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# STIM1 and Orai in cardiac hypertrophy and vascular proliferative diseases

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## 1. ABSTRACT

Cardiac hypertrophy and vascular proliferative diseases are associated with major alterations in calcium homeostasis and calcium signaling. The recent discovery of STIM and Orai has generated great enthusiasm concerning the role of these proteins in the cardiovascular system. We will review the major results concerning the existence and the role of these proteins in the cardiovascular system in normal and pathological situations and their implication in cardiovascular remodeling.

## 2. INTRODUCTION

In cardiac myocytes, calcium ($Ca^{2+}$) is the central regulator of excitation-contraction coupling (EC coupling), which controls muscle contraction on a beat-to-beat basis. EC coupling is the process of electrical excitation of myocytes leading to contraction of the heart (1). Defective intracellular $Ca^{2+}$ homeostasis has consistently been reported in heart failure and several defects in the intracellular $Ca^{2+}$ homeostasis have been reported including depressed $Ca^{2+}$ uptake, decreased storage and release of the

sarcoplasmic reticulum (SR) associated with diastolic SR $Ca^{2+}$ leak (2). It is clear now that beyond its effect on contraction, $Ca^{2+}$ is also involved in activation of new transcription pathways leading to cardiac growth (3).

Multiple alterations in $Ca^{2+}$ cycling that may precede changes in phenotype have also been described in vascular remodeling (4-7). Quiescent VSMC are present in normal vessel whereas proliferation is associated with neointimal thickening in diseases such as atherosclerosis, systemic or pulmonary artery hypertension and injury-induced restenosis which occurs after percutaneous vessel intervention. We will review recent data concerning the role of STIM and its partners in the cardiovascular system in normal and diseased conditions.

## 3. STIM1 AND ORAI IN CARDIAC PATHOPHYSIOLOGY

### 3.1. Roles and regulation of calcium in cardiac myocytes

EC coupling consists of processes involved in $Ca^{2+}$ activation of contractile proteins and the subsequent removal of calcium, thereby facilitating relaxation (1, 8). At any moment in time, the level of intracellular $Ca^{2+}$ is tightly regulated by a balance between the release of $Ca^{2+}$ into the cytosol and the removal of $Ca^{2+}$ by the combined action of buffers, pumps and channels. The endoplasmic reticulum (ER) and the sarcoplasmic reticulum (SR) which surrounds the contractile apparatus and extends to the plasma-membrane and to T-tubules in muscle cells play an essential role as a signal-transducing organelle.

In addition to its critical role in EC coupling, it is now well acknowledged that $Ca^{2+}$ ions are also fundamental in the activation of signaling pathways that can induce and promote cardiac hypertrophy and heart failure. Even though significant advances have been made in understanding the molecular mechanisms that regulate $Ca^{2+}$ cycling in heart failure, there are a number of pathways that need to be elucidated to understand the spatio-temporal control of $Ca^{2+}$-mediated signaling pathways. In many cell types, the activation of $Ca^{2+}$-mediated pathways is due to a sudden rise in cytosolic $Ca^{2+}$ levels. The $Ca^{2+}$ can be released by internal stores or can enter the cells from the outside through channel activation. Cardiac myocytes are however characterized by large swings of intracellular calcium levels that occur during EC coupling. This raises questions about how cardiac myocytes distinguish different $Ca^{2+}$ signaling pathways during the large repetitive $Ca^{2+}$ transients during E-C coupling. An interesting and comprehensive review of the double life of $Ca^{2+}$ (contractile versus signaling $Ca^{2+}$) in a cardiac myocyte has been published recently (9).

