



BRAZILIAN JOURNAL
OF MEDICAL AND BIOLOGICAL RESEARCH

www.bjournal.com.br

ISSN 0100-879X
Volume 44 (11) 1070-1193 November 2011

BIOMEDICAL SCIENCES
AND
CLINICAL INVESTIGATION

Braz J Med Biol Res, November 2011, Volume 44(11) 1080-1087

doi: 10.1590/S0100-879X2011007500133

STIM1/Orai1-mediated store-operated Ca²⁺ entry: the tip of the iceberg

F.R. Giachini, V.V. Lima, J.L. Hannan, F.S. Carneiro, R.C. Webb and R.C. Tostes

The Brazilian Journal of Medical and Biological Research is partially financed by



Ministério
da Ciência e Tecnologia



Ministério
da Educação



Institutional Sponsors



Explore High - Performance MS
Orbitrap Technology
In Proteomics & Metabolomics



All the contents of this journal, except where otherwise noted, is licensed under a [Creative Commons Attribution License](http://creativecommons.org/licenses/by-nc/4.0/)

STIM1/Orai1-mediated store-operated Ca²⁺ entry: the tip of the iceberg

F.R. Giachini¹, V.V. Lima^{1,2}, J.L. Hannan¹, F.S. Carneiro^{1,2},
R.C. Webb¹ and R.C. Tostes²

¹Department of Physiology, Georgia Health Science University, Augusta, GA, USA

²Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Abstract

Highly efficient mechanisms regulate intracellular calcium (Ca²⁺) levels. The recent discovery of new components linking intracellular Ca²⁺ stores to plasma membrane Ca²⁺ entry channels has brought new insight into the understanding of Ca²⁺ homeostasis. Stromal interaction molecule 1 (STIM1) was identified as a Ca²⁺ sensor essential for Ca²⁺ store depletion-triggered Ca²⁺ influx. Orai1 was recognized as being an essential component for the Ca²⁺ release-activated Ca²⁺ (CRAC) channel. Together, these proteins participate in store-operated Ca²⁺ channel function. Defective regulation of intracellular Ca²⁺ is a hallmark of several diseases. In this review, we focus on Ca²⁺ regulation by the STIM1/Orai1 pathway and review evidence that implicates STIM1/Orai1 in several pathological conditions including cardiovascular and pulmonary diseases, among others.

Key words: Ca²⁺ release-activated Ca²⁺ channel; Sarcoplasmic reticulum; Intracellular Ca²⁺ store; Ca²⁺ influx

Introduction

Nearly all cell types depend on the generation of cytoplasmic calcium (Ca²⁺) signals to regulate cell function or trigger specific responses. The use of Ca²⁺ for intracellular signaling implies a tight local and global control of cytoplasmic concentration and mechanisms for maintaining the cellular Ca²⁺ balance. Cytosolic Ca²⁺ signals are adjusted by intracellular sources such as the endo-/sarcoplasmic reticulum, Ca²⁺ binding proteins and plasma membrane Ca²⁺ permeable channels. Therefore, Ca²⁺ pumps, along with Ca²⁺ release channels, are key components in Ca²⁺ homeostasis. The endo-/sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) pumps have the highest affinity for Ca²⁺ removal from the cytosol. SERCA, along with plasma membrane Ca²⁺ ATPases and other transporters, determine the resting cytosolic Ca²⁺ concentration (1).

Upon stimulation, most excitable cells display a biphasic increase in cytosolic Ca²⁺ concentration. The initial transient increase is due to inositol triphosphate (IP₃)-mediated release of endoplasmic reticulum (ER) Ca²⁺. The subsequent prolonged increase requires extracellular Ca²⁺ influx through various pathways, which include store-operated Ca²⁺ channels (SOCC), voltage-operated Ca²⁺ channels, purinergic receptors, transient receptor membrane potential

channels (TRPC), and the Na⁺/Ca²⁺ exchanger (NCX), among others (1).

In this review, we will focus on new components of store-operated Ca²⁺ entry (SOCE) and of the stromal interaction molecule 1 (STIM1)/Orai1 system, and review evidence suggesting that alterations in this system may contribute to several pathological conditions. We will first briefly describe mechanisms that contribute to Ca²⁺ entry in the cell, with special focus on SOCE. Then, a major review addressing the STIM1-Orai1 pathway will be presented. Finally, we will describe how alterations in the STIM1-Orai1 pathway have been correlated directly with some pathological conditions.

Store-operated Ca²⁺ entry

One of the pathways that contribute to extracellular Ca²⁺ influx relies on depletion of Ca²⁺ from the ER, which causes activation of plasma membrane Ca²⁺ channels to refill internal stores. This mechanism, by which the ER acts as a capacitor, has led to the term SOCE (2). In many cell types, SOCE carries a highly Ca²⁺-selective, non-voltage-gated, inwardly rectifying current termed Ca²⁺ release-activated

Correspondence: F.R. Giachini, Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, USP, Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brasil. E-mail: fernandagiachini@hotmail.com

Received January 14, 2011. Accepted September 5, 2011. Available online October 14, 2011. Published November 14, 2011.

