT-PCR was performed to determine the cells measured by patch clamp were successfully transfected with the desired cDNA using the previously described method with modification (Rabe et al. 1998). In this

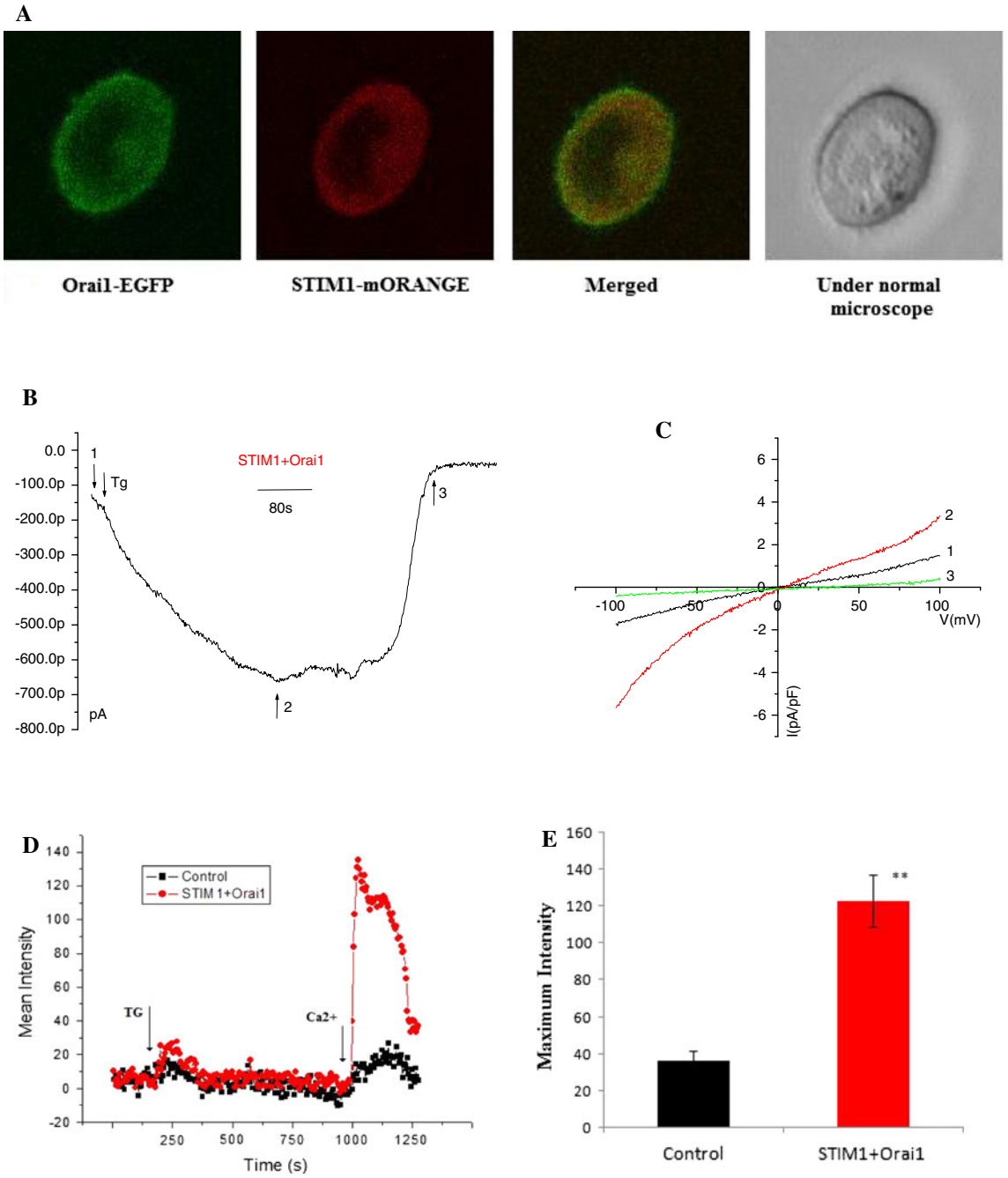

Fig. 3 STIM1 and Orai1 enhance SOC function in HL-7702 cells. **a** Images of transfected cells were captured using confocal laser microscopy. Successful co-overexpression of STIM1 and Orai1 in the same cell was verified. **b** Whole cell currents elicited by Tg at − 100 mV in cells transfected with STIM1 + Orai1. **c** I–V curves at the three time points indicated in panel B: *1*, at the beginning of the experiment, SOCs were partially activated by 10 mM EGTA in the pipette solution, *2*, at the peak of current development after Tg stimulation and *3*, after current inactivation as time elapsed. **d** $[Ca^{2+}]_i$ measurement of control and transfected cells. Cells were loaded with fluo-4 and placed in a nominally Ca^{2+}-free extracellular solution. 2 μM Tg was added to deplete Ca^{2+} stores and this causes a transient rise in $[Ca^{2+}]_i$. When $[Ca^{2+}]_i$ returned baseline, 2 mM Ca^{2+} was added extracellularly and this caused $[Ca^{2+}]_i$ to increase again rapidly. Confocal images of fluo-4 fluorescence were used to record the $[Ca^{2+}]_i$ change. **e** The maximal fluorescence intensities acquired after Ca^{2+} readdition in control and transfected HL-7702 cells. Means ± SD for $n = 6$, ** $p < 0.01$

experiment, harvesting cytoplasm of patched cells was performed by a gentle suction applied to the pipette. The tip of the pipette was then broken in a PCR tube containing the required reagents for reverse transcription, which was followed by PCR using specific primers described in Table 1. The first PCR amplification was performed as follows: a pre-denaturation at 94°C for 3 min, followed by 25 (for STIM1 and Orai1) or 30 (for TRPC1) cycles of

denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and synthesis at 72°C for 60 s, and then a last extension step at 72°C for 5 min. The second round of PCR was then performed using 5 μl of the products of the first amplification as templates. Each gene fragment was amplified individually using its specific primer pair by performing 35 cycles for TRPC1 and 30 cycles for STIM1 and Orai1 (as described above). PCR products were analyzed using agarose gel electrophoresis.

