

1-1-2016

TRPC1 ion channel gene regulates store-operated calcium entry and proliferation in human aortic smooth muscle cells

YASEMİN ERAC

CIGDEM SELLI

METİNER TOSUN

Follow this and additional works at: <https://journals.tubitak.gov.tr/biology>



Part of the [Biology Commons](#)

Recommended Citation

ERAC, YASEMİN; SELLI, CIGDEM; and TOSUN, METİNER (2016) "TRPC1 ion channel gene regulates store-operated calcium entry and proliferation in human aortic smooth muscle cells," *Turkish Journal of Biology*. Vol. 40: No. 6, Article 24. <https://doi.org/10.3906/biy-1602-57>
Available at: <https://journals.tubitak.gov.tr/biology/vol40/iss6/24>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Biology by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

TRPC1 ion channel gene regulates store-operated calcium entry and proliferation in human aortic smooth muscle cells

Yasemin ERAÇ¹, Çiğdem SELLİ^{1,2,*}, Metiner TOSUN^{1,3}

¹Department of Pharmacology, Faculty of Pharmacy, Ege University, İzmir, Turkey

²Applied Bioinformatics of Cancer, Edinburgh Cancer Research Centre, Institute of Genetics and Molecular Medicine, Edinburgh, UK

³Faculty of Medicine, İzmir University of Economics, İzmir, Turkey

Received: 15.02.2016 • Accepted/Published Online: 07.05.2016 • Final Version: 16.12.2016

Abstract: This study investigates whether the reciprocal changes in transient receptor potential canonical (TRPC)1 and TRPC6 expressions, which have previously been observed in aging rat aorta, are functional in store-operated calcium (SOC) entry and proliferation in human vascular smooth muscle cells. TRPC1 levels were modulated via silencing and overexpression vectors in human primary aortic smooth muscle cells. Following *TRPC1* gene modulation, TRPC1 and TRPC6 expression levels were measured using quantitative real-time RT-PCR. In functional analyses, real-time changes in intracellular calcium levels and cell proliferation were determined. Microarray analysis was performed to identify genes associated with functional alterations following TRPC1 silencing. TRPC1 expression was significantly increased in *TRPC1*-overexpressing cells and inhibited in *TRPC1*-silenced cells, as expected. TRPC6 expression was significantly decreased in *TRPC1*-overexpressing cells but not affected by *TRPC1* silencing. SOC entry was significantly enhanced in *TRPC1*-silenced cells but not altered by *TRPC1*-overexpression. Furthermore, cell proliferation was correlated with changes in TRPC1 expression. Microarray analysis revealed that cell cycle-associated genes were significantly differentially expressed in *TRPC1*-silenced cells. In addition, STIM1 levels were downregulated significantly following *TRPC1* silencing. Data suggest that TRPC1 has a functional role in SOC entry regulation as well as in human aortic smooth muscle cell proliferation.

Key words: Aorta, store-operated calcium, vascular smooth muscle, TRPC

1. Introduction

The transient receptor potential (TRP) family, a large group of ion channels, was initially discovered in *Drosophila melanogaster* and has been shown to be involved in phototransduction (Cosens and Perry, 1972). Mammalian TRP channels are divided into six subfamilies, TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPP (polycystin), and TRPML (mucolipin), based on amino acid homologies (Montell, 2005). They have been shown to take part in a wide range of physiological processes such as cold sensation (Peier et al., 2002) and vascular cell functions (Dietrich et al., 2010), as well as in pathological conditions such as cardiovascular diseases (Nilius et al., 2007). The calcium-permeable TRPC channel subfamily has seven members, which are activated by agonist-induced inositol-3-phosphate (IP₃) formation. TRPC1 and TRPC6 have been shown to be expressed in vascular smooth muscle (VSM) cells and regulate VSM cell contraction and proliferation (Yu et al., 2003; Kumar et al., 2006). It was reported that TRPC6 may function as an essential component of α_1 -adrenoceptor-activated

nonselective cation channels (Inoue et al., 2001). TRPC6 has also been related to pulmonary artery smooth muscle cell proliferation (Yu et al., 2004) and chronic hypoxic pulmonary hypertension (Lin et al., 2004).

Store-operated Ca²⁺ (SOC) entry is activated following the depletion of the sarcoplasmic reticulum (SR) by IP₃, allowing SR Ca²⁺ refilling, which is essential for cellular processes such as cell growth and proliferation (Leung et al., 2008). Although its functional role in SOC entry is still controversial, TRPC1 has been reported to be a molecular component of SOC entry (Ambudkar et al., 2007). Aside from TRPC1, STIM1, a Ca²⁺ sensor located in the SR membrane (Collins et al., 2013), and a transmembrane channel protein Orai1 (Cheng et al., 2011), have also been suggested to be critical components of SOC entry. Orai1 also modulates SOC entry by modulation and recruitment of ion channels to the plasma membrane (Cheng et al., 2011). Local Ca²⁺ elevations via Orai1 have been shown to be required in the activation of TRPC1 following Ca²⁺ store depletion, suggesting the importance of Orai1 in a variety of cell functions initiated by SOC entry (Cheng et

* Correspondence: cigdemselli@gmail.com

al., 2011). Furthermore, Orai1 was proposed to be essential for activation of TRPC1/STIM channels (De Souza and Ambudkar, 2014). Orai1- and STIM1-mediated SOC entry was also suggested to be critical for angiotensin-induced VSM cell proliferation, suggesting their role in cardiovascular diseases (Guo et al., 2012). The role of STIM1/Orai1-mediated SOC entry in cardiac function and pathology was recently reviewed (Collins et al., 2013).

We previously showed that TRPC1 and TRPC6 expression levels were downregulated and upregulated, respectively, in rat thoracic aorta during aging (Erac et al., 2010). In addition, TRPC6 expression and SOC entry were enhanced in *TRPC1*-silenced A7r5 embryonic rat thoracic aortic smooth muscle cells, suggesting the regulatory role of TRPC1 in SOC entry in rat VSM cells (Selli et al., 2009). Furthermore, silencing of TRPC1 has been shown to regulate both SOC entry and cell proliferation in hepatocellular carcinoma cells (Selli et al., 2015).

