Emerging roles of Orai3 in pathophysiology

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Calcium (Ca²⁺) is a ubiquitous second messenger that regulates a plethora of physiological functions. Deregulation of calcium homeostasis has been reported in a wide variety of pathological conditions including cardiovascular disorders, cancer and neurodegenerative diseases. One of the most ubiquitous pathways involved in regulated Ca²⁺ influx into cells is the store-operated Ca²⁺ entry (SOCE) pathway. In 2006, Orai1 was identified as the channel protein that mediates SOCE in immune cells. Orai1 has two mammalian homologs, Orai2 and Orai3. Although Orai1 has been the most widely studied Orai isoform, Orai3 has recently received significant attention. Under native conditions, Orai3 was demonstrated to be an important component of store-independent arachidonate-regulated Ca²⁺ (ARC) entry in HEK293 cells, and more recently of a store-independent leukotrieneC₄-regulated Ca²⁺ (LRC) entry pathway in vascular smooth muscle cells. Recent studies have shown upregulation of Orai3 in estrogen receptor-expressing breast cancers and a critical role for Orai3 in breast cancer development in immune-compromised mice. Orai3 upregulation was also shown to contribute to vascular smooth muscle remodeling and neointimal hyperplasia caused by vascular injury. Furthermore, Orai3 has been shown to contribute to proliferation of effector T-lymphocytes under oxidative stress. In this review, we will discuss the role of Orai3 in reported pathophysiological conditions and will contribute ideas on the potential role of Orai3 in native Ca²⁺ signaling pathways and human disease.

Introduction

Ca²⁺ is a versatile second messenger that regulates a large number of cellular functions by acting on various proteins and signaling pathways. Ca²⁺ binding can trigger changes in both protein conformation and charge, which in turn modulates downstream protein function. Cellular Ca²⁺ signals are spatially and temporally diverse and are tightly regulated through dynamic buffers, ion channels, transporters and pumps. Rapid Ca²⁺ oscillations regulate fast responses such as exocytosis, contraction and cellular metabolism,1,2 whereas global Ca²⁺ transients or intracellular waves regulate long-term effects such as proliferation, apoptosis, fertilization and transcription.1 Deregulations in intracellular Ca²⁺ signaling can lead to severe deleterious effects on cellular homeostasis and as a result cause disease. This review will highlight the unique role of a highly Ca²⁺ selective protein, Orai3, in mediating Ca²⁺ signaling, regulating physiological functions and contributing to human pathologies.

Store-Operated Ca²⁺ Entry (SOCE)

Store-operated Ca²⁺ entry (SOCE) is one of the most ubiquitous routes of receptor-regulated Ca²⁺ influx into non-excitable cells. SOCE is activated upon depletion of intracellular Ca²⁺ stores, mainly the endoplasmic reticulum (ER), leading to the opening of plasma membrane store-operated Ca²⁺ (SOC) channels.3,4 The current mediating SOCE is called Ca²⁺ release-activated Ca²⁺ (CRAC) current.5 The molecular machinery mediating SOCE had remained elusive for over two decades. Only recently, the stromal interaction molecule 1 (STIM1) and Orai proteins were discovered as the molecular players mediating SOCE.6–14 STIM1 is the Ca²⁺ sensor in the ER, which senses depletion of Ca²⁺ from the stores and transmits the signal to plasma membrane Orai proteins, resulting in channel activation. This Ca²⁺ influx pathway regulates various cell functions including proliferation and migration.15,16 Although mammals possess three Orai proteins (Orai1/2/3),6 Orai1 was uniformly shown to mediate native SOCE and CRAC currents in various cell types. Orai3 is an exclusively mammalian protein that contributes, along with Orai1, to store-independent conductances discussed below.17,20 (Fig. 1). There are reported instances where Orai3 was shown to encode SOCE in a subset of breast cancer cells, expressing the estrogen receptors21,22 and in mature effector T cells,23 but in general SOCE is mediated by Orai1 homomultimeric channels. In fact, in the absence of functional Orai1 channels, ectopically expressed Orai3 can only partially compensate for Orai1 in mediating SOCE in HEK 293 and human fibroblasts.24 Earlier studies using ectopic expression of concatenated oligomers, chemical crosslinking and imaging methods suggested that functional human CRAC channels are formed of Orai1 tetramers.25,26 These findings have been challenged recently by X-ray crystallography data resolving the structure of drosophila Orai at 3.35 Å resolution and revealing that it forms hexamers.27