Statistical analysis

Unless specially noted, all current traces were corrected for leak currents. Data analysis was conducted using IGOR Pro 5.01 (Wavemetrics, Portland, OR, USA). Means standard deviation (SD) were calculated according to standard procedures. Statistical significance of difference between test samples and controls was determined by using the DUNNET *t* test. Asterisks denote statistically significant difference with p values less than 0.05 (*) and 0.01 (**).

Results

Native store-operated Ca^{2+} currents in HL-7702 cells

It has been shown that I_{SOC} is present in rat liver cells and human hepatomas (Rychkov et al. 2001; Jiang et al. 2001; El Boustany et al. 2008). In this study, current response to Ca^{2+} store depletion induced by Tg was tested in wild type HL-7702 cells by whole-cell patch clamp recording. As shown in Fig. 1a, the store-depletion activated Ca^{2+} currents have a linear current–voltage relationship at negative potentials and a slight outward rectification at positive potentials, with a reversal potential at near 0 mV. The peak current density was 0.62 ± 0.07 pA/pF at -100 mV ($n = 6$), and it was reduced to 0.29 ± 0.04 pA/pF and 0.29 ± 0.02 pA/pF by La^{3+} (100 μM) and 2-APB (50 μM), respectively (Fig. 1b).

STIM1, Orai1 and TRPC1 expression in HL-7702 cells

To determine if STIM1, Orai1 and TRPC1 are expressed in the HL-7702 cells, we first used RT-PCR. Using gene-specific primers described in Table 1, the corresponding products of STIM1 (343 bps), Orai1 (342 bps) and TRPC1 (455 bps), were amplified, confirming the expression of these three genes in HL-7702 cells. Second, we tested the expressions of the three proteins by western-blot analysis using specific antibodies. The results showing molecular mass of 77 kDa for STIM1, 35 kDa for Orai1 and 88 kDa for TRPC1 are in agreement with the expected sizes of these proteins, demonstrating that all three proteins are constitutively expressed in HL-7702 cells (Fig. 2a, c, e–h).

