STIM1 and Orai1: Novel Targets for Vascular Diseases?

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Abstract

The past five years have witnessed the discovery of the endoplasmic reticulum calcium (Ca^{2+}) sensor STIM1 and the plasma membrane Ca^{2+} channel Orai1 as the *bona fide* molecular components of the store-operated Ca^{2+} entry (SOCE) and the Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}). It has been known for two decades that SOCE and I_{CRAC} are required for lymphocyte activation as evidenced by severe immunodeficient phenotypes in patients lacking I_{CRAC}. In recent years however, studies have uncovered expression of STIM1 and Orai1 proteins in various tissues and described additional roles for these proteins in physiological functions and pathophysiological conditions. Here, we will summarize novel findings pertaining to the role of STIM1 and Orai1 in the vascular system and discuss their potential use as targets in the therapy of vascular disease.

Keywords

Calcium signaling; Calcium channels; Orai1; STIM1; CRAC channels; Vascular disease

Introduction

Calcium (Ca^{2+}) ions are universal second messengers and, unlike other second messengers, they are generally located outside the cells or stored in cell organelles such as the endoplasmic reticulum (ER). Resting cytoplasmic Ca^{2+} concentrations are maintained at 100-300nM[20,22,56], orders of magnitude lower than the extracellular Ca^{2+} concentrations (1-2mM). The ER lumen for instance usually maintains Ca^{2+} concentrations of around 500μM and is responsible for transient cytoplasmic Ca^{2+} increases upon extracellular stimulations. However, a sustained Ca^{2+} elevation requires Ca^{2+} entry from the extracellular space through Ca^{2+} channels located on the plasma membrane (PM). The Ca^{2+} entry happens concomitantly to the Ca^{2+} release from ER lumen and, in some cases, Ca^{2+} entry is triggered by Ca^{2+} release. The Ca^{2+} entry pathway that is specifically activated as a consequence of store emptying is called store-operated Ca^{2+} entry (SOCE) or capacitative Ca^{2+} entry[14,38,40-42], and the best characterized store-operated Ca^{2+} current is the calcium release-activated calcium current (I_{CRAC})[21]. Recent studies have identified the major protein components of the SOCE pathway: the Ca^{2+} sensor protein STIM1[26,43] and the Ca^{2+} channel protein Orai1[12,54].

Calcium release from ER lumen is achieved either actively through physiological agonist-mediated IP$_3$ production and subsequent activation of IP$_3$ receptors or passively by pharmacological inhibition of the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) on the ER membrane by drugs such as thapsigargin. Store depletion causes the Ca^{2+} sensor STIM1 on the ER membrane to oligomerize and move to regions of close ER-PM junctions.

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Orai1 channels on the plasma membrane move to the same ER-PM junctions and are activated by STIM1 oligomers through direct interaction of a minimal C-terminal 100 amino acid region of STIM1 to the C- and N-termini of Orai1[8]. Ectopic expression of the minimal 100 amino acid region of STIM1 which was termed SOAR (for STIM Orai Activating Region) or CAD (for CRAC activation Domain)[36,59] is sufficient to activate Orai1 channels and induce Ca^{2+} entry independently of store depletion.

There are other members of the STIM and Orai families encoded by separate genes: STIM2, Orai2 and Orai3. STIM2 appears to be involved in maintaining resting Ca^{2+} concentrations. Orai2 and Orai3 are activated by STIM proteins in a similar manner to Orai1 when co-expressed in HEK293 cells[8,13,47] . Orai3 has been shown to encode a native I_{CRAC}-like conductance in breast cancer cells[29], and to contribute to heteromultimeric Orai3/Orai1 store-independent arachidonate-regulated Ca^{2+} channels[28]. The role of Orai2 in encoding native Ca^{2+} entry pathways remains unclear. Ca^{2+} signaling and SOCE play an essential role in diverse cell functions and are involved in cellular dysfunctions, including those of the vascular system.