Orai3 and Store-Independent Ca²⁺ Channels

Under physiological conditions, agonists that bind to their specific phospholipase C (PLC)-coupled receptors, such as G-protein coupled receptors or receptor tyrosine kinases, induce PLC-mediated breakdown of phosphatidylinositol-4,5-bisphosphate...
Similarly, the knockdown of Orai3 using siRNA also caused significant reduction in ARC current in HEK293 cells. Additionally, ectopic expression of Orai3 along with STIM1 in HEK293 cells resulted in 50% increase in store-independent ARC currents.

Interestingly, unlike CRAC channel activation which requires ER-resident STIM1, the involvement of STIM1 in ARC channel activation is contingent on the minor pool of STIM1 located in the plasma membrane. Using various preassembled concatenated Orai1-Orai3 multimers, the Shuttleworth group further reported that the molecular architecture of ARC channels is a pentameric assembly of three Orai1 and two Orai3 subunits. They showed that ectopic expression of these concatenated pentamers increases by 2-fold the amplitude of ARC currents activated by exogenous AA. They generated a number of concatenated constructs with different permutations and suggested that functional ARC pentamers are in the following configurations: 31311 and 31113 (where 1 and 3 correspond to Orai1 and Orai3, respectively). In their most recent work, the Shuttleworth group proposed that Orai3 confers sensitivity to AA and that the cytosolic N-terminal domain of Orai3 is responsible for sensing AA.

(PIP)3 into two second messengers, diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3). In one hand, IP3 mediates depletion of ER Ca2+ stores leading to the activation of SOCE. On the other hand, DAG can also activate non-selective cation channels at the plasma membrane, such as members of transient receptor potential canonical 3/6/7 (TRPC3/6/7) channels and can also generate other second messengers such as arachidonic acid (AA) through the action of the enzyme DAG lipase. AA, which can also be synthesized by phospholipase A2 (PLA2), has been implicated in the activation of a distinct store-independent Ca2+ entry pathway mediated by the arachidonate-regulated Ca2+(ARC) channels. ARC channels are Ca2+ selective and have similar biophysical properties to CRAC channels, although unlike CRAC channels they are intriguingly insensitive to inhibition with the drug 2-aminoethoxydiphenyl borane (2-APB). Shuttleworth and colleagues reported that STIM1 is required for ARC channel activation and that both Orai1 and Orai3 contribute subunits to ARC channels (Fig. 1). Using overexpression, knockdown, and dominant-negative strategies, they showed that Orai3 is uniquely involved in ARC channels in HEK293 cells. The introduction of dominant-negative Orai3 (E81Q) in HEK293 cells stably expressing STIM1 and Orai1 resulted in complete abrogation of ARC current without significant changes in the CRAC current. Similarly, the knockdown of Orai3 using siRNA also caused significant reduction in ARC current in HEK293 cells. Additionally, ectopic expression of Orai3 along with STIM1 in HEK293 cells resulted in 50% increase in store-independent ARC currents. Interestingly, unlike CRAC channel activation which requires ER-resident STIM1, the involvement of STIM1 in ARC channel activation is contingent on the minor pool of STIM1 located in the plasma membrane.

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Using preassembled Orai1/ARCs in ER+ breast cancer cells, SOCE is mediated by Orai3 instead of Orai1; ERα enhances Orai3 expression in ER+ breast cancer cells. Bottom: Differences in the amino acid sequence of Orai1 and Orai3 subunits of CRAC and ARC/LRC channels bring about differences in channel gating properties and pharmacology (see Table 2 for details). Amino acids indicated by colored circles indicate essential residues for ion selectivity and lanthanide sensitivity (green) or oxidation sensitivity (yellow).
Orai3 concatenates, they reported that only one Orai3 subunit in the hetero-pentameric Orai1/Orai3 concatenate is sufficient to sense AA, but two Orai3 subunits are required to confer preferential activation by AA over store-depletion. A heteropentamer of Orai1/Orai3 containing four Orai and one Orai3 subunit could activate ARC currents if the N-terminus of one Orai subunit is replaced with the N-terminus of Orai3. These studies on the ARC channel oligomeric state contrast with the aforementioned crystal structure showing hexameric assembly of drosophila Orai. For more in-depth details on the mechanisms of activation of ARC channels, the reader is directed to a number of review articles.