Fig. 4 Significant role of TRPC1 on the activation of SOCs in HL-7702 cells. **a** I–V curves in cultured HL-7702 cells (*black trace*) and TRPC1 transfected cells (*red trace*). Transfection of TRPC1 resulted in an approximate 2.5-fold increase in I_{SOC} at -100 mV. **b** Appearance of a patched cell before (*left picture*) and after (*right picture*) suction to extract the cellular content. The shrinkage of cell membrane can be seen in the right image. **c** Whole cell currents at -100 mV elicited by Tg in cells co-transfected with STIM1 + TRPC1. **d** I–V curves at the two time points indicated in panel C. At the first time point, I_{SOC} emerged and increased slowly because of the existence of EGTA in pipette solution; after Tg was added, the current developed more rapidly and reached the peak in 90 s (curve 2). **e** Bar graph of the mean current density of maximal I_{SOC} of control cells and STIM1 + TRPC1 transfected cells at -100 mV ($n = 6$, ** $p < 0.01$). **f** Whole cell currents at -100 mV elicited by Tg in cells transfected with STIM1 + Orai1 + TRPC1. **g** I–V curves at the two time points indicated in panel F. **h** Bar graph of mean current densities of maximal I_{SOC} of control cells and STIM1 + Orai1 + TRPC1 transfected cells at -100 mV ($n = 6$, ** $p < 0.01$) ▶

STIM1 and Orai1 enhance SOC functions in HL-7702 cells

Co-overexpression of STIM1 and Orai1 reconstituted I_{CRAC} in several different cell types, including HEK293, lymphocytes, mast cells, etc. (Barritt et al. 2009; Zhang et al. 2006; Soboloff et al. 2006; Peinelt et al. 2006). However, overexpressing either STIM1 or Orai1 failed to increase I_{CRAC}. We observed similar phenomena in HL-7702 cells. Overexpression of STIM1 or Orai1 alone did not induce any change in I_{SOC} (the peak current density of STIM1-transfected cells was 0.64 ± 0.06 pA/pF and it remained as 0.60 ± 0.08 pA/pF when Orai1 was overexpressed in HL-7702 cells), while co-expression of STIM1-mORANGE and Orai1-EGFP together in HL-7702 cells, as shown by RT-PCR and western blotting (Fig. 2a–d) as well as images taken for the same HL-7702 cell by confocal laser microscopy (Fig. 3a), resulted in an increase in the Tg-evoked I_{SOC} with obvious inward rectification at negative potentials, reminiscent of that of I_{CRAC} (Fig. 3b, c). However, there is also an obvious increase in the outward current and the reversal potential was again near 0, suggesting that the newly formed channels due to STIM1 and Orai1 co-expression in the liver cells are different from the CRAC channel, in terms of ion selectivity. In calcium imaging experiments, we show that overexpressing STIM1 + Orai1 caused an increase in SOCE (Fig. 3d, e).

Significant role of TRPC1 on the activation of SOCs in HL-7702 cells

Unlike STIM1 and Orai1, transfection of TRPC1 alone substantially increased I_{SOC}. As shown in Fig. 4a, an