One of the answers might lie in different sources (or pools) of $Ca^{2+}$ that create localized $Ca^{2+}$ signaling complexes with strategic locations and time frames of action. One of these microdomains could be the caveolae where calcineurin is docked to the plasma-membrane $Ca^{2+}$-ATPase type 4 (PMCA 4) (10). Another subdomain is likely the nuclear envelope through a mechanism involving $IP_3$-dependent release of $Ca^{2+}$ and/or CamKII-dependent activation of PKD and nuclear HDAC-activity (11). Moreover, the activation of $Ca^{2+}$-mediated signaling pathways occurs at early stages of cardiac remodeling when EC coupling and diastolic $Ca^{2+}$ concentrations are in the physiological range. Beyond this evidence, the specific mechanisms controlling $Ca^{2+}$-mediated signaling pathways in cardiac myocytes remain largely undetermined. The discovery of STIM1 as an ER $Ca^{2+}$ sensor and an essential component of SOCE (Store Operated Calcium Entry) (12, 13) and later on of Orai1 as the pore of the calcium release activated channel $I_{CRAC}$ (14-17), led to some excitement concerning the role of these two proteins in cardiovascular diseases.

### 3.2. SOCE, STIM1 and Orai in normal and pathological cardiac myocytes

In many non-excitable cell types, STIM1 is a key protein for $Ca^{2+}$-dependent signal transduction. In these cells, STIM1 is able to sense the depletion of ER-based $Ca^{2+}$ and to subsequently dimerize to activate plasmalemmal $Ca^{2+}$ entry also called capacitative calcium entry (CCE) or SOCE.

The existence of SOCE in excitable cells such as cardiac myocytes has however been debated until recently. Several studies have reported that SOCE is abundant and robust in neonatal cardiac myocytes (18-20). In these cells, STIM1 plays a critical role in SOCE activation as demonstrated by STIM1 knock down experiments that largely reduces SOCE. Reciprocally, STIM1 overexpression increases SOCE. These results were also confirmed in isolated neonatal cardiac myocytes from STIM1 deficient mice that exhibit a strong reduction in SOCE as compared to controls (19). Neonatal cardiac myocytes however lack some of the characteristics observed in adult myocytes including the development of T-tubules and the expression of an adult cardiac gene program. SOCE was also observed in adult cardiac myocytes (21), but only at a marginal level in healthy isolated adult rat cardiac myocytes suggesting that once the cardiac myocyte is matured, SOCE does not play a role in the $Ca^{2+}$ homeostasis (19, 20). To support this observation, lower STIM1 mRNA and protein levels were observed in adult myocytes compared to neonatal ones (19, 20). Furthermore, SOCE re-appears in cardiac myocytes placed in pathological conditions such as myocytes isolated from hypertrophied hearts of rat that underwent aortic constriction, or isolated cardiac myocytes stimulated with hypertrophic agonists. Interestingly, STIM1 protein level increased in the heart of mice and rat after pathological stimuli (19, 20). This overexpression occurs rapidly suggesting that STIM1 expression and function re-emerges in diseased cardiac myocytes.

When first discovered in non-excitable cells such as T-lymphocytes, the mechanism of STIM1 activation has been primarily linked to ER calcium store depletion (13). The EF hand of STIM1 is located in the ER lumen where it acts as a sensor of $Ca^{2+}$ concentration. When a decrease is measured, STIM1 dimerizes, and gets closer to the plasma membrane where it forms punctae and activates its channel partners to induce a $Ca^{2+}$ entry and refill the stores. In

cardiac myocytes, intracellular $Ca^{2+}$ is hugely modified at each cardiac beat raising concerns on whether STIM1 might be functional in such cells. During cardiac hypertrophy, STIM1 controls 2 separate currents (19). The first STIM1-dependent current is a large, nonselective cation current activated by the depletion of $Ca^{2+}$ stores with thapsigargin. This store-dependent current is concordant with previous understanding of STIM1 and SOCE in non excitable cells. The second current shows both electrophysiological and pharmacological profiles of Orai-activated currents. Interestingly, this current activated under basal conditions, in absence of drug-induced store depletion, meets criteria of Isoce and is abolished after STIM1 silencing. In addition, SR $Ca^{2+}$ load and release are not significantly affected in the early stages of the cardiac hypertrophic remodeling, suggesting that STIM1 activation in cardiac myocytes can occur in a $Ca^{2+}$-store independent mode.