Ca²⁺ (CRAC) current, or I_{CRAC}. SOCE also plays a crucial role in most cells by being the main source of Ca²⁺ to refill the ER after its depletion (2).

Several factors have been considered to be SOCE activators. It has been proposed that a Ca²⁺-influx factor (CIF) is released upon Ca²⁺ store depletion, resulting in restoration of calmodulin activity, generation of lysophospholipids and consequently, activation of SOCE. The identity of CIF is unknown, but its presence and biological activity have been detected in several studies. In addition, it seems that CIF is important for the regulation of some types of SOCE, but very little is known about the molecular mechanism of CIF production in the stores (3).

The NCX represents another pathway for extracellular Ca²⁺ influx and there is evidence that NCX contributes to SOCE. It has been reported that NCX participates in store depletion when this exchanger is operating in the reverse mode. This seems particularly important for Ca²⁺ homeostasis in the mitochondria. This organelle was found to be essential for the activation and maintenance of SOCE, but the molecular mechanisms by which mitochondria control SOCE are unknown. It has been recently reported that mitochondrial NCX is fundamental for STIM1/Orai1-dependent SOCE (4).

Before the discovery of STIM1, the coupling process between ER and plasma membrane to mediate SOCE was a mechanistic mystery. Recently, two research groups independently identified STIM1 as a protein that functions as a Ca²⁺ sensor (5,6). It was shown that knockdown of STIM1 in *Drosophila* S2 cells significantly reduced thapsigargin-dependent Ca²⁺ entry and completely suppressed I_{CRAC}. Similar observations were made in Jurkat T cells, human embryonic kidney (HEK293) (6) and airway smooth muscle cells (7). At the same time, Spassova et al. (8) demonstrated that, in addition to being an ER Ca²⁺ sensor, STIM1 functions within the plasma membrane to control the operation of the Ca²⁺ entry channel itself. They showed that the overexpression of STIM1 increased the activity of CRAC channels whereas mutations in the helix-loop-helix structural domain and cytoplasmic C terminus of STIM1 altered operational parameters of CRAC channels, including the pharmacological profile and inactivation properties (8).

There are two members in the STIM family: STIM1 and STIM2. They present 61% homology and are expressed widely. Co-expression of both STIM proteins favors translocation of STIM2 upon store depletion. STIM translocation occurs in specific areas where the ER comes in close proximity to the plasma membrane, the so-called puncta formations. However, STIM1 and STIM2 seem to work in different ranges of ER Ca²⁺ concentration (9). The reason why cells display two sensors of Ca²⁺ to set basal and stimulated Ca²⁺ separately is not known.

The signaling breakthrough represented by the discovery of the STIM1 system has led to a better understanding of how STIM1, by interacting with other components in

the plasma membrane, initiates the process to refill the intracellular Ca²⁺ stores.

Store-operated activation of STIM1/Orai1

Better comprehension of the molecular mechanisms associated with STIM1 function came after the report of a new plasma membrane protein, Orai1, which was named on the basis of Greek mythology. In Greek mythology, the Orai are the keepers of the gates of heaven: Eunomia (Order or Harmony), Dike (Justice) and Eirene (Peace) (10). Patch-clamp studies have shown that CRAC modulator 1, also known as Orai1, modulates I_{CRAC} in *Drosophila* cells (11). This discovery was followed by the identification of a mutation in Orai1 in cells of patients with one form of hereditary severe combined immune deficiency syndrome. The Orai1 mutation resulted in defective SOCE and CRAC channel function (10). Thus, the possibility that Orai1 could be the CRAC channel itself, a subunit of it or a component of the CRAC signaling machinery made this protein a candidate for interaction with STIM1.

Following these seminal findings, it was shown that the combined overexpression of STIM1 and Orai1 markedly increases I_{CRAC} (12). In addition, the interaction between STIM1 and Orai1 is greatly enhanced after thapsigargin treatment (12), which acts as a selective inhibitor of the SERCA resulting in depletion of ER Ca²⁺ stores (13). At the same time, another research group showed that substitutions in the transmembrane helices of Orai1 resulted in decreased Ca²⁺ influx and proposed that Orai1 is an essential pore subunit of the CRAC channel (14). These data were confirmed by another study showing that, upon depletion of ER Ca²⁺ stores, STIM1 and Orai1 move in a coordinated fashion to form closely opposed clusters in the ER and plasma membranes, thereby creating the elementary unit of SOCE (15). Combined expression of STIM1 and Orai1 resulted in a huge gain of SOCE function and indicated that the two proteins are probably sufficient to mediate the operation of SOCE (16). Peel et al. (7) showed that Orai1 small interference RNA (siRNA) transfection on whole airway smooth muscle cells blocks inward currents initiated by Ca²⁺ store depletion. They found a similar result by using inhibitors of SOCE, including 2-aminoethoxydiphenyl borate (2-APB), gadolinium (Gd³⁺) and lanthanum (La³⁺) (7).

In addition, mutations in Orai1 also result in significant changes to the electrophysiological properties of I_{CRAC}, rendering the current less Ca²⁺ selective with outward rectification (11). Such studies have provided evidence for the theory that Orai1 forms the pore forming subunit of the CRAC channel. Orai1 and STIM1 may also contribute to the function of other SOCE channels (17).