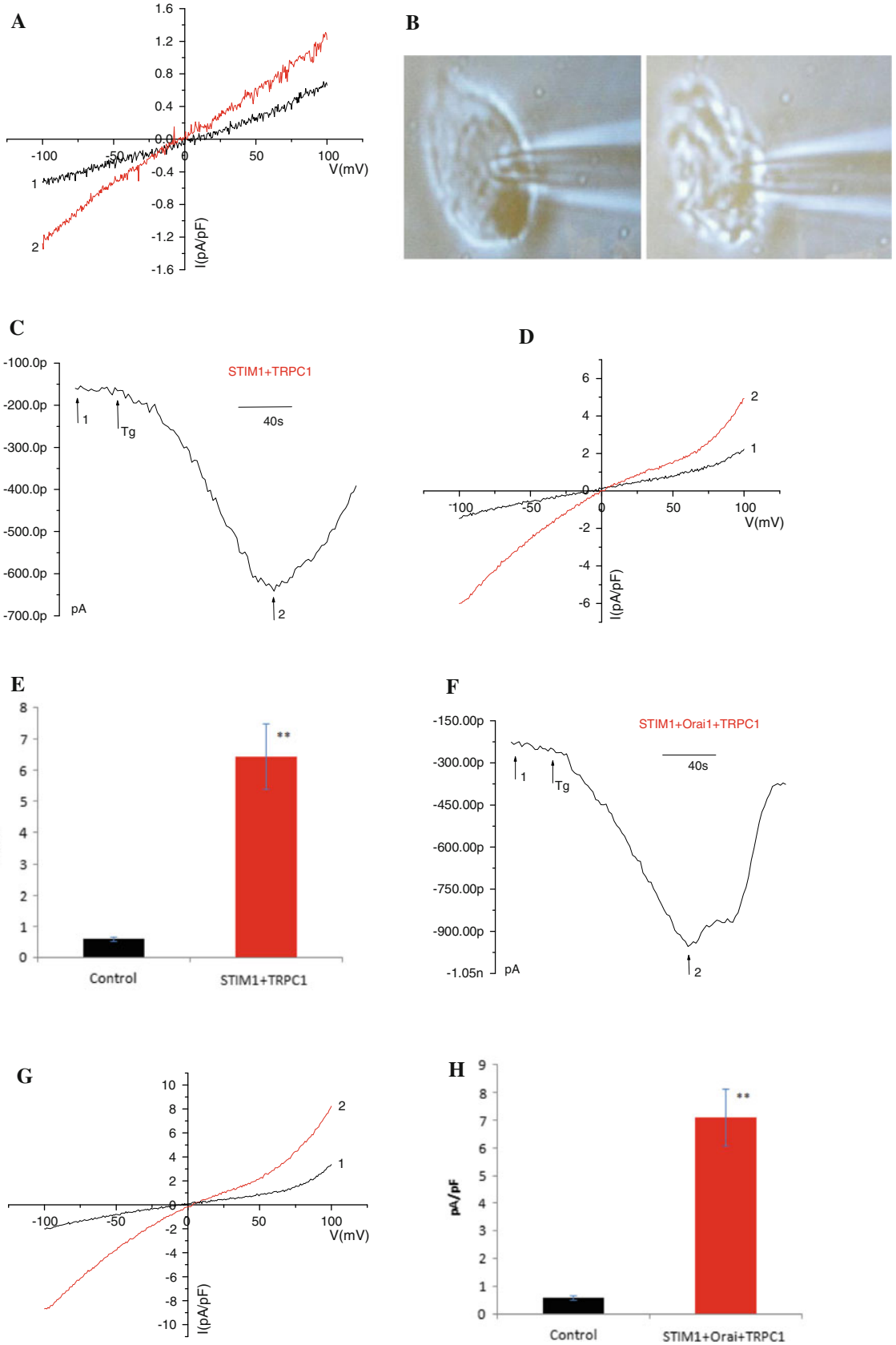
A
B
C
D
E
F
G
H
STIM1+TRPC1
40s
Tg
pA
V(mV)
I(pA/pF)
STIM1+Orai1+TRPC1
Control
STIM1+TRPC1
STIM1+Orai1+TRPC1
pA/pF
**

Table 2 Maximal current densities for I_{SOC} at −100 mV in control and transfected HL-7702 cells (pA/pF)

	Control cells	STIM1 + TRPC1 transfected cells (**)	STIM1 + Orai1 + TRPC1 transfected cells (**)
1	0.554	6.857	8.682
2	0.681	5.602	6.296
3	0.717	5.915	6.814
4	0.533	6.041	7.304
5	0.595	5.932	5.888
6	0.614	8.419	7.708

** $p < 0.01$ versus control cells group

approximate 2.5-fold increase in peak current density of I_{SOC} was caused by TRPC1 overexpression. Single cell RT-PCR experiments were performed to confirm that the expression levels of TRPC1 were elevated by transfection in the patched cells. Figure 4b shows the images of a cell after electrophysiological recording before (left) and after (right) a suction was applied to extract the cell content. The shrinkage of cell membrane was recognized as successful harvesting of cell cytoplasm.