Although Orai3 has been implicated in ARC channel function, the precise role of Orai3 proteins or ARC-mediated Ca\(^{2+}\) signals in cellular functions remain largely unexplored. Likewise, the identity of physiological agonists that specifically use Orai3 to mediate either store-dependent or store-independent Ca\(^{2+}\) entry pathways is also largely unknown. Mignen et al. reported the activation of ARC channels in parotid and pancreatic acinar cells by carbachol (in the parotid) and both carbachol and cholecystokinin (in the pancreas). We have recently reported a store-independent ARC-like Ca\(^{2+}\) selective entry channel contributed by Orai1 and Orai3 and activated by the agonist thrombin in primary vascular smooth muscle through generation of the second messenger leukotrieneC\(_4\) (LTC\(_4\)) downstream receptor stimulation. This LTC\(_4\) -regulated Ca\(^{2+}\) (LRC) channel requires STIM1 downstream of LTC\(_4\). LTC\(_4\) acts on LRC channels from the cytosolic side in a manner analogous to a second messenger; the pool of STIM1 involved, the exact role of STIM1 in the activation of this channel and whether LTC\(_4\) acts directly or through intermediary factors remain unknown.

**Orai3 in Breast Cancer**

Breast cancer is one of the most commonly diagnosed cancers in the world. Numerous factors contribute to the development and progression of breast cancer such as genetic mutations, growth factors, and the presence or absence of estrogen receptors. A large body of evidence suggests that Ca\(^{2+}\) channels contribute to cancer progression. When Orai channel isoforms are co-expressed with STIM1 in the HEK293 expression system, several groups have shown that these channels form store depletion-activated Ca\(^{2+}\) channels. Furthermore, these studies showed that the drug 2-APB blocks Orai1-mediated SOCE and CRAC currents at concentrations of 30–50 μM, while at these concentrations 2-APB potentiates Orai3 channel activity. These pharmacological differences between Orai1 and Orai3 were exploited by Motiani et al. to reveal that under native conditions 2-APB inhibited SOCE in estrogen receptor negative (ER\(^{-}\)) breast cancer cell lines but potentiated SOCE in estrogen receptor expressing (ER\(^{+}\)) breast cancer cells, suggesting that Orai3 is likely the isoform mediating SOCE in ER\(^{+}\) breast cancer cells. Molecular knockdown confirmed that in ER\(^{+}\) breast cancer cells (MCF7), pharmacological store depletion with the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) blocker thapsigargin or intracellular dialysis of the Ca\(^{2+}\)-chelator BAPTA activates a SOCE/CRAC channel encoded by Orai3 whereas ER\(^{-}\) breast cancer cells use the canonical Orai1 pathway for mediating SOCE and CRAC currents. Indeed, Orai1 has been reported to mediate SOCE in the ER\(^{-}\) breast cancer cell line MDA-MB231 and was shown to contribute to breast cancer metastasis. Studies on Orai3 were subsequently confirmed by other groups; Faouzi et al. showed that store depletion activated SOCE through Orai3 channels in ER\(^{+}\) MCF7 cells, whereas an earlier report by Feng et al. reported that Orai1 is not involved in mediating SOCE in MCF7 cells.