These astonishing data brought a new paradigm to the concept of inter-organelle communication and extensive studies have been performed in order to further character-

ize how STIM1 couples store depletion to the functioning of Orai1 in the plasma membrane. Accordingly, it is well known that STIM1 and Orai1 accumulate and colocalize in puncta formations.

Orai1 clusters are formed only in the presence of STIM1, but not expressed alone (18). Following store depletion, STIM1 moves from locations throughout the ER to accumulate in subregions of the ER positioned within 10-25 nm of the plasma membrane. STIM1 must first oligomerize to enable its accumulation at the ER-plasma membrane junctions. Simultaneously, Orai1 gathers at discrete sites in the plasma membrane directly opposite to STIM1, resulting in local CRAC channel activation (19).

Under basal conditions, Orai1 is predominantly a dimer in the plasma membrane. Interaction with the C-terminus of STIM1 causes redimerization of Orai dimers and formation of tetramers that constitute the Ca^{2+} -selective pore (Figure 1A) (20). Single-molecule imaging techniques made it possible to determine the stoichiometry of each CRAC channel complex. Four Orai1 molecules and two STIM1 must associate to form an active CRAC channel (18). This represents a new mechanism by which assembly and activation of the functional ion channel are mediated by the same triggering molecules (Figure 1B).

The relationship between STIM1 and Orai1 expression, puncta formation and I_{CRAC} activation has been examined. It was shown that STIM1 accumulates in puncta equally well in the presence or absence of Orai1 expression. In addition, STIM1 migration is not sufficient for Orai1 accumulation in the same area and normal I_{CRAC} can be activated in STIM1-deficient cells. These data challenge the view of direct conformational coupling between STIM1 and Orai1 as a viable mechanism of puncta formation and SOCE activation and uncover greater complexity in their relationship, which may require additional intermediate elements (21).

Recent information indicates that STIM1 and Orai proteins can interact with and functionally connect to TRPC channels (17,22). As reviewed by Wang et al. (23), TRPC channels may act as "store-operated" channels (SOC) in smooth muscle and it has been an issue with much evidence supporting and denying such a role (23). The clusters formed by STIM1 and Orai1 are also required for TRPC1-SOC channel activation, suggesting that SOC and CRAC channels are regulated by similar molecular components (17,22). These data indicate that the STIM1-Orai1 system interacts with multiple proteins and activates channels in a coordinate way. The association between TRPC channels and STIM1/Orai proteins is also an area of intense investigation.

Taken together, these data show that STIM1 is a key regulator and its activity was initially described for SOCE through an Orai-dependent pathway. However, it is important to remember that there is now considerable evidence supporting a role for TRP proteins in the conduction of Ca^{2+}