### 3.3. Role of STIM1 in controlling cardiac myocyte growth

The detection of STIM1-dependent currents in hypertrophic cardiac myocytes was largely unanticipated and pointed out a potential contribution of STIM1 in the adult heart. Ohba *et al.* gave the first piece of evidence by showing that in rat neonatal cardiac myocytes, STIM1 silencing with siRNA prevented hormonal induced hypertrophy (22). Silencing STIM1 also reduced BNP expression in cardiac myocytes after hypertrophic stimulus (phenylephrine, endothelin1 or angiotensinII). Silencing STIM1 or Orai1 by siRNA in neonatal cardiac myocytes resulted in abrogated SOCE and prevented phenylephrine-induced hypertrophy (18). This prevention of hypertrophic response in neonatal myocytes came along with a decrease in MCIP and ANP mRNA. In line with these results, silencing of either STIM1 or Orai1 prevented activation of CaMKII and ERK1/2, but surprisingly only Orai1 knock-down prevented calcineurin activation in this study. Recently, Orai1 deficiency was shown to result in heart failure and skeletal myopathy in zebrafish. In this study the absence of Orai1 was associated with loss of the calcineurin-associated protein calsarcin from the z-discs and impairment of NFAT activation (23). Recently, we did not only confirm that silencing STIM1 *in vitro* prevents hypertrophy of isolated rat neonatal cardiac myocytes but we also showed that overexpressing STIM1 in these cells is sufficient to induce SOCE and hypertrophy (19). Genetic manipulation of STIM1 *in vivo* by the use of loss-of-function strategies greatly influences the development of pressure-overload induced cardiac hypertrophy. Indeed, in rats infected with AAV9 encoding either shSTIM1 or shscrambled prior to abdominal aortic constriction, we found that shSTIM1-treated hearts displayed reduced left ventricular mass and thickness. All these data suggest a strong link between STIM1 activity, STIM1-dependent currents and activation of hypertrophic signaling pathways. Conversely, there was no evidence that STIM1 manipulation has an influence on cardiac EC coupling.

### 3.4. TRPC versus Orai

Different studies have reported that SOCE mainly results from the interaction between STIM1 and the $Ca^{2+}$ release-activated channel of the Orai protein family at the plasma membrane (14-17). The expression of Orai1 as well as Orai3 has been reported in rat ventricular cardiac myocytes (24). Voelkers *et al.* have shown that knocking down Orai1 in neonatal rat cardiac myocytes mimics almost all the effects of STIM1 knock down in terms of preventing cell hypertrophy (18). In zebrafish, *in vivo* Orai1 deficiency also led to cardiac dystrophy (23). In a recent study, 2-APB (2-aminoethoxydiphenyl borate), a classical non-specific Orai inhibitor, was shown to provoke arrhythmias in rat hearts perfused with a Langendorff system (24). When we analyzed SOC currents from isolated adult rat cardiac myocytes, the curve shape displayed characteristics of gating by Orai1 proteins but also present significant discrepancies with previous descriptions in non-excitable cells. Firstly, STIM1-dependent currents are mainly activated through a store-independent mechanism. Secondly, the kinetics of Isoce activation is more rapid in cardiac myocytes than in non-excitable cells. It is thus likely that the occurrence of SOCE may not be recapitulated by the model described in other cell types but rather have some specificities. First data report a constitutive association of STIM1 and Orai1 at the membrane as seen in the more immature myotubes (25).