Based on this, in the present study, we further investigated the functional role of TRPC1 and TRPC6 in the regulation of SOC entry and proliferation of human VSM cells. For this purpose, TRPC1 and TRPC6 expression along with SOC entry and cell proliferation were monitored in *TRPC1*-silenced and *TRPC1*-overexpressing primary human VSM cells.

2. Materials and methods

2.1. Cell culture

Primary human aortic smooth muscle cells (Invitrogen, C-007-5C) were cultured in Medium 231 (Invitrogen) supplemented with smooth muscle growth supplement (Invitrogen). When they reached 70% confluency, cells were subcultured (1:2) using 0.5% trypsin-EDTA (GIBCO). The cell phenotype was confirmed by alpha-actin staining. Cells at passages 3–6 were used in expression as well as functional analyses.

2.2. TRPC1 silencing and overexpression

A detailed protocol for the construction of the TRPC1 silencing vector (pSUPERIOR.shTRPC1) was recently published (Selli et al., 2015). For TRPC1 silencing, cells were transfected with 2 µg of pSUPERIOR.shTRPC1 and an empty vector as a negative control via electroporation (Neon, Invitrogen) with 2 pulses at 1350 V for 20 ms. Cells were transfected with 0.5 µg of pCMV6-AC-GFP-TRPC1 (Origene) and an empty vector as a negative control using the same electroporation protocol to overexpress TRPC1. Optimal electroporation conditions including vector concentrations were determined in preliminary experiments. Transfection efficiencies were determined by monitoring the GFP signal using fluorescence microscopy (IX71, Olympus). Cells with transfection efficiency over 70% were used in further gene expression and functional analysis.

2.3. Quantitative real-time RT-PCR

Following 48 h of vector incubation, TRPC1 and TRPC6 expressions were measured by quantitative real-time RT-PCR. The High Pure RNA Isolation Kit (Roche Applied Science) was used to isolate total RNA and reverse transcription was performed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Applied Science). To determine the expression levels of TRPC1 and TRPC6, quantitative real-time RT-PCR experiments were performed using the FastStart DNA Master SYBR Green I Kit and LightCycler 1.5 (Roche Applied Science). Primer sequences are given in the Table. PCR product sizes were confirmed by agarose gel electrophoresis. A serially diluted standard 18S rRNA cDNA-containing plasmid with known copy number was used with each PCR to determine the linear regression. All expression levels were normalized to that of internal 18S rRNA ($[TRPCx] / [18S\ rRNA] \times 10^4$).

Table. Oligonucleotide sequences of RT-PCR primers and amplicon sizes.

| Target (Accession no.) | | Sequence (5'-3') | Amplicon size |
|------------------------------|----|--------------------------|---------------|
| <i>hTRPC1</i> (NM_003304) | F: | TGCGACAAGGGTGACTATTA | 176 bp |
| | R: | TCCATTAGTTTCTGACAACCG | |
| <i>hTRPC6</i> (NM_004621) | F: | GATATCTTCAAATTCATGGTCATA | 317 bp |
| | R: | ATCCGCATCATCCTCAATTTC | |
| <i>h18S rRNA</i> (NR_003286) | F: | CGACGACCCATTTCGAACGTCT | 312 bp |
| | R: | GCTATTGGAGCTGGAATTACCG | |

F: forward; R: reverse; bp: base pair.

2.4. Intracellular Ca²⁺ measurements

Cells were grown on round glass coverslips and imaging was performed at 72 h after vector transfection, a time-point at which a significant decrease in TRPC1 protein levels was observed in our previous studies (Selli et al., 2015). Changes in intracellular Ca²⁺ levels were monitored indirectly using a dual wavelength front surface spectrofluorometer (PTI QM8 / 2005) as described previously (Selli et al., 2009). Briefly, cells were incubated in HEPES-buffered saline with 2.5 µM Fura-2 (Molecular Probes). Fluorescence emission at 510 nm was monitored with excitation at 340 and 380 nm and changes in intracellular Ca²⁺ levels were expressed as ratiometric changes in emission intensity at 510 nm ratio (340/380). Background fluorescence was determined at the end of the experiment by quenching the Fura-2 fluorescence with MnCl₂ (5 mM) in the presence of 10 µM ionomycin in Ca²⁺-free solution containing 2 mM EGTA. Cyclopiazonic acid (CPA) at a 10 µM concentration that depletes SR-stored Ca²⁺ was used to activate SOC entry. Cells were exposed to Ca²⁺-free solution, and then CPA was applied. Following a transient increase in Ca²⁺ levels due to store depletion, Ca²⁺ was readded to initiate SOC entry.

2.5. Real-time analysis of cell proliferation

Changes in cell proliferation were monitored using a real-time cellular analysis system (xCELLigence, Roche Applied Science) using 96-well microtiter plates (E-Plate 96, Roche Applied Science). Following 48 h of vector incubation, 5000 cells/well were seeded into the E-plate 96. Cell proliferation was monitored every 30 min for 96 h and cells were fed with 200 µL/well fresh medium every 48 h. Data were expressed as “cell index”, defined as $(R_n - R_b)/15$, where R_b is the background impedance and R_n is the impedance of the well with cells. Negative control groups (wells containing 200 µL of culture medium without cells with cell index values around zero) were tested in every experiment; however, they are not shown in the figures in order to simplify the representations.

2.6. Microarray experiments

Total RNA (500 ng), isolated using the High Pure RNA Isolation Kit (Roche Applied Science), was amplified and labeled with biotin using the Illumina Total Prep RNA Amplification Kit (Ambion). Biotinylated cRNA (750 ng) was hybridized at 58 °C for 16 h to HumanHT-12 v4 expression BeadChip (Direct Hybridization Assay Kit, Illumina). BeadChip was washed, blocked, and scanned using the Illumina BeadArray Reader, and Cy3 signal intensity was measured.