Vascular Smooth Muscle Cells and Vascular Proliferative Diseases

Vascular smooth muscle cells (VSMCs) are the most prominent cell type in blood vessel wall and are responsible for the maintenance of vascular tone[57,58]. VSMCs disorders, usually manifested as a phenotype change, are involved in the pathogenesis of major vascular diseases such as atherosclerosis, hypertension and restenosis. Mechanical or inflammatory stimuli to the blood vessel site cause VSMCs to dedifferentiate from the fully mature quiescent contractile phenotype to an immature synthetic, proliferative and migratory phenotype. Synthetic VSMCs are mainly responsible for the narrowing of blood vessels in atherosclerosis[35,44] and restenosis[27] due to the proliferative/migratory VSMCs accumulation within the innermost layer of blood vessel wall. VSMC proliferation also occurs during vessel remodeling characteristic of development and progression of hypertension as increased presence of immature VSMC was found in the arterioles of hypertensive patients[39].

Ca^{2+} Signaling modulates VSMC function, including phenotypic change, proliferation and migration. SOCE was found increased in synthetic VSMCs compared to the quiescent freshly isolated cells and this correlated with increased protein expression of STIM1 and Orai1[5,37]. Our lab has demonstrated STIM1 and Orai1 are essential components of SOCE and I_{CRAC} in rat VSMCs[37]. The knockdown of STIM1 or Orai1 greatly reduced thapsigargin- or platelet derived growth factor (PDGF)-activated SOCE, as well as VSMC proliferation and migration[6,37]. STIM2, Orai2, Orai3, TRPC1, TRPC4 or TRPC6 knockdown did not show any effect on SOCE. Studies from other groups have also demonstrated that STIM1 is the major component of SOCE and plays an essential role in the proliferation of various types of VSMCs[24,49]. Using an *in vivo* model of VSMC proliferation and migration, STIM1 was found up-regulated in injured rat carotid arteries subjected to balloon injury[3,6,18]. We reported that the protein expression of Orai1 is upregulated in media and neointima of carotid arteries from balloon-injured rats[6]. When an adenoviral vector encoding a short hairpin RNA (shRNA) against STIM1 was applied at the balloon-injured site to prevent STIM1upregulation, neointima formation and the activation of transcription factor NFAT were prevented[3,18]. A recent report from our group demonstrated that prevention of Orai1 upregulation and I_{CRAC} activation in the balloon-injured carotid artery of rats using a lentiviral vector encoding shRNA inhibits NFAT nuclear translocation and activity, VSMC proliferation and neointima formation[60].

SOCE was also found increased in metabolic syndrome (MetS) swine coronary smooth muscle cells. MetS is a combination of medical disorders and MetS patients are at higher risk for coronary artery disease, stroke and type 2 diabetes. The swine MetS model consists of 40 weeks on atherogenic diets and stent placement in coronary artery of male Ossabaw pigs[9,32]. The increase of SOCE in MetS smooth muscle cells was associated with increased STIM1, Orai1 and TRPC1 mRNA and protein levels[9]. However, the increase of SOCE and STIM1/TRPC1 protein overexpression were attenuated by subjecting the pigs to exercise training[9].

Giachini and colleagues has discovered a role for STIM1 and Orai1 in hypertensive rat aorta[15]. Twenty-four weeks old stroke-prone spontaneously hypertensive rats (SHR) displayed higher systolic blood pressure, compared to Wistar-Kyoto (WKY) rats. Endothelium-denuded aortic rings from SHR displayed greater contractions than WKY rats during the Ca^{2+} store re-loading period following store depletion. Caffeine-induced SHR aortic contraction was greater than WKY rats', and both contractions were dramatically suppressed by CRAC channel blockers 2-aminophenyl borane (2-APB) and gadolinium (Gd^{3+}). Application of Orai1 or STIM1 antibodies reduced the SR Ca^{2+} loading capacity and the transient aortic contractions of both SHR and WKY rats induced by caffeine and abolished the differences in contractions between SHR and WKY rats. These authors showed higher protein and mRNA levels of STIM1 and Orai1 in SHR aortas. Immuno-fluorescence analysis confirmed the increased expression of STIM1 and Orai1 proteins in SHR aortas[15]. Collectively, the data presented above supports a role for the SOCE pathway and STIM1/Orai1 proteins in driving smooth muscle proliferation and migration and suggests the potential use of these proteins as targets for vascular occlusive diseases.