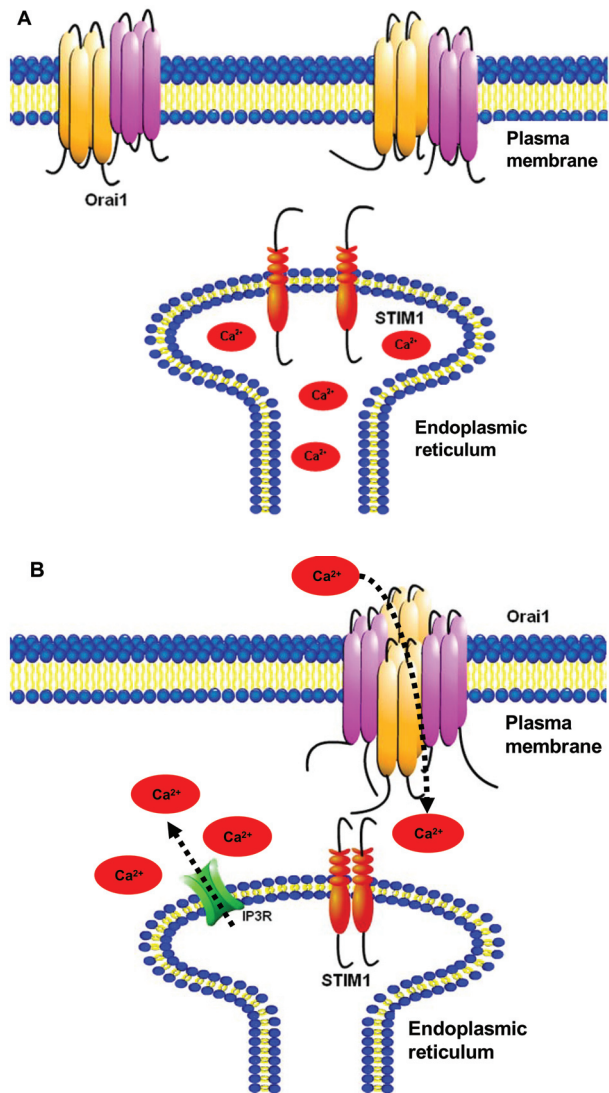


Figure 1. Ca^{2+} entry pathways can be mediated by Orai1, by activation of STIM1. **A**, Orai1 is a protein with four transmembrane regions in the plasma membrane. Under basal condition, when Ca^{2+} is abundant in the endoplasmic reticulum, Orai1 is predominantly a dimer in the plasma membrane. **B**, STIM1 is a single transmembrane-spanning protein located in the endoplasmic reticulum and plasma membrane. Under Ca^{2+} store depletion, e.g., when 1,4,5- inositol trisphosphate (IP_3) is formed and binds to its receptor (IP_3R) on the endoplasmic membrane, and Ca^{2+} is released from stores, the reduced luminal Ca^{2+} causes dissociation of Ca^{2+} bound to the low-affinity helix-loop-helix Ca^{2+} -binding site domain on the N-terminus of STIM1. This Ca^{2+} dissociation causes STIM1 molecules to aggregate and be translocated to regions of the endoplasmic reticulum in close proximity to the plasma membrane. Interaction with the C-terminus of STIM1 induces Orai dimers to dimerize, forming tetramers that constitute the selective Ca^{2+} pore of the channel, which is a highly Ca^{2+} selective store-operated channel moiety. Association of STIM1/Orai1 initiates Ca^{2+} influx and consequently I_{CRAC} . STIM1 = stromal interaction molecule 1; I_{CRAC} = Ca^{2+} release-activated Ca^{2+} .

during SOCE. In this regard, members of the canonical TRP subfamily of cationic channels are especially important.

Orai1, Orai2 and Orai3

Three members of the Orai family have been described: Orai1, Orai2 and Orai3. They are inhibited similarly by extracellular Ca^{2+} , indicating similar affinities for Ca^{2+} within the selectivity filter. However, expression of Orai1 and Orai2, but not Orai3, results in a substantial increase in I_{CRAC} . Orai3 channels appear to differ from Orai1 and Orai2 in being somewhat resistant to the process of Ca^{2+} potentiation (24).

Co-expression of Orai2 with STIM1 increases the activity of the CRAC channels (16), although the currents induced by Orai2 are smaller than those induced by Orai1. Orai3, on the other hand, does not synergistically enhance Ca^{2+} entry when co-expressed with STIM1 (16,25).

In transient transfection experiments, the rank order of Ca^{2+} influx efficacy of Orai family members appears to be Orai1 > Orai2 > Orai3 (26). The differential efficacy of the members of the Orai family may be determined by the N-terminal tail region. It was shown that Orai1 without its N-terminal tail displayed a small effect on SOCE. In addition, the N-terminus region of Orai1 was important for pore-formation of the channel (27). Furthermore, the C-terminus region appears to be a key element for communication between Orai1, but not Orai2 or Orai3, and STIM1 (28).

Orai1 and Orai3, but not Orai2, seem to participate in STIM1-mediated activation in arachidonic acid-regulated Ca^{2+} entry, which is a store-independent process that follows a distinct regulation from the regular CRAC channel activation (29).

Role of STIM1 and Orai1 in pathological conditions

Ca^{2+} concentration is tightly regulated in order to control cell function, and it is well known that an imbalance in Ca^{2+} homeostasis is associated with several diseases. Since STIM1 and Orai1 proteins play a fundamental role in intracellular Ca^{2+} regulation, their contribution to pathological conditions represents an area under intense investigation (30). In this last part of the review, we will discuss evidence suggesting that alterations in the STIM1-Orai1 pathway may play a role in some pathological conditions.

Immune response

The first evidence describing abnormalities in the STIM1/Orai1 pathway was identified in a genetic defect, present in patients with one form of hereditary severe combined immune deficiency (SCID) syndrome. Patients with SCID are homozygous for a single-missense mutation in Orai1 and display a defect in SOCE and CRAC channel function. Feske et al. (10) demonstrated that when T-cells from af-

ected patients were transfected with a functional isoform of Orai1, this restored SOCE and I_{CRAC} .

In T-cells, Ca^{2+} influx through CRAC channels and the formation of an immunological synapse with the antigen-presenting cell are required for the development of an immune response. During T-cell activation, STIM1 and Orai1 are up-regulated and accumulate in the area of contact between resting or super-antigen-pretreated cells, where they colocalize with T-cell receptors (31). Additionally, it was reported that patients with a homozygous nonsense mutation in STIM1, which abrogates the expression of STIM1 and Ca^{2+} influx, exhibit immunodeficiency and autoimmunity (32). This may be of clinical importance and has caught the attention of pharmaceutical companies that currently investigate CRAC channel blockers as an option to reduce the toxicity of immunosuppressant drugs such as cyclosporine A (12).

Cardiovascular disease

Ca^{2+} homeostasis plays an important role, particularly in the vasculature, by regulating the function of the different vascular cell types. Recent studies on STIM1/Orai1 have contributed to enhancing knowledge in this field, especially regarding vascular smooth muscle cell (VSMC) proliferation, platelet aggregation and hypertension.

Recent reports have shown that CRAC seems to be the most important source of SOCE in VSMC, being a significant contributor to proliferation and migration (33). Disruption of Orai1 expression resulted in reduced SOCE and attenuated cell proliferation in human VSMCs, demonstrating that Orai1 is critical to this process (34). Additionally, the STIM1/Orai1 pathway seems to play a role in the SOCE-induced proliferative activity of endothelial cells (35) and STIM1 siRNA abrogated both proliferation and migration of endothelial progenitor cells (36).