Data quality was assessed using GenomeStudio; all system control values were within the expected ranges. Background fluorescence representing signals from nonspecific dye binding and cross-hybridization was subtracted from all other probe intensities using

GenomeStudio. The R and BioConductor packages were used for analysis. Following quantile normalization using *lumi*, rank product analysis (Breitling et al., 2004) was performed using the RankProd package to determine differentially expressed genes. Pathway analysis was performed using DAVID Bioinformatics Resources 6.7 (Functional Annotation Clustering) (Huang et al., 2009). Raw and processed microarray data were submitted to the GEO database (GSE77618).

2.7. Statistical analysis

Data are expressed as mean ± standard error of the mean. “n” represents the number of samples. Statistical significance between the means of two groups was evaluated using Student’s t-test. $P \leq 0.05$ was considered significant. Data analyses and graphical presentations were performed using GraphPad Prism 5.

3. Results

3.1. TRPC expression following *TRPC1* silencing and overexpression

Based on the quantitative real-time RT-PCR analyses, both TRPC1 and TRPC6 were shown to be expressed in primary human VSM cells. *TRPC1* silencing decreased TRPC1 mRNA levels significantly (46%, $P \leq 0.05$, $n = 4$) without affecting the mRNA levels of TRPC6 (Figure 1A). In *TRPC1*-overexpressing cells, TRPC1 mRNA levels increased drastically by over 2000-fold ($P \leq 0.01$, $n = 7-8$, Figure 1B) while TRPC6 levels changed reciprocally, with a 41% decrease ($P \leq 0.05$, $n = 4$, Figure 1B).

3.2. Changes in intracellular Ca²⁺ levels following *TRPC1* gene modulation

At 72 h following vector transfection, real-time changes in intracellular Ca²⁺ levels were monitored in cells grown on polystyrene coverslips and incubated with Fura-2. In *TRPC1*-silenced cells, SOC entry induced by CPA was enhanced significantly ($P \leq 0.05$, $n = 9-14$) without a change in SR Ca²⁺ release (Figure 2A). Both CPA-induced SR Ca²⁺ release and SOC entry did not alter in *TRPC1*-overexpressing cells ($n = 8-9$, Figure 2B).

3.3. Cell proliferation following *TRPC1* gene modulation

Proliferation curves at five different seeding densities (20,000–1250 cells/well) were monitored to determine the optimum cell density before examining the effects of *TRPC1* gene modulation. Cell index values increased proportionally to cell density as expected (not shown). Real-time changes in proliferation were monitored at optimum seeding density (5000 cells/well) in *TRPC1*-silenced and *TRPC1*-overexpressing cells. *TRPC1* silencing significantly inhibited the proliferation of human VSM cells for 96 h compared to control cells transfected with the empty vector ($P \leq 0.05$, $P \leq 0.01$; $n = 5-8$, Figure 3A). In *TRPC1*-overexpressing cells, proliferation rates were

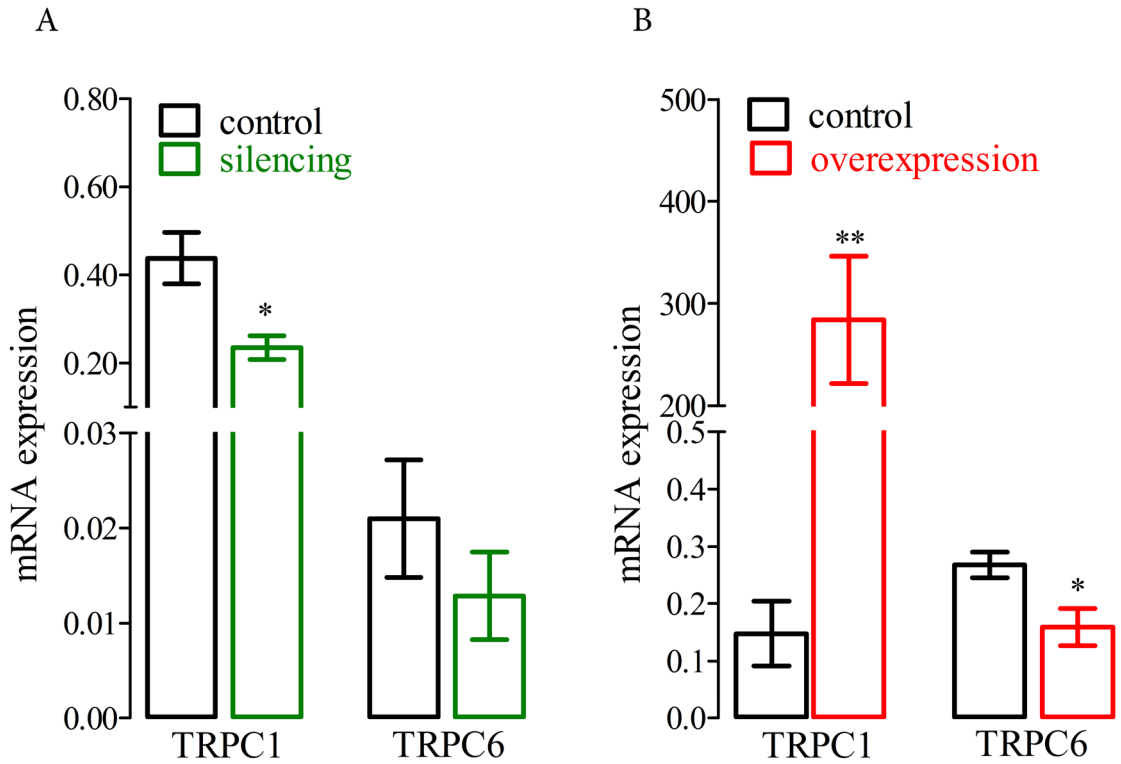


Figure 1. The effects of *TRPC1* gene modulation on TRPC expression in human vascular smooth muscle cells. Expression levels, determined 48 h following vector transfection, were normalized to that of internal 18S rRNA ($TRPC/18S\ rRNA\ mRNA \times 10^4$). TRPC1 and TRPC6 mRNA expression in *TRPC1*-silenced (A) and -overexpressing (B) cells are shown (* $P \leq 0.05$; ** $P \leq 0.01$; $n = 4-8$).

