

# Emerging roles of store-operated $\text{Ca}^{2+}$ entry through STIM and ORAI proteins in immunity, hemostasis and cancer

Wolfgang Bergmeier,<sup>1,†</sup> Carl Weidinger,<sup>2,†</sup> Isabelle Zee<sup>2</sup> and Stefan Feske<sup>2,\*</sup>

<sup>1</sup>Department of Biochemistry and Biophysics and McAllister Heart Institute; University of North Carolina; Chapel Hill, NC USA; <sup>2</sup>Department of Pathology; New York University Langone Medical Center; New York, NY USA

<sup>†</sup>These authors contributed equally to this work.

**Keywords:** ORAI1, STIM1, CRAC, calcium,  $\text{Ca}^{2+}$ , platelets, hemostasis, lymphocytes, immunodeficiency, autoimmunity, allergy, cancer, tumor

Store-operated  $\text{Ca}^{2+}$  entry (SOCE) is an important  $\text{Ca}^{2+}$  influx pathway, which is defined by the fact that depletion of intracellular  $\text{Ca}^{2+}$  stores, mainly the endoplasmic reticulum (ER), triggers the opening of  $\text{Ca}^{2+}$  channels in the plasma membrane. The best characterized SOC channel is the  $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  (CRAC) channel, which was first described in cells of the immune system but has since been reported in many different cell types. CRAC channels are multimers of ORAI family proteins, of which ORAI1 is the best characterized. They are activated by stromal interaction molecules (STIM) 1 and 2, which respond to the depletion of intracellular  $\text{Ca}^{2+}$  stores with oligomerization and binding to ORAI proteins. The resulting SOCE is critical for the physiological function of many cell types including immune cells and platelets. Recent studies using cell lines, animal models and primary cells from human patients with defects in SOCE have highlighted the importance of this  $\text{Ca}^{2+}$  entry mechanism in a variety of pathophysiological processes. This review focuses on the role of SOCE in immunity to infection, allergy, hemostasis, and cancer.

## Introduction

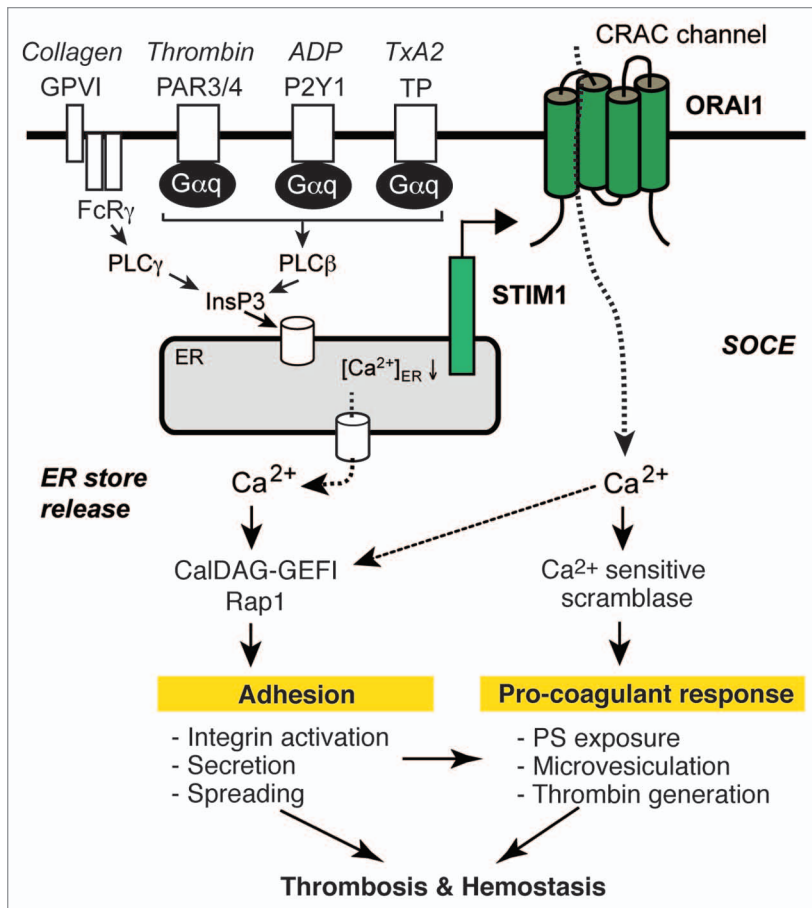
CRAC channel opening and SOCE are triggered by the engagement of a variety of cell surface receptors including immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors on T cells (TCR), B cells (BCR) and platelets (GPVI, CLEC2) as well as G protein coupled receptors (GPCR) such as chemokine receptors on immune cells and the receptors for thrombin and ADP on platelets (Fig. 1). Cellular stimulation via immunoreceptors and GPCRs leads to the activation of phospholipase  $\text{C}\gamma$  and  $\text{C}\beta$ , respectively, both of which result in the production of the second messenger inositol-1,4,5-triphosphate ( $\text{InsP}_3$ ).  $\text{InsP}_3$  binds to and opens  $\text{InsP}_3$  receptor channels in the membrane of the ER causing the efflux of  $\text{Ca}^{2+}$  from the ER and a reduction of the

$\text{Ca}^{2+}$  concentration in the ER, or  $[\text{Ca}^{2+}]_{\text{ER}}$ . This triggers the opening of CRAC channels in the plasma membrane and SOCE. The pore forming subunit of the CRAC channel is ORAI1,<sup>1-3</sup> which is a tetraspanning plasma membrane (PM) protein. It is unrelated to other ion channels and was discovered by RNAi screens and positional cloning in patients with inherited CRAC channel dysfunction.<sup>4-6</sup> Two homologs, ORAI2 and ORAI3, conduct  $\text{Ca}^{2+}$  currents when ectopically expressed in cells and may contribute to CRAC channel function and SOCE. Loss-of-function mutations in the human *ORAI1* gene and genetically engineered mice lacking functional ORAI1 channels have established its essential role in SOCE in many cell types.

ORAI1/CRAC channels are gated by stromal interaction molecule 1 (STIM1), a single-pass transmembrane protein located in the membrane of the ER that is activated in response to depletion of ER  $\text{Ca}^{2+}$  stores.<sup>7,8</sup> STIM1 senses the depletion of ER  $\text{Ca}^{2+}$  stores via paired EF-hand  $\text{Ca}^{2+}$  binding domains in its ER luminal N-terminus. Dissociation of  $\text{Ca}^{2+}$  from the EF-hands of STIM1 results in its oligomerization and translocation to ER-plasma membrane (PM) junctions, where it forms large protein clusters referred to as STIM1 puncta.<sup>9</sup> STIM1 recruits ORAI1 to these puncta by binding to its intracellular N and C termini,<sup>10-12</sup> thereby opening CRAC channels and promoting  $\text{Ca}^{2+}$  influx. STIM2, like its homolog STIM1, also mediates SOCE but has slower kinetics of activation and is activated by weaker depletion of ER  $\text{Ca}^{2+}$  stores.<sup>13,14</sup> While ORAI1 and STIM1 are necessary and sufficient to mediate SOCE,<sup>15,16</sup> several proteins have been identified that modulate CRAC channel function and SOCE both positively and negatively.<sup>17</sup> The molecular mechanisms underlying CRAC channel function have been reviewed in detail elsewhere.<sup>17-21</sup>

SOCE is critical for the activation and function of many non-excitabile cells, and emerging more recently, excitable cells such as neurons and muscle cells as well. Cell-based experiments in vitro, animal models and studies in human patients with defects in SOCE have revealed important roles of ORAI and STIM proteins in a variety of physiological and pathophysiological processes including hypoxic neuronal cell death,<sup>22</sup> cardiac hypertrophy<sup>23</sup> and the function of vascular smooth muscle and endothelial cells

\*Correspondence to: Stefan Feske; Email: feskes01@nyumc.org  
Submitted: 01/21/13; Accepted: 03/14/13  
<http://dx.doi.org/10.4161/chan.24302>



**Figure 1.** STIM1 and ORAI1 in platelet function. Stimulation of platelets with agonists such as thrombin, thromboxane A2, ADP, or collagen results in Ca<sup>2+</sup> release from ER stores and SOCE. Ca<sup>2+</sup> released from the ER activates the guanine nucleotide exchange factor, CalDAG-GEFI, which controls the activity of the small GTPase Rap1, a critical regulator of platelet responses such as integrin activation, secretion and spreading. STIM1 and ORAI1 are the major homologs mediating SOCE in platelets. Sustained elevated [Ca<sup>2+</sup>]<sub>i</sub>, resulting from SOCE is essential for activation of the enzyme scramblase, which exposes phosphatidylserine (PS) on the outer surface of the cell membrane. PS exposure allows for the binding of coagulation factors on the platelet surface, thereby stimulating the generation of thrombin at sites of vascular injury. Both the adhesive and the pro-coagulant activity of platelets are critical for hemostasis and thrombosis.

during vascular injury.<sup>24-26</sup> In this review, we will discuss the phenotype of CRAC channel deficient patients, the role of SOCE in immune cell function and immunity to infection,<sup>4,27-34</sup> in platelet function and hemostasis<sup>35-39</sup> as well as carcinogenesis.<sup>40,41</sup>

### CRAC Channels in Immune Function

#### SOCE is essential for immunity to infection

The role of SOCE in immunity to infection is underlined by the severe, recurrent infections with viral, bacterial, and fungal pathogens affecting patients with mutations in *ORAI1* and *STIM1* genes that abolish SOCE.<sup>42,43</sup> Patients are susceptible to chronic and recurrent viral infections, especially with herpes viruses such as cytomegalovirus (CMV), Epstein Barr virus (EBV) and human herpes virus (HHV) 8.<sup>44-46</sup> SOCE is impaired in cells of both the innate and adaptive immune system

in these patients, and defective immune responses by both systems are likely to contribute to their immunodeficiency.

Several loss-of-function or null mutations that abolish ORAI1 protein expression or function in T cells, B cells and neutrophils have been described.<sup>47-49</sup> The first mutation identified was a missense mutation in ORAI1 that results in the substitution of a highly conserved arginine residue in the first TM domain of ORAI1 (ORAI1-R91W). It abolishes CRAC channel function despite normal ORAI1 expression.<sup>4,50,51</sup> R91 is located at the cytoplasmic face of the CRAC channel pore and its substitution with tryptophan is likely to establish a hydrophobic seal that occludes the channel pore.<sup>52</sup> Other immunodeficient patients lacking SOCE were found to be homozygous for nonsense (A88SfsX25) or missense (A103E, L194P) mutations in the *ORAI1* gene that abolish protein expression.<sup>47-49</sup> In addition to ORAI1 mutations, patients with loss-of-function and null mutations in the *STIM1* gene lacked SOCE in immune cells. STIM1 protein expression was absent in patients with frameshift (E128RfsX9) or splice site (1,538-1 G > A) mutations in STIM1.<sup>44,53</sup> In addition, patients homozygous for a missense mutation in STIM1 (R429C) express a nonfunctional form of STIM1 protein that abolishes Ca<sup>2+</sup> influx in T cells and fibroblasts and results in severely impaired T cell function.<sup>46</sup> ORAI1 and STIM1 deficient patients suffer from an almost identical form of immunodeficiency, which we named CRAC channelopathy to emphasize the causative role of impaired CRAC channel function in its pathogenesis. Besides immunodeficiency, disease in these patients is characterized by autoimmunity, ectodermal dysplasia (impaired dental enamel calcification and eccrine sweat gland function) and muscular hypotonia (reviewed in refs. 42 and 43).