Faouzi et al. also showed that Orai3 plays an important role in cell cycle regulation of ER\(^{+}\) breast cancer cells but not normal breast epithelial cells. Using MCF7 and T47D (another ER\(^{+}\) breast cancer cell line), they reported that Orai3 knockdown results in cell cycle arrest in the G1 phase. This cell cycle arrest was suggested to be due to a reduction in the expression of cyclin D and E, as well as in cyclin-dependent kinase (CDK 2/4) expression. The same authors showed that Orai3 knockdown caused an unexpected increase in the levels of p53 (a well-established tumor suppressor) and p21\(^{Waf1/Cip1}\) (a cyclin-dependent kinase inhibitor), suggesting that accumulation of these tumor suppressors could contribute to cell cycle arrest upon Orai3 knockdown. Orai3 knockdown induced apoptosis and caused an increase in the ratio of Bax/Bcl2 expression in MCF7 ER\(^{+}\) breast cancers but not in normal breast epithelial cells. A follow-up study by the same group reported a correlation between Orai3 and c-myc expression in tumor tissues and ER\(^{+}\) MCF-7 cells and that Orai3 knockdown inhibits both expression and activity of the proto-oncogene c-myc, presumably through the MAP Kinase pathway. The role of Orai3 in ER\(^{+}\) breast cancer cell survival was confirmed by Pangburn et al. using nanoparticle-mediated delivery of siRNA targeting Orai3. These authors encapsulated siRNA targeting Orai3 in polymersomes and showed that these polymersomes were able to deliver siRNA to T47D cells and MCF10A cells (“normal” breast epithelial cells). This group reported that the viability of T47D cells was decreased upon Orai3 siRNA delivery whereas no effect on the viability of MCF10A was noted. Data from our lab showed that SOCE mediated through Orai3 is required for activation of pro-proliferative and pro-migratory pathways; Orai3 knockdown inhibited NFAT transcriptional activity, as well as Ca\(^{2+}\)-dependent phosphorylation of ERK1/2 and focal adhesion kinase (FAK).

As mentioned above, STIM1 and Orai1 were implicated in mediating breast cancer cell migration in vitro using ER\(^{-}\)-MBA-MD231 breast cancer cells as well as in mediating metastasis in vivo in immune-compromised mice. Although STIM1 and Orai1 knockdown result in inhibition of ER\(^{+}\) breast cancer metastasis in nude mice, their potential use as therapeutic targets is questionable since functional STIM1/Orai1-mediated CRAC pathway is essential for proper immunological responses. Human patients with mutations in either STIM1 or Orai1 have impaired SOCE/CRAC in their T-cells, mast cells and platelets and suffer from a Severe Combined Immunodeficiency (SCID) syndrome. This indicates that targeting STIM1 or Orai1 could compromise the immune system, and thus Orai3 might be a better therapeutic target. We showed that Orai3 plays a critical role of Orai3 in breast cancer.
role in ERα breast cancer cell tumorigenesis both in vitro and in vivo in immune-compromised mice. An siRNA against Orai3 that achieved -54% knockdown of Orai3 protein levels affected in vitro invasion of MCF7 cells by -44% with no effect on the invasive capabilities of ERα-MDA-MB231 cells.26 Greater levels of Orai3 knockdown (~68%) were achieved using lentivirus-encoding shRNA against Orai3. Stable knockdown of Orai3 was achieved in MCF7 cells using lentiviral infection, and these cells were subsequently injected into the mammary fat pads of severe combined immunodeficient (SCID) mice. This Orai3 knockdown led to a significant reduction in ERα-dependent in vivo tumor growth in SCID mice and caused an average reduction in tumor incidence, reduction of tumor volume by -66% and tumor weight by -63% compared with control mice.26

Recent data from our group has shown that physiological agonists relevant to breast cancer cell proliferation and migration, such as the Epidermal Growth Factor (EGF) and thrombin activate Ca2+ influx in MCF7 cells through Orai3 channels.26 The potential connection between the presence of estrogen receptors and the function of Orai3 as SOCE channels in ERα breast cancer cells was also explored. We showed that ERα regulates Orai3 expression; ERα knockdown resulted in a significant decrease in Orai3 mRNA and protein levels with no effect on Orai1.26 Interestingly, although we achieved -82% knockdown of ERα protein, Orai3 protein levels, SOCE magnitude and CRAC currents in MCF7 were decreased by approximately 40%.26 suggesting additional regulatory mechanisms for control of Orai3 expression in MCF7 cells. The decrease in SOCE in MCF7 cells upon ERα knockdown could be rescued by ectopic expression of human Orai3 and the activation of ERα by 17β-estradiol increased Orai3 mRNA levels by 3-fold, suggesting that ERα likely regulates Orai3 at the transcriptional level. However, it remains to be determined whether this regulation is direct (i.e., through binding of ERα to ERα-responsive elements on the Orai3 promoter) or an indirect one. While the sequence and location of the Orai3 promoter remain unknown, there are two putative ERα binding sites in the 3′UTR and six in the intronic sequence of the Orai3 gene. Future studies are required to determine whether these putative ERα-binding sequences are involved in directly controlling Orai3 transcriptional activity. Interestingly, a recent study by Flourakis et al. showed that androgen receptor (AR) knockdown in the prostate cancer cell line LNCaP results in reduction in Orai1 protein levels. AR knockdown in these cells also reduced Orai1-mediated Ca2+ entry and currents, suggesting that Orai1 expression and function are regulated by androgen receptors.70 An interesting observation is that Orai1 expression appears higher in males compared with females, and the suggestion that higher Orai1 levels in males might explain the known propensity of males to develop cardiovascular diseases compared with females and the well-documented protective cardiovascular effects of female sex hormones in pre-menopausal women.71,72 Whether an increased expression of Orai3 relative to Orai1 in the cardiovascular system is a characteristic of females is unknown. If this is indeed the case, the functional outcome of Orai3 upregulation in the cardiovascular system is an important question that warrants further investigations.