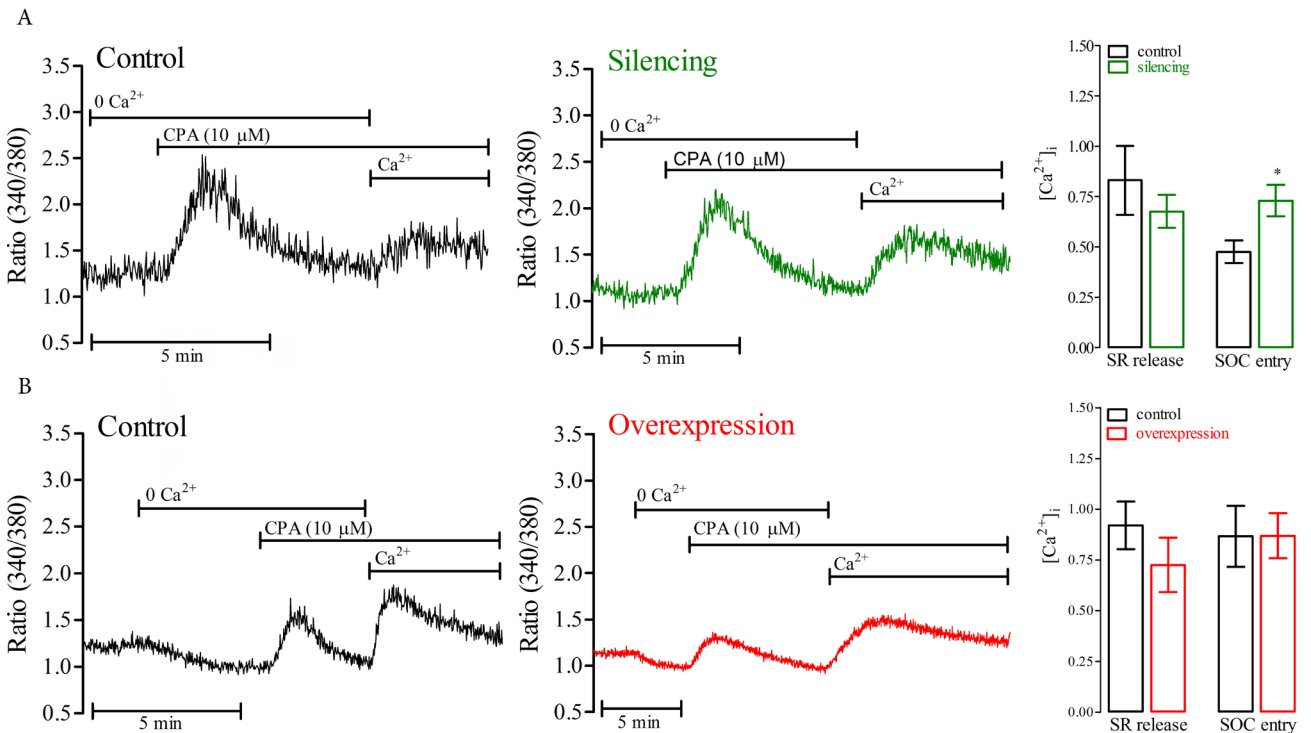
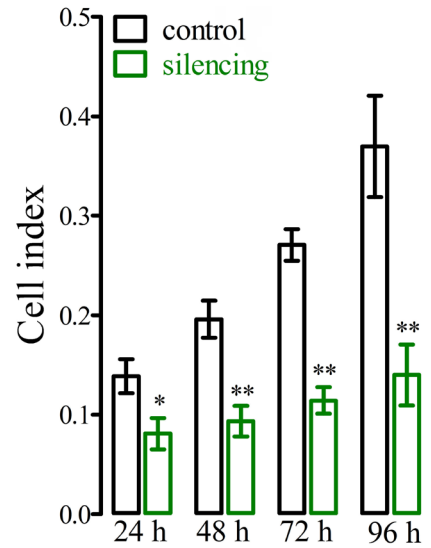
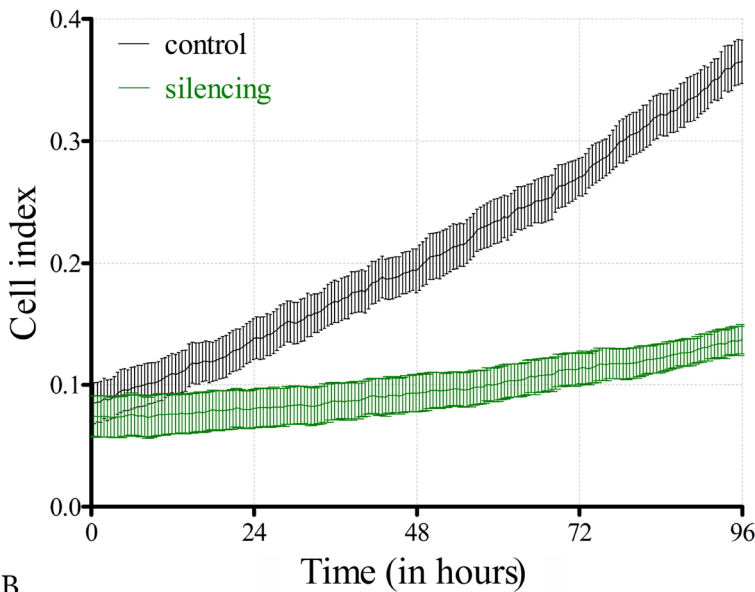


Figure 2. The effects of *TRPC1* gene modulation on intracellular Ca^{2+} elevations in human vascular smooth muscle cells. Changes in intracellular Ca^{2+} were monitored in Fura-2 loaded cells 72 h following vector transfection. CPA-induced Ca^{2+} elevations and the cumulative data of SR-released Ca^{2+} and SOC entry in *TRPC1*-silenced (A, $n = 9-14$) and -overexpressing (B, $n = 8-9$) cells are shown (* $P \leq 0.05$).