### SOCE Regulates Adaptive Immune Function

Infections in CRAC channel deficient patients are caused by impaired T cell and NK cell activation as a consequence of abolished SOCE.<sup>44,47-49,53-55</sup> In T cells, SOCE is induced after antigen recognition by the T cell receptor and regulates many processes including T cell proliferation, apoptosis, cytokine gene expression and T cell differentiation. TCR mediated activation of T cells is severely impaired in the absence of SOCE as apparent from impaired proliferation and expression of cytokines such as IL-2, IL-4, TNFα and IFNγ in T cells from ORAI1 or STIM1 deficient patients.<sup>47,53-55</sup> Recently, SOCE was shown to be important for the regulation of T cell survival and activation-induced cell death. T cells from *Orai1*<sup>-/-</sup> mice were more resistant to apoptosis in vitro and in vivo due to reduced mitochondrial Ca<sup>2+</sup> uptake and altered expression of proapoptotic and antiapoptotic factors

such as Fas ligand (CD178), Noxa and Mcl-1.<sup>56</sup> In vivo studies using mice with complete or conditional, T-cell-specific deletion of *Orai1* or *Stim1* genes confirmed that immune responses by CD4<sup>+</sup> and CD8<sup>+</sup> T cells are dependent on functional CRAC channels.<sup>31,32,34,57</sup> T cell-specific deletion of *Stim2*, *Stim1* or both *Stim1* and *Stim2* (*Stim1,2*) genes resulted in progressively greater defects in SOCE and T cell function. Lack of STIM2 in CD4<sup>+</sup> T cells did not impair the acute peak of Ca<sup>2+</sup> influx in the first minutes after T cell stimulation, but abolished sustained Ca<sup>2+</sup> influx and activation of the Ca<sup>2+</sup> dependent transcription factor NFAT measured over several hours after stimulation.<sup>32</sup> By contrast, T cells lacking STIM1 or both STIM1,2 had strongly reduced peak and sustained Ca<sup>2+</sup> levels.<sup>32</sup> Accordingly, their production of IL-2, IFN- $\gamma$  and other cytokines was abolished, whereas STIM2 deficient T cells had a partial defect in cytokine production. Therefore, both STIM1 and STIM2 contribute to SOCE in T cells, albeit to a different extent and with different kinetics. ORAI1 is indispensable for CRAC channel function and SOCE in human T cells because T cells from patients with loss-of-function or null mutations in *ORAI1* lacked I<sub>CRAC</sub> and SOCE, failed to proliferate in vitro and to produce cytokines. In contrast to human T cells, ORAI1 appears to be more redundant in naïve mouse T cells, suggesting potential contributions of ORAI2 and ORAI3 to CRAC channel function. Ca<sup>2+</sup> influx was reduced but not absent in naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells from *Orai1*<sup>-/-</sup> mice and *Orai1*<sup>KIKI</sup> knockin mice, which express the non-functional ORAI1-R93W mutant.<sup>31,36</sup> In another study, SOCE was normal in T cells from *Orai1*<sup>-/-</sup> mice.<sup>34</sup> When cultured in vitro, ORAI1-deficient T cells proliferated normally despite almost completely impaired SOCE in differentiated CD4<sup>+</sup> T helper cells and CD8<sup>+</sup> cytotoxic T cells.<sup>31,58</sup> These findings suggest that the composition of CRAC channels in murine T cells may change during T cell differentiation and that naïve T cells may utilize not only ORAI1 but also other ORAI homologs to conduct Ca<sup>2+</sup>. Regardless, T-cell-mediated immune responses in vivo require ORAI1 function because *Orai1*<sup>KIKI</sup> mice failed to mount a hypersensitivity response in response to contact allergen exposure of the skin and showed impaired rejection of HLA mismatched skin allografts.<sup>31</sup> In addition, naïve CD4<sup>+</sup> CD45RB<sup>+</sup> T cells from *Orai1*<sup>KIKI</sup> mice failed to induce inflammatory bowel disease (IBD) when adoptively transferred to T cell deficient *Rag2*<sup>-/-</sup> mice, indicating that the differentiation and/or function of proinflammatory T cells depends on functional CRAC channels.<sup>31</sup>

Like in T cells, SOCE in B cells is induced by antigen binding to the B cell receptor (BCR) and subsequent activation of protein tyrosine kinases and PLC $\gamma$ .<sup>59</sup> While cytokine production and proliferation of murine B cells in vitro depended on SOCE mediated by ORAI1,<sup>58</sup> STIM1 and STIM2,<sup>30</sup> respectively, the production of immunoglobulins by B cells was independent of CRAC channels. Mice lacking either *Stim1* alone or both *Stim1* and *Stim2* showed normal or slightly increased levels of IgM and IgG following immunization in vivo.<sup>30,57</sup> In humans, ORAI1 and STIM1 deficiency was associated with normal serum Ig levels, whereas antigen specific antibodies were absent in some patients.<sup>53,54</sup> Given the many Ca<sup>2+</sup> dependent signaling cascades and transcription factors in B cells<sup>59</sup> and

almost completely impaired SOCE in *Stim1,2* deficient mice,<sup>30</sup> it is surprising that lack of SOCE in these mice and human patients lacking ORAI1 or STIM1 does not have a greater impact on B cell development or function. It is conceivable that small increases in [Ca<sup>2+</sup>]<sub>i</sub> suffice for signal propagation or that other Ca<sup>2+</sup> channels besides STIM1,2-activated channels mediate Ca<sup>2+</sup> influx in B cells.

### SOCE Regulates the Function of Innate Immune Cells

Besides lymphocytes, CRAC channel currents and SOCE have also been observed in a variety of cells of the innate immune system such as neutrophils, monocytes/macrophages and natural killer (NK) cells. Neutrophils and NK cells from ORAI1 deficient patients lack SOCE in response to store depletion and antigen receptor stimulation.<sup>49,51</sup> NK cells are cytotoxic lymphocytes that are essential for immune responses against many viral infections and antitumor immunity. Intriguingly, ORAI1- and STIM1-deficient NK cells from patients showed impaired cytokine production, failed to exocytose cytotoxic granules and were unable to lyse tumor target cells when cocultured in vitro.<sup>46,51</sup> Consistent with these findings, NK cells from *Orai1*<sup>KIKI</sup> mice also showed reduced degranulation and cytotoxic function in vitro (SF, unpublished data). In addition to functional defects in NK cells, a STIM1 deficient patient completely lacked CD3<sup>+</sup> Va24<sup>+</sup> Vb11<sup>+</sup> NKT cells in their peripheral blood. Likewise, NKT cell development was impaired in STIM1,2-deficient mice.<sup>142</sup> These findings suggest that SOCE is important for the development of this subset of cytotoxic lymphocytes.<sup>46</sup> NKT cells express a TCR that recognizes lipid and glycolipid antigens presented by CD1d molecules. They share properties with NK cells such as the ability to produce IFN- $\gamma$ . Impaired NKT cell development in the absence of SOCE is consistent with earlier findings that the transcription factor early growth response protein 2 (Egr2) regulates NKT cell development.<sup>60</sup> Since Egr2 expression is regulated by the Ca<sup>2+</sup> dependent transcription factor NFAT,<sup>61</sup> SOCE is likely to contribute to NKT cell development through its regulation of NFAT activation. Impaired function and development of NK and NKT cells, respectively, may contribute to impaired antiviral immunity in CRAC channel deficient patients, although the precise role of SOCE in these cells remains to be investigated.

As cells of the innate immune system, neutrophils and macrophages have important functions in the killing of bacteria and other pathogens through their ability to phagocytose pathogens, to produce reactive oxygen species (ROS) and other effector mechanisms. SOCE in these cells is triggered by binding of IgG antibodies to Fc receptors (Fc $\gamma$ R) or chemokines such as fMLP to chemokine receptors. SOCE mediated by ORAI1 channels regulates integrin-mediated arrest of neutrophils and thus potentially the ability of neutrophils to migrate to sites of inflammation. Inhibition of SOCE in human neutrophils with 2-APB or deletion of *Orai1* in mouse neutrophils delayed and impaired the arrest of neutrophils under shear stress conditions.<sup>62</sup> This effect of Ca<sup>2+</sup> influx via ORAI1 was proposed to be mediated by



Kindlin-3 and ORAI1/Ca<sup>2+</sup> dependent consolidation of the integrin LFA-1 into macroclusters at focal sites of adhesion.<sup>63</sup>

After arriving at sites of infection, neutrophils and macrophages phagocytose bacteria, which is an important mechanism to remove and subsequently kill pathogens. In neutrophils, phagocytosis following binding of opsonized particles to FcγR appears to be dependent on Ca<sup>2+</sup> as chelating intracellular Ca<sup>2+</sup> with BAPTA reduced phagocytosis in human neutrophils and HL-60 cells.<sup>64,65</sup> However, phagocytosis is independent of SOCE as inhibition of Ca<sup>2+</sup> influx in HL-60 cells with La<sup>3+</sup>, Gd<sup>3+</sup> or the CRAC channel inhibitor BTP-2 did not impair phagocytosis and bacterial killing.<sup>65,66</sup> In macrophages, conflicting results had been obtained regarding the role of Ca<sup>2+</sup> in phagocytosis.<sup>67-69</sup> More recently, a severe defect in FcγR-dependent phagocytosis was described in SOCE-deficient peritoneal macrophages isolated from *Stim1*<sup>-/-</sup> mice when these were incubated with opsonized red blood cells *in vitro*.<sup>70</sup> When *Stim1*<sup>-/-</sup> mice were injected with antibody-opsonized red blood cells (RBC) or platelets—common animal models of autoimmune hemolytic anemia and thrombocytopenia, respectively—they developed less severe disease, which was interpreted as a defect in phagocytosis of RBC and platelets by SOCE deficient macrophages.<sup>70</sup> Intriguingly, CRAC channel-deficient patients with mutations in *STIM1* and *ORAI1* genes suffer from autoimmune hemolytic anemia and thrombocytopenia due to autoantibodies directed against RBC and platelets,<sup>43,46,47,53</sup> indicating that SOCE may not be required for phagocytosis by cells of the mononuclear phagocyte system in humans, which is responsible for the removal of cells and pathogens. SOCE in macrophages from human ORAI1/STIM1 deficient patients could not be evaluated and it is unclear how impaired SOCE affects human macrophage function.

Pathogens sequestered within phagosomes of macrophages and neutrophils are killed following fusion of phagosomes with lysosomes. The phagosomal production of reactive oxygen species (ROS) by NADPH oxidase is dependent on SOCE.<sup>66</sup> When neutrophil granulocytes derived from the promyelocytic leukemia cell line HL-60 were stimulated with fMLF in the presence of the CRAC channel inhibitor BTP2, their ROS production was significantly decreased. Follow-up studies showed that ROS production is dependent on STIM1 and ORAI1.<sup>65,71</sup> The precise mechanisms through which SOCE regulates NADPH oxidase function and ROS production remain to be established. They have been suggested to involve several kinases including protein kinase C (PKC), phosphoinositide 3-kinase (PI3K) and sphingosine kinase<sup>71,72</sup> as well as the translocation of the Ca<sup>2+</sup> binding proteins S100A8-A9 to the phagosomal membrane.<sup>65</sup> Like in neutrophils, ROS production in macrophages is dependent on elevated [Ca<sup>2+</sup>]<sub>i</sub>, as were the production of nitric oxide (NO), secretion of microbicidal proteins and proinflammatory mediators.<sup>73-75</sup>

Given the emerging role of ORAI1 and STIM1 in NK cells, neutrophils, macrophages and potentially other cells in the innate immune system, it is likely that the immunodeficiency observed in CRAC channel deficient patients is due to a combination of defects in innate and adaptive immunity. In the future, it will be

important to define the role of SOCE in the differentiation and function of distinct immune cell populations through the use of gene-targeted mice, selective CRAC channel inhibitors and the analysis of SOCE-deficient patients.

#### SOCE in mast cell function and allergy

Mast cells are cells of the innate immune cells that are located predominantly at mucosal surfaces where they mediate a first-line response to infections with bacteria and parasites.<sup>76</sup> In addition, mast cells are central players in (type I) hypersensitivity responses and allergic diseases such as rhinoconjunctivitis, asthma and anaphylaxis. Recognition of pathogens and allergens by the high affinity IgE receptor (FcεRI) at the mast cell surface triggers an increase in [Ca<sup>2+</sup>]<sub>i</sub> and the release of granules containing a wide variety of proinflammatory mediators. Early studies had suggested that Ca<sup>2+</sup> influx is required for or modulates the extent of release of histamine containing granules from mast cells.<sup>77-79</sup> More recently, it has been shown that the microtubule-dependent translocation of cytoplasmic granules to the plasma membrane is Ca<sup>2+</sup> independent, whereas their release by exocytosis requires Ca<sup>2+</sup> signals.<sup>80</sup>

In murine mast cells, FcεRI mediated Ca<sup>2+</sup> influx is mediated by ORAI1 and STIM1. Mast cells from *Stim1*<sup>-/-</sup> and *Orai1*<sup>-/-</sup> mice showed impaired SOCE in response to thapsigargin stimulation or FcεRI crosslinking.<sup>27,34</sup> Importantly, the degranulation of mast cells lacking ORAI1 or STIM1 following FcεRI crosslinking was strongly reduced.<sup>27,34</sup> In addition, they failed to secrete proinflammatory cytokines (IL-6, TNF-α) and lipid mediators such as leukotriene (LT) C<sub>4</sub>. As a consequence, the passive cutaneous anaphylaxis response *in vivo*, which depends on mast cell activation, was attenuated,<sup>27,34</sup> suggesting that SOCE may be essential for mast cell activation in allergic diseases.<sup>81</sup> Indeed, ORAI1 was upregulated in cells of the nasal mucosa of mice subjected to ovalbumin (OVA)-induced allergic rhinitis.<sup>82</sup> Inhibition of SOCE in mice with allergic rhinitis by injection of 2-APB or by inhalation of siRNA against ORAI1 reduced the levels of inflammatory mediators and clinical symptoms in mice.<sup>83</sup> In humans, SOCE was shown to play a role in mast cell activation in nasal polyposis, a chronic inflammatory disease of the upper respiratory tract. Polyps are rich in mast cells and eosinophils, which produce high levels of proinflammatory mediators such as LTC<sub>4</sub>. Blocking CRAC channel function with La<sup>3+</sup> showed a strong synergistic effect with low dose LT receptor antagonists on inhibiting mast cell function,<sup>28</sup> further indicating that SOCE is essential for mediating mast cell mediated inflammation in allergy.