Compared to TRPC1 overexpression alone, co-overexpression of STIM1 and TRPC1 induced a further increase in I_{SOC}. The time course in current development at −100 mV in Fig. 4c and I–V curves in Fig. 4d show representative traces of I_{SOC} developed in cells co-transfected with TRPC1 and STIM1. An approximate 10-fold increase in I_{SOC} was obtained by the co-expression of TRPC1 + STIM1. Notably, no further increase in I_{SOC} was detected with the addition of Orai1. In cells that co-expressed STIM1, Orai1 and TRPC1, the time course of I_{SOC} development and the shape of the I–V curves at the peak current are not different from those that co-expressed STIM1 and TRPC1 (Fig. 4f, g). The peak current density of the triple expressing cells is also similar to cells that expressed STIM1 and TRPC1 (Fig. 4h; Table 2). For all cells, we also carried out single cell RT-PCR experiments after the electrophysiological recording to confirm that mRNAs for the three proteins were indeed overexpressed in the patched cells (Fig. 2h). Peak current densities at −100 mV for individual cells are listed in Table 2.

Discussion

The present study provides the first direct evidence that functional interactions among STIM1, Orai1 and TRPC1 activate SOCs in HL-7702 cells. This was indicated by the inhibitory effects of SOC inhibitors and the enhancement of I_{SOC} in cells that overexpressed the three proteins. With a near zero reversal potential, I_{SOC} recorded in this study is not identical to the traditional I_{CRAC}. Furthermore, co-overexpression of the STIM1 and Orai1 proteins resulted in an increase in I_{SOC} not only at negative but also positive potentials. It is possible that the outward currents at the positive potentials represent activation of Ca^{2+}-dependent Cl^- channels, which had been found in rat liver cells (Arornataris et al. 2006).

It was reported that the overexpression of STIM1 alone caused a zero-to-modest increase of I_{CRAC} in HEK293 and Jurkat cells, whereas Orai1 overexpression alone did not affect I_{CRAC} induced by store depletion in HEK293 cells, and instead, caused a small reduction in I_{CRAC} in Jurkat cells (Roos et al. 2005; Cheng et al. 2008; Spassova et al. 2006). Our findings in HL-7702 cells are consistent with these previous results, indicating that STIM1 or Orai1 alone was either insufficient to generate large I_{SOC} or they are stoichiometrically linked limiting each others' ability to generate an increased I_{SOC} (Peinelt et al. 2006). Among all TRP channels, evidence for TRPC1 to form SOCs is perhaps the strongest. It has been shown that functional expression of human TRPC1 resulted in a modest increase in Tg-evoked Ca^{2+} influx in COS cells (Zhu et al. 1996) and HEK293 cells (Cheng et al. 2008). In the present study, the experimental results also show association of TRPC1 overexpression with an increase of I_{SOC} in HL-7702 cells. This supports the view that TRPC1 is involved in the formation of SOCs. A possible mechanism to account for this (as shown in Fig. 5) is that SOCs in the plasma membrane consist of TRPC1 and other regulatory subunits (including Orai1); store depletion causes STIM1 aggregation and binding to either TRPC1 or a regulatory subunit, resulting in conformation change and channel opening.

There is also increasing evidence in several different cell types that co-overexpression of two proteins (STIM1 + Orai1 or STIM1 + TRPC1) or three proteins (STIM1 + Orai1 + TRPC1) leads to enormous I_{SOC}, which is significantly larger than the corresponding native currents evoked using the same experimental protocol (Ong et al. 2007; Peinelt et al. 2006; Cheng et al. 2008). This suggests that either Orai1 or TRPC1 may interact with STIM1 and respond to store depletion signal, and each may be sufficient to control the magnitude of I_{SOC}, although the coexistence of the three proteins is not detrimental to activate SOCE. This hypothesis is, however, distorted by the inconsistent data in the existing literature where the enhancement of I_{SOC} caused by co-overexpression is not fully equal in different experiments, even in the same type of cells (Li et al. 2007; Cheng et al. 2008). Presumably, variable transfection efficiencies may partly be responsible for the differences in the degree of I_{SOC} increase. This argues for the importance of maintaining the appropriate stoichiometry of all key proteins and factors in the regulation of SOCs.