The atherosclerotic process leads to alterations in the vascular wall that impair its mechanical properties, resulting in vascular dysfunction. Percutaneous coronary intervention decreases atherosclerosis but the reoccurrence of stenosis after this procedure has been widely reported (37). The restenosis process after vascular injury is characterized by proliferation and migration of medial VSMC, a process that is highly regulated by cytoplasmic Ca^{2+} levels. STIM1 knockdown by siRNA significantly suppressed neointimal hyperplasia in a rat carotid artery balloon injury model and in cultured rat aortic VSMC. Re-expression of STIM1 reversed the effect of STIM1 knockdown in neointimal formation (38). Similar results were obtained in a study using human coronary artery smooth muscle cells (39).

STIM1 and Orai1 have also been reported to be the primary pathway for agonist-evoked Ca^{2+} influx in platelets (40), and consequently for pathological thrombus formation (41). Orai1-deficient mice display severely defective SOCE associated with impairment of both platelet activation and thrombus formation. As a direct consequence, this defi-

ciency results in resistance to pulmonary thromboembolism, arterial thrombosis and ischemic brain infarction, with only a limited bleeding time prolongation (41,42). Furthermore, mice expressing a mutated inactive form of Orai1 in blood cells only [Orai(R93W)] exhibit reduced SOCE and impaired agonist-induced increases in Ca^{2+} , showing that Orai1 is crucial for the pro-coagulant response of platelets (43). The incidence of stroke in diabetic patients is very high. Increased Ca^{2+} entry in platelets from diabetic patients as a consequence of augmented STIM1/Orai1 has been reported (44).

These studies initiated the new concept that the STIM1/Orai1 system is a crucial mediator of ischemic cardio- and cerebrovascular events. Recent evidence suggested that STIM1 may be a new therapeutic target in occlusive vascular disease, including the clinical problem of restenosis after angioplasty (45).

Our group has demonstrated that STIM1/Orai1 is important for the vascular dysfunction associated with arterial hypertension. Aortas from stroke-prone spontaneously hypertensive rats exhibit augmented vascular contractions in response to Ca^{2+} influx, compared to aortas from normotensive rats. In addition, ER store depletion induces greater SOCE activation in arteries from hypertensive rats and this is blocked both by inhibiting STIM1 and Orai1. Furthermore, expression of STIM1 and Orai1 proteins is increased in aortas from the hypertensive rat (46).

The above-mentioned studies show a positive correlation between disturbances in CRAC channel and SOCE function, via the STIM1/Orai1 pathway, indicating that CRAC channel and SOCE inhibitors may represent a new therapeutic approach to the treatment of these conditions. Although there is increasing evidence that the STIM1/Orai1 pathway plays a major role in (patho)physiological processes, many aspects require further investigation. Accordingly, it is not clear whether abnormal activation of STIM1/Orai1 leads to activation of protein kinases that ultimately stimulate VSMC contraction (47). Since defects in Ca^{2+} handling by VSMC are candidates for the pathogenesis of hypertension and that STIM1-Orai1 represent important components for intracellular Ca^{2+} regulation, it seems plausible that augmented activation of STIM1-Orai1 contributes to increased vascular reactivity in hypertension. We recently tested this hypothesis and showed that extracellular Ca^{2+} influx is increased in VSMC during hypertension, partially by STIM1-Orai1-dependent mechanisms (46). However, it remains to be shown whether increased Ca^{2+} influx through the STIM1-Orai1 machinery results in greater activation of Ca^{2+} -dependent proteins, such as protein kinase C (PKC), and therefore contributes to increased vascular reactivity in hypertension. Since increased activation of Ca^{2+} -dependent isoforms of PKC results in increased vascular resistance and hypertension, it would be interesting to determine whether this results from increased STIM1/Orai1 activation. It is possible that an intervention at the molecular level,

targeting STIM1-Orai1 system activation, could improve vascular function in hypertensive conditions.

Sexual dysfunction

Recent studies have examined the role of Ca^{2+} signaling through STIM1/Orai1 in erectile dysfunction. At the 2010 Sexual Medicine Society of North America meeting, two abstracts were presented, which characterized the presence and role of STIM1 and Orai1 in human penile corporal smooth muscle cells (CSMC) and internal pudendal arteries from hypertensive rats (48,49). A common mechanism of erectile dysfunction is the inability of the penile cavernous tissue to relax. In diseased states such as hypertension or diabetes, the cavernous tissue and vasculature supplying the penis have increased contractile responses to agonists such as endothelin-1, phenylephrine, angiotensin, etc. (50,51). It has been hypothesized that the augmented contraction is a result of increased sensitivity of SOCE leading to greater cavernous smooth muscle tone. In both human and cultured CSMC, the endogenous expression of STIM1, Orai1 and TRPC proteins was confirmed via RT-PCR (52). Additionally, after depletion of Ca^{2+} stores with thapsigargin or cyclopiazonic acid, Ca^{2+} re-entry was reduced in the presence of SOCE blockers. The internal pudendal artery is the main arterial supply to the penis and contributes to 70% of the total penile vascular resistance during an erectile response (52). In angiotensin-II-infused hypertensive rats, after depletion and reloading of Ca^{2+} stores, the caffeine-mediated contractions were greater in pudendal arteries from hypertensive animals (49). Furthermore, these contractions were diminished in the presence of thapsigargin. Thus, increased activation of STIM1/Orai1 may lead to impaired intracellular Ca^{2+} control, excessive constriction of the pudendal arteries of hypertensive rats and contribute to erectile dysfunction. The ability to modulate the control of the activity of STIM1/Orai1 may provide a novel therapeutic target for the treatment of erectile dysfunction.

