SOCE and cancer: Recent progress and new perspectives

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Ca^{2+} acts as a universal and versatile second messenger in the regulation of a myriad of biological processes, including cell proliferation, differentiation, migration and apoptosis. Store-operated Ca^{2+} entry (SOCE) mediated by ORAI and the stromal interaction molecule (STIM) constitutes one of the major routes of calcium entry in nonexcitable cells, in which the depletion of intracellular Ca^{2+} stores triggers activation of the endoplasmic reticulum (ER)-resident Ca^{2+} sensor protein STIM to gate and open the ORAI Ca^{2+} channels in the plasma membrane (PM). Accumulating evidence indicates that SOCE plays critical roles in cancer cell proliferation, metastasis and tumor neovascularization, as well as in antitumor immunity. We summarize herein the recent advances in our understanding of the function of SOCE in various types of tumor cells, vascular endothelial cells and cells of the immune system. Finally, the therapeutic potential of SOCE inhibitors in the treatment of cancer is also discussed.

Mini Review

Ca^{2+}, one of the most versatile and universal signaling molecules, is known to regulate numerous cellular activities, ranging from short-term responses such as contraction and secretion to long-term control of transcription, cell division and cell death.[1,2] Ca^{2+} homeostasis in mammalian cells is maintained through the coordinated actions of a repertoire of Ca^{2+} signaling components, including Ca^{2+} channels, pumps and exchangers, that are situated in the plasma membrane (PM) or in intracellular organelles such as the endoplasmic

reticulum (ER) and mitochondria.[3–5] Interruptions in Ca^{2+} homeostasis may result in human diseases such as cardiovascular disease, immunodeficiency, diabetes and cancer.[6,7]

Invasive cancer is the leading cause of death.[8] Accumulating evidence suggests that Ca^{2+}-related signaling pathways represent promising new therapeutic targets for cancer.[2] Compared with nonmalignant cells, cancer cells undergo constant remodeling of Ca^{2+} signaling, with remarkable alterations in the expression and/or activity of calcium channels and pumps,[9] as well as in intracellular Ca^{2+}-dependent signaling components. Cancer cells do this to sustain their own proliferation and to avoid cell death response.[10] An understanding of the remodeling of Ca^{2+} signaling and of channel proteins in cancer is certainly anticipated to provide novel opportunities for therapeutic intervention. Store-operated calcium channels (SOCCs) are some of the most abundantly expressed channels in nonexcitable cells in which the emptying of intracellular Ca^{2+} stores activates Ca^{2+} influx through the PM.[11,12] In this review, we primarily focus on the role of store-operated Ca^{2+} entry (SOCE) in cancer.