A



B

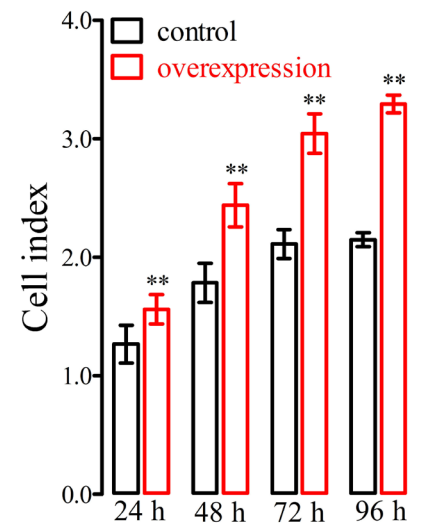
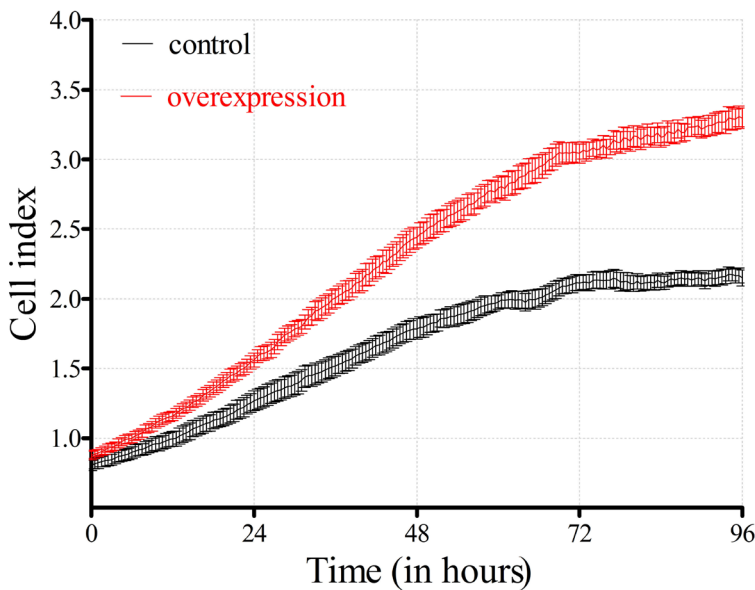


Figure 3. The effects of *TRPC1* gene modulation on the proliferation of human vascular smooth muscle cells. At 48 h following vector transfection, 5000 cells/well were seeded on the E-plate 96 and real-time cell proliferation was monitored in real time for 4 days. The proliferation curves and the cumulative data in *TRPC1*-silenced (A) and -overexpressing (B) cells are shown (* $P \leq 0.05$, ** $P \leq 0.01$; n = 5–8).

enhanced significantly throughout the monitored period ($P \leq 0.01$; n = 5–8, Figure 3B).

3.4. Differential gene expression following *TRPC1* silencing

Differentially expressed genes between *TRPC1*-silenced and parental cells were determined using rank product analysis (FDR, 5%). A total of 206 genes were significantly differentially regulated (61 up and 145 down) between

TRPC1-silenced and control cells. Proliferation-associated genes in the differentially expressed gene list were further examined. A total of 10 cell cycle-associated genes (*DSN1*, *E2F2*, *RAB11B*, *TRNP1*, *SKA1*, *CCNF*, *KILLIN*, *KIFC1*, *NCAPG*, and *PARD3*) were classified into a functional subgroup following enrichment analysis ($P = 0.0016$, fold enrichment = 3.7, FDR = 2%).

3.5. Microarray expression of SOC entry-related genes following *TRPC1* silencing

The TRPC family and other SOC entry-related genes were not among the differentially expressed genes. Therefore, we further analyzed the changes in their expression. A heat map illustrating *TRPC1*, *STIM1*, and *ORAI* gene expression in control and *TRPC1*-silenced cells is shown in Figure 4A. *TRPC1* levels decreased significantly ($P \leq 0.05$, $n = 6$) in *TRPC1*-silenced cells compared to that of control, as expected (Figure 4B). Other TRPC family members failed to pass the microarray detection threshold. In addition, *STIM1* levels were downregulated significantly ($P \leq 0.05$, $n = 5$) following *TRPC1* silencing (Figure 2B).

4. Discussion

The present study provides evidence for the role of *TRPC1* in the regulation of SOC entry in human VSM cells and a possible causative relationship between altered *TRPC1*, *TRPC6*, and *STIM1* expressions. We also suggest that *TRPC1* regulates human VSM cell proliferation via alterations in cell cycle genes. *TRPC1* and *TRPC6* expressions have been previously shown to change reciprocally in aging rat aorta; *TRPC1* expression was downregulated along with *TRPC6* upregulation (Erac et al., 2010). Mimicking the reciprocal regulation of *TRPC1* and *TRPC6* ion channel genes in A7r5 rat VSM cells, RNA interference-mediated *TRPC1* knockdown significantly increased *TRPC6* protein expression and