STIM1 is also known to activate several canonical transient receptor potential (TRPC) channels. Many of their subclasses are expressed in the heart and they are clearly implicated in hypertrophic responses (26). Whether a TRPC channel contributes to STIM1-dependent currents and to the consequent hypertrophic response remains unknown. Nakayama *et al.* demonstrated that TRPC3 in mice could increase activation of calcineurin-NFAT pathway (27), which is also the consequence of STIM1 over-expression. In isolated neonatal rat cardiac myocytes, endothelin-1 treatment induced hypertrophy, increased SOCE and also TRPC1 upregulation (28) but TRPC1 -/- mice fail to induce cardiac hypertrophy and NFAT activation after TAC surgery (29). TRPC6 expression was upregulated in the hearts of mice expressing constitutively active calcineurin and subjected to pressure overload by thoracic aortic banding. The promoter of the TRPC6 gene contains 2 conserved NFAT-binding sites that confer responsiveness to calcineurin-NFAT signaling. Cardiac-specific overexpression of TRPC6 in transgenic mice sensitized the heart to stress when expressed at low levels and resulted in fatal cardiomyopathy when expressed at high levels (30). In neonatal rat cardiac myocytes, silencing of TRPC3 and TRPC6 reduced the AngII dependent NFAT activation (31). Evidence seems to suggest that TRPC1, 3 and 6 are involved in calcineurin-NFAT induced hypertrophy. These studies suggest also that SOCE protein complex in cardiac myocytes is more complex than imagined first: STIM1 seems clearly as the main regulator of the system but the nature of plasma membrane partners have still to be identified. In the figure 1 we present a scheme of a putative role for STIM1 and SOCE in cardiac myocytes.

## 4. STIM1 AND ORAI IN VASCULAR PROLIFERATIVE DISEASES

Vascular diseases include pathologies such as atherosclerosis, systemic and pulmonary hypertension, aortic aneurysm and dissection, posttransplant

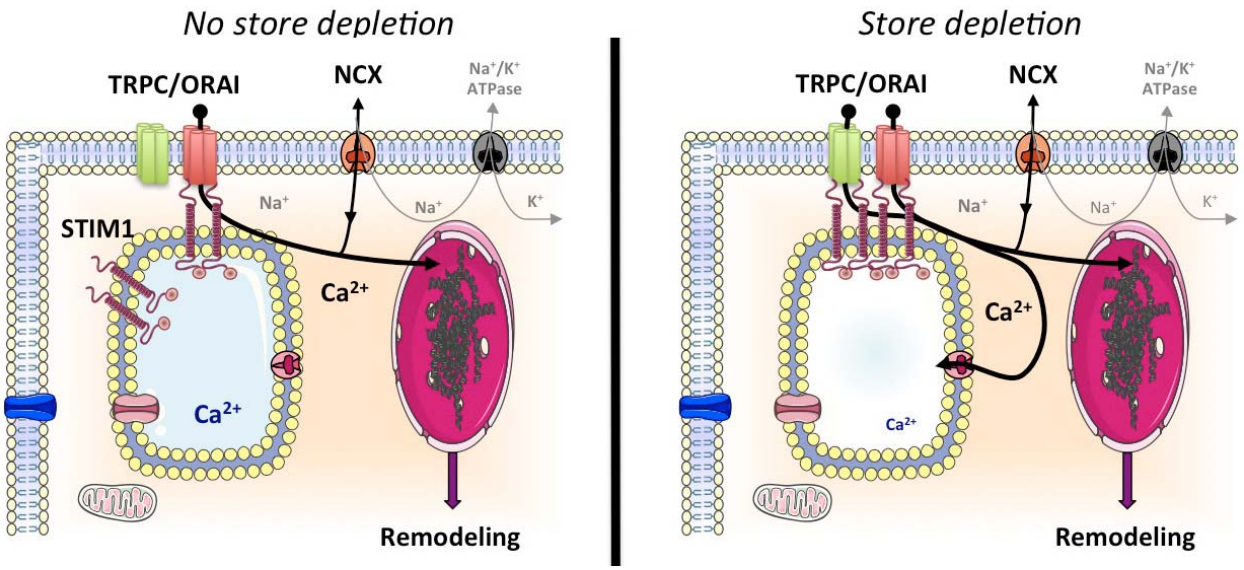


**Figure 1.** Hypothetic scheme in pathological situation of STIM1-induced $Ca^{2+}$ entry in absence or after a massive store depletion. In hypertrophic cardiomyocytes, in absence of store depletion, a non specific cationic current which resembled to a current carried by Orai channels, is present in basal condition. Pharmacological store depletion reveals a second component that resembles to TRPC activation. Both components are absent after STIM1 knock down (19).