Platelets and Thrombosis

Platelets are small anucleated cells derived from bone marrow megakaryocytes. They are circulating within the blood, being prepared to respond to any damage to the blood vessels. The stimuli released from the injured site of blood vessel walls triggers platelets activation and aggregation, which causes blood coagulation to maintain blood in the circulatory system (hemostasis). However, when the platelets aggregation and blood coagulation are enhanced in response to severe damage and overwhelming stimuli, the blood clot formed inside the vessel lumen obstructs the blood flow rather than heals the injury, and this process is a pathological one called thrombosis. Platelets activation is the major cause of thrombosis and is therefore the major target of antithrombotic therapy. Platelets activation and aggregation requires intracellular Ca^{2+} influx during the processes of their adhesion to the exposed subendothelium, aggregation and formation of clots in blood vessel lumen. Diverse agonists are involved in platelets activation through elevations of Ca^{2+} concentrations, mainly through two different pathways [24]. Soluble agonists (thrombin, ADP and thromboxaneA_2; TXA_2) activate platelets through G protein-coupled receptors (P2Y, PAR, and TP respectively). G protein then leads to the activation of phospholipase Cβ (PLCβ) and IP_3 production[19,25]. Adhesive ligands (von Willebrand factor; vWF, collagen, fibronection, etc) activate platelet surface receptors such as GPIb/V/IX, GPVI, and integrins, and trigger the production of IP_3 by activation of phospholipase Cγ (PLCγ)[53]. Although both pathways can presumably activate SOCE by the production of IP_3, the G protein pathway mainly triggers platelet-platelet interaction, while GPIb/V/XI and GPVI pathways mediate platelets aggregation through platelet-matrix adhesion. The cellular mechanisms of these processes remain largely unknown.

Both STIM1 and Orai1 are found highly expressed in platelets, suggesting they might play an important role in platelet function. Bernhard Nieswandt group has generated three types of SOCE-impaired mice line: STIM1$^{sax/sax}$ (an activating STIM1 EF hand D84G mutant),

STIM1$^{-/-}$, and Orai1$^{-/-}$ [7,17,52]. The STIM1$^{sax/sax}$ homozygous mice embryos had severe hemorrhage and only a few of them survived until E13-E14. Due to the high lethality and low survival of homozygous animals (1 out of 72 offspring), most of the work was done in the STIM1$^{sax/+}$ heterozygous mice in which the impairment of SOCE and platelets function was also observed. Platelets from STIM1$^{sax/+}$ mice were pre-activated, displayed elevated basal Ca^{2+} concentrations and a short life span. Ca^{2+} store release evoked by thapsgargin (TG) was reduced by 60% in the platelets from these heterozygous animals and the subsequent Ca^{2+} influx (in the presence of 2mM extracellular Ca^{2+}) was reduced by 70% in compared with wild type. One important mechanism was discovered when the authors compared the Ca^{2+} influx in response to agonists in STIM1$^{sax/+}$ and wild type platelets. They found that, Ca^{2+} influx in STIM1 mutant platelets was reduced only upon stimulation with collagen receptor-specific agonists (such as collagen related peptide; CRP and rhodocytin; RC)[17]. Those receptors are associated with the immunoreceptor tyrosine-based activating motif (ITAM) and triggers Ca^{2+} influx through activation of the PLCγ pathway, reminiscent of T cell receptor activation[33]. In contrast, when G protein coupled agonists such as thrombin and ADP were applied, levels of Ca^{2+} rise were similar in both mutant and wild type platelets[17]. The STIM1$^{sax/+}$ mice blood also showed less adhesion to the collagen surface than wild type. Tail bleeding times were significantly prolonged in STIM1$^{sax/+}$ mice. One specific injury model was used, in which the thrombus formation is mainly driven by thrombin, and the formation times of occlusion were similar in STIM1$^{sax/+}$ mice and wild type mice[17].