#### ORAI1 and STIM1 in Platelet Function

##### Calcium and platelet activation

Hemostasis at sites of vascular injury depends on the coordinated activation of platelets and the clotting system. In platelets, the second messenger Ca<sup>2+</sup> controls a variety of cellular responses, including integrin activation, secretion, shape change and procoagulant activity.<sup>84-87</sup> Engagement of platelet surface receptors with agonists such as thrombin, thromboxane A<sub>2</sub>, ADP or collagen triggers the activation of phospholipase C, followed by

generation of InsP3 and release of Ca<sup>2+</sup> from intracellular stores of the endoplasmic reticulum (ER) (Fig. 1). Like in immune cells this depletion of Ca<sup>2+</sup> stores is linked to the opening of CRAC channels in the plasma membrane and SOCE.<sup>88-90</sup> Elevated [Ca<sup>2+</sup>]<sub>i</sub> leads to the activation of CalDAG-GEFI, a Ca<sup>2+</sup>-sensitive guanine nucleotide exchange factor for small GTPases of the Rap family.<sup>91,92</sup> Rap GTPases in turn modulate the activity of other signaling cascades important for platelet activation, such as MAP kinases<sup>93</sup> or Rac GTPases.<sup>94</sup> While these signaling responses are efficiently triggered in the absence of extracellular Ca<sup>2+</sup>, the conversion of a pro-adhesive to a pro-coagulant platelet strongly depends on agonist-induced Ca<sup>2+</sup> entry.<sup>95,96</sup> To achieve this pro-coagulant state, platelets have to lose their membrane asymmetry and express phosphatidylserine (PS) on the outer leaflet of the lipid bilayer. This negatively charged surface provides docking sites critical for the assembly and activation of plasma clotting factors, thus promoting the propagation of coagulation and the generation of a fibrin plug at sites of vascular injury.

Although SOCE had been established as a main mechanism for Ca<sup>2+</sup> entry in platelets, the molecular machinery regulating this process has only recently been identified. In this part of the review, we will summarize important studies that (a) identified STIM1 and ORAI1 as regulators of SOCE in platelets, and (b) investigated the role of STIM1 and ORAI1 in platelet function *in vitro* and *in vivo*.

#### STIM1 and ORAI1 regulate SOCE in platelets

The first evidence supporting a role for STIM1 in platelet SOCE came from studies in mice expressing a gain-of-function mutant of STIM1 (named Saxcoburggotski, *Stim1<sup>Sax</sup>*).<sup>38</sup> In these mice, an aspartate at position 84 in the Ca<sup>2+</sup> binding EF hand domain of STIM1 is replaced by a glycine, leading to a reduced ability of STIM1 to bind Ca<sup>2+</sup>, thereby mimicking store depletion and resulting in constitutively active STIM1. Homozygosity for the D84G mutation in mice is lethal *in utero* due to disseminated bleeding. Mice heterozygous for the mutation (*Stim1<sup>Sax/+</sup>*) are viable but are severely thrombocytopenic due to increased platelet turnover (platelet counts < 10% of that in wild-type controls). *Stim1<sup>Sax/+</sup>* platelets are characterized by increased baseline [Ca<sup>2+</sup>]<sub>i</sub> and mild pre-activation of the major integrin, αIIbβ3. When stimulated with thapsigargin, *Stim1<sup>Sax/+</sup>* platelets showed faster store release but markedly impaired calcium entry (~70% reduction). Interestingly, *Stim1<sup>Sax/+</sup>* platelets responded normally to stimulation via G protein-coupled receptors (GPCRs), while they were unresponsive to agonists of immunoreceptor tyrosine-activation motif (ITAM)-based receptors. Thus, enhanced SOCE pre-activates platelets, leading to increased platelet clearance, but it also desensitizes platelets toward stimulation via ITAM receptors. The consequence of the low platelet count and the ITAM activation defect is a significant protection of *Stim1<sup>Sax/+</sup>* mice from arterial thrombosis.

Studies by Tolhurst and colleagues supported a critical role of STIM1 and ORAI1 in platelet SOCE.<sup>97</sup> They showed that RNA transcripts for STIM1 and ORAI1 are expressed at much higher levels in platelets and megakaryocytes when compared with non-selective cation channels such as TRPC1, TRPC6, or TRPM2. Electrophysiological recordings on activated megakaryocytes

further identified CRAC currents with characteristics similar to those shown for ORAI1 channels in immune cells.

Four different mouse models have been used to evaluate the role of STIM1 and ORAI1 in platelet activation: mice lacking expression of *Stim1* (*Stim1<sup>-/-</sup>*)<sup>39</sup> or *Orai1* (*Orai1<sup>-/-</sup>*)<sup>37</sup> genes, knock-in mice expressing a loss-of-function mutant of *Orai1* (*Orai1<sup>R93W</sup>*),<sup>36</sup> and conditional knockout mice lacking *Stim1* expression selectively in platelets/megakaryocytes (*Stim1<sup>fl/fl</sup> PF4-Cre*).<sup>35</sup> To circumvent the perinatal lethality associated with the systemic loss of *Stim1* or *Orai1* gene expression, bone marrow chimeras were generated to delete STIM1 or ORAI1 in blood cells only.<sup>37,39</sup> A similar approach was taken to generate chimeric mice that express ORAI1<sup>R93W</sup> in blood cells.<sup>36</sup> Common to platelets from all four strains was an almost complete defect in SOCE. Deficiency or loss-of-function of ORAI1 did not affect Ca<sup>2+</sup> mobilization from intracellular stores. Interestingly, platelets from *Stim1<sup>-/-</sup>* mice also showed a defect in Ca<sup>2+</sup> release from intracellular stores, suggesting that STIM1 is important for proper store refilling as well as SOCE in these cells. Platelets with impaired SOCE showed only minor defects in their ability to activate integrin αIIbβ3 or to release their granules in response to cellular stimulation, in particular when the cells were stimulated via GPCRs. A more pronounced dependence on SOCE was observed for ITAM-dependent platelet activation. This is likely the result of weak activation of PLCγ2 downstream of ITAM-coupled receptors, i.e., the very limited mobilization of Ca<sup>2+</sup> from intracellular stores induced by such agonists.

While studies on the platelet activation response under static conditions yielded consistent results between the four mouse models, marked differences were seen in the ability of platelets with impaired function of STIM1 or ORAI1 to form thrombi under flow conditions *ex vivo*. In these studies, anticoagulated whole blood is perfused over a collagen surface in a microfluidics device to mimic pathophysiological shear stress. Thrombus formation was significantly impaired in blood from both *Stim1<sup>-/-</sup>* or *Orai1<sup>-/-</sup>* mice.<sup>37,39</sup> In contrast, thrombus formation in this *ex vivo* system was normal in blood isolated from *Stim1<sup>fl/fl</sup> PF4-Cre* or *Orai1<sup>R93W</sup>* mice.<sup>35,36</sup> Possible explanations for the discrepancy between these studies include variations in the experimental setups, different levels of residual SOCE in platelets from *Stim1<sup>fl/fl</sup> PF4-Cre* and *Orai1<sup>R93W</sup>* vs. *Stim1<sup>-/-</sup>* and *Orai1<sup>-/-</sup>* mice, or adverse effects of irradiation (during the generation of bone marrow chimeric mice) on circulating platelets.

In contrast to the relatively minor defects in integrin activation and granule release, platelets with impaired STIM1 or ORAI1 function showed a more profound defect in their ability to switch to a pro-coagulant state. Compared with WT controls, phosphatidylserine (PS) exposure on the surface of activated platelets from *Stim1<sup>-/-</sup>*, *Orai1<sup>-/-</sup>*, *Stim1<sup>fl/fl</sup> PF4-Cre*, or *Orai1<sup>R93W</sup>* mice was markedly reduced.<sup>35,36,98</sup> This defect was particularly strong when anticoagulated blood was perfused over collagen *ex vivo*, where the number of PS-positive cells adherent to collagen was reduced by 50–95% in the absence of functional STIM1 or ORAI1. Gilio and colleagues further demonstrated that this defect in PS exposure correlated with impaired collagen-induced pro-coagulant activity of platelets from *Stim1<sup>-/-</sup>* or *Orai1<sup>-/-</sup>* mice

in vitro. Under coagulant conditions, however, PS exposure and thrombin-dependent generation of fibrin-rich thrombi were normalized, suggesting that in vivo this defect may be compensated for by additional agonists such as thrombin.

*Stim1*<sup>-/-</sup> and *Orai1*<sup>-/-</sup> mice have been subjected to various in vivo models of thrombosis, including arterial thrombosis in the microcirculation and the carotid artery. In all models, impaired SOCE led to a significant protection of mice from vessel occlusion, presumably due to impaired platelet function. However, these studies were done with chimeric mice lacking *Stim1* or *Orai1* gene expression in all hematopoietic cells, including inflammatory cells known to play a role in arterial thrombosis<sup>99</sup> and ischemia reperfusion injury in stroke.<sup>100</sup> To specifically address whether SOCE in platelets is critical for arterial thrombosis, we studied thrombus formation in *Stim1*<sup>fl/fl</sup> *PF4-Cre* mice by intravital microscopy.<sup>35</sup> Similar to previous work with chimeric mice, we found thrombi in *Stim1*<sup>fl/fl</sup> *PF4-Cre* mice to be less stable. Thrombus instability in *Stim1*<sup>fl/fl</sup> *PF4-Cre* mice correlated with the delayed formation of fibrin at sites of vascular injury, suggesting that the pro-coagulant role of STIM1 and SOCE in platelets is critical for thrombus stability and growth. Thrombus instability observed in *Stim1*<sup>-/-</sup>, *Orai1*<sup>-/-</sup>, and *Stim1*<sup>fl/fl</sup> *PF4-Cre* mice, however, was not associated with a markedly increased bleeding risk as tested in a tail bleeding time assay. In summary, these studies suggest STIM1 and ORAI1 as novel targets for anti-platelet therapy.

#### STIM1- and ORAI1-independent Ca<sup>2+</sup> entry in platelets

STIM1 and ORAI1 are the major homologs expressed in platelets, both at the RNA and protein level.<sup>97</sup> However, platelets also express significant levels of STIM2, ORAI2, and ORAI3. So far, only mice lacking STIM2 were analyzed for platelet function. No defects in platelet activation in vitro and thrombus formation in vivo were observed in *Stim2*<sup>-/-</sup> mice.<sup>98</sup> Studies in human platelets also suggested a critical role of TRPC1 as a SOC channel in platelets. While it was initially assumed that TRPC1 is directly regulated by InsP<sub>3</sub>R-II,<sup>101</sup> more recent studies suggested that TRPC1 channel activity is regulated by STIM1 and ORAI1.<sup>102</sup> Genetic deletion of TRPC1, however, did not affect platelet Ca<sup>2+</sup> signaling and hemostasis in mice.<sup>103</sup> Furthermore, Hassock and colleagues showed that TRPC1 expression in platelets is low and that it is predominantly found in internal membranes.<sup>104</sup>

While SOCE is the main mechanism of Ca<sup>2+</sup> entry in platelets, receptor-operated Ca<sup>2+</sup> entry (ROCE) also contributes to this process. The ligand-gated cation channel P2X1 mediates fast Ca<sup>2+</sup> entry induced by ATP. Ca<sup>2+</sup> entry following selective activation of P2X1 is not sufficient to cause platelet aggregation. However, P2X1 contributes to collagen- and shear-induced platelet activation, and deficiency in this channel protects mice from experimental thrombosis.<sup>105,106</sup> Studies in humans and mice identified TRPC6 as a diacylglycerol (DAG) activated ROC channel in platelets.<sup>104,107</sup> Interestingly, genetic deletion of TRPC6 in mice had no effect on platelet function in vitro and in vivo, suggesting that this ROC channel is dispensable for the hemostatic function of platelets at sites of vascular injury.

Lastly, the small-conductance Ca<sup>2+</sup>-activated nonselective channel (SCAN) channel TMEM16F was recently identified as a

critical regulator of platelet pro-coagulant activity in humans<sup>108</sup> and mice.<sup>109</sup> The studies by Yang and colleagues elegantly documented that TMEM16F is a SCAN channel with a large pore that is permeable for cations, in particular Ca<sup>2+</sup> and Ba<sup>2+</sup>. Heterologously expressed TMEM16F showed a small single channel conductance for Ca<sup>2+</sup>, suggesting that this SCAN channel may contribute to Ca<sup>2+</sup> entry in platelets. Deficiency in TMEM16F in mice led to reduced platelet PS exposure, prolonged tail bleeding times, and protection from experimental thrombosis.<sup>109</sup> Consistently, TMEM16F was identified as the gene affected in patients with Scott Syndrome, a rare bleeding disorder caused by impaired platelet pro-coagulant activity.<sup>108</sup> At this point it is unclear how TMEM16F affects Ca<sup>2+</sup>-dependent scramblase activity in platelets.

#### Blockers of SOCE as antithrombotic agents

To date, only one study investigated the effects of inhibitors of SOCE on platelet activation and thrombosis in vivo.<sup>110</sup> In their work, van Kruchten and colleagues demonstrate that various established and novel SOCE inhibitors significantly reduce platelet PS exposure ex vivo. Compared with vehicle-treated controls, thrombus formation on collagen was slightly but significantly reduced. Importantly, mice treated with the SOCE inhibitor 2-aminoethyl diphenylborate (2-APB) were characterized by a reduced platelet activation response and partial protection from experimental stroke after transient cerebral arterial occlusion. It should be noted, however, that 2-APB is a non-selective blocker of ORAI1 and SOCE and that it also inhibits the function of a variety of ion channels including Ins(1,4,5)P<sub>3</sub>R and TRP channels. Although 2-APB itself is therefore not useful as an anti-thrombotic drug, more specific SOCE inhibitors may be suitable for the treatment of thrombosis.