Although Orai3 is expressed in normal breast epithelial cells and a number of different cell types including estrogen responsive smooth muscle cells, it remains puzzling why Orai3 is not functionally active as a SOCE channel in these cell types? Another intriguing question that remains unanswered is why Orai3 is functional as a SOCE/CRAC channel only in ERα breast cancer cells? We compared the expression of Orai3 between five ERα and five ERβ breast cancer cells and could clearly detect that Orai3 protein expression is consistently higher in ERα breast cancer cells compared with ERα cells.25 Faouzi et al. reported similar results showing higher Orai3 expression in ERα-MCF7 and T47D cells compared with normal breast epithelial cells and further showed that over 70% of human breast cancer samples showed upregulation of Orai3 mRNA in comparison to control breast tissue samples.23 These data suggest that Orai3 upregulation in ERα cells might tip the balance of stoichiometry in favor of an interaction between STIM1/Orai3 instead of STIM1/Orai1 (Fig. 1, inset). In support of this concept, we used ectopic expression to show that the relative Orai isoform expression levels regulate their functional recruitment as SOCE channels in ERα and ERβ breast cancer cells. Data shown in Figure 2 shows that stimulation of ERα breast cancer cells, MDA-MB231 with thapsigargin activates SOCE that is subsequently inhibited with application of 30 μM 2-APB to the bath solution (Fig. 2A), consistent with the pharmacological properties of Orai1-mediated SOCE.73 Ectopic expression of Orai3 in these ERα breast cancer cells results in a smaller thapsigargin-activated SOCE and its potentiation by 2-APB (Fig. 2B), suggesting functional activation of Orai3-mediated SOCE in these cells (potentiation by 2-APB is a characteristic of SOCE mediated by Orai3).70,72 Conversely, stimulation of ERβ breast cancer cells, MCF7 with thapsigargin activates SOCE mediated by Orai3,25,26 which is potentiated by addition of 30 μM 2-APB to the bath solution (Fig. 2C). Ectopic expression of Orai1 in ERβ breast cancer cells results in enhanced thapsigargin-activated SOCE and apparent inhibition by 2-APB, suggesting that overexpressed Orai1 in these cells are functionally recruited to compete with endogenous Orai3 for mediating SOCE (Fig. 2D).

However, it is quite intriguing that upon Orai3 knockdown, endogenous Orai1 does not substitute for Orai3 in mediating SOCE and CRAC channel function in ERα MCF7 cells,25,26 suggesting a more complex scenario occurs under native conditions that cannot be explained by changes in STIM1/Orai stoichiometry alone. One possible reason for the inability of native Orai3 to compensate for absence of Orai3 and form a functional CRAC channel in MCF7 cells could be the recently reported constitutive binding of Orai1 to the secretory pathway Ca2+-ATPase 2 (SPCA2) in MCF7 cells.65 These Orai1 molecules engaged in STIM1-independent constitutive Ca2+ mobilization in MCF7 cells might not be freely available to interact with STIM1 and mediate store depletion-activated CRAC channels. Independently of Orai1 interaction with SPCA2 in MCF7 cells, Orai1 might be localized within a cellular signaling microdomain such that it is not accessible to STIM1 molecules; further experimentation is required to test these hypotheses.
Orai3 in Leukemia