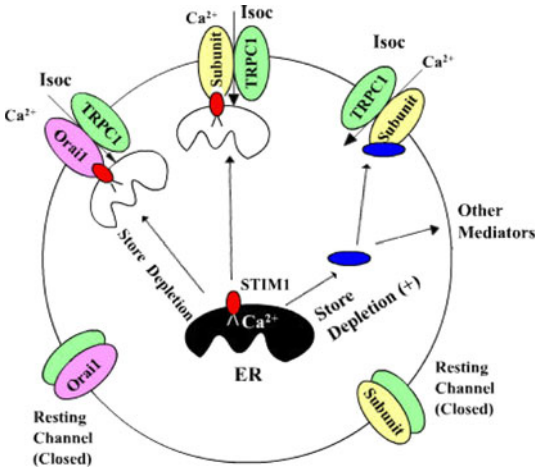

Fig. 5 A possible mechanism of the interplay among STIM1, Orai1 and TRPC1. TRPC1 is depicted as being stabilized in an inactive state by interacting with Orai1 or other subunits, e.g. TRPC4 and TRPC5, etc. Located in ER, STIM1 can interact with Orai1 and the undefined Subunit (may be TRPC4, TRPC5 or other proteins) directly. As shown in the left two pathways, when Ca^{2+} store is depleted, ER approaches plasma membrane and STIM1 aggregates with Orai1/Subunit. Conformation of the Ca^{2+} channels changes and I_{SOC} is elicited. It should be noted that aggregation of STIM1 + Orai1 is the main cause of activation of SOCs formed by Orai1 and TRPC1. Increasing any single one is not able to generate a large $I_{SOC,}$ whereas co-overexpression of STIM1 + Orai1 promotes Ca^{2+} entry obviously. On the other hand, despite coupling with STIM1, TRPC1-subunit channels have another opening mechanism. As described in the right pathway, store depletion is capable of inducing other mediators (may be IP_3, ATP or cGMP, etc.) to evoke $I_{SOC.}$ This maybe why store-operated Ca^{2+} currents were moderately enhanced when TRPC1 expression level was elevated alone, and enormous I_{SOC} emerged in the case of co-overexpression of STIM1 + TRPC1 or STIM1 + Orai1 + TRPC1

Taken together, STIM1, Orai1 and TRPC1 are all important in activating I_{SOC} and it can infer from our experimental findings that overexpression of STIM1 or Orai1 alone will not increase I_{SOC} while overexpression of TRPC1 will, co-overexpression of STIM1 + Orai1, STIM1 + TRPC1 or STIM1 + Orai1 + TRPC1 can all dramatically increase $I_{SOC.}$ Here we assume at least three distinct possible pathways of SOC activation in HL-7702 cells. The first pathway proposes the existence of a protein channel consisting of Orai1 and TRPC1, and although it is kept inactive at rest, once Ca^{2+} store is depleted, it is detected by the Ca^{2+} sensor in the ER and STIM1 will then couple to Orai1, promoting a change in conformation of SOCs to open the channels. The other two pathways are more complex. They support the channel is consisted of TRPC1 and another regulatory subunit (may be TRPC4, TRPC5 or other proteins), either STIM1 or other mediators (IP_3, ATP or cGMP, etc.) may interact with the subunit and activate SOCE. Thus, opening of Orai1-based SOCs highly depend on STIM1, whereas STIM1 is only one of the activators in the latter two pathways.

In conclusion, STIM1, Orai1 and TRPC1, the three proteins that regulate normal functions and pathological processes in liver cells since Ca^{2+} acts as an important second messenger. In wild type cells, variant Ca^{2+} channels constitute an equilibrious system and maintain physiological state. Under pathological conditions, the expression of the three proteins may change and thereby impairs the intracellular Ca^{2+} concentration regulation and hence the functions of hepatocytes. Thus, STIM1, Orai1 and TRPC1 may be new targets for disease prevention or therapy of the ion channel disorders or channelopathies of liver in clinic.