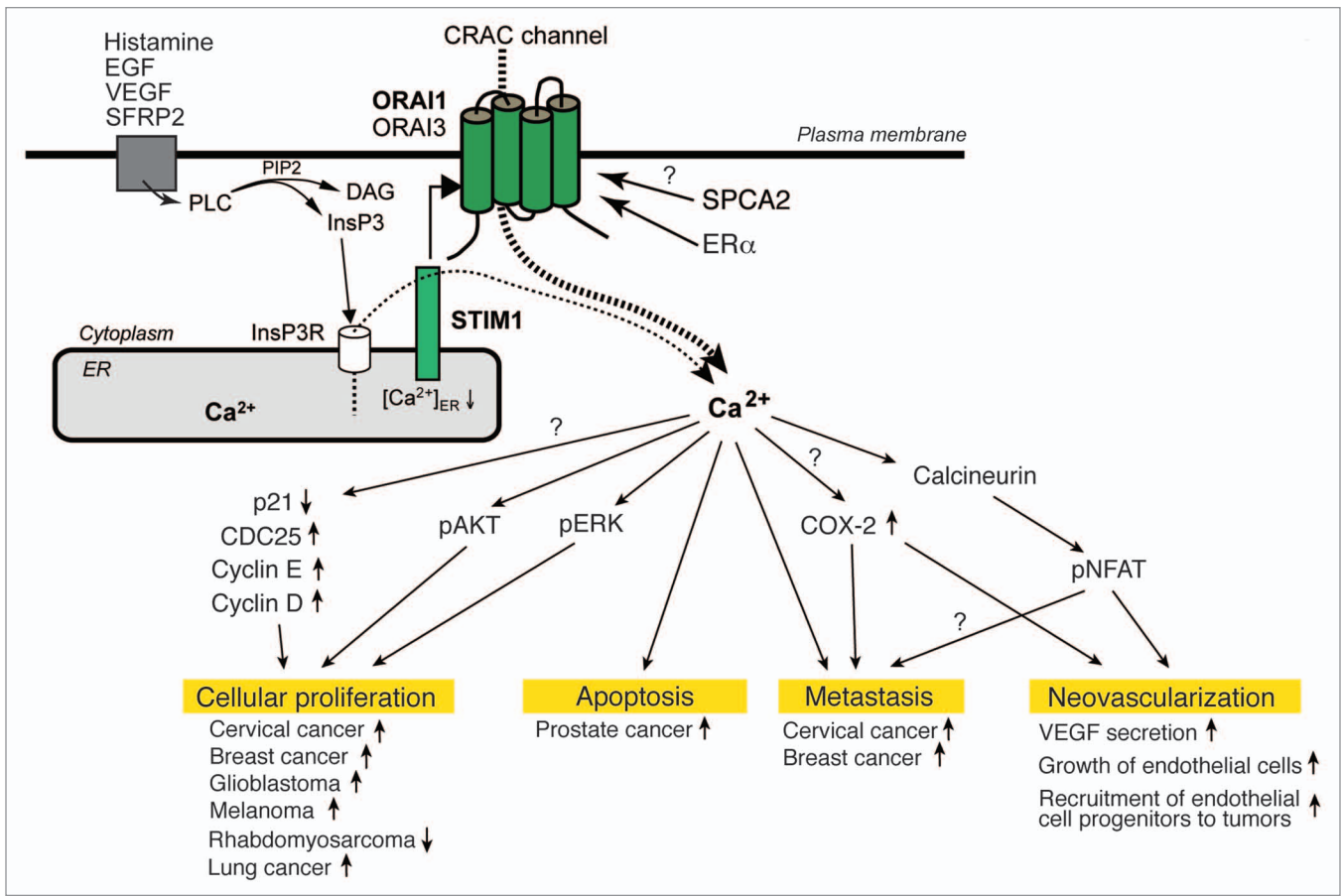
#### STIM1 and ORAI1 in Cancer

SOCE mediated by ORAI and STIM proteins has recently been implicated in various cellular processes associated with carcinogenesis. These include, for instance, increased proliferation, escape from apoptosis, evasion of growth restriction and metastasis.<sup>111</sup> Dysregulated expression of Ca<sup>2+</sup> channels and other molecules involved in Ca<sup>2+</sup> homeostasis has been observed in several forms of cancer.<sup>112</sup> In the following section, we will discuss recent evidence indicating that SOCE mediated by ORAI1 and STIM1 may control key processes in carcinogenesis.

#### STIM1 and ORAI1 regulate proliferation and apoptosis of cancer cells

Initial studies linking STIM1 to cancer observed that the gene locus encoding *STIM1* on chromosome 11p15 was deleted in human rhabdomyosarcoma and rhabdoid tumor cell lines. Ectopic overexpression of STIM1 in vitro in G401 rhabdomyosarcoma cells resulted in morphologic changes and ultimately in cell death.<sup>113,114</sup> These findings, made long before the essential function of STIM1 in SOCE had been discovered, led to the hypothesis that STIM1 might function as a tumor suppressor. More recently, a tumor suppressive function of SOCE was described by Flourakis et al., who showed that SOCE mediated by ORAI1 induces apoptosis in human prostate cancer cells and





**Figure 2.** The role of ORAI and STIM proteins in carcinogenesis. The second messenger  $\text{Ca}^{2+}$  plays an important role in regulating many signaling pathways related to cancer development and progression. SOCE in tumor and endothelial cells results from the activation of cell surface receptors such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and secreted frizzled-related protein 2 (SFRP2). The subsequent activation of phospholipase C (PLC) leads to the production of  $\text{InsP}_3$  and release of  $\text{Ca}^{2+}$  from ER  $\text{Ca}^{2+}$  stores via the opening of  $\text{InsP}_3$  receptor channels. The reduced  $\text{Ca}^{2+}$  concentration in the ER, or  $[\text{Ca}^{2+}]_{\text{ER}}$ , is sensed by stromal interaction molecule (STIM) 1, which binds to ORAI1, the pore-forming subunit of the  $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  (CRAC) channel. Opening of CRAC channels results in  $\text{Ca}^{2+}$  influx and a sustained increase in intracellular  $\text{Ca}^{2+}$  levels,  $[\text{Ca}^{2+}]_i$ . Several cytoplasmic enzymes associated with cancer development and progression are regulated by  $\text{Ca}^{2+}$ . ERK and AKT are phosphorylated in a  $\text{Ca}^{2+}$ -dependent manner, whereas mRNA and protein levels of others molecules such as COX2 and cyclins are controlled by  $[\text{Ca}^{2+}]_i$ . SOCE has been linked to the regulation of cancer cell proliferation, apoptosis and metastasis as well as neovascularization of tumors. Abbreviations: SPCA2, secretory pathway  $\text{Ca}^{2+}$  ATPase 2;  $\text{ER}\alpha$ , estrogen receptor- $\alpha$ .

that ORAI1 expression is regulated by androgens.<sup>115</sup> Androgen deprivation of human LNCaP prostate cancer cells resulted in significant reduction of ORAI1 protein levels and diminished SOC currents when compared with androgen-stimulated LNCaP cells. Moreover, knockdown of ORAI1 by siRNA decreased thapsigargin-mediated SOCE and significantly lowered the susceptibility of LNCaP cells to a variety of apoptosis-inducing cytostatic drugs such as Cisplatin and Oxalplatin.<sup>115</sup>

In contrast, several studies in a variety of cancer types find that SOCE – mediated by ORAI and STIM family proteins—promotes tumor growth through a variety of mechanisms (Fig. 2). Fedida-Metula et al. showed that in murine B16-BL6 melanoma cells, SOCE promotes cell proliferation by activating calmodulin and SRC kinase residing in lipid rafts.<sup>116</sup> SRC kinase, in turn, inactivated the phosphatase PP2A, an important negative regulator of the growth promoting AKT/protein kinase B (PKB) signaling cascade. Knock down of STIM1 reduced

thapsigargin-induced SOCE and decreased SRC and AKT activity. Ablation of lipid raft formation in vivo by treatment of mice with methyl- $\beta$ -cyclodextrin resulted in decreased tumor growth and prolonged survival in a B16 transfer model of melanoma.<sup>116</sup> A tumor growth-promoting role of SOCE was also shown in glioblastoma. Knockdown of ORAI1 or STIM1 reduced  $\text{Ca}^{2+}$  influx in C6 rat and U251 human glioblastoma cells, inhibited tumor cell proliferation and induced apoptosis.<sup>117</sup> Interestingly, a genome-wide gene expression analysis in 20 patients with primary glioblastoma found increased expression of several genes including STIM2, but the functional consequences were not further analyzed.<sup>118</sup>

Better documented is the role of STIM1 in cervical cancer. A study investigating 24 patients with cervical cancer found that the expression of STIM1 correlates with tumor size and clinical outcome.<sup>40</sup> 71% of patients showed increased expression of STIM1 protein in primary cervical cancer tissue compared with

adjacent non-cancerous tissue. The amount of STIM1 expression directly correlated with the size of the primary tumor and metastasis; furthermore, the five year survival rate was significantly lower in patients with high levels of STIM1 expression. In vitro, stimulation of SiHa cervical cancer cells with epidermal growth factor (EGF) resulted in co-localization of STIM1 and ORAI1 and the induction of  $Ca^{2+}$  oscillations. Knock-down of STIM1 by siRNA not only abrogated  $Ca^{2+}$  influx in response to EGF but also inhibited the proliferation of SiHA cells. The latter was associated with downregulation of the cell cycle promoter CDC25C and upregulation of the anti-proliferative protein p21.<sup>40</sup> Ectopic overexpression of STIM1 in SiHA cells, by contrast, resulted in increased tumor growth in a xenograft model of cervical cancer in mice.<sup>40</sup> A similar correlation between STIM1 expression and tumor growth was also shown in a cohort of 295 breast cancer patients.<sup>119</sup> The survival of breast cancer patients with tumors expressing high STIM1 mRNA levels was significantly reduced compared with control groups. Together, these data indicate that STIM1 may play an important role in carcinogenesis.

In line with these findings, Faouzi et al. report a role of ORAI3 in breast cancer. The expression of ORAI3 mRNA is increased in breast cancer tissue in the majority (76.9%) of patients from a small cohort of patients compared with healthy control tissue.<sup>120</sup> Knockdown of ORAI3 by siRNA in the human breast cancer cell lines MCF-7 and T47D reduced thapsigargin (TG)-induced SOCE, decreased cellular proliferation and promoted cell cycle arrest at the G1 stage. The latter was associated with upregulation of p21 and downregulation of cyclin E, cyclin D, CDCK2 and CDCK4. Reduced ORAI3 expression resulted in increased apoptosis of MCF-7 cancer cells but not non-malignant control MCF-10a epithelial cells.<sup>120</sup> A similar increase in ORAI3 expression in breast cancer cell lines was noted by Motiani et al., especially those expressing estrogen receptors (ER<sup>+</sup>).<sup>121</sup> SOCE in ER<sup>+</sup> breast cancer cells was found to be mediated by ORAI3 (and STIM1), whereas ER<sup>-</sup> breast cancer cells or normal epithelial cells depended on ORAI1 (and STIM1) for SOCE. Expression of ORAI3, but not that of ORAI1, appears to be directly regulated by ER, as knockdown of ER $\alpha$  resulted in a selective decrease in ORAI3 levels and SOCE, whereas treatment of ER<sup>+</sup> MCF7 breast cancer cells with 17 $\beta$ -estradiol enhanced ORAI3 expression and SOCE. Interfering with ORAI3 expression in MCF7 cells inhibited their anchorage-independent growth in soft agar assays and tumor cell invasion in matrigel assays in vitro.<sup>122</sup> Importantly, the growth of breast tumors in vivo was significantly reduced when ORAI3 expression in MCF7 cells was knocked down by shRNA before transfer to recipient SCID mice, suggesting that ORAI3 controls SOCE in ER $\alpha$ <sup>+</sup> breast cancer cells and their growth in vivo.<sup>122</sup> It is noteworthy, however, that a similar predominance of ORAI3 channels in ER<sup>+</sup> MCF7 or ER<sup>-</sup> MDA-MB-231 breast cancer cell lines was not observed in another study, in which ORAI1 knock-down impaired SOCE and cancer cell growth.<sup>119</sup> Nevertheless, the data discussed above suggest an intriguing connection between ORAI3 and cancer development and provide a first insight into the endogenous role of ORAI3 channels.