Pulmonary disease

The bronchoconstriction response of the asthmatic airway depends on airway smooth muscle contraction and this response is directly associated with increased cytoplasmic Ca^{2+} through SOCE. Orai1, together with STIM1, has been shown to be a major mediator of SOCE in human airway smooth muscle cells and modulation of this pathway was proposed to be useful in the treatment of bronchoconstriction (7,53,54). Knockdown of STIM1 resulted in reduced SOCE during acute hypoxia in pulmonary arterial smooth muscle (55).

Cancer

A better understanding of the proliferative process is particularly important to understand the pathophysiology of cancers. In 2004, before the discovery of the role of STIM1 in Ca^{2+} regulation, one specific study showed that

the STIM1 gene, at that time classified as a transmembrane glycoprotein gene, was a candidate as a metastasis-related gene. The initial observations suggested that STIM1 worked as a tumor suppressor (56), but recent data indicated the contrary (30), which may be a result of the complexity of the pathophysiology of cancer.

More recently, it has been shown that Orai1 and STIM1 are essential for breast tumor cell migration and Orai1 and STIM1 siRNA was able to decrease tumor metastasis in animal models, suggesting that SOCE may be a very important target for cancer therapy (57). One study conducted on various breast cell lines showed a differential contribution of STIM1/2 and Orai3 among different cells. SOCE was mediated by STIM1/2 and Orai3 in breast cell lines expressing estrogen receptors, whereas breast cell lines lacking estrogen receptors used STIM1/Orai1 to induce SOCE, suggesting a potential role for Orai3 as a target for breast cancer expressing estrogen receptors (58). The store-independent activation of Orai1 was described in mammary tumors, and it seems that the secretory pathway of Ca^{2+} -ATPase is involved in this process (59).

Perspectives

Over the past 4 years, a long-standing mystery in the cell biology of Ca^{2+} channel regulation has begun to be

uncovered. Components of the signaling pathway linking intracellular Ca^{2+} stores to plasma membrane Ca^{2+} entry channels were discovered. STIM1 was identified as a Ca^{2+} sensor essential for Ca^{2+} store depletion-triggered Ca^{2+} influx. Orai1 was recognized as being an essential component of the CRAC channel. Orai1 combined with STIM1 was shown to reconstitute store-operated Ca^{2+} channel function and STIM1 and Orai1 were biochemically and functionally identified. The molecular dissection of the CRAC channel complex, moving the field beyond I_{CRAC} signature to structure, was partially revealed. Numerous studies have shown an abundance of sites where Ca^{2+} signaling through its sensor, STIM1 and Orai1, might be controlled to modulate (patho)physiological conditions.

The metabolism of Ca^{2+} is profoundly disturbed in hypertension. Considering that STIM1 and the Orai1 pathway participates in ER Ca^{2+} regulation, it seems plausible that alterations in these proteins play a role in arterial hypertension-associated vascular dysfunction. Therefore, STIM and Orai proteins represent highly promising new pharmacological targets that should be investigated further.

Acknowledgments

Research supported by a grant from the American Heart Association (AHA #09GRNT2250383) and FAPESP.

References

- Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 2003; 4: 517-529.
- Parekh AB, Putney JW Jr. Store-operated calcium channels. *Physiol Rev* 2005; 85: 757-810.
- Bolotina VM. Orai, STIM1 and iPLA2beta: a view from a different perspective. *J Physiol* 2008; 586: 3035-3042.
- Naghdhi S, Waldeck-Weiermair M, Fertschai I, Poteser M, Graier WF, Malli R. Mitochondrial Ca^{2+} uptake and not mitochondrial motility is required for STIM1-Orai1-dependent store-operated Ca^{2+} entry. *J Cell Sci* 2010; 123: 2553-2564.
- Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, et al. STIM is a Ca^{2+} sensor essential for Ca^{2+} -store-depletion-triggered Ca^{2+} influx. *Curr Biol* 2005; 15: 1235-1241.
- Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, et al. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol* 2005; 169: 435-445.
- Peel SE, Liu B, Hall IP. ORAI and store-operated calcium influx in human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2008; 38: 744-749.
- Spassova MA, Soboloff J, He LP, Xu W, Dziadek MA, Gill DL. STIM1 has a plasma membrane role in the activation of store-operated Ca^{2+} channels. *Proc Natl Acad Sci U S A* 2006; 103: 4040-4045.
- Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM reconstitute store-operated calcium channel function. *J Biol Chem* 2006; 281: 20661-20665.
- Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 2006; 441: 179-185.
- Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* 2006; 312: 1220-1223.
- Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 2006; 443: 226-229.
- Leung FP, Yung LM, Yao X, Laher I, Huang Y. Store-operated calcium entry in vascular smooth muscle. *Br J Pharmacol* 2008; 153: 846-857.
- Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006; 443: 230-233.
- Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol* 2006; 174: 815-825.
- Mercer JC, Dehaven WI, Smyth JT, Wedel B, Boyles RR, Bird GS, et al. Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1. *J Biol Chem* 2006; 281: 24979-24990.