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References

Albert AP, Saleh SN, Peppiatt-Wildman CM, Large WA (2007) Multiple activation mechanisms of store-operated TRPC channels in smooth muscle cells. J Physiol 583:25–36

Aromataris EC, Roberts ML, Barritt GJ, Rychkov GY (2006) Glucagon activates Ca^{2+} and Cl^- channels in rat hepatocytes. J Physiol 573:611–625

Aromataris EC, Castro J, Rychkov GY, Barritt GJ (2008) Store-operated Ca^{2+} channels and Stromal Interaction Molecule 1 (STIM1) are targets for the actions of bile acids on liver cells. Biochim Biophys Acta 1783:874–885

Auld A, Chen J, Brereton HM, Wang YJ, Gregory RB, Barritt GJ (2000) Store-operated Ca^{2+} inflow in Reuber hepatoma cells is inhibited by voltage-operated Ca^{2+} channel antagonists and, in contrast to freshly isolated hepatocytes, does not require a pertussis toxin-sensitive trimeric GTP-binding protein. Biochim Biophys Acta 1497:11–26

Barritt GJ (1999) Receptor activated Ca^{2+} inflow in animal cells: a variety of pathways tailored to meet different intracellular Ca^{2+} signaling requirements. Biochem J 337:153–169

Barritt GJ, Parker JC, Wadsworth JC (1981) A kinetic analysis of the effects of adrenaline on calcium distribution in isolated rat liver parenchymal cells. J Physiol 312:29–55

Barritt GJ, Litjens TL, Castro J, Aromataris E, Rychkov GY (2009) Store-operated Ca^{2+} channels and microdomains of Ca^{2+} in liver cells. Clin Exp Pharmacol Physiol 36:77–83

Brereton HM, Chen J, Rychkov G, Harland ML, Barritt GJ (2001) Maitotoxin activates an endogenous non-selective cation channel and is an effective initiator of the activation of the heterologously expressed hTRPC-1 (transient receptor potential) non-selective cation channel in H4-IIE liver cells. Biochim Biophys Acta 1540:107–126

Chen J, Barritt GJ (2003) Evidence that TRPC1 (transient receptor potential canonical 1) forms a Ca^{2+}-permeable channel linked to the regulation of cell volume in liver cells obtained using small

interfering RNA targeted against TRPC1. Biochem J 373:327–336

Cheng KT, Liu X, Ong HL, Ambudkar IS (2008) Functional requirement for Orai1 in store-operated TRPC1-STIM1 channels. J Biol Chem 283:12935–12940

El Boustany C, Bidaux G, Enfissi A, Delcourt P, Prevarskaya N, Capiod T (2008) Capacitative calcium entry and transient receptor potential canonical 6 expression control human hepatoma cell proliferation. Hepatology 47:2068–2077

Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A (2006) A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 441:179–185

Gaspers LD, Thomas AP (2005) Calcium signaling in liver. Cell Calcium 38:329–342

Graf J, Häussinger D (1996) Ion transport in hepatocytes: mechanisms and correlations to cell volume, hormone actions and metabolism. J Hepatol 24(Suppl 1):53–77

Huang GN, Zeng W, Kim JY, Yuan JP, Han L, Muallem S, Worley PF (2006) STIM1 carboxyl-terminus activates native SOC, I(crac) and TRPC1 channels. Nat Cell Biol 8:1003–1010

Jardin I, Lopez JJ, Salido GM, Rosado JA (2008) Orai1 mediates the interaction between STIM1 and hTRPC1 and regulates the mode of activation of hTRPC1-forming Ca^{2+} channels. J Biol Chem 283:25296–25304

Jiang JL, Yu MK, Chen ZN, Chan HC (2001) cGMP-regulated store-operated calcium entry in human hepatoma cells. Cell Biol Int 25:993–995

Li Z, Lu J, Xu P, Xie X, Chen L, Xu T (2007) Mapping the interacting domains of STIM1 and Orai1 in Ca^{2+} release-activated Ca^{2+} channel activation. J Biol Chem 282:29448–29456

Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, Meyer T (2005) STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion triggered Ca^{2+} influx. Curr Biol 15:1235–1241

Nieuwenhuijs VB, De Bruijn MT, Padbury RT, Barritt GJ (2006) Hepatic ischemia-reperfusion injury: roles of Ca^{2+} and other intracellular mediators of impaired bile flow and hepatocyte damage. Dig Dis Sci 51:1087–1102