A potentially important role for ORAI1 in breast cancer pathogenesis was also shown by Feng et al.<sup>123</sup> In contrast to the studies

discussed above, ORAI1 in this study was shown to mediate  $Ca^{2+}$  influx independent of  $Ca^{2+}$  store depletion and STIM1. Instead, the authors argue that ORAI1 is activated directly by binding of the secretory pathway calcium ATPase (SPCA2) to ORAI1 as both proteins could be co-immunoprecipitated. Expression of SPCA2, known to regulate  $Ca^{2+}$  sequestration in the Golgi apparatus, was found to be higher in the MCF-7 adenocarcinoma cell line and primary breast tumor tissue compared with non-malignant MCF-10A epithelial cells. Knockdown of SPCA2 in MCF-7 cells resulted in a small reduction of basal  $Ca^{2+}$  levels (without affecting SOCE) but significantly inhibited tumor cell proliferation in vitro and enhanced survival of mice injected with MCF7 breast cancer cells in a xenograft tumor model.<sup>123</sup> The mechanisms by which SPCA2 regulates  $Ca^{2+}$  homeostasis and carcinogenesis remain to be elucidated.

#### SOCE controls migration and metastasis of cancer cells

Besides its role in regulating tumor cell proliferation and apoptosis, STIM1 and ORAI1 have been found to affect the metastasis of cancer cells.<sup>41</sup> Knockdown of STIM1 and ORAI1 in human MDA-MB-231 breast cancer cells strongly decreased serum-induced cell migration in vitro despite only partial reduction in SOCE. In addition, ORAI1 or STIM1 knock-down impaired the turnover of focal adhesion molecules such as vinculin and impaired generation of traction force in migrating MDA-MB-231 cells. In vivo, MDA-MB-231 cells depleted of ORAI1 or STIM1 and injected i.v. into SCID mice showed reduced numbers of lung metastases compared with untreated tumor cells. A similar reduction in metastatic lung foci was also observed when mice that had received MDA-MB-231 cells by i.v. injection were treated with the non-selective SOCE inhibitor SKF 96365.<sup>41</sup>

Recent findings by Hu et al. suggest that STIM1 and ORAI1 may also be involved in the epithelial-to-mesenchymal transition (EMT), which is a critical step in the tissue evasion and metastasis of tumor cells.<sup>124</sup> The induction of EMT in human MCF-7 breast cancer cells by TGF- $\beta$ 1 was associated with downregulation of the transcription factor Oct4 and increased expression of STIM1 and ORAI1 as well as augmented  $Ca^{2+}$  influx. Direct silencing of Oct4 resulted in ORAI1 and STIM1 upregulation, increased SOCE and enhanced migratory potential of MCF-7 cells in a transwell assay in vitro. Conversely, siRNA-mediated silencing of STIM1 expression in MCF-7 cells or inhibition of SOCE by the non-selective inhibitor 2-APB prevented EMT in breast cancer cells and reduced their migratory capacity.<sup>124</sup> In addition to breast cancer cells, STIM1 may also regulate the metastatic potential of human cervical cancer cells. Of 24 patients with cervical cancer, those with pelvic lymph node metastasis had significantly higher levels of STIM1 expression in their primary tumors.<sup>40</sup> Knock down of STIM1 in human SiHa cervical cancer cells decreased their ability to migrate in vitro by reducing the turnover and activation of focal adhesion proteins such as focal adhesion kinase (FAK).<sup>40</sup>

The  $Ca^{2+}$  dependent mechanisms that control tumor cell migration and metastasis are not yet fully understood. Expression of a constitutively active form of the  $Ca^{2+}$  dependent transcription factor NFAT1 promoted the migration and invasion of



breast cancer cells in vitro.<sup>125</sup> NFAT regulates the expression of genes that play important roles in EMT including autotaxin and cyclooxygenase 2 (COX2). Both are secreted enzymes that mediate the production of lysophosphatidic acid (LPA) and prostaglandin E2 (PGE2), respectively. LPA and PGE2 bind to receptors on breast epithelial or other cells and promote their migration and tissue invasion.<sup>126</sup> siRNA mediated knockdown of COX2 in highly metastatic breast cancer cells, for instance, reduced transmigration of tumor cells across an artificial blood brain barrier in vitro and decreased brain metastasis in mice in vivo.<sup>127</sup> Huang et al. recently showed that histamine stimulation evokes SOCE in human lung cancer cells and results in COX2 expression, indicating a potential link between COX2 and Ca<sup>2+</sup> influx. Following the depletion of ORAI1 by siRNA or inhibition of SOCE by 2-APB, COX2 expression and NF-κB activation were inhibited.<sup>128</sup> Furthermore, deletion of the distal part of the COX2 promoter containing two NF-κB binding sites abolished COX2 expression suggesting that histamine/Ca<sup>2+</sup> dependent COX2 expression is mediated by NF-κB. It is likely that other Ca<sup>2+</sup> regulated factors besides NFAT and NF-κB regulate EMT and metastasis as well.

#### SOCE in neovascularization of tumors

Chen et al. observed that SOCE is important for the formation of blood vessels inside tumors by controlling the secretion of vascular endothelial growth factor (VEGF) by cervical cancer cells.<sup>40</sup> Knockdown of STIM1 in human cervical cancer cells resulted in diminished secretion of VEGF. In vivo, cervical tumors in SCID mice injected with SiHA tumor cells showed decreased neovascularization after i.v. injection with the non-selective SOCE inhibitors 2-APB and SKF 96365.<sup>40</sup> Interestingly, not only did SOCE control VEGF secretion by tumor cells, but VEGF itself is known to increase [Ca<sup>2+</sup>]<sub>i</sub> after binding to its receptor VEGFR through activation of PLCγ and induction of SOCE in human endothelial cells. Silencing of STIM1 or ORAI1 by siRNA reduced VEGF-mediated Ca<sup>2+</sup> influx in human HUVEC cells and impaired their proliferation.<sup>129</sup> Consistent with these findings, Dragoni et al. observed that stimulation of human endothelial progenitor cells (EPC) with VEGF induced Ca<sup>2+</sup> oscillations. Treatment of cells with the CRAC channel inhibitor BTP-2 reduced Ca<sup>2+</sup> oscillations, cell proliferation and tubulogenesis in vitro.<sup>130</sup> The Ca<sup>2+</sup> dependent mechanisms controlling neovascularization in tumors have not been studied directly, but are likely to involve Ca<sup>2+</sup> dependent transcription factors such as NFAT.<sup>126,131</sup> NFAT was shown to regulate expression of molecules essential for endothelial cell migration, tube formation and angiogenesis such as tissue factor (TF)<sup>132,133</sup> and cyclooxygenase (COX) 2.<sup>127,134,135</sup> Increased expression of inhibitors of the calcineurin-NFAT pathway such as the calcineurin suppressor DSCR1 results in decreased tumor angiogenesis and tumor growth due to attenuated NFAT activation.<sup>136</sup> In addition, shRNA-mediated knock down of NFAT4 (NFATc3) in 2H11 endothelial cells prevented endothelial tube formation in vitro in response to the pro-angiogenic factor secreted frizzled-related protein 2 (SFRP2).<sup>137</sup> Treatment of MMTV-neu transgenic mice, a rodent breast cancer model, with the NFAT inhibitor tacrolimus significantly decreased the density of blood vessels in tumors.<sup>137</sup>

## Summary and Outlook

The emerging role of SOCE mediated by ORAI and STIM proteins in a variety of disease processes has important clinical implications as it suggests that CRAC channel inhibition can potentially be used for the treatment of, for instance, autoimmune diseases and inflammation, thrombosis or cancer progression and metastasis. Inhibition of SOCE by genetic deletion of *Stim1* protected mice from T-cell mediated CNS inflammation in an animal model of multiple sclerosis (MS) and intestinal inflammation in a model of Crohn's disease.<sup>29,31,33</sup> This protection was due to impaired function of Th1 and Th17 cells, which produce proinflammatory cytokines such as IFN-γ and IL-17A. It is noteworthy that STIM2-deficient mice were partially resistant to EAE consistent with their partial defect in SOCE and cytokine production in T cells.<sup>29</sup> These findings suggest that pro-inflammatory Th1 and Th17 cells require strong and sustained SOCE to induce cytokine expression and to mediate autoimmune inflammation. By contrast, the function of other immune cells such as Foxp3<sup>+</sup> Treg cells and CD8<sup>+</sup> cytotoxic T cells (CTL) is less dependent on CRAC channels as only complete deletion of SOCE in STIM1,2-deficient mice impaired their function<sup>32</sup> (SF, CW, unpublished observation), thereby potentially providing a large therapeutic window for CRAC channel inhibition in inflammatory diseases.

In platelets, SOCE depends predominantly on STIM1 and ORAI1. Studies in mice lacking functional STIM1 or ORAI1 demonstrated that inhibition of SOCE could be an effective strategy to prevent thrombosis without increasing the risk of bleeding. Such novel therapies, however, would have to be targeted specifically toward platelets in order to avoid adverse effects on the various other cells that depend on SOCE. Such specificity toward platelets could be achieved by (a) targeted delivery of the drug to circulating platelets, or (b) the identification of compounds that predominantly affect the function of STIM1 or ORAI1 in platelets. The latter will depend on a better understanding of the fine-tuning of SOCE in platelets and other cells, i.e., the identification of posttranslational modifications in STIM1 and ORAI1 that are unique to platelets. Another line of research will have to investigate why SOCE is critical for the pro-coagulant rather than the pro-adhesive response of platelets, even though Ca<sup>2+</sup> signaling via CalDAG-GEFI is a central event in the inside-out activation of the major platelet adhesion receptor, integrin αIIbβ3. Our studies so far suggest that Ca<sup>2+</sup> released from intracellular stores in response to stimulation by well-known platelet agonists is the main trigger of CalDAG-GEFI-dependent platelet activation. It will be interesting to see if there are pathophysiological situations where SOCE rather than store release controls platelet adhesion. Lastly, we need a better understanding of the molecular mechanisms linking SOCE to platelet pro-coagulant responses. Calcium is known to regulate proteins like calpain and Ca<sup>2+</sup>-sensitive scramblase, which are critical for surface expression of PS and the binding of soluble clotting factors. Calcium overload also leads to the disruption of mitochondrial integrity and the formation of the mitochondrial transition pore, another requirement for the conversion

of platelets to a pro-coagulant state.<sup>138</sup> The contribution of STIM1 and ORAI1 to these processes, however, is not fully understood.

Recent evidence suggests that the expression of STIM and ORAI proteins is dysregulated in several types of human cancer and that SOCE may be a critical regulator of carcinogenesis. For instance, SOCE is involved in controlling proliferation and apoptosis of cancer cells, tumor neovascularization and expression of enzymes such as COX2 and autotaxin – thereby regulating the migration and tissue invasion of tumor cells. While the majority of studies suggest that SOCE is pro-oncogenic, it is too early for a conclusive interpretation of how SOCE affects carcinogenesis. To date, most experiments investigating the role of SOCE in cancer have been performed in cell lines; future studies will have to analyze how ORAI and STIM affect carcinogenesis in vivo. Initial in vivo studies demonstrated that SOCE controls the growth and metastasis of breast and cervical cancer; whether this is a general feature of carcinogenesis or specific to these tumors remains to be investigated. Furthermore, the molecular mechanisms by which SOCE regulates tumor growth and metastasis are still poorly understood. The multiple roles for SOCE in carcinogenesis and metastasis make CRAC channels an attractive drug target for tumor therapy. It is important to keep in mind, however, that STIM1 was initially found to be a tumor suppressor in rhabdomyosarcoma and prostate cancer cell lines. In addition, the positive effects of CRAC channel inhibition on the growth or invasive behavior of certain tumors types

may be offset by the concomittant suppression of antitumor immunity by cytotoxic CD8<sup>+</sup> T cells and natural killer (NK) cells that require SOCE for their function.<sup>51,139</sup> Calcineurin inhibitors (cyclosporin, tacrolimus) have been considered as potential cancer therapeutics because they inhibit NFAT activation, which regulates expression of tumor promoting factors such as COX2, autotaxin and VEGFR.<sup>126</sup> Nevertheless, long-term immunosuppressive treatment with cyclosporine accelerates the development of skin cancers and lymphoproliferative disorders in human patients.<sup>140</sup> This may be due to effects of calcineurin inhibition on antitumor immunity or cancer cell intrinsic effects as cyclosporine was shown to increase the motility and invasive growth of adenocarcinoma cells.<sup>141</sup> For the reasons discussed above, additional studies are required to better understand the role of SOCE and ORAI/STIM proteins in carcinogenesis.

#### Disclosure of Potential Conflicts of Interest

Feske S is a co-founder of Calcimedica; the other authors declare no potential conflicts of interest.

#### Acknowledgments

We thank members of our labs for critically reading the manuscript and helpful suggestions. This work was funded by NIH grants AI097302 (to Feske S) and HL094594 and HL106009 (to Bergmeier) and a postdoctoral fellowship by the Deutsche Forschungsgemeinschaft (DFG; WE 5303/1-1) to Weidinger C.