Overview of SOCE

Back in 1980 sec, Putney proposed that the depletion of Ca^{2+} store directly results in the activation of Ca^{2+} channels in the PM, a process now referred to as SOCE.[13] SOCE can be activated by ligand-induced activation of membrane receptors or by pharmacological manipulations that empty the intracellular Ca^{2+} stores that are located primarily in the ER. The most well-studied example of store-operated channel (SOC) is the calcium release-activated calcium (CRAC) channel that was initially characterized in human T cells and mast cells.[14,15] Under physiological conditions, stimulation of diverse PM receptors such as G-protein-coupled receptors (GPCR) activates phospholipase C (PLC), which hydrolyzes
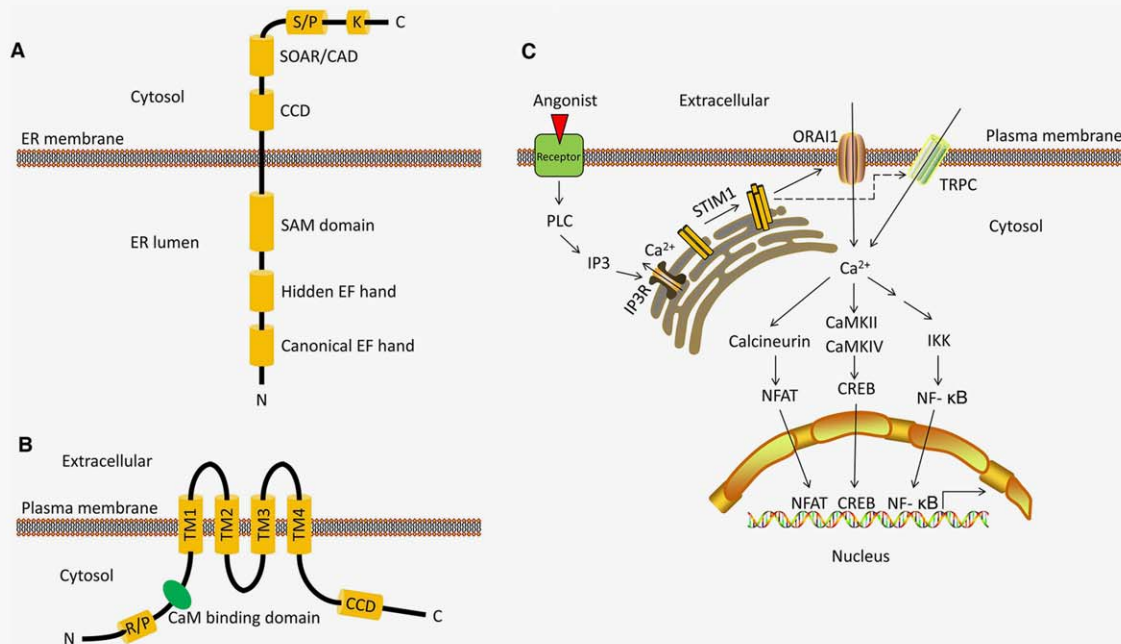
Figure 1. Schematic diagram of SOCE mediated by ORAI1 and STIM1. (*a*) STIM1 protein consists of a canonical EF hand, a hidden EF hand and a SAM domain in the ER luminal domain, and CCD, SOAR and ERM domains as well as serine- or proline- and lysine-rich clusters on the cytosolic side. (*b*) The ORAI1 protein contains four membrane-spanning regions and intracellular N- and C-termini. It also has a unique R/P-rich region in the N-terminus and a putative coiled-coil domain in its intracellular C-terminus. (*c*) The stimulation of PM receptors activates PLC, which leads to the production of the second messenger IP3. IP3 binds to the IP3R and elicits rapid Ca^{2+} release from the ER lumen. STIM1 senses Ca^{2+} decrease in ER and undergoes conformational changes to mediate ORAI gating, which results in Ca^{2+} influx through ORAI channels. The Ca^{2+} increase activates NFAT and a number of other transcription factors such as NF-κB and CREB, among others, which play crucial roles in cancer cells, endothelial cells, cells of the immune system and other nonhematopoietic cells.

phosphatidylinositol-4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol trisphosphate (IP3). IP3 binds to the Ca^{2+}-permeable IP3 receptor (IP3R) on the ER membrane and evokes Ca^{2+} release from the stores into the cytosol.[16] Once the stromal interaction molecule (STIM) protein senses a drop in ER Ca^{2+} levels, it undergoes conformational changes with subsequent migration toward the PM to open ORAI channels through direct physical coupling.[12,17–20] Functional interactions between the IP3 receptors and transient receptor potential (TRP) channels have also been reported to play modulatory roles during SOCE in certain types of cells.[21,22]

Implications of STIM-ORAI in SOCE

During the last decade, the two major protein families STIM and ORAI[12,17–19] were identified. STIM proteins (STIM1 and STIM2) are type I single-pass transmembrane proteins that are located predominantly in the ER.[23] As shown in Figure 1*a*, the domain structure of STIM1 includes the following: an N-terminal signal peptide, a canonical EF hand Ca^{2+}-binding motif, a hidden non-Ca^{2+}-binding EF hand and a sterile α-motif (SAM) domain in the ER luminal region, a putative coiled-coil domain (CCD), a STIM-ORAI activating region (SOAR) or CRAC activation domain (CAD), a serine- or proline-rich clusters and a polybasic C-tail in the cytoplasmic region.[24] The EF-hand domain is responsible for sensing the