In the STIM1$^{-/-}$ mice platelets, Ca^{2+} influx evoked by either CRP or by the G protein-coupled agonists (ADP, thrombin and TXA$_2$ analogue U46619) was suppressed. However, in a manner similar to STIM1$^{sax/+}$ mice, platelets aggregation was only diminished in STIM1$^{-/-}$ blood when triggered by collagen-related agonists, and did not change in STIM1$^{-/-}$ blood when triggered by G protein-coupled agonists, compared to wild type. The experiments of three-dimensional growth of thrombi on collagen-coated surface showed that, STIM1$^{-/-}$ platelets formed less thrombus than the wild type did. Surface area covered by mutant platelets was reduced by ~42%, and the total volume of thrombus that formed by mutant platelets was reduced by ~81%. *In vivo* experiments showed a mild prolongation of tail bleeding time, a significant delay in vessel occlusion time and a high resistance to ischemic brain infarction in STIM1$^{-/-}$ mice[52].

Orai1 was found to be the predominant member of the Orai family in both human and mice platelets. Because the Orai1 knockout mice showed very high mortality, Braun et al transplanted Orai1$^{-/-}$ bone marrow to irradiated wild-type mice and generated platelets Orai1$^{-/-}$ chimeric mice[7]. Thapsigargin-evoked SOCE was dramatically suppressed in platelets, indicating that Orai1 is the essential component of SOCE in these cells. Very similar to STIM1$^{-/-}$ platelets, the Ca^{2+} influx of Orai1$^{-/-}$ platelets was inhibited in response to CRP, ADP and thrombin. However, the Orai1$^{-/-}$ platelets aggregation in response to G protein-coupled agonists (ADP and thrombin) was comparable to wild type, but was diminished in response to low concentration of collagen or CRP. Intravenous injection of collagen/epinephrine caused death of wild type mice within 20 minutes by pulmonary thromboembolism, whereas most Orai1$^{-/-}$ mice (6 out of 7) survived[7]. In an arterial thrombosis model, whereas all the wild type mice got complete occlusion, 4 out of 10 Orai1-knockout mice had maintained blood flow. In a FeCl$_3$-induced arterioles injury model, where the thrombus formation mainly depends on thrombin, 14 out of 15 Orai1$^{-/-}$ mice had occlusive thrombi and the process was similar to wild type mice. Similar to the STIM1$^{-/-}$ mice, the Orai1$^{-/-}$ mice showed high resistance to ischemic brain infarction[7]. Around the same time, Stefan Feske's group generated chimeric mice expressing Orai1^{R93W} exclusively in blood cells[4]; Orai1^{R93W} is the mutation of Orai1 found in severe combined immunodeficient patients (SCID) characterized by abrogated SOCE and I_{CRAC}[11]. Ca^{2+}

entry was impaired when $Orai1^{R93W}$ platelets were stimulated by either thapsigargin or agonists (PAR4p or convulxin to active PAR4 or GPVI respectively). However, platelet aggregation and thrombus formation was not apparently affected[4]. Since mice harboring $Orai1^{R93W}$ mutation are equivalent to human SCID patients with $Orai1^{R91W}$ mutation and these patients did not show any obvious bleeding or clotting issues, these results are consistent with the clinical observations. However, these authors found that exposure of phosphatidylserine (PS) on cell surface of $Orai1^{R91W}$ platelets was reduced by 80%[4]. The cell surface exposure of PS is required for platelet procoagulant activity and is triggered by cytosolic Ca^{2+} elevation. These results were confirmed by the Nieswandt group where PS exposure and thrombus generation was suppressed in $STIM1^{-/-}$ and $Orai1^{-/-}$ mice when triggered through the GPVI pathway (using convulxin as an agonist) but not through thrombin stimulation; STIM2 did not show any apparent role in these processes[16].

To summarize, STIM1 and Orai1 are the major components of SOCE in platelets and dominate the Ca^{2+} Entry through GPIb-GPVI-ITAM signaling pathway. Deficiency of STIM1 or Orai1 reduced thrombosis formation in arteries (subjected to high shear flow conditions) when triggered mainly by collagen (through GPVI/ PLCγ pathway). In contrast, STIM1 and Orai1 did not show any evident effect in hemostasis at the wound bleeding site, where abundant tissue factors are involved and thrombus formation is triggered mainly by thrombin or other tissue factors acting through G protein coupled receptors involving the activation of the PLCβ pathway.