#### References

- Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006; 443:230-3; PMID:16921383; <http://dx.doi.org/10.1038/nature05122>.
- Vig M, Beck A, Billingsley JM, Lis A, Parvez S, Peinelt C, et al. CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr Biol* 2006; 16:2073-9; PMID:16978865; <http://dx.doi.org/10.1016/j.cub.2006.08.085>.
- 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; 443:226-9; PMID:16921385; <http://dx.doi.org/10.1038/nature05108>.
- 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; 441:179-85; PMID:16582901; <http://dx.doi.org/10.1038/nature04702>.
- 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<sup>2+</sup> entry. *Science* 2006; 312:1220-3; PMID:16645049; <http://dx.doi.org/10.1126/science.1127883>.
- Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, et al. Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. *Proc Natl Acad Sci U S A* 2006; 103:9357-62; PMID:16751269; <http://dx.doi.org/10.1073/pnas.0603161103>.
- Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, et al. STIM1 is a Ca<sup>2+</sup> sensor essential for Ca<sup>2+</sup>-store-depletion-triggered Ca<sup>2+</sup> influx. *Curr Biol* 2005; 15:1235-41; PMID:16005298; <http://dx.doi.org/10.1016/j.cub.2005.05.055>.
- Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, et al. STIM1, an essential and conserved component of store-operated Ca<sup>2+</sup> channel function. *J Cell Biol* 2005; 169:435-45; PMID:15866891; <http://dx.doi.org/10.1083/jcb.200502019>.
- Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca<sup>2+</sup> entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol* 2006; 174:815-25; PMID:16966423; <http://dx.doi.org/10.1083/jcb.200604015>.
- Muik M, Frischauf I, Derler I, Fahrner M, Bergmann J, Eder P, et al. Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. *J Biol Chem* 2008; 283:8014-22; PMID:18187424; <http://dx.doi.org/10.1074/jbc.M708898200>.
- Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, et al. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 2009; 136:876-90; PMID:19249086; <http://dx.doi.org/10.1016/j.cell.2009.02.014>.
- Zhou Y, Meraner P, Kwon HT, Machnes D, Oh-hora M, Zimmer J, et al. STIM1 gates the store-operated calcium channel ORAI1 in vitro. *Nat Struct Mol Biol* 2010; 17:112-6; PMID:20037597; <http://dx.doi.org/10.1038/nsmb.1724>.
- Stathopoulos PB, Zheng L, Ikura M. Stromal interaction molecule (STIM) 1 and STIM2 calcium sensing regions exhibit distinct unfolding and oligomerization kinetics. *J Biol Chem* 2009; 284:728-32; PMID:19019825; <http://dx.doi.org/10.1074/jbc.C800178200>.
- Brandman O, Liou J, Park WS, Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca<sup>2+</sup> levels. *Cell* 2007; 131:1327-39; PMID:18160041; <http://dx.doi.org/10.1016/j.cell.2007.11.039>.
- Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, et al. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol* 2006; 8:771-3; PMID:16733527; <http://dx.doi.org/10.1038/ncb1435>.
- Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM1 reconstitute store-operated calcium channel function. *J Biol Chem* 2006; 281:20661-5; PMID:16766533; <http://dx.doi.org/10.1074/jbc.C600126200>.
- Shaw PJ, Qu B, Hoth M, Feske S. Molecular regulation of CRAC channels and their role in lymphocyte function. *Cell Mol Life Sci* 2012; PMID:23052215; <http://dx.doi.org/10.1007/s00018-012-1175-2>.
- Derler I, Madl J, Schütz G, Romanin C. Structure, regulation and biophysics of I(CRAC), STIM/Orai1. *Adv Exp Med Biol* 2012; 740:383-410; PMID:22453951; [http://dx.doi.org/10.1007/978-94-007-2888-2\\_16](http://dx.doi.org/10.1007/978-94-007-2888-2_16).
- Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* 2010; 28:491-533; PMID:20307213; <http://dx.doi.org/10.1146/annurev.immunol.021908.132550>.
- Soboloff J, Rothberg BS, Madesh M, Gill DL. STIM proteins: dynamic calcium signal transducers. *Nat Rev Mol Cell Biol* 2012; 13:549-65; PMID:22914293; <http://dx.doi.org/10.1038/nrm3414>.
- Cahalan MD. Cell biology. How to STIMulate calcium channels. *Science* 2010; 330:43-4; PMID:20929798; <http://dx.doi.org/10.1126/science.1196348>.
- Berna-Ero A, Braun A, Kraft R, Kleinschnitz C, Schuhmann MK, Stegner D, et al. STIM2 regulates capacitive Ca<sup>2+</sup> entry in neurons and plays a key role in hypoxic neuronal cell death. *Sci Signal* 2009; 2:ra67; PMID:19843959; <http://dx.doi.org/10.1126/scisignal.2000522>.

23. Hulot JS, Fauconnier J, Ramanujam D, Chaanine A, Aubart F, Sassi Y, et al. Critical role for stromal interaction molecule 1 in cardiac hypertrophy. *Circulation* 2011; 124:796-805; PMID:21810664; <http://dx.doi.org/10.1161/CIRCULATIONAHA.111.031229>.
24. Zhang W, Halligan KE, Zhang X, Bisailon JM, Gonzalez-Cobos JC, Motiani RK, et al. Orai1-mediated I (CRAC) is essential for neointima formation after vascular injury. *Circ Res* 2011; 109:534-42; PMID:21737791; <http://dx.doi.org/10.1161/CIRCRESAHA.111.246777>.
25. 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; 81:660-8; PMID:19052075; <http://dx.doi.org/10.1093/cvr/cvn338>.
26. Guo RW, Yang LX, Li MQ, Pan XH, Liu B, Deng YL. Stim1- and Orai1-mediated store-operated calcium entry is critical for angiotensin II-induced vascular smooth muscle cell proliferation. *Cardiovasc Res* 2012; 93:360-70; PMID:22108917; <http://dx.doi.org/10.1093/cvr/cvr307>.
27. Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nat Immunol* 2008; 9:81-8; PMID:18059272; <http://dx.doi.org/10.1038/ni1546>.
28. Di Capite J, Nelson C, Bates G, Parekh AB. Targeting Ca<sup>2+</sup> release-activated Ca<sup>2+</sup> channel channels and leukotriene receptors provides a novel combination strategy for treating nasal polyposis. *J Allergy Clin Immunol* 2009; 124:1014-21, e1-3; PMID:19895990; <http://dx.doi.org/10.1016/j.jaci.2009.08.030>.
29. Ma J, McCarl CA, Khalil S, Lüthy K, Feske S. T-cell-specific deletion of STIM1 and STIM2 protects mice from EAE by impairing the effector functions of Th1 and Th17 cells. *Eur J Immunol* 2010; 40:3028-42; PMID:21061435; <http://dx.doi.org/10.1002/eji.201040614>.
30. Matsumoto M, Fujii Y, Baba A, Hikida M, Kurosaki T, Baba Y. The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. *Immunity* 2011; 34:703-14; PMID:21530328; <http://dx.doi.org/10.1016/j.immuni.2011.03.016>.
31. McCarl CA, Khalil S, Ma J, Oh-hora M, Yamashita M, Roether J, et al. Store-operated Ca<sup>2+</sup> entry through ORAI1 is critical for T cell-mediated autoimmunity and allograft rejection. *J Immunol* 2010; 185:5845-58; PMID:20956344; <http://dx.doi.org/10.4049/jimmunol.1001796>.
32. Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, et al. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol* 2008; 9:432-43; PMID:18327260; <http://dx.doi.org/10.1038/ni1574>.
33. Schuhmann MK, Stegner D, Berna-Ero A, Bittner S, Braun A, Kleinschnitz C, et al. Stromal interaction molecules 1 and 2 are key regulators of autoreactive T cell activation in murine autoimmune central nervous system inflammation. *J Immunol* 2010; 184:1536-42; PMID:20028655; <http://dx.doi.org/10.4049/jimmunol.0902161>.
34. Vig M, DeHaven WI, Bird GS, Billingsley JM, Wang H, Rao PE, et al. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat Immunol* 2008; 9:89-96; PMID:18059270; <http://dx.doi.org/10.1038/ni1550>.
35. Ahmad F, Boulaftali Y, Greene TK, Ouellette TD, Poncz M, Feske S, et al. Relative contributions of stromal interaction molecule 1 and CalDAG-GEF1 to calcium-dependent platelet activation and thrombosis. *J Thromb Haemost* 2011; 9:2077-86; PMID:21848641; <http://dx.doi.org/10.1111/j.1538-7836.2011.04474.x>.
36. Bergmeier W, Oh-Hora M, McCarl CA, Roden RC, Bray PF, Feske S. R93W mutation in Orai1 causes impaired calcium influx in platelets. *Blood* 2009; 113:675-8; PMID:18952890; <http://dx.doi.org/10.1182/blood-2008-08-174516>.
37. 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; 113:2056-63; PMID:18832659; <http://dx.doi.org/10.1182/blood-2008-07-171611>.
38. Grosse J, Braun A, Varga-Szabo D, Beyersdorf N, Schneider B, Zeitlmann L, et al. An EF hand mutation in Stim1 causes premature platelet activation and bleeding in mice. *J Clin Invest* 2007; 117:3540-50; PMID:17965774; <http://dx.doi.org/10.1172/JCI32312>.
39. 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; 205:1583-91; PMID:18559454; <http://dx.doi.org/10.1084/jem.20080302>.
40. Chen YF, Chiu WT, Chen YT, Lin PY, Huang HJ, Chou CY, et al. Calcium store sensor stromal-interaction molecule 1-dependent signaling plays an important role in cervical cancer growth, migration, and angiogenesis. *Proc Natl Acad Sci U S A* 2011; 108:15225-30; PMID:21876174; <http://dx.doi.org/10.1073/pnas.1103315108>.
41. Yang S, Zhang JJ, Huang XY. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 2009; 15:124-34; PMID:19185847; <http://dx.doi.org/10.1016/j.ccr.2008.12.019>.
42. Feske S. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca<sup>2+</sup> entry in the immune system and beyond. *Immunol Rev* 2009; 231:189-209; PMID:19754898; <http://dx.doi.org/10.1111/j.1600-065X.2009.00818.x>.
43. Feske S. Immunodeficiency due to defects in store-operated calcium entry. *Ann N Y Acad Sci* 2011; 1238:74-90; PMID:22129055; <http://dx.doi.org/10.1111/j.1749-6632.2011.06240.x>.
44. Byun M, Abhyankar A, Lelarge V, Plancoulaine S, Palanduz A, Telhan L, et al. Whole-exome sequencing-based discovery of STIM1 deficiency in a child with fatal classic Kaposi sarcoma. *J Exp Med* 2010; 207:2307-12; PMID:20876309; <http://dx.doi.org/10.1084/jem.20101597>.
45. Sahin G, Palanduz A, Aydogan G, Cassar O, Ertem AU, Telhan L, et al. Classic Kaposi sarcoma in 3 unrelated Turkish children born to consanguineous kindreds. *Pediatrics* 2010; 125:e704-8; PMID:20156905; <http://dx.doi.org/10.1542/peds.2009-2224>.
46. Fuchs S, Rensing-Ehl A, Speckmann C, Bensch B, Schmitt-Graeff A, Bondzio I, et al. Antiviral and regulatory T cell immunity in a patient with stromal interaction molecule 1 deficiency. *J Immunol* 2012; 188:1523-33; PMID:22190180; <http://dx.doi.org/10.4049/jimmunol.1102507>.
47. McCarl CA, Picard C, Khalil S, Kawasaki T, Röther J, Papolos A, et al. ORAI1 deficiency and lack of store-operated Ca<sup>2+</sup> entry cause immunodeficiency, myopathy, and ectodermal dysplasia. *J Allergy Clin Immunol* 2009; 124:1311-8, e7; PMID:20004786; <http://dx.doi.org/10.1016/j.jaci.2009.10.007>.
48. Partiseti M, Le Deist F, Hivroz C, Fischer A, Korn H, Choquet D. The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodeficiency. *J Biol Chem* 1994; 269:32327-35; PMID:7798233.
49. Le Deist F, Hivroz C, Partiseti M, Thomas C, Buc HA, Oleastro M, et al. A primary T-cell immunodeficiency associated with defective transmembrane calcium influx. *Blood* 1995; 85:1053-62; PMID:7531512.
50. Feske S, Prakriya M, Rao A, Lewis RS. A severe defect in CRAC Ca<sup>2+</sup> channel activation and altered K<sup>+</sup> channel gating in T cells from immunodeficient patients. *J Exp Med* 2005; 202:651-62; PMID:16147976; <http://dx.doi.org/10.1084/jem.20050687>.
51. Maul-Pavicic A, Chiang SC, Rensing-Ehl A, Jessen B, Fauriat C, Wood SM, et al. ORAI1-mediated calcium influx is required for human cytotoxic lymphocyte degranulation and target cell lysis. *Proc Natl Acad Sci U S A* 2011; 108:3324-9; PMID:21300876; <http://dx.doi.org/10.1073/pnas.1013285108>.
52. Hou X, Pedit L, Diver MM, Long SB. Crystal structure of the calcium release-activated calcium channel Orai. *Science* 2012; 338:1308-13; PMID:23180775; <http://dx.doi.org/10.1126/science.1228757>.
53. Picard C, McCarl CA, Papolos A, Khalil S, Lüthy K, Hivroz C, et al. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med* 2009; 360:1971-80; PMID:19420366; <http://dx.doi.org/10.1056/NEJMoa0900082>.
54. Feske S, Müller JM, Graf D, Kroczeck RA, Dräger R, Niemeyer C, et al. Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male siblings. *Eur J Immunol* 1996; 26:2119-26; PMID:8814256; <http://dx.doi.org/10.1002/eji.1830260924>.
55. Feske S, Giltne J, Dolmetsch R, Staudt LM, Rao A. Gene regulation mediated by calcium signals in T lymphocytes. *Nat Immunol* 2001; 2:316-24; PMID:11276202; <http://dx.doi.org/10.1038/86318>.
56. Kim KD, Srikanth S, Yee MK, Mock DC, Lawson GW, Gwack Y. ORAI1 deficiency impairs activated T cell death and enhances T cell survival. *J Immunol* 2011; 187:3620-30; PMID:21873530; <http://dx.doi.org/10.4049/jimmunol.1100847>.
57. Beyersdorf N, Braun A, Vögtle T, Varga-Szabo D, Galdos RR, Kissler S, et al. STIM1-independent T cell development and effector function in vivo. *J Immunol* 2009; 182:3390-7; PMID:19265116; <http://dx.doi.org/10.4049/jimmunol.0802888>.
58. Gwack Y, Srikanth S, Oh-Hora M, Hogan PG, Lamperti ED, Yamashita M, et al. Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol Cell Biol* 2008; 28:5209-22; PMID:18591248; <http://dx.doi.org/10.1128/MCB.00360-08>.
59. Engelke M, Engels N, Dittmann K, Stork B, Wienands J. Ca<sup>2+</sup> signaling in antigen receptor-activated B lymphocytes. *Immunol Rev* 2007; 218:235-46; PMID:17624956; <http://dx.doi.org/10.1111/j.1600-065X.2007.00539.x>.
60. Lazarevic V, Zullo AJ, Schweitzer MN, Staton TL, Gallo EM, Crabtree GR, et al. The gene encoding early growth response 2, a target of the transcription factor NFAT, is required for the development and maturation of natural killer T cells. *Nat Immunol* 2009; 10:306-13; PMID:19169262; <http://dx.doi.org/10.1038/ni.1696>.
61. Rengarajan J, Mittelstadt PR, Mages HW, Gerth AJ, Kroczeck RA, Ashwell JD, et al. Sequential involvement of NFAT and Egr transcription factors in FasL regulation. *Immunity* 2000; 12:293-300; PMID:10755616; [http://dx.doi.org/10.1016/S1074-7613\(00\)80182-X](http://dx.doi.org/10.1016/S1074-7613(00)80182-X).
62. Schaff UY, Dixit N, Procyk E, Yamayoshi I, Tse T, Simon SI. Orai1 regulates intracellular calcium, arrest, and shape polarization during neutrophil recruitment in shear flow. *Blood* 2010; 115:657-66; PMID:19965684; <http://dx.doi.org/10.1182/blood-2009-05-224659>.
63. Dixit N, Kim MH, Rossaint J, Yamayoshi I, Zarbock A, Simon SI. Leukocyte function antigen-1, kindlin-3, and calcium flux orchestrate neutrophil recruitment during inflammation. *J Immunol* 2012; 189:5954-64; PMID:23144497; <http://dx.doi.org/10.4049/jimmunol.1201638>.
64. Kobayashi K, Takahashi K, Nagasawa S. The role of tyrosine phosphorylation and Ca<sup>2+</sup> accumulation in Fc gamma-receptor-mediated phagocytosis of human neutrophils. *J Biochem* 1995; 117:1156-61; PMID:7490254.