17. Ambudkar IS, Ong HL, Liu X, Bandyopadhyay BC, Cheng KT. TRPC1: the link between functionally distinct store-operated calcium channels. *Cell Calcium* 2007; 42: 213-223.
18. Ji W, Xu P, Li Z, Lu J, Liu L, Zhan Y, et al. Functional stoichiometry of the unitary calcium-release-activated calcium channel. *Proc Natl Acad Sci U S A* 2008; 105: 13668-13673.
19. Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature* 2008; 454: 538-542.
20. Penna A, Demuro A, Yeromin AV, Zhang SL, Safrina O, Parker I, et al. The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature* 2008; 456: 116-120.
21. Gwozdz T, Dutko-Gwozdz J, Zarayskiy V, Peter K, Bolotina VM. How strict is the correlation between STIM1 and Orai1 expression, puncta formation, and ICRAC activation? *Am J Physiol Cell Physiol* 2008; 295: C1133-C1140.
22. Liao Y, Exleben C, Yildirim E, Abramowitz J, Armstrong DL, Birnbaumer L. Orai proteins interact with TRPC channels and confer responsiveness to store depletion. *Proc Natl Acad Sci U S A* 2007; 104: 4682-4687.
23. Deng X, Wang Y, Zhou Y, Soboloff J, Gill DL. STIM and Orai: dynamic intermembrane coupling to control cellular calcium signals. *J Biol Chem* 2009; 284: 22501-22505.
24. Dehaven WI, Smyth JT, Boyles RR, Putney JW Jr. Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem* 2007; 282: 17548-17556.
25. Gross SA, Wissenbach U, Philipp SE, Freichel M, Cavalie A, Flockerzi V. Murine ORAI2 splice variants form functional Ca²⁺ release-activated Ca²⁺ (CRAC) channels. *J Biol Chem* 2007; 282: 19375-19384.
26. Guo RW, Huang L. New insights into the activation mechanism of store-operated calcium channels: roles of STIM and Orai. *J Zhejiang Univ Sci B* 2008; 9: 591-601.
27. Takahashi Y, Watanabe H, Murakami M, Ono K, Munehisa Y, Koyama T, et al. Functional role of stromal interaction molecule 1 (STIM1) in vascular smooth muscle cells. *Biochem Biophys Res Commun* 2007; 361: 934-940.
28. Frischauf I, Muik M, Derler I, Bergsmann J, Fahrner M, Schindl R, et al. Molecular determinants of the coupling between STIM1 and Orai channels: differential activation of Orai1-3 channels by a STIM1 coiled-coil mutant. *J Biol Chem* 2009; 284: 21696-21706.
29. Mignen O, Thompson JL, Shuttleworth TJ. Orai1 subunit stoichiometry of the mammalian CRAC channel pore. *J Physiol* 2008; 586: 419-425.
30. Roberts-Thomson SJ, Peters AA, Grice DM, Monteith GR. ORAI-mediated calcium entry: mechanism and roles, diseases and pharmacology. *Pharmacol Ther* 2010; 127: 121-130.
31. Lioudyno MI, Kozak JA, Penna A, Safrina O, Zhang SL, Sen D, et al. Orai1 and STIM1 move to the immunological synapse and are up-regulated during T cell activation. *Proc Natl Acad Sci U S A* 2008; 105: 2011-2016.
32. Picard C, McCarl CA, Papolos A, Khalil S, Luthy K, Hivroz C, et al. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med* 2009; 360: 1971-1980.
33. Potier M, Gonzalez JC, Motiani RK, Abdullaev IF, Bisailon JM, Singer HA, et al. Evidence for STIM1- and Orai1-dependent store-operated calcium influx through I_{CRAC} in vascular smooth muscle cells: role in proliferation and migration *FASEB J* 2009; 23: 2425-2437.
34. Baryshnikov SG, Pulina MV, Zulian A, Linde CI, Golovina VA. Orai1, a critical component of store-operated Ca²⁺ entry, is functionally associated with Na⁺/Ca²⁺ exchanger and plasma membrane Ca²⁺ pump in proliferating human arterial myocytes. *Am J Physiol Cell Physiol* 2009; 297: C1103-C1112.
35. Abdullaev IF, Bisailon JM, Potier M, Gonzalez JC, Motiani RK, Trebak M. Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ Res* 2008; 103: 1289-1299.
36. Kuang CY, Yu Y, Guo RW, Qian DH, Wang K, Den MY, et al. Silencing stromal interaction molecule 1 by RNA interference inhibits the proliferation and migration of endothelial progenitor cells. *Biochem Biophys Res Commun* 2010; 398: 315-320.
37. Glass CK, Witztum JL. Atherosclerosis. The road ahead. *Cell* 2001; 104: 503-516.
38. Guo RW, Wang H, Gao P, Li MQ, Zeng CY, Yu Y, et al. An essential role for stromal interaction molecule 1 in neointima formation following arterial injury. *Cardiovasc Res* 2009; 81: 660-668.
39. Aubart FC, Sassi Y, Coulombe A, Mougnot N, Vrignaud C, Leprince P, et al. RNA interference targeting STIM1 suppresses vascular smooth muscle cell proliferation and neointima formation in the rat. *Mol Ther* 2009; 17: 455-462.
40. Galan C, Zbidi H, Bartegi A, Salido GM, Rosado JA. STIM1, Orai1 and hTRPC1 are important for thrombin- and ADP-induced aggregation in human platelets. *Arch Biochem Biophys* 2009; 490: 137-144.
41. Varga-Szabo D, Braun A, Kleinschnitz C, Bender M, Pleines I, Pham M, et al. The calcium sensor STIM1 is an essential mediator of arterial thrombosis and ischemic brain infarction. *J Exp Med* 2008; 205: 1583-1591.
42. Braun A, Varga-Szabo D, Kleinschnitz C, Pleines I, Bender M, Austinat M, et al. Orai1 (CRACM1) is the platelet SOC channel and essential for pathological thrombus formation. *Blood* 2009; 113: 2056-2063.
43. Bergmeier W, Chauhan AK, Wagner DD. Glycoprotein Ibalpha and von Willebrand factor in primary platelet adhesion and thrombus formation: lessons from mutant mice. *Thromb Haemost* 2008; 99: 264-270.
44. Zbidi H, Lopez JJ, Amor NB, Bartegi A, Salido GM, Rosado JA. Enhanced expression of STIM1/Orai1 and TRPC3 in platelets from patients with type 2 diabetes mellitus. *Blood Cells Mol Dis* 2009; 43: 211-213.
45. Agrotis A, Koulis C. STIM1: a new therapeutic target in occlusive vascular disease? *Cardiovasc Res* 2009; 81: 627-628.
46. Giachini FR, Chiao CW, Carneiro FS, Lima VV, Carneiro ZN, Dorrance AM, et al. Increased activation of stromal interaction molecule-1/Orai-1 in aorta from hypertensive rats: a novel insight into vascular dysfunction. *Hypertension* 2009; 53: 409-416.
47. Salamanca DA, Khalil RA. Protein kinase C isoforms as specific targets for modulation of vascular smooth muscle function in hypertension. *Biochem Pharmacol* 2005; 70: 1537-1547.
48. Lee SW, Chae MR, Lee JH, Park JK. Molecular and functional characterization of store-operated Ca²⁺ channel in