Work by Yanamandra and colleagues suggested that Ca\(^{2+}\) influx through Orai3 can contribute to apoptosis in acute myeloid leukemia and multi-myeloma cell lines treated with tipifarnib. Tipifarnib is a non-peptidomimetic farnesyltransferase inhibitor that inhibits Ras kinase and is used in clinical trial for treatment of certain types of leukemia and breast cancers. This group reported that some leukemic cell lines possess a tipifarnib-activated Ca\(^{2+}\) influx pathway with a pharmacological profile similar to that of SOCE mediated by Orai3 in ER+ breast cancer cells; tipifarnib-induced Ca\(^{2+}\) influx is potentiated by 2-APB and blocked by lanthanides. These authors correlated the extent of Ca\(^{2+}\) entry with cell death and found that application of tipifarnib alone resulted in apoptosis; co-application of 2-APB increased apoptosis whereas co-application of lanthanides decreased tipifarnib-induced apoptosis. They examined Orai3 mRNA levels in these cell lines and reported that tipifarnib-sensitive cells express higher levels of Orai3 mRNA compared with tipifarnib-resistant cells, suggesting that the difference in mRNA expression might explain Orai3 involvement in these cells. Although the study by Yanamandra et al. reported some interesting observations, it is entirely based on pharmacological agents (e.g., 2-APB) that might have effects on cell apoptosis in a Ca\(^{2+}\) or SOCE-independent mechanism. Furthermore, these drugs were used at much higher doses (e.g., 2-APB at 100 \(\mu\)M) than normally used to block SOCE (30–50 \(\mu\)M). Therefore, future studies that employ molecular tools to determine the effects of either knockdown or overexpression of Orai3 on apoptosis of acute myeloid leukemia and multiple myeloma cell lines are warranted.

The study of Yanamandra et al. in leukemia contrasts with the studies of Orai3 in breast cancer and raises an intriguing question regarding the role of Orai3 in cancer. Faouzi et al. suggested that Orai3-mediated Ca\(^{2+}\) influx is important for cell survival of breast cancer cells, while Orai3-mediated Ca\(^{2+}\) influx was proposed to act as a pro-apoptotic signal in leukemic cells. Yanamandra et al. showed that tipifarnib-induced Ca\(^{2+}\) influx results in ER stress-mediated apoptosis of leukemic cells as indicated by increase of caspases 3/4/12 activities, whereas Faouzi et al. showed that Orai3-mediated Ca\(^{2+}\) entry is involved in survival of breast cancer cells through inhibition of the mitochondrial pro-apoptotic pathways (Bax/Bcl2 ratio was increased upon Orai3 knockdown). This apparent discrepancy could be explained by the differences in spatial localization of Orai3 in...
breast cancer vs. leukemic cells resulting in differential activation of downstream molecular pathways by Orai3-mediated Ca$^{2+}$-gradients. Alternatively, differences in the relative levels of Orai3 expression between breast cancer and leukemic cells and the resulting differences in the extent of Orai3-mediated Ca$^{2+}$ signals might couple to different functional outcomes. Interestingly, the same situation has been reported for Orai1-mediated SOCE in breast cancer vs. prostate cancer. Orai1 was reported to drive proliferation and in vivo metastasis of the ER$^+$ breast cancer cell line, MDA-MB231.$^{19}$ However, the progression of prostate cancer to androgen-independent phenotype was characterized by downregulation of Orai1 and decrease in SOCE activity.$^{70}$ The decrease in SOCE was proposed to enhance the resistance of prostate cancer cells (LNCaP) to Ca$^{2+}$-dependent apoptosis; ectopic expression of Orai1 into these cells re-established SOCE and restored the normal rate of apoptosis.$^{70}$