65. Steinckwich N, Schenten V, Melchior C, Brécharard S, Tschirhart EJ. An essential role of STIM1, Orai1, and S100A8-A9 proteins for Ca<sup>2+</sup> signaling and FcγR-mediated phagosomal oxidative activity. *J Immunol* 2011; 186:2182-91; PMID:21239714; <http://dx.doi.org/10.4049/jimmunol.1001338>.
66. Steinckwich N, Frippiat JP, Stasia MJ, Erard M, Boxio R, Tankosic C, et al. Potent inhibition of store-operated Ca<sup>2+</sup> influx and superoxide production in HL60 cells and polymorphonuclear neutrophils by the pyrazole derivative BTP2. *J Leukoc Biol* 2007; 81:1054-64; PMID:17261548; <http://dx.doi.org/10.1189/jlb.0406248>.
67. Greenberg S, el Khoury J, di Virgilio F, Kaplan EM, Silverstein SC. Ca(2+)-independent F-actin assembly and disassembly during Fc receptor-mediated phagocytosis in mouse macrophages. *J Cell Biol* 1991; 113:757-67; PMID:2026648; <http://dx.doi.org/10.1083/jcb.113.4.757>.
68. Hishikawa T, Cheung JY, Yelamarty RV, Knutson DW. Calcium transients during Fc receptor-mediated and nonspecific phagocytosis by murine peritoneal macrophages. *J Cell Biol* 1991; 115:59-66; PMID:1918139; <http://dx.doi.org/10.1083/jcb.115.1.59>.
69. McNeil PL, Swanson JA, Wright SD, Silverstein SC, Taylor DL. Fc-receptor-mediated phagocytosis occurs in macrophages without an increase in average [Ca<sup>++</sup>] i. *J Cell Biol* 1986; 102:1586-92; PMID:3700467; <http://dx.doi.org/10.1083/jcb.102.5.1586>.
70. Braun A, Gessner JE, Varga-Szabo D, Syed SN, Konrad S, Stegner D, et al. STIM1 is essential for Fcγ<sub>3</sub> receptor activation and autoimmune inflammation. *Blood* 2009; 113:1097-104; PMID:18941110; <http://dx.doi.org/10.1182/blood-2008-05-158477>.
71. Brécharard S, Plañçon S, Melchior C, Tschirhart EJ. STIM1 but not STIM2 is an essential regulator of Ca<sup>2+</sup> influx-mediated NADPH oxidase activity in neutrophil-like HL-60 cells. *Biochem Pharmacol* 2009; 78:504-13; PMID:19433064; <http://dx.doi.org/10.1016/j.bcp.2009.05.006>.
72. Schenten V, Melchior C, Steinckwich N, Tschirhart EJ, Brécharard S. Sphingosine kinases regulate NOX2 activity via p38 MAPK-dependent translocation of S100A8/A9. *J Leukoc Biol* 2011; 89:587-96; PMID:21233411; <http://dx.doi.org/10.1189/jlb.0510304>.
73. Chen BC, Chou CF, Lin WW. Pyrimidinoneceptor-mediated potentiation of inducible nitric-oxide synthase induction in J774 macrophages. Role of intracellular calcium. *J Biol Chem* 1998; 273:29754-63; PMID:9792689; <http://dx.doi.org/10.1074/jbc.273.45.29754>.
74. Tapper H. The secretion of preformed granules by macrophages and neutrophils. *J Leukoc Biol* 1996; 59:613-22; PMID:8656045.
75. Watanabe N, Suzuki J, Kobayashi Y. Role of calcium in tumor necrosis factor-α production by activated macrophages. *J Biochem* 1996; 120:1190-5; PMID:9010769; <http://dx.doi.org/10.1093/oxford-journals.jbchem.a021540>.
76. Dawicki W, Marshall JS. New and emerging roles for mast cells in host defence. *Curr Opin Immunol* 2007; 19:31-8; PMID:17126541; <http://dx.doi.org/10.1016/j.coi.2006.11.006>.
77. Hide M, Beaven MA. Calcium influx in a rat mast cell (RBL-2H3) line. Use of multivalent metal ions to define its characteristics and role in exocytosis. *J Biol Chem* 1991; 266:15221-9; PMID:1869551.
78. Penner R, Neher E. The role of calcium in stimulus-secretion coupling in excitable and non-excitable cells. *J Exp Biol* 1988; 139:329-45; PMID:2850338.
79. Takei M, Urashima H, Endo K, Muramatsu M. Role of calcium in histamine release from rat mast cells activated by various secretagogues; intracellular calcium mobilization correlates with histamine release. *Biol Chem Hoppe Seyler* 1989; 370:1-10; PMID:2469433; <http://dx.doi.org/10.1515/bchm3.1989.370.1.1>.
80. Nishida K, Yamasaki S, Ito Y, Kabu K, Hattori K, Tezuka T, et al. Fcε-silencing-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane. *J Cell Biol* 2005; 170:115-26; PMID:15998803; <http://dx.doi.org/10.1083/jcb.200501111>.
81. Di Capite JL, Bates GJ, Parekh AB. Mast cell CRAC channel as a novel therapeutic target in allergy. *Curr Opin Allergy Clin Immunol* 2011; 11:33-8; PMID:21150433; <http://dx.doi.org/10.1097/ACI.0b013e32834232b0>.
82. Lin L, Zheng C, Zhang L, Da C, Zhao K. Up-regulation of Orai1 in murine allergic rhinitis. *Histochem Cell Biol* 2010; 134:93-102; PMID:20552218; <http://dx.doi.org/10.1007/s00418-010-0717-7>.
83. Wang Y, Lin L, Zheng C. Downregulation of Orai1 expression in the airway alleviates murine allergic rhinitis. *Exp Mol Med* 2012; 44:177-90; PMID:22170034; <http://dx.doi.org/10.3858/emmm.2012.44.3.013>.
84. Feinstein MB, Becker EL, Fraser C. Thrombin, collagen and A23187 stimulated endogenous platelet arachidonate metabolism: differential inhibition by PGE1, local anesthetics and a serine-protease inhibitor. *Prostaglandins* 1977; 14:1075-93; PMID:341226; [http://dx.doi.org/10.1016/0090-6980\(77\)90286-6](http://dx.doi.org/10.1016/0090-6980(77)90286-6).
85. White GC 2<sup>nd</sup>, Scarborough DE, Brinkhous KM. Morphological study of early phases of platelet adhesion to foreign surfaces: effect of calcium. *Ann N Y Acad Sci* 1983; 416:351-62; PMID:6587812; <http://dx.doi.org/10.1111/j.1749-6632.1983.tb35198.x>.
86. Wörner P, Brossmer R. Platelet aggregation and the release induced by inophores for divalent cations. *Thromb Res* 1975; 6:295-305; PMID:1094583; [http://dx.doi.org/10.1016/0049-3848\(75\)90079-1](http://dx.doi.org/10.1016/0049-3848(75)90079-1).
87. Bevers EM, Comfurius R, Zwaal RF. Changes in membrane phospholipid distribution during platelet activation. *Biochim Biophys Acta* 1983; 736:57-66; PMID:6418205; [http://dx.doi.org/10.1016/0005-2736\(83\)90169-4](http://dx.doi.org/10.1016/0005-2736(83)90169-4).
88. Alonso MT, Alvarez J, Montero M, Sanchez A, Garcia-Sancho J. Agonist-induced Ca<sup>2+</sup> influx into human platelets is secondary to the emptying of intracellular Ca<sup>2+</sup> stores. *Biochem J* 1991; 280:783-9; PMID:1764041.
89. Sage SO, Pintado E, Mahaut-Smith MP, Merritt JE. Rapid kinetics of agonist-evoked changes in cytosolic free Ca<sup>2+</sup> concentration in fura-2-loaded human neutrophils. *Biochem J* 1990; 265:915-8; PMID:2306226.
90. Sage SO, Reast R, Rink TJ. ADP evokes biphasic Ca<sup>2+</sup> influx in fura-2-loaded human platelets. Evidence for Ca<sup>2+</sup> entry regulated by the intracellular Ca<sup>2+</sup> store. *Biochem J* 1990; 265:675-80; PMID:2306207.
91. Stefanini L, Bergmeier W. CalDAG-GEFI and platelet activation. *Platelets* 2010; 21:239-43; PMID:20218908; <http://dx.doi.org/10.3109/09537101003639931>.
92. Crittenden JR, Bergmeier W, Zhang Y, Piffath CL, Liang Y, Wagner DD, et al. CalDAG-GEFI integrates signaling for platelet aggregation and thrombus formation. *Nat Med* 2004; 10:982-6; PMID:15334074; <http://dx.doi.org/10.1038/nm1098>.
93. Stefanini L, Roden RC, Bergmeier W. CalDAG-GEFI is at the nexus of calcium-dependent platelet activation. *Blood* 2009; 114:2506-14; PMID:19628710; <http://dx.doi.org/10.1182/blood-2009-04-218768>.
94. Stefanini L, Boulaftali Y, Ouellette TD, Holinstar M, Désiré L, Leblond B, et al. Rap1-Rac1 circuits potentiate platelet activation. *Arterioscler Thromb Vasc Biol* 2012; 32:434-41; PMID:22075250; <http://dx.doi.org/10.1161/ATVBAHA.111.239194>.
95. Henderson NS, So SS, Martin C, Kulkarni R, Thanassi DG. Topology of the outer membrane usher PapC determined by site-directed fluorescence labeling. *J Biol Chem* 2004; 279:53747-54; PMID:15485883; <http://dx.doi.org/10.1074/jbc.M409192200>.
96. Kulkarni S, Jackson SP. Platelet factor XIII and calpain negatively regulate integrin αIIbβ3 adhesive function and thrombus growth. *J Biol Chem* 2004; 279:30697-706; PMID:15131115; <http://dx.doi.org/10.1074/jbc.M403559200>.
97. Tolhurst G, Carter RN, Amisten S, Holdich JP, Erlinge D, Mahaut-Smith MP. Expression profiling and electrophysiological studies suggest a major role for Orai1 in the store-operated Ca<sup>2+</sup> influx pathway of platelets and megakaryocytes. *Platelets* 2008; 19:308-13; PMID:18569867; <http://dx.doi.org/10.1080/09537100801935710>.
98. Gilio K, van Kruchten R, Braun A, Berna-Erro A, Feijge MA, Stegner D, et al. Roles of platelet STIM1 and Orai1 in glycoprotein VI- and thrombin-dependent procoagulant activity and thrombus formation. *J Biol Chem* 2010; 285:23629-38; PMID:20519511; <http://dx.doi.org/10.1074/jbc.M110.108696>.
99. Darbousset R, Thomas GM, Mezouar S, Frère C, Bonier R, Mackman N, et al. Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation. *Blood* 2012; 120:2133-43; PMID:22837532; <http://dx.doi.org/10.1182/blood-2012-06-437772>.
100. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. *J Leukoc Biol* 2010; 87:779-89; PMID:20130219; <http://dx.doi.org/10.1189/jlb.1109766>.
101. Rosado JA, Sage SO. Coupling between inositol 1,4,5-trisphosphate receptors and human transient receptor potential channel 1 when intracellular Ca<sup>2+</sup> stores are depleted. *Biochem J* 2000; 350:631-5; PMID:10970773; <http://dx.doi.org/10.1042/0264-6021:3500631>.
102. Jardin I, Lopez JJ, Salido GM, Rosado JA. Orai1 mediates the interaction between STIM1 and hTRPC1 and regulates the mode of activation of hTRPC1-forming Ca<sup>2+</sup> channels. *J Biol Chem* 2008; 283:25296-304; PMID:18644792; <http://dx.doi.org/10.1074/jbc.M802904200>.
103. Varga-Szabo D, Authi KS, Braun A, Bender M, Ambily A, Hassock SR, et al. Store-operated Ca(2+) entry in platelets occurs independently of transient receptor potential (TRP) C1. *Pflügers Arch* 2008; 457:377-87; PMID:18546016; <http://dx.doi.org/10.1007/s00424-008-0531-4>.
104. Hassock SR, Zhu MX, Trost C, Flockner V, Authi KS. Expression and role of TRPC proteins in human platelets: evidence that TRPC6 forms the store-independent calcium entry channel. *Blood* 2002; 100:2801-11; PMID:12351388; <http://dx.doi.org/10.1182/blood-2002-03-0723>.
105. Hechler B, Lenain N, Marchese P, Vial C, Heim V, Freund M, et al. A role of the fast ATP-gated P2X1 cation channel in thrombosis of small arteries in vivo. *J Exp Med* 2003; 198:661-7; PMID:12913094; <http://dx.doi.org/10.1084/jem.20030144>.
106. Hu H, Hoylaerts MF. The P2X1 ion channel in platelet function. *Platelets* 2010; 21:153-66; PMID:20201633; <http://dx.doi.org/10.3109/09537101003599549>.
107. Ramanathan G, Gupta S, Thielmann I, Pleines I, Varga-Szabo D, May F, et al. Defective diacylglycerol-induced Ca<sup>2+</sup> entry but normal agonist-induced activation responses in TRPC6-deficient mouse platelets. *J Thromb Haemost* 2012; 10:419-29; PMID:22176814; <http://dx.doi.org/10.1111/j.1538-7836.2011.04596.x>.
108. Suzuki J, Umeda M, Sims PJ, Nagata S. Calcium-dependent phospholipid scrambling by TMEM16F. *Nature* 2010; 468:834-8; PMID:21107324; <http://dx.doi.org/10.1038/nature09583>.
109. Yang H, Kim A, David T, Palmer D, Jin T, Tien J, et al. TMEM16F forms a Ca<sup>2+</sup>-activated cation channel required for lipid scrambling in platelets during blood coagulation. *Cell* 2012; 151:111-22; PMID:23021219; <http://dx.doi.org/10.1016/j.cell.2012.07.036>.