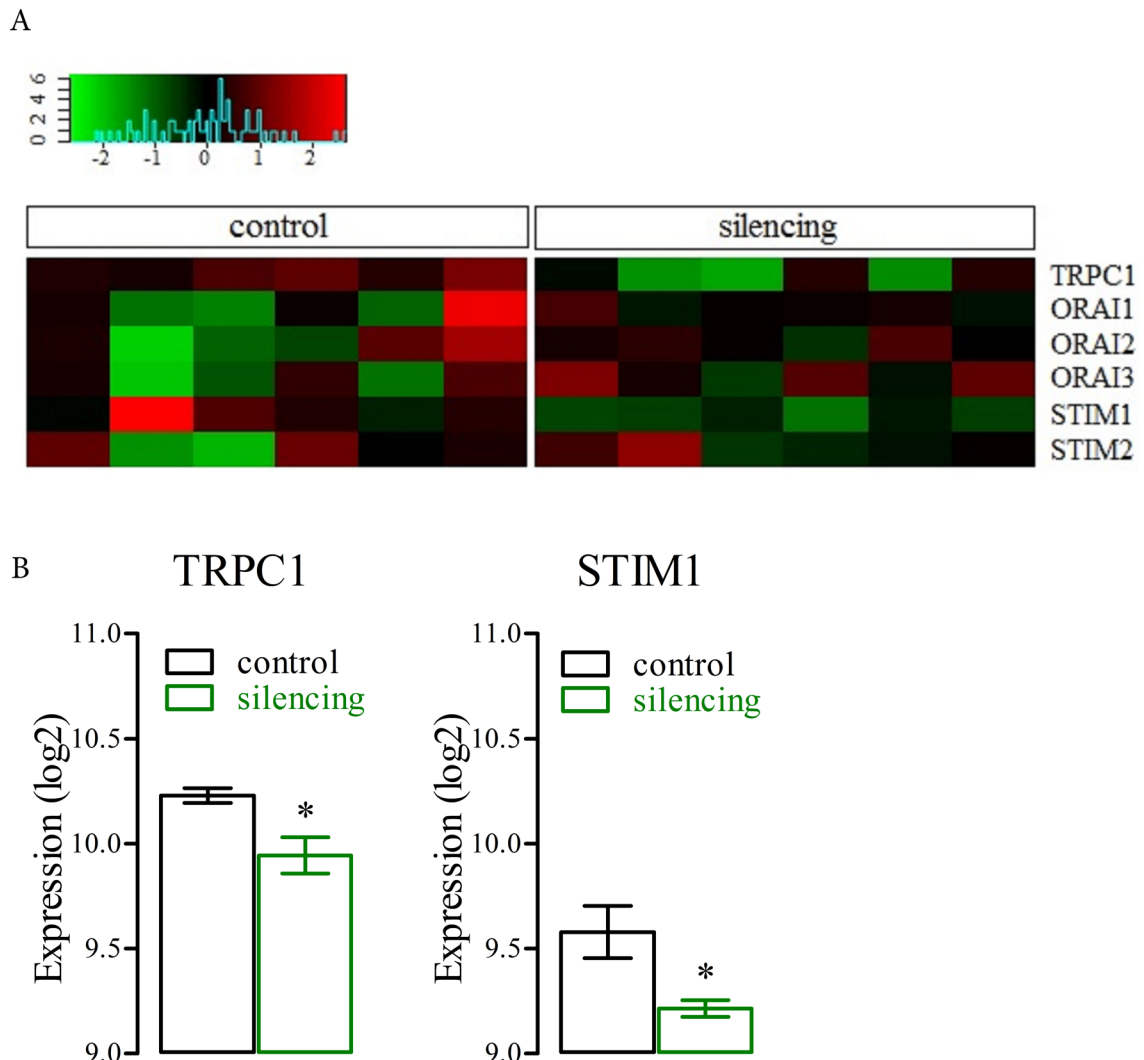


Figure 4. Changes in SOC entry-related genes following *TRPC1* silencing. (A) Heat map showing the SOC entry-related gene expression (log2 values) following *TRPC1* silencing. (B) Cumulative changes in *TRPC1* and *STIM1* expression in control and *TRPC1*-silenced (siTRPC1) cells (* $P \leq 0.05$; $n = 6$).

SOC entry in rat VSM cells, suggesting the regulatory role of TRPC1 in SOC entry (Selli et al., 2009). Furthermore, *TRPC1* downregulation significantly elevated SOC entry in Huh-7 hepatocellular carcinoma cells without altering TRPC6 levels (Selli et al., 2015). Similar to our previous results in rat VSM cells and hepatocellular carcinoma cells, elevation of SOC entry following *TRPC1* downregulation in the present study suggests the negative regulatory role of TRPC1 in SOC entry in primary human VSM cells. Furthermore, SOC entry remained unchanged in *TRPC1*-overexpressing cells, supporting the negative regulatory role of TRPC1 in heteromeric SOC channel composition. Lack of SOC entry upregulation may also be explained by poor localization of TRPC1 to the plasma membrane. Since Orai-mediated local Ca^{2+} entry has been shown to be essential for recruitment of TRPC1 to the plasma membrane (Cheng et al., 2011), overexpressed TRPC1 proteins may not be properly localized to membranes required for the formation of a functional SOC channel. A novel activation mechanism for TRPC1 through store depletion-induced phospholipase C beta 1 activity was recently reported in VSM cells (Shi et al., 2016). The potential negative regulatory role of TRPC1 in SOC entry was also recently reviewed by Dietrich et al. (2014). The negative regulatory role and the functional localization of TRPC1 in human VSM cells await further investigation using imaging techniques such as total internal reflection fluorescence.

TRPC1 mRNA expression has been shown to decrease along with TRPC6 upregulation during organ culture in cultured rat mesenteric artery (Tai et al., 2009).

In contrast to rat VSM cells (Selli et al., 2009) and similar to hepatocellular carcinoma cells (Selli et al., 2015), TRPC6 levels were unchanged in *TRPC1*-silenced human VSM cells (present study). However, TRPC6 levels were downregulated in *TRPC1*-overexpressing human VSM cells, confirming the reciprocal relationship between TRPC1 and TRPC6 (Selli et al., 2009; Erac et al., 2010).

The functional interaction between TRPC1 and STIM1 occurs within plasma membrane lipid raft domains and has been shown to be dynamically regulated by endoplasmic reticulum Ca^{2+} levels (Pani et al., 2008). TRPC1 appears to be operational in SOC entry by interacting with STIM1 in human coronary artery smooth muscle cells (Takahashi et al., 2007). The silencing of TRPC1 diminished STIM1-mediated SOC entry, suggesting the participation of TRPC1 in STIM1-mediated SOC entry, which was also associated with proliferation (Takahashi et al., 2007). In addition, increased levels of TRPC1 and STIM1 were suggested to participate in the modulation of cultured primary human aortic myocytes from the contractile to the proliferative phenotype (Berra-Romani et al., 2008). Downregulation of STIM1 in *TRPC1*-silenced VSM cells in the present study suggests a mutual interaction between

STIM1 and TRPC1 in the regulation of SOC entry and cell proliferation.