Ca^{2+} fluctuation in the ER lumen, whereas the cytoplasmic domain, in particular the SOAR/CAD domain,[25,26] directly gates and opens the ORAI channels. At rest, STIM1 is evenly distributed throughout the ER membrane. On depletion of Ca^{2+} stores, STIM1 undergoes rapid dimerization/oligomerization and moves into regions at the ER–PM junctions (termed puncta) so that they can physically interact with ORAI channels and elicits Ca^{2+} influx within a few seconds.[27] The STIM1 homologue STIM2 acts as a weaker activator of ORAI channels and is responsible for the maintenance of stable cytosolic and ER Ca^{2+} concentrations to prevent uncontrolled activation of SOCE.[28,29]

ORAI1 is a four-pass transmembrane protein whose N- and C- termini face the cytoplasm and is postulated to assemble as a tetramer or hexamer in the PM (Fig. 1*b*).[30–32] The first transmembrane segment of ORAI1 forms the ion-conducting pathway, which mediates Ca^{2+} influx.[32–34] The intracellular C-terminus of ORAI1 has been shown to form a coiled-coil structure, which interacts with the SOAR/CAD domain of STIM1.[19,26,30,35–37] Disruption of coiled-coil formations in the C-tail of ORAI1 impairs STIM1-mediated activation of ORAI channels.[35] The N-terminus of ORAI1 is also essential for STIM1-mediated gating. It contains a calmodulin (CaM)-binding domain, which is involved in fast Ca^{2+}-dependent inactivation of ORAI channels.[38] In mammals, ORAI1 has two other homologues (ORAI2 and 3) that

can also be gated by STIM1 following store depletion. However, the ORAI1 protein has the highest potency in the conduction of Ca^{2+} currents, and thus the genetic depletion of ORAI1 substantially impairs SOCE.[39]

Sustained elevation of intracellular Ca^{2+} through CRAC channels leads to the activation of the ubiquitous Ca^{2+} sensor CaM, which further activates the Ca^{2+}/CaM-dependent phosphatase calcineurin.[40] Calcineurin dephosphorylates multiple phosphoserines in the regulatory domain of the nuclear factor of activated T cells (NFAT), which leads to the nuclear translocation of NFAT within minutes (Fig. 1c). In the nucleus, NFAT can cooperate with multiple transcription factors such as the activator protein 1 (AP1), forkhead box P-family protein (FOXP) and GATA to initiate the expression of multiple genes. These genes can then regulate diverse cellular functions,[40] including cell survival, proliferation, migration, invasion and angiogenesis. SOCE could also activate a number of other transcription factors such as cAMP-responsive element-binding protein (CREB) and nuclear factor-κB (NF-κB) *via* the activation of calmodulin-dependent protein kinase II/IV (CaMKII/IV) and IκB kinase (IKK), respectively.[41–43] It has been shown that SOCE-mediated CREB activation promotes the proliferation of vascular smooth muscle cells (VSMCs).[44] NF-κB, which is stimulated by SOCE, is well known for its function in innate immunity, inflammation and oncogenesis.[45,46] In turn, NF-κB stimulates the transcription of *ORAI1* and *STIM1*, which play important roles in the regulation of platelet secretion, aggregation and thrombus formation.[47]

Implications of TRP Channels in SOCE

The TRP proteins are nonselective cation channels that were first identified in the *trp* mutant of *Drosophila*.[48] To date, approximately 30 different mammalian TRP channels have been identified according to their sequence homology. The TRP proteins have a common structure, including six transmembrane-spanning domains (S1-S6) with a loop region between the S5 and S6 domains and intracellular N- and C-termini.[49] The role of *Drosophila* TRP proteins in SOCE was controversial as they were later found to behave as non-SOCs.[50] However, as the identification of TRP homologues in mammals, a body of evidence has supported a role for TRP channels in the conduction of SOCE, especially the transient receptor potential canonical (TRPC) subfamily members; these can be activated in response to stimuli, which results in PIP2 hydrolysis.[51] For example, the inhibition of transcription of native TRPC1 and TRPC3 channels in HEK cells could reduce Ca^{2+} influx after the depletion of Ca^{2+} stores.[52] The knockdown of other TRPC channels such as TRPC4 can inhibit SOCE in human corneal epithelial cells.[53] Together, these findings provide evidence to support a possible implication of TRP channels in SOCE in certain types of cells.[53–55]