vasculopathy, and restenosis after percutaneous coronary intervention. Various cell types are involved in these diseases such as T-cells, mast cells, macrophages. The role of STIM1 and Orai in these cell types has been extensively reviewed by Shaw and Feske in a previous issue of this journal (32). Furthermore, an interesting review of the role of STIM1 and Orai in thrombosis has been published by Nieswandt's team (33). Thus, we will focus this review on vascular proliferative disorders. One main vascular proliferative disorder is induced by injury of the endothelial layer of the vessel during percutaneous coronary intervention and stent placement. The vascular smooth muscle cells are then exposed to growth factors and start proliferating and migrating toward the lumen of the vessel giving rise to the neointima and leading to restenosis. VSMC stop proliferating when the endothelial layer is restored. Another major vascular proliferative disease is pulmonary hypertension which also involves proliferation of smooth muscle cells and in some cases proliferation of endothelial cells to form a plexiform lesion.

### 4.1. SOCE in vascular in proliferative diseases

Alterations in expression or activity of the major partners of $Ca^{2+}$ signaling including channels and pumps have been described during the dedifferentiation process associated with VSMC proliferation, (4-7).

Here we have focused on the role of SOCE in vascular proliferative diseases. SOCE was found in both contractile and synthetic smooth muscle cells (SMC) (for a review see (34)). A marked increase in SOCE was observed during proliferation of human pulmonary artery smooth cells (35). SOCE was also shown to play a role in vasoconstriction (36). Store depletion caused SOCE, in several rat small muscular arteries but a corresponding contraction was observed only in pulmonary artery, suggesting that coupling between SOCE and contraction is of particular importance in this vessel (37). Enhanced SOCE activity was observed in monocrotaline-induced pulmonary hypertensive rats (38) and in pulmonary smooth muscle cells from patients with pulmonary artery hypertension (39).

SOCE was also reported in endothelial cells in many animal species (40) and in endothelial progenitor cells (EPCs) (41) which is an important cell population recruited to the site of injury after acute vascular injury. The nature of SOCE in this cell type is still matter of debate (see below).

### 4.2. Implication of STIM and ORAI in vascular proliferative diseases

With the discovery of STIM1 and Orai as molecular players of SOCE, their importance in vascular proliferative disorders was then analyzed. STIM1 is induced during VSMC proliferation and silencing STIM1 using shRNA prevents neointimal thickening in a model of balloon injury of the carotid artery in the rat (42-44). Orai1 is also upregulated in synthetic/proliferative VSMC and during neointimal thickening after carotid injury (43-46). Protein knockdown of either STIM1 or Orai1 in synthetic VSMCs greatly reduced SOCE and VSMC proliferation as well as PDGF-induced migration whereas Orai2, Orai3, TRPC1, TRPC4, and TRPC6 knockdown had no effect (45, 46). *In vivo* knock-down of either STIM1 or Orai1 prevented mechanical injury-induced neointimal thickening (44, 47) and angiotensin II-induced proliferation in injured vessels (47). In human pulmonary arterial smooth muscle cells, PDGF enhanced SOCE by upregulating STIM1 and Orai1 (48). Coronary endothelial cells isolated from

diabetic mice exhibited downregulation of STIM1 in comparison to controls and overexpression of STIM1 restored endothelium-dependent vasorelaxation that was attenuated in diabetic coronary arteries(49). STIM1 knock-down inhibits proliferation and migration of endothelial cells and EPCs (50-52). STIM1 was found to be necessary and sufficient for the SOCE entry (50, 53) but its partners are not clearly defined and both TRPC1/4 and Orai1 were suggested (see 4.3).