The present data further suggest the presence of a common transcriptional control mechanism over TRPC channels and STIM1, which may play a critical role in the regulation of intracellular Ca^{2+} homeostasis in excitable cells. A limitation of our study is the lack of protein expression data that would further support the findings. The changes in levels of other channel proteins and Ca^{2+} entry pathways in SOC entry upregulated human VSM cells need further investigation.

The role of TRPC1 in vascular injury and occlusive vascular diseases has been reported (Kumar et al., 2006). In addition, TRPC upregulation has been associated with increased pulmonary artery smooth muscle cell proliferation induced by chronic hypoxia (Malczyk et al., 2013), supporting its role in pulmonary vascular remodeling and hypoxia-induced pulmonary hypertension (Lin et al., 2004). Pathological alterations in TRPC channels and intracellular Ca^{2+} levels may be prevented by potential or conventional drugs. For example, phosphodiesterase type 5 inhibitor sildenafil has been shown to prevent endothelin-1-induced primary aortic smooth muscle cell proliferation and TRPC1 and TRPC6 upregulation (Wang et al., 2008). Furthermore, the upregulation of TRPC channels and SOC entry has been shown to be prevented by sildenafil in rat pulmonary arterial smooth muscle (Lu et al., 2010) and rat hypertrophied cardiomyocytes (Kiso et al., 2013). Significantly suppressed and enhanced proliferation rates observed in *TRPC1*-silenced and -overexpressing cells, respectively (present study), suggest the potential regulatory role of TRPC1 in human VSM cell proliferation. Microarray analysis showed alterations in cell cycle genes in *TRPC1*-silenced cells, further supporting the role of TRPC1 in cell proliferation.

In contrast to our results, SOC entry was shown to be significantly reduced along with cell growth inhibition in *TRPC1*-downregulated pulmonary artery smooth muscle cells (Sweeney et al., 2002). Since Ca^{2+} permeability was significantly decreased in TRPC1 containing heterotetrameric SOCE channels (Storch et al., 2012), tissue-specific TRPC expression levels may determine the SOC entry channel composition and Ca^{2+} permeability underlying this discrepancy. In addition, SOC entry has been shown to be at control levels during G1, upregulated during the S phase, and suppressed during mitosis (Tani et al., 2007). Regulation of both SOC entry and channel composition during different phases of the cell cycle may lead to these controversial findings. The roles of TRPC1 and SOC entry in VSM cell proliferation require further investigation, such as analysis of cell cycle distribution and measuring of newly synthesized DNA strands of proliferating cells.

In conclusion, this study provides evidence for the negative regulatory role of TRPC1 in SOC entry in human VSM cells. Our results showed for the first time that TRPC1 may be involved in the proliferation of human VSM cells. The causative relationship between altered TRPC1, TRPC6, and STIM1 expression and SOC entry and VSM cell proliferation requires further investigation using cardiovascular disease models. Moreover, alterations in cell cycle genes may contribute to TRPC1 and SOC entry-mediated regulation of cell proliferation. The functional link between the proposed cellular processes and TRPC channels awaits further investigation.

Acknowledgments

This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK, 110S096 to MT),

References

- Ambudkar IS, Ong HL, Liu X, Bandyopadhyay B, Cheng KT (2007). TRPC1: The link between functionally distinct store-operated calcium channels. *Cell Calcium* 44: 213-223.
- Berra-Romani R, Mazzocco-Spezia A, Pulina MV, Golovina VA (2008). Ca²⁺ handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture. *Am J Physiol-Cell Ph* 295: C779-C790.
- Breitling R, Armengaud P, Amtmann A, Herzyk P (2004). Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett* 573: 83-92.
- Cheng KT, Liu X, Ong HL, Swaim W, Ambudkar IS (2011). Local Ca(2)⁺ entry via Orai1 regulates plasma membrane recruitment of TRPC1 and controls cytosolic Ca(2)⁺ signals required for specific cell functions. *PLoS Biol* 9: e1001025.
- Collins HE, Zhu-Mauldin X, Marchase RB, Chatham JC (2013). STIM1/Orai1-mediated SOCE: current perspectives and potential roles in cardiac function and pathology. *Am J Physiol-Heart C* 305: H446-458.
- Cosens D, Perry MM (1972). The fine structure of the eye of a visual mutant, A-type of *Drosophila melanogaster*. *J Insect Physiol* 18: 1773-1786.
- De Souza LB, Ambudkar IS (2014). Trafficking mechanisms and regulation of TRPC channels. *Cell Calcium* 56: 43-50.
- Dietrich A, Fahlbusch M, Gudermann T (2014). Classical Transient Receptor Potential 1 (TRPC1): Channel or channel regulator? *Cells* 3: 939-962.
- Dietrich A, Kalwa H, Gudermann T (2010). TRPC channels in vascular cell function. *Thromb Haemost* 103: 262-270.
- Erac Y, Selli C, Kosova B, Akcali KC, Tosun M (2010). Expression levels of TRPC1 and TRPC6 ion channels are reciprocally altered in aging rat aorta: implications for age-related vasospastic disorders. *Age (Dordr)* 32: 223-230.
- and in part by Ege University (EBİLTEM 11BİL004 to MT). Support to conduct bioinformatics analyses at the Edinburgh Cancer Research Centre was provided by a European Molecular Biology Organization (EMBO) Short-Term Fellowship (ASTF 115-2013 to ÇS). ÇS would like to thank Dr Andrew H Sims, Edinburgh University, UK, for his supervision during the EMBO Fellowship. Research activities were performed at the Pharmaceutical Sciences Research Laboratory (FABAL) with the support of the State Planning Organization Infrastructure Project (DPT 2009K120640 to Faculty of Pharmacy, Ege University). The authors would like to thank Dr Petek Ballar Kırmızıbayrak (Department of Biochemistry, Faculty of Pharmacy, Ege University) and Dr Buket Kosova (Department of Medical Biology, Faculty of Medicine, Ege University) for their comments on the study.
- Guo RW, Yang LX, Li MQ, Pan XH, Liu B, Deng YL (2012). Stim1- and Orai1-mediated store-operated calcium entry is critical for angiotensin II-induced vascular smooth muscle cell proliferation. *Cardiovasc Res* 93: 360-370.
- Huang DW, Sherman BT, Lempicki RA (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols* 4: 44-57.
- Inoue R, Okada T, Onoue H, Hara Y, Shimizu S, Naitoh S, Ito Y, Mori Y (2001). The transient receptor potential homologue TRP6 is the essential component of vascular alpha(1)-adrenoceptor-activated Ca(2+)-permeable cation channel. *Circ Res* 88: 325-332.
- Kiso H, Ohba T, Iino K, Sato K, Terata Y, Murakami M, Ono K, Watanabe H, Ito H (2013). Sildenafil prevents the up-regulation of transient receptor potential canonical channels in the development of cardiomyocyte hypertrophy. *Biochem Biophys Res Commun* 436: 514-518.
- Kumar B, Dreja K, Shah SS, Cheong A, Xu SZ, Sukumar P, Naylor J, Forte A, Cipollaro M, McHugh D et al. (2006). Upregulated TRPC1 channel in vascular injury in vivo and its role in human neointimal hyperplasia. *Circ Res* 98: 557-563.
- Leung FP, Yung LM, Yao X, Laher I, Huang Y (2008). Store-operated calcium entry in vascular smooth muscle. *Br J Pharmacol* 153: 846-857.
- Lin MJ, Leung GP, Zhang WM, Yang XR, Yip KP, Tse CM, Sham JS (2004). Chronic hypoxia-induced upregulation of store-operated and receptor-operated Ca²⁺ channels in pulmonary arterial smooth muscle cells: a novel mechanism of hypoxic pulmonary hypertension. *Circ Res* 95: 496-505.
- Lu W, Ran P, Zhang D, Peng G, Li B, Zhong N, Wang J (2010). Sildenafil inhibits chronically hypoxic upregulation of canonical transient receptor potential expression in rat pulmonary arterial smooth muscle. *Am J Physiol-Cell Ph* 298: C114-123.