Interaction Between STIM1, ORAI and TRPC Proteins

STIM1 can interact with all three ORAI proteins to induce SOCE.[56] Following the depletion of Ca^{2+} stores, the EF-SAM domains of STIM1 undergo oligomerization and initiate the translocation of STIM1 into the ER–PM junctions, which activates ORAI channels.[27] As a precise feedback mechanism, an elevation in the intracellular Ca^{2+} concentration leads to rapid Ca^{2+}-dependent inactivation (CDI) of the ORAI channel or dissociation of the STIM1–ORAI complex, which protects cells from ER Ca^{2+} overload.[57]

The activation of ORAI channels is strictly dependent on STIM1, while the involvement of STIM1 in TRPC activation remains controversial.[58] It was reported that STIM1 could activate TRPC1, 2 and 4, where the ezrin/radixin/moesin (ERM) domain and the cationic lysine-rich region of STIM1 are required for the binding and gating of TRPC channels, respectively.[59] STIM1 does not interact with TRPC3 directly as it mediates the heteromultimerization of TRPC1 with TRPC3.[60] DeHaven *et al.* also reported that TRPC3 functions as a STIM1-dependent channel in the presence of TRPC1.[61]

Overall, current evidence suggests that the depletion of Ca^{2+} stores results in a dynamic interplay between STIM1, ORAI and the TRPC proteins, where STIM1 communicates information from the ER lumen to the Ca^{2+} channels at the PM.[62] ORAI channels may mediate Ca^{2+} influx either independently or together with the TRPC proteins.[63,64] The coordination of the STIM1, ORAI and TRPC proteins in mediating SOCE, as well as their possible regulatory mechanisms, is still a topic of debate and warrants further investigation.

Role of SOCE in Cancer

SOCE mediated by the STIM and ORAI proteins has recently been implicated in various processes during oncogenic transformation such as malignant transformation, apoptosis, proliferation, angiogenesis, metastasis and antitumor immunity. At the tumor initiation stage, Ca^{2+} signaling mediated by SOCE is needed to induce genetic changes in premalignant cells. These genetic alterations ultimately reprogram cells and cause them to undergo malignant transformation.[65] At the tumor development stage, blood vessels are necessary for tumor nutritional support. In cancer cells, SOCE promotes the secretion of vascular endothelial growth factor (VEGF),[66] which activates SOCE in endothelial cells by binding to its receptor; this subsequently promotes the proliferation of endothelial cells.[67] Interestingly, calcium signaling mediated by SOCE also plays a critical role in the antitumor activity of cytotoxic T lymphocytes (CTLs).[68] The following sections summarize recent advances in our understanding of SOCE in tumor cells as well as in other cell types within the tumor environment.

SOCE Regulates Apoptotic Cell Death, Proliferation and Metastasis of Cancer Cells

Sabbioni *et al.* reported that mRNA expression of STIM1 was absent in human rhabdomyosarcoma and rhabdoid tumor cell lines, and the forced expression of STIM1 caused growth arrest in these cells.[69,70] More recently, Flourakis *et al.* demonstrated that the endogenous SOCE mediated by ORAI1 is the principal source of Ca^{2+} influx that triggers apoptotic cell