At the moment there are very few results concerning the involvement of STIM2, Orai 2 and Orai3 in vessels. Higher STIM2 and Orai2 expression was observed in pulmonary artery SMC from idiopathic pulmonary artery hypertensive (IPAH) patients as compared to controls. Overexpression of STIM2 is necessary for increased SOCE and proliferation of SMC from IPAH patients but increasing STIM2 expression is not sufficient to increase SOCE and proliferation in PASMC from control patients. This suggests that overexpression of another partner such as Orai2 (or a TRPC channel) is involved in IPAH (39). Similarly, knock-down of STIM2, Orai2 and Orai3 had no effect on proliferation and migration of rat aortic smooth muscle suggesting that the effect of STIM2 and Orai2 may be cell type or disease-specific. Of note, expression of both STIM2 and Orai2 is increased during hypoxia (39).

### 4.3. TRPC versus Orai

Extensive discussion on the roles of TRPCs in smooth muscle and endothelial cells in health and disease has been already published (54-56). Thus we will only discuss recent data. SMC isolated from thoracic aortas and cerebral arteries of TRPC1-/- mice showed no change in store-operated cation influx induced by thapsigargin, inositol-1,4,5 trisphosphate, and cyclopiazonic acid compared to cells from wild-type mice. Decreasing the expression of STIM1by small interference RNAs inhibited thapsigargin-induced SOCE, demonstrating that STIM1 and TRPC1 are mutually independent (57, 58). In lung endothelial cells from mice, TRPC4 was necessary to form functional SOCE in association with TRPC1 and STIM1 (53) whereas in human umbilical vein and pulmonary artery endothelial cells, Orai1 alone was found to mediate SOCE (50). Heterogeneity in the vascular cell types as well as compensation of the loss of one the partners of the channel (Orai, TRPC1 or TRPC4) by other TRPCs or Orai in knock-down as well as in transgenic models could have explained the results. Thus, the implication of TRPC1 in SOCE remains still unclear. More recently, Cioffi *et al.* proposed that Orai1 determines calcium selectivity of an endogenous TRPC1/4 heterotretramer channel (59). Thus, the picture is not yet complete and especially the role of other partners of STIM1 in the store-dependent and independent $Ca^{2+}$ entry remains to be studied.

## 5. STIM CONTROLS A NEW TRANSCRIPTION PATHWAY

The link between STIM1-dependent SOCE and NFAT activation has been well described in different cell types including lymphocytes (13, 60). In both cardiac and vascular myocytes STIM1 expression is associated with the activation of the calcineurin–NFAT pathway. Silencing of STIM1 in neonatal cardiac myocytes prevented agonist-induced NFAT activation (18-20) and *in vivo* STIM1 knock-down in adult rat heart resulted also in prevention of pressure overload-induced NFAT activation (19). Similarly, in vascular myocytes STIM1 knock-down induced NFAT inactivation (42, 44). It is not yet clear what is (are) the channel(s) involved in $Ca^{2+}$ entry leading to NFAT activation. In smooth muscle cells, silencing Orai1 is sufficient to block NFAT activation induced by store depletion induced by thapsigargin (44). In neonatal cardiac myocytes Orai1 knock-down also completely inhibited Gq-dependent NFAT activation (18, 23). Besides, TRPC1, 3 and 6 were also shown to control NFAT activity in the heart (29-31). Different agonists were shown to activate different STIM proteins to sustain $Ca^{2+}$ signals and downstream responses (61). STIM2 (which has a lower affinity for $Ca^{2+}$) contributes to $Ca^{2+}$ signals in response to low levels of receptor stimulation that deplete stores modestly. This could also be true for Orais and TRPCs.

## 6. CONCLUSIONS

STIM1 is present in healthy cardiac or vascular myocytes but has a marginal role. Its expression and role become important during cardiac growth or vascular proliferation because they activate a new transcription pathway. It is not yet clear what is (are) the partner(s) of STIM1 in the heart. In the vascular myocyte STIM1 and Orai1, are involved in proliferation but the implication of TRPC in SOCE and vascular function is more controversial. STIM1 and Orai1 do not seem to regulate contraction but rather to induce a new gene expression program through NFAT activation and may be other pathways. We still have a lot to learn about the role of STIM2 and of the different Orais in these cell types.

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