**Orai3 and Oxidative Stress**

During inflammation, T-helper lymphocytes migrate to the site of inflammation and differentiate into effector T lymphocytes. The effector T lymphocytes then proliferate and secrete cytokines at the site of inflammation. Under such inflammatory conditions, T lymphocytes are exposed to high levels of reactive oxygen species (ROS) and thus must survive under extreme oxidative stress. It is well established that under normal conditions, Orai1-mediated Ca$^{2+}$ influx is required for activation, proliferation and differentiation of T lymphocytes. Bogeski et al. has reported an interesting role for Orai3 in lymphocytes exposed to high oxidative stress.$^{27}$ These authors suggested that during high oxidative stress, Orai1 channels become inhibited when ROS acts on a reactive cysteine residue at position 195 present in the second predicted extracellular loop of Orai1 (Fig. 1). These authors further reported that mutation of this cysteine residue to serine (C195S) decreases Orai1 redox sensitivity. Interestingly, Orai3 does not have a homologous cysteine residue in this position, and remarkably remains functional even under high oxidative stress. To confirm the significance of the reactive cysteine residue in Orai3, Bogeski et al. introduced a reactive cysteine residue into Orai3 (G170C) at the position homologous to Cys195 in Orai1; introduction of this cysteine residue made Orai3 sensitive to ROS and Orai3 channel activity was thus inhibited by H$_2$O$_2$. Bogeski et al. further showed that Orai3 expression is higher in T-effector lymphocytes compared with T-helper lymphocytes, suggesting that T-effector lymphocytes use Orai3 under inflammatory conditions to ensure Ca$^{2+}$ influx required for proliferation and cytokine secretion. This switch to Orai3 expression is coupled with a decrease in Orai1/Orai3 expression ratio, making T-effector lymphocytes less redox-sensitive and therefore functionally active. The authors validated this role for Orai3 in T-effector lymphocytes by showing that Orai3 knockdown made T-effector lymphocytes sensitive to oxidative stress, evidenced by a decrease in Ca$^{2+}$ influx in these cells upon treatment with H$_2$O$_2$. Therefore, it appears that Orai3 offers T effector lymphocytes an advantage by reducing their redox sensitivity, thus allowing these cells to function in highly oxidative environments.$^{27}$

**Orai3 in Vascular Proliferative Diseases**

Under normal physiological conditions, Vascular Smooth Muscle Cells (VSMCs) are in a quiescent contractile state that is non-proliferative and non-migratory.$^{73,76}$ VSMCs primary function is to maintain blood pressure and vascular tone. Upon mechanical injury, diet-induced injury or insults from exposure to chronic high blood pressure or inflammatory molecules, VSMCs switch to a highly proliferative and migratory phenotype, called synthetic phenotype. These synthetic VSMCs contribute to neointimal hyperplasia and atherosclerotic lesions that cause the narrowing of blood vessels common to vascular occlusive diseases such as atherosclerosis, hypertension and restenosis. CRAC currents mediated by Orai1 and STIM1 have recently emerged as important players in VSMC proliferation and migration in vitro and remodeling in vivo.$^{18,77-85}$ STIM1 and Orai1 protein expression are increased in synthetic VSMCs compared with quiescent VSMCs. STIM1 and Orai1 mediate SOCE and CRAC currents in response to the potent proliferative and migratory VSMC agonist, the platelet-derived growth factor (PDGF);$^{18,79,84}$ this Ca$^{2+}$ entry is required for nuclear translocation and transcriptional activity of nuclear factor for activated T cells (NFAT).$^{82,84}$ We recently reported that Orai3 proteins are also upregulated when VSMC switch to a synthetic phenotype.$^{21}$ Interestingly, Orai3 did not contribute to Ca$^{2+}$ entry activated by either passive store depletion or physiological stimulation with PDGF; instead, Orai3 was shown to play a crucial role in mediating Ca$^{2+}$ entry upon stimulation with another VSMC agonist, thrombin.$^{21}$ In synthetic VSMCs, thrombin activated a store-independent pathway contributed by both Orai3 and Orai1 and requiring STIM1. This ARClke store-independent pathway was highly Ca$^{2+}$ selective and required intracellular leukotriene C$_4$ (LTC$_4$) produced by LTC$_4$ synthase downstream of thrombin receptor ligation.$^{21}$ Furthermore, using an in vivo model of vascular injury we showed that Orai3-mediated Ca$^{2+}$ selective currents were required for neointimal hyperplasia, suggesting the potential use of Orai3 or LTC$_4$-regulated Ca$^{2+}$ (LRC) channels as potential targets for therapy of vascular occlusive diseases. The exact stoichiometry of LRC channels, the mechanisms of actions of LTC$_4$, how STIM1 regulates LRC channel function and the downstream pathways and transcriptions factors activated by LRC-mediated Ca$^{2+}$ signals are important questions that remain unanswered.