- Malczyk M, Veith C, Fuchs B, Hofmann K, Storch U, Schermuly RT, Witzentrath M, Ahlbrecht K, Fecher-Trost C, Flockerzi V et al. (2013). Classical transient receptor potential channel 1 in hypoxia-induced pulmonary hypertension. *Am J Respir Crit Care Med* 188: 1451-1459.
- Montell C (2005). The TRP superfamily of cation channels. *Sci STKE* 2005: re3.
- Nilius B, Owsianik G, Voets T, Peters JA (2007). Transient receptor potential cation channels in disease. *Physiol Rev* 87: 165-217.
- Pani B, Ong HL, Liu X, Rauser K, Ambudkar IS, Singh BB (2008). Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca²⁺ entry (SOCE). *J Biol Chem* 283: 17333-17340.
- Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S et al. (2002). A TRP channel that senses cold stimuli and menthol. *Cell* 108: 705-715.
- Selli C, Erac Y, Kosova B, Erdal ES, Tosun M (2015). Silencing of TRPC1 regulates store-operated calcium entry and proliferation in Huh7 hepatocellular carcinoma cells. *Biomed Pharmacother* 71: 194-200.
- Selli C, Erac Y, Kosova B, Tosun M (2009). Post-transcriptional silencing of TRPC1 ion channel gene by RNA interference upregulates TRPC6 expression and store-operated Ca²⁺ entry in A7r5 vascular smooth muscle cells. *Vascul Pharmacol* 51: 96-100.
- Shi J, Miralles F, Birnbaumer L, Large WA, Albert AP (2016). Store depletion induces Galphaq-mediated PLCbeta1 activity to stimulate TRPC1 channels in vascular smooth muscle cells. *FASEB J* 30: 702-715.
- Storch U, Forst AL, Philipp M, Gudermann T, Mederos y Schnitzler M (2012). Transient receptor potential channel 1 (TRPC1) reduces calcium permeability in heteromeric channel complexes. *J Biol Chem* 287: 3530-3540.
- Sweeney M, Yu Y, Platoshyn O, Zhang S, McDaniel SS, Yuan JX (2002). Inhibition of endogenous TRP1 decreases capacitative Ca²⁺ entry and attenuates pulmonary artery smooth muscle cell proliferation. *Am J Physiol Lung Cell Mol Physiol* 283: L144-155.
- Tai K, Vandenberg G, Hamaide MC, Wibó M, Morel N (2009). Effect of organ culture on noradrenaline-evoked contraction, calcium signalling and TRPC expression in rat mesenteric artery. *J Vasc Res* 46: 353-364.
- Takahashi Y, Watanabe H, Murakami M, Ono K, Munehisa Y, Koyama T, Nobori K, Iijima T, Ito H (2007). Functional role of stromal interaction molecule 1 (STIM1) in vascular smooth muscle cells. *Biochem Biophys Res Commun* 361: 934-940.
- Tani D, Monteilh-Zoller MK, Fleig A, Penner R (2007). Cell cycle-dependent regulation of store-operated I(CRAC) and Mg²⁺-nucleotide-regulated MagNum (TRPM7) currents. *Cell Calcium* 41: 249-260.
- Wang C, Wang J, Zhao L, Wang Y, Liu J, Shi L, Xu M, Wang C (2008). Sildenafil inhibits human pulmonary artery smooth muscle cell proliferation by decreasing capacitative Ca²⁺ entry. *J Pharmacol Sci* 108: 71-78.
- Yu Y, Fantozzi I, Remillard CV, Landsberg JW, Kunichika N, Platoshyn O, Tigno DD, Thistlethwaite PA, Rubin LJ, Yuan JX (2004). Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *P Natl Acad Sci USA* 101: 13861-13866.
- Yu Y, Sweeney M, Zhang S, Platoshyn O, Landsberg J, Rothman A, Yuan JX (2003). PDGF stimulates pulmonary vascular smooth muscle cell proliferation by upregulating TRPC6 expression. *Am J Physiol Cell Physiol* 284: C316-330.