Endothelial Cells and Angiogenesis

Endothelial cells line the inner side of blood vessels and help maintain vascular tone through production of nitric oxide and acts as a barrier between the circulating blood and the surrounding tissue. Endothelial dysfunction is involved in various cardiovascular diseases.

Endothelial cells have been known for a while to express the SOCE pathway[10,46] and the elevation of Ca^{2+} concentration in cytoplasm plays an important role in a variety of endothelial cell functions, such as barrier function, motility, proliferation, migration and angiogenesis[2,30,31,50,51]. Work in our lab showed that endothelial cells from different vascular beds, human umbilical vein endothelial cells (HUVEC) and human pulmonary artery endothelial cells (HPAEC) express STIM and Orai proteins and display SOCE and I_{CRAC} that are encoded by STIM1 and Orai1[1]. Interestingly, endothelial agonists such as vascular endothelial growth factor (VEGF) or thrombin regulate intracellular calcium entry through STIM1/Orai1-mediated SOCE[1]. STIM1/Orai1-mediated SOCE plays an essential role in HUVEC proliferation; STIM1 or Orai1 knockdown using silencing RNA (siRNA) lead to cell cycle arrest at S and G2/M phases of the cell cycle[1]. Orai1 and SOCE are also functional in HUVEC angiogenesis[23]. Knockdown of Orai1 or STIM1 by siRNA suppressed VEGF-evoked Ca^{2+} entry and inhibited HUVEC migration through 8-μm pores. Orai1 was pivotal for tube formation of HUVEC in Matrigel, suggesting Orai1 as a potential target in angiogenesis. A CRAC channel inhibitor, S66, showed similar effect on tube formation and also inhibited vessel growth in an *in vivo* model of angiogenesis[23]. SOCE was also found expressed in human Endothelial Progenitor Cells (EPC)[45], including those harvested from Peripheral blood (PB-EPC) and umbilical cord blood (UCB-EPC). Orai1 and STIM1 were more abundant in both types of EPCs, and were proposed as the major components of SOCE. Knockdown of STIM1 attenuated hepatocyte growth factor (HGF)-induced SOCE and proliferation in EPC[48].

Cardiac function and hypertrophy

Ohba et al demonstrated that rat cardiomyocytes express STIM1 and that knockdown of STIM1 inhibited Ca^{2+} entry in response to thapsigargin and endothelin-1[34]. Importantly,

STIM1 knockdown inhibited endothelin1-mediated nuclear factor for activated T-cells (NFAT) activation and prevented the increase of cardiac fetal genes induced by hypertrophic stimuli such as endothelin-1, phenyl-epinephrine[34]. Voelkers et al demonstrated that STIM1 and Orai1 proteins are required for thapsigargin-mediated SOCE and Ca^{2+} transients in neonatal cardiomyocytes[55]. These authors also showed that knockdown of Orai1 caused a significant decrease in cardiac fetal genes expression and size of neonatal cardiomyocytes and a reduction in ERK1/2 phosphorylation and calcineurin activation under resting conditions and hypertrophic stimulation with phenylepinephrine [55]. However, STIM1 knockdown caused a significant decrease in cell size only in response to hypertrophic stimulation with phenylephrine, but had no effect under resting conditions. These data support a role for STIM1 and Orai1 in hypertrophic cardiac remodeling.[55]

Conclusions

STIM1 and Orai1 have recently emerged as mediators for SOCE in several cell types including those of the cardiovascular system. The contribution of STIM1 and Orai1 to cardiovascular diseases is also beginning to emerge. As of this writing, there is no indication for a role of Orai2 or Orai3 in the cardiovascular system but future research is likely to unravel novel roles for these isoforms. Orai isoforms are known to heteromultimerize suggesting means to enhance the diversity of calcium signals induced by specific agonists to control specific physiological functions. We look forward future work to unravel additional roles, oligomerization patterns and regulation mechanisms for native STIM and Orai proteins in different cells of the cardiovascular system. Deciphering subtle differences of Orai channel organization and regulation between different cell types might make selective targeting of these molecules in treatment of cardiovascular diseases a reality.

Acknowledgments

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