**Concluding Remarks**

Since their discovery in 2006, Orai channels have emerged as important players in cancers, cardiovascular disease and autoimmune diseases which raise the possibility that, with proper understanding of their precise regulation mechanisms within their native environments, they might be targeted for future disease therapy. As of this writing, little is known about Orai3 proteins and the channels they contribute to under native conditions. Table 1 summarizes the evidence for Orai3 expression and contribution to various pathologies. Table 2 summarizes the different Ca$^{2+}$ channels contributed by Orai3 and Orai1 in different cell types. Emerging evidence however suggests that while Orai3 can form
Orai3 regulates proliferation and viability

Orai3 mediates cell proliferation and cytokine secretion from Orai1 to Orai3 is also needed. While Orai3 upregulation in VSMCs is required for neointimal hyperplasia after vascular injury, a moderate enhancement of Orai3 expression (relative to that of Orai1) by female sex hormones in vascular cells might be beneficial. Indeed, Orai3 regulation by female sex hormones is intriguing and additional studies on Orai3 within the context of cardiovascular disorders might connect Orai3 function under either store-dependent or store-independent modes to the lessened susceptibility of females to cardiovascular diseases. Furthermore, while Orai1 and STIM1-deficient mice have been generated and studied within the context of several diseases, Orai3 knockout mice are currently unavailable. Orai3 tissue-specific knockout mice are likely to enhance our understanding of the function of this protein in physiology and pathophysiology and will bring us closer to the targeting of Orai3 for the purpose of therapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Table 2. Properties of Ca\(^{2+}\) selective channels formed by Orai3 and Orai1

| Composition (#units) | CRAC | ARC | LRC | References |
|----------------------|------|-----|-----|------------|
| Orai3 (n.d.)         |      |     |     |            |
| Orai1 (4 or 67)      |      |     |     | 1, 5, 21, 25, 85 |
| Orai1/Orai3 (3:2?)   |      |     |     | 1, 5, 86  |

### Biophysical properties

- Highly Ca\(^{2+}\)-selective
- Small currents
- Inwardly rectifying I/V relationship
- Reversal potential of greater than +60 mV
- Permeability to monovalent cations upon removal of extracellular divalent cations

Small Ca\(^{2+}\) currents (smaller than Orai1)

### Gating properties

| Store-dependent | Activation | AA (8 \(\mu\)M) requires PM STIM1 | Intracellular LTC (100 \(nM\)); requires STIM1 (ER or PM pool unknown) |
|-----------------|------------|-----------------------------------|---------------------------------------------------------------------|
| Yes             | Store depletion, requires ER STIM1 | n.d.                              | 5, 21, 25, 44, 85, 89, 90                                            |

### Negative regulation

Ca\(^{2+}\)-dependent inactivation (CDI) mediated by Ca\(^{2+}\) and calmodulin actions on STIM1 and Orai

The protein SARAF

| Positive modulators | Potentiation of CRAC and SOCE at low concentrations (5 \(\mu\)M); inhibition at higher concentrations (30–50 \(\mu\)M) |
|--------------------|-----------------------------------------------------------------------------------------------------------------|
| 2-APB              | n.d.                                                                                                             |

### Pharmacology

| Lanthanides | Inhibition of CRAC and SOCE by 1–10 \(\mu\)M Gd\(^{3+}\), La\(^{3+}\) | Inhibition by 10 \(\mu\)M Gd\(^{3+}\), 50 \(\mu\)M La\(^{3+}\) |
|-------------|------------------------------------------------------------------|-------------------------------------------------|
| 2-APB       | Potentiation of CRAC and SOCE                                   | Insensitive                                     |
| pH          | Sensitive to decrease in pH                                     | Less sensitive toward lowering external pH     |
| Lanthanides |                                                                  | n.d.                                           |
| pH          |                                                                  | n.d.                                           |

ER, endoplasmic reticulum; PM, plasma membrane; STIM1, stromal interaction molecule 1; n.d., not determined.

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