110. van Kruchten R, Braun A, Feijge MA, Kuijpers MJ, Rivera-Galdos R, Kraft P, et al. Antithrombotic potential of blockers of store-operated calcium channels in platelets. *Arterioscler Thromb Vasc Biol* 2012; 32:1717-23; PMID:22580895; <http://dx.doi.org/10.1161/ATVBAHA.111.243907>.
111. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; 100:57-70; PMID:10647931; [http://dx.doi.org/10.1016/S0092-8674\(00\)81683-9](http://dx.doi.org/10.1016/S0092-8674(00)81683-9).
112. Monteith GR, McAndrew D, Faddy HM, Roberts-Thomson SJ. Calcium and cancer: targeting Ca<sup>2+</sup> transport. *Nat Rev Cancer* 2007; 7:519-30; PMID:17585332; <http://dx.doi.org/10.1038/nrc2171>.
113. Sabbioni S, Barbanti-Brodano G, Croce CM, Negrini M. GOK: a gene at 11p15 involved in rhabdomyosarcoma and rhabdoid tumor development. *Cancer Res* 1997; 57:4493-7; PMID:9377559.
114. Sabbioni S, Veronese A, Trubia M, Taramelli R, Barbanti-Brodano G, Croce CM, et al. Exon structure and promoter identification of STIM1 (alias GOK), a human gene causing growth arrest of the human tumor cell lines G401 and RD. *Cytogenet Cell Genet* 1999; 86:214-8; PMID:10575208; <http://dx.doi.org/10.1159/000015341>.
115. Flourakis M, Lehen'kyi V, Beck B, Raphaël M, Vandenberghe M, Abele FV, et al. Orai1 contributes to the establishment of an apoptosis-resistant phenotype in prostate cancer cells. *Cell Death Dis* 2010; 1:e75; PMID:21364678; <http://dx.doi.org/10.1038/cddis.2010.52>.
116. Fedida-Metula S, Feldman B, Koshelev V, Levin-Gromiko U, Voronov E, Fishman D. Lipid rafts couple store-operated Ca<sup>2+</sup> entry to constitutive activation of PKB/Akt in a Ca<sup>2+</sup>/calmodulin-, Src- and PP2A-mediated pathway and promote melanoma tumor growth. *Carcinogenesis* 2012; 33:740-50; PMID:22287561; <http://dx.doi.org/10.1093/carcin/bgs021>.
117. Liu H, Hughes JD, Rollins S, Chen B, Perkins E. Calcium entry via ORAI1 regulates glioblastoma cell proliferation and apoptosis. *Exp Mol Pathol* 2011; 91:753-60; PMID:21945734; <http://dx.doi.org/10.1016/j.yexmp.2011.09.005>.
118. Ruano Y, Mollejo M, Ribalta T, Fiaño C, Camacho FI, Gómez E, et al. Identification of novel candidate target genes in amplicons of Glioblastoma multiforme tumors detected by expression and CGH microarray profiling. *Mol Cancer* 2006; 5:39; PMID:17002787; <http://dx.doi.org/10.1186/1476-4598-5-39>.
119. McAndrew D, Grice DM, Peters AA, Davis FM, Stewart T, Rice M, et al. ORAI1-mediated calcium influx in lactation and in breast cancer. *Mol Cancer Ther* 2011; 10:448-60; PMID:21224390; <http://dx.doi.org/10.1158/1535-7163.MCT-10-0923>.
120. Faouzi M, Hague F, Potier M, Ahidouch A, Sevestre H, Ouidid-Ahidouch H. Down-regulation of Orai3 arrests cell-cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells. *J Cell Physiol* 2011; 226:542-51; PMID:20683915; <http://dx.doi.org/10.1002/jcp.22363>.
121. 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; 285:19173-83; PMID:20395295; <http://dx.doi.org/10.1074/jbc.M110.102582>.
122. Motiani RK, et al. Orai3 is an estrogen receptor alpha-regulated Ca<sup>2+</sup> channel that promotes tumorigenesis. *FASEB J* 2012; 2012:19.
123. 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; PMID:20887894; <http://dx.doi.org/10.1016/j.cell.2010.08.040>.
124. Hu J, Qin K, Zhang Y, Gong J, Li N, Lv D, et al. Downregulation of transcription factor Oct4 induces an epithelial-to-mesenchymal transition via enhancement of Ca<sup>2+</sup> influx in breast cancer cells. *Biochem Biophys Res Commun* 2011; 411:786-91; PMID:21798248; <http://dx.doi.org/10.1016/j.bbrc.2011.07.025>.
125. Jauliac S, López-Rodríguez C, Shaw LM, Brown LF, Rao A, Tokar A. The role of NFAT transcription factors in integrin-mediated carcinoma invasion. *Nat Cell Biol* 2002; 4:540-4; PMID:12080349; <http://dx.doi.org/10.1038/ncb816>.
126. Mancini M, Tokar A. NFAT proteins: emerging roles in cancer progression. *Nat Rev Cancer* 2009; 9:810-20; PMID:19851316; <http://dx.doi.org/10.1038/nrc2735>.
127. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. *Nature* 2009; 459:1005-9; PMID:19421193; <http://dx.doi.org/10.1038/nature08021>.
128. Huang WC, Chai CY, Chen WC, Hou MF, Wang YS, Chiu YC, et al. Histamine regulates cyclooxygenase 2 gene activation through Orai1-mediated NFκB activation in lung cancer cells. *Cell Calcium* 2011; 50:27-35; PMID:21605904; <http://dx.doi.org/10.1016/j.ceca.2011.04.004>.
129. 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; 103:1289-99; PMID:18845811; <http://dx.doi.org/10.1161/01.RES.0000338496.95579.56>.
130. Dragoni S, Laforenza U, Bonetti E, Lodola F, Bottino C, Berra-Romani R, et al. Vascular endothelial growth factor stimulates endothelial colony forming cells proliferation and tubulogenesis by inducing oscillations in intracellular Ca<sup>2+</sup> concentration. *Stem Cells* 2011; 29:1898-907; PMID:21905169; <http://dx.doi.org/10.1002/stem.734>.
131. Müller MR, Rao A. NFAT, immunity and cancer: a transcription factor comes of age. *Nat Rev Immunol* 2010; 10:645-56; PMID:20725108; <http://dx.doi.org/10.1038/nri2818>.
132. Armesilla AL, Lorenzo E, Gómez del Arco P, Martínez-Martínez S, Alfranca A, Redondo JM. Vascular endothelial growth factor activates nuclear factor of activated T cells in human endothelial cells: a role for tissue factor gene expression. *Mol Cell Biol* 1999; 19:2032-43; PMID:10022890.
133. van den Berg YW, Osanto S, Reitsma PH, Versteeg HH. The relationship between tissue factor and cancer progression: insights from bench and bedside. *Blood* 2012; 119:924-32; PMID:22065595; <http://dx.doi.org/10.1182/blood-2011-06-317685>.
134. Gupta KH, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C, et al. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* 2007; 446:765-70; PMID:17429393; <http://dx.doi.org/10.1038/nature05760>.
135. Hernández GL, Volpert OV, Iñiguez MA, Lorenzo E, Martínez-Martínez S, Grau R, et al. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J Exp Med* 2001; 193:607-20; PMID:11238591; <http://dx.doi.org/10.1084/jem.193.5.607>.
136. Baek KH, Zaslavsky A, Lynch RC, Britt C, Okada Y, Siarey RJ, et al. Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. *Nature* 2009; 459:1126-30; PMID:19458618; <http://dx.doi.org/10.1038/nature08062>.
137. Siamakpour-Reihani S, Caster J, Bandhu Nepal D, Courtwright A, Hilliard E, Usary J, et al. The role of calcineurin/NFAT in SFRP2 induced angiogenesis—a rationale for breast cancer treatment with the calcineurin inhibitor tacrolimus. *PLoS One* 2011; 6:e20412; PMID:21673995; <http://dx.doi.org/10.1371/journal.pone.0020412>.
138. Jackson SP, Schoenwaelder SM. Procoagulant platelets: are they necrotic? *Blood* 2010; 116:2011-8; PMID:20538794; <http://dx.doi.org/10.1182/blood-2010-01-261669>.
139. Pores-Fernando AT, Zweifach A. Calcium influx and signaling in cytotoxic T-lymphocyte lytic granule exocytosis. *Immunol Rev* 2009; 231:160-73; PMID:19754896; <http://dx.doi.org/10.1111/j.1600-065X.2009.00809.x>.
140. Dantal J, Souillou JP. Immunosuppressive drugs and the risk of cancer after organ transplantation. *N Engl J Med* 2005; 352:1371-3; PMID:15800234; <http://dx.doi.org/10.1056/NEJMe058018>.
141. Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 1999; 397:530-4; PMID:10028970; <http://dx.doi.org/10.1038/17401>.
142. Oh-Hora M, Komatsu N, Pishyareh M, Feske S, Hori S, Taniguchi M, Rao A, Takayanagi H. Agonist-selected T cell development requires strong T cell receptor signaling and store-operated calcium entry. *Immunity* 2013; 38:881-895; PMID:23499491; <http://dx.doi.org/10.1016/j.immuni.2013.02.008>.