- corporal smooth muscle cells. *J Sexual Med* 2011 (in press).
49. Hannan JL, Hardy DM, Giachini FR, Webb RC. Intracellular calcium is regulated by stromal interaction molecule-1/Orai-1 in pudendal arteries from hypertensive rats. *J Sexual Med* 2011 (in press).
 50. Okabe H, Hale TM, Kumon H, Heaton JP, Adams MA. The penis is not protected - in hypertension there are vascular changes in the penis which are similar to those in other vascular beds. *Int J Impot Res* 1999; 11: 133-140.
 51. Carneiro FS, Giachini FR, Lima VV, Carneiro ZN, Nunes KP, Ergul A, et al. DOCA-salt treatment enhances responses to endothelin-1 in murine corpus cavernosum. *Can J Physiol Pharmacol* 2008; 86: 320-328.
 52. Hannan JL, Blaser MC, Oldfield L, Pang JJ, Adams SM, Pang SC, et al. Morphological and functional evidence for the contribution of the pudendal artery in aging-induced erectile dysfunction. *J Sex Med* 2010; 7: 3373-3384.
 53. Lu W, Wang J, Shimoda LA, Sylvester JT. Differences in STIM1 and TRPC expression in proximal and distal pulmonary arterial smooth muscle are associated with differences in Ca²⁺ responses to hypoxia. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L104-L113.
 54. Ng LC, Ramduny D, Airey JA, Singer CA, Keller PS, Shen XM, et al. Orai1 interacts with STIM1 and mediates capacitative Ca²⁺ entry in mouse pulmonary arterial smooth muscle cells. *Am J Physiol Cell Physiol* 2010; 299: C1079-C1090.
 55. Lu W, Wang J, Peng G, Shimoda LA, Sylvester JT. Knock-down of stromal interaction molecule 1 attenuates store-operated Ca²⁺ entry and Ca²⁺ responses to acute hypoxia in pulmonary arterial smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2009; 297: L17-L25.
 56. Suyama E, Wadhwa R, Kaur K, Miyagishi M, Kaul SC, Kawasaki H, et al. Identification of metastasis-related genes in a mouse model using a library of randomized ribozymes. *J Biol Chem* 2004; 279: 38083-38086.
 57. Yang S, Zhang JJ, Huang XY. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 2009; 15: 124-134.
 58. Motiani RK, Abdullaev IF, Trebak M. A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. *J Biol Chem* 2010; 285: 19173-19183.
 59. Feng M, Grice DM, Faddy HM, Nguyen N, Leitch S, Wang Y, et al. Store-independent activation of Orai1 by SPCA2 in mammary tumors. *Cell* 2010; 143: 84-98.