death of human prostate cancer (PCa) cells. The inhibition of SOCE through the knockdown of ORAI1 protects PCa cells from death induced by diverse apoptosis-inducing stimuli.[71] These findings led to the hypothesis that SOCE might serve as part of a tumor-suppressing mechanism. Nonetheless, accumulating evidence indicates that STIM/ORAI-mediated SOCE promotes tumor growth and metastasis in a variety of cancer types. Microarray data from 295 breast cancer samples showed that breast cancer patients with a STIM1^{high}/STIM2-[low] profile demonstrated abnormally augmented SOCE and displayed a significantly poorer prognosis.[72] Enhanced expression of ORAI1 and STIM1 as well as enhanced SOCE were also reported in therapy-resistant ovarian carcinoma cells.[73] Furthermore, STIM1 or ORAI1 was overexpressed in tumor tissues when compared with precancerous tissues in patients with colorectal,[74,75] cervical,[66] liver,[76] lung[77] and clear cell renal cancers.[78] The potential anticancer and therapeutic value of targeting SOCE is strongly supported by the fact that the knockdown or pharmacological inhibition of STIM1–ORAI1 can efficiently restrain the growth and metastasis of breast,[79] colorectal,[74,75] cervical,[66] liver,[76] nasopharyngeal,[80] epidermoid,[81] glioma,[82] melanoma[83] and clear cell renal cancer cells.[78] In addition, the blockage of STIM1-mediated SOCE can significantly enhance chemotherapy-induced apoptosis in lung and pancreatic cancer cells.[77,84] Recent studies also indicate that ORAI3-mediated SOCE is involved in the tumorigenesis of estrogen receptor-positive breast cancer and non-small cell lung cancer.[85,86]

The molecular mechanisms of SOCE in the regulation of cancer cell proliferation and migration can be summarized as follows: (i) Inhibition of SOCE through STIM1 knockdown results in the upregulation of p21 and the downregulation of Cdc25C, cyclin E, cyclin D, CDCK2 and CDCK4, which eventually elicits cell cycle arrest.[66,82] (ii) SOCE blockade through STIM1 or ORAI1 knockdown impairs focal adhesion turnover and cell migration.[66,76,79] Aberrant SOCE causes the activation of the Ca^{2+}-regulated protease calpain and the cytoplasmic kinase Pyk2, which regulate the focal adhesion dynamics of migratory cervical cancer cells.[66] The small GTPases Ras and Rac are also regulators of focal adhesion turnover. The expression of constitutively active Ras and Rac could rescue the defects of focal adhesion turnover and migration induced by the inhibition of SOCE in breast cancer cells.[79] (iii) Enhanced SOCE through STIM1 overexpression promotes the migration of colorectal cancer cells via an increase in the expression of cyclooxygenase-2 (COX-2) and the production of prostaglandin E2 (PGE2).[74] Ca^{2+}-dependent transcription factor NFAT may play an important role in this process.[87] NFAT also induces the transcription of many other proinvasive genes such as autotoxin.[88] As described above, STIM/ORAI-mediated SOCE plays important roles in the proliferation and metastasis of cancer cells. The exact function and mechanism are complicated and context-dependent, as summarized in Table 1 below.

SOCE Regulates Tumor Angiogenesis

The recruitment of new blood vessels, or angiogenesis, represents one of the central hallmarks of cancer that is necessary to support tumor growth and metastasis. During angiogenesis, endothelial cells proliferate so that new capillary blood vessels can develop from preexisting microvessels; they then form tubes and connect the tips of these tubes to create loops to tolerate blood flow.[94,95] VEGF and its receptors function as critical regulators during angiogenesis.[96] After binding to its receptor, VEGF can elicit Ca^{2+} entry via PLCγ activation, thereby inducing SOCE in human endothelial cells. The knockdown of STIM or ORAI could reduce VEGF-induced Ca^{2+} influx in human umbilical vein endothelial cells (HUVECs), and consequently inhibit cell proliferation, migration and tube formation.[67,97] On the other hand, in cancer cells, SOCE can control the secretion of VEGF. For example, in a mouse xenograft model, the inhibition of SOCE resulted in decreased VEGF secretion in cervical cancer cells, which led to a reduction in neovascularization and tumor growth.[66]