Ong HL, Cheng KT, Liu X, Bandyopadhyay BC, Paria BC, Soboloff J, Pani B, Gwack Y, Srikanth S, Singh BB, Gill DL, Ambudkar IS (2007) Dynamic assembly of TRPC1-STIM1-Orai1 ternary complex is involved in store-operated calcium influx. Evidence for similarities in store-operated and calcium release-activated calcium channel components. J Biol Chem 282:9105–9116

Parekh AB (2006) On the activation mechanism of store-operated calcium channels. Pflugers Arch 453:303–311

Parekh AB, Putney JW Jr (2005) Store-operated calcium channels. Physiol Rev 85:757–810

Pedersen SF, Owsianik G, Nilius B (2005) TRP channels: an overview. Cell Calcium 38:233–252

Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, Lis A, Fleig A, Penner R, Kinet JP (2006) Amplification of CRAC current by STIM1 and CRACM1 (Orai1). Nat Cell Biol 8:771–773

Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG (2006) Orai1 is an essential pore subunit of the CRAC channel. Nature 443:230–233

Putney JW Jr (1986) A model for receptor-regulated calcium entry. Cell Calcium 7:1–12

Rabe H, Ritz HJ, Jeserich G (1998) Voltage-gated potassium channels of schwann cells from trout lateral line nerve: a combined electrophysiological and molecular characterization. Gila 23:329–338

Rao JN, Platoshyn O, Golovina VA, Liu L, Zou T, Marasa BS, Turner DJ, Yuan JX, Wang JY (2006) TRPC1 functions as a store-operated Ca^{2+} channel in intestinal epithelial cells and regulates early mucosal restitution after wounding. Am J Physiol Gastrointest Liver Physiol 290:G782–G792

Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Veliçelebi G, Stauderman KA (2005) STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. J Cell Biol 69:435–445

Rychkov G, Brereton HM, Harland ML, Barritt GJ (2001) Plasma membrane Ca^{2+} release-activated Ca^{2+} channels with a high selectivity for Ca^{2+} identified by patch-clamp recording in rat liver cells. Hepatology 33:938–947

Saleh SN, Albert AP, Peppiatt CM, Large WA (2006) Angiotensin II activates two cation conductances with distinct TRPC1 and TRPC6 channel properties in rabbit mesenteric artery myocytes. J Physiol 577:479–495

Saleh SN, Albert AP, Peppiatt-Wildman CM, Large WA (2008) Diverse properties of store-operated TRPC channels activated by protein kinase C in vascular myocytes. J Physiol 586:2463–2476

Smyth JT, Dehaven WI, Jones BF, Mercer JC, Trebak M, Vazquez G, Putney JW Jr (2006) Emerging perspectives in store-operated Ca^{2+} entry: roles of Orai, Stim and TRP. Biochim Biophys Acta 1763:1147–1160

Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL (2006) Orai1 and STIM reconstitute store-operated calcium channel function. J Biol Chem 281:20661–20665

Spassova MA, Soboloff J, He LP, Xu W, Dziadek MA, Gill DL (2006) STIM1 has a plasma membrane role in the activation of store-operated Ca^{2+} channels. Proc Natl Acad Sci USA 103:4040–4045

Takahashi Y, Watanabe H, Murakami M, Ohba T, Radovanovic M, Ono K, Iijima T, Ito H (2007) Involvement of transient receptor potential canonical 1 (TRPC1) in angiotensin II-induced vascular smooth muscle cell hypertrophy. Atherosclerosis 195:287–296

Teoh NC, Farrell GC (2003) Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. J Gastroenterol Hepatol 18:891–902

Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP (2006) CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. Science 312:1220–1223

Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Stauderman KA, Cahalan MD (2005) STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane. Nature 437:902–905

Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, Roos J, Stauderman KA, Cahalan MD (2006) Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity. Proc Natl Acad Sci USA 103:9357–9362

Zhu X, Jiang M, Peyton M, Boulay G, Hurst R, Stefani E, Birnbaumer L (1996) Trp, a novel mammalian gene family essential for agonist-activated capacitative Ca^{2+} entry. Cell 85:661–671