Endothelial progenitor cells (EPCs) are a population of progenitor cells that may proliferate, migrate and acquire a mature endothelial phenotype.[98] After acute vascular injury, EPCs are recruited from the bone marrow (BM) to sites of tissue regeneration where they sustain neovascularization, which is triggered by the increased availability of angiogenic growth factors or chemokines such as VEGF and angiopoietin.[99] Emerging evidence indicates that EPCs contribute to the sprouting of new tumor vessels to accelerate tumor proliferation and metastasis.[100,101] SOCE is essential for the proliferation, motility and tubulogenesis of EPCs.[102–104] A recent study revealed that SOCE is upregulated and controls proliferation and tubulogenesis in renal cellular carcinoma (RCC)-EPCs.[105] The higher amplitude of SOCE in these cells is associated with the overexpression of STIM1, ORAI1 and TRPC1. The suppression of SOCE in RCC-EPCs represents a novel and promising method for ameliorating vascularization in RCC.[105]

Collectively, augmented SOCE appears to promote angiogenesis through the following possible mechanisms: (i) SOCE in cancer cells regulates the production of VEGF, which is critical for the formation of new blood vessels[66]; (ii) SOCE in endothelial cells regulates the cell cycle and proliferation of these cells[97]; (iii) Ca^{2+}-dependent activation of NFAT regulates the expression of molecules such as tissue factor (TF) and COX-2, which are essential for endothelial cell migration, tube formation and angiogenesis.[106–108]

SOCE Regulates Antitumor Immunity

An increase in the intracellular Ca^{2+} concentration is required for a variety of cellular immune functions. Ca^{2+} influx in immune cells occurs predominantly through the SOCE process. Defects in SOCE that are caused by mutations in the STIM or ORAI genes result in dysfunction of immune

Table 1. Aberrant SOCE in different cancer cells

Ca^{2+} channel	Cancer type	Major effects	Possible mechanisms	Ref.
STIM1–ORAI1	Cervical cancer SiHa and Caski cells	Promotion of tumor cell growth, migration and invasion	STIM1 knockdown induces cell cycle arrest, abolishes focal adhesion and actomyosin formation, and tumorigenesis	66,89
STIM1–ORAI1	Ovarian cancer A2780 cells	Contribution to cisplatin resistance	ORAI1/STIM1 enhances AKT activity	73
STIM1–ORAI1	Breast cancer MDA-MB-231 and 4T1 cells	Promotion of tumor metastasis	Blockade of SOCE impairs focal adhesion turnover	79
STIM1–ORAI1	Colorectal cancer cells	Promotion of cell motility	STIM1 overexpression causes upregulated expression of COX-2 and PGE2 and promotes EMT	74,75
STIM1–ORAI1	Hepatocarcinoma HCC-LM3 cells	Promotion of cell migration and invasion	STIM1 knockdown impairs focal adhesion turnover	76
STIM1–ORAI1	Glioblastoma U251 cells	Promotion of cell proliferation and invasion	STIM1 suppression induces cell cycle arrest	82
STIM1–ORAI1	Epidermoid carcinoma A431 cells	Promotion of cell and tumor growth	STIM1 knockdown inhibits DNA synthesis and decreases EGFR phosphorylation	81
STIM1–ORAI1	Melanoma cells	Promotion of cell proliferation and migration	SOCE activates the ERK signaling pathway	83
ORAI3	Breast cancer MCF-7 cells	Promotion of cell growth, invasion and tumorigenesis	ORAI3 knockdown reduces c-Myc expression and activity	90
TRPC3	Ovarian cancer SKOV-3 cells	Increase in cell proliferation and tumor formation	TRPC3 inhibition dephosphorylates Cdc2 and induces G2/M phase arrest	91
TRPC6	Gastric cancer AGS and MKN45 cells	Increase in cell growth and tumor formation in mice	TRPC6 blockade induces G2/M phase arrest	92
TRPC6	Glioblastoma U373 MG and HMEC-1 cells	Promotion of cell growth, invasion and angiogenesis	TRPC6 is coupled to the activation of the calcineurin–NFAT pathway	93

cells, including T cells, B cells, NK cells, dendritic cells, mast cells, macrophages and neutrophils.[109] For example, SOCE-deficient NK cells display defective exocytosis of cytotoxic granules. Moreover, pharmacological inhibition of SOCE severely impaired the cytotoxicity mediated by primary NK cells and CD8$^+$ effector T cells.[110] Recently, Weidinger *et al.* found that the normal function of SOCE in cytotoxic CD8$^+$ T cells is required to retard tumor growth in xenograft models of melanoma and colon carcinoma. This is because STIM1 and STIM2 deficiency in CD8$^+$ T cells significantly impairs their production of IFN-γ and TNF-α, their expression of Fas ligand and degranulation of CTLs.[68] SOCE was also shown to participate in the cellular immune response as the blockage of SOCE by BTP2 significantly inhibited the antihost CTL response, donor T-cell expansion and IFN-γ production in mouse models of graft-*versus*-host disease (GvHD).[111] Fuchs *et al.* reported that a homozygous R429C point mutation in *STIM1* in two patients completely abolished SOCE in their T cells. This loss-of-function mutation of STIM1 resulted in a lack of NKT cells and in abnormal functions of NK- and FOXP3-positive regulatory T (Treg) cells, and eventually impaired antiviral immunity, which helps prevent chronic CMV and EBV infections.[112] Shaw *et al.* reported that STIM1/STIM2-mediated SOCE contributed to the differentiation and function of effector CD8$^+$ T cells during early acute virus infection.[113] More importantly,

SOCE was found to regulate CD40L expression in CD4$^+$ T cells, which is essential for the maintenance of memory CD8$^+$ T cells and their ability to mediate recall responses and protection against secondary viral infections.[113] Recurrent and chronic viral infections such as EBV, CMV and human herpes virus 8 (HHV-8) result in the development of virus-associated tumors in SOCE-deficient patients.[113] For example, it was shown that T-cell immunodeficiency caused by STIM1 mutation accelerated the development of lethal Kaposi sarcoma (KS) upon infection with HHV-8.[114]

ORAI- and STIM-mediated SOCE is also required for the cell differentiation and functions of traditional effector CD4$^+$ T cells, including Th1 and Th2 cells.[109] Notably, the proliferation of Th17 cells also appears to require SOCE.[115] SOCE-deficient CD4$^+$ T cells grown under Th17 polarizing conditions *in vitro* failed to produce IL-17, which acts as a proinflammatory molecule to induce angiogenesis during tumor progression.[115] The production of IFN-γ and IL-2 by Th1 cells is also decreased in the absence of SOCE.[116] However, IFN-γ and IL-2 are commonly regarded as tumor inhibitory cytokines.[117,118] Thus, the exact role of CD4$^+$ T-helper cells in tumor development is determined by its differentiation status. Interestingly, effector CD4+ T cells appear to require varying degrees of SOCE for their maximal activity and exhibit differential sensitivity toward SOC inhibitors. For instance, the levels of SOCE required for the differentiation

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and function of Th17 cells are higher than that of Th1 and Th2 cells.[119] Kim *et al.* reported that Th17 cells showed higher sensitivity to the SOCE inhibitor than Th1 and Th2 cells.[120]

Until now, the possible mechanism of the regulation of antitumor immunity by SOCE could be summarized as follows: (*i*) SOCE induces the activation of Ca^{2+}-dependent transcription factors, which are essential for the development and normal function of immune cells such as NFAT,[121] CREB[122] or activating transcription factor (ATF) 2[123] and (*ii*) SOCE mediates the production of cytokines and chemokines that can directly kill cancer cells.

Because the function of the immune system during cancer progression is paradoxical,[124] it is necessary to be aware that not all of the immune responses that are regulated by SOCE are cancer protective. Thus, a clarification of the mechanisms and functions of SOCE in different types of immune cells in different stages of tumor development is important for a deeper understanding of the role of SOCE in antitumor immunity. In the early stages of tumorigenesis, SOCE is important for the normal function of immune surveillance system, which can prevent tumorigenesis. Once pathogens or cells escape immune surveillance, more immune cells are recruited and an inflammatory microenvironment is formed. It has been shown that chronic inflammation is the cause of various human cancers.[125] For example, patients with inflammatory bowel disease (IBD) have an increased risk for colorectal cancer.[126] Sustained Ca^{2+} flux *via* SOCE is necessary for the activation of immune cells in cases of chronic inflammation. Therefore, it is possible to avoid the initiation of tumor formation by inhibiting SOCE-involved chronic inflammation. However, as mentioned above, the functions of different types of immune cells are diverse, and their role in tumor immunity is sometimes different even for cells of the same cell type. For example, Th17 cells can promote tumor growth through IL-17 secretion, but it also prevents tumor development *via* the induction of the recruitment and activation of cytotoxic CD8^{+} T cells within tumors.[127] Briefly, the role of SOCE in antitumor immunity is complicated and requires investigation in specific conditions. In particular, Weidinger *et al.* demonstrated that SOCE in CD8^{+} T cells is critical for their cytotoxic activity against tumor cells.[68] The model used in this study was the engraftment of melanoma and colon carcinoma cells into conditional gene knockout mice, rather than a spontaneous tumor formation model. The function of CD8^{+} T cells against xenogeneic and allogeneic tumor cells may be different. The results should therefore be explained cautiously.

As mentioned above, distinct T-cell types need different amounts of Ca^{2+} influx for their function. Th17 and cancer cells appear to require relatively large amounts of Ca^{2+} influx. For example, genetic depletion of STIM1 alone can readily lead to impaired Th17 cell function,[128,129] suggesting that Th17 cells react very sensitively to even partial blockade of SOCE. By contrast, CTLs requires very little residual Ca^{2+}

influx for their function. Thus, the cytotoxicity of CTLs and antiviral immunity mediated by CD8^{+} T cells are only impaired if both STIM1 and STIM2 are deleted in mice, in which no residual Ca^{2+} influx is present.[68,113] Such CTL defects were not observed in either STIM1 or STIM2 single knockout mice, where moderate SOCE is still present in CD8^{+} T cells. The differential sensitivity of T lymphocytes and cancer cells toward Ca^{2+} influx might open a therapeutic window, in which SOCE of cancer cells and proinflammatory Th17 cells can be targeted without affecting antitumor functions of CTLs, thereby maximizing the antitumor efficacy.

In summary, SOCE can directly regulate apoptotic cell death, proliferation and metastasis of cancer cells. SOCE also promotes VEGF secretion, which subsequently stimulates SOCE in vascular endothelial cells and leads to their proliferation for angiogenesis. Interestingly, in immune cells, SOCE plays a paradoxical role during cancer development. Figure 2 presents an overview of the primary signaling pathways that are regulated by SOCE in cancer cells and in cells of the tumor microenvironment. Because the function of immune cells in cancer is complicated, the xenograft model that was used in the latest work[68] could not fully mimic the tumor initiation and development process *in vivo*. Therefore, other well-designed experiments should be conducted before a final conclusion can be made with regard to the role of SOCE in modulating antitumor immunity.

Therapeutic Potential of SOCE in the Treatment of Cancer

As knowledge of SOCE in human disease accumulates, there has been an increased interest in the development of SOCE inhibitors that can be used to fight against cancer. Several small-molecule SOCE inhibitors have been developed over the past decades, which hold promise in the treatment of cancer.

SKF-96365

Primarily introduced as an inhibitor of receptor-operated calcium entry (ROCE),[130] the imidazole compound SKF-96365 was found to block SOCE in various cells such as mast, rat basophilic leukemia (RBL) and Jurkat cells.[131–133] SKF-96365 could block STIM1 overexpression-induced SOCE augmentation and NFAT nuclear translocation; this indicates that STIM1 is one of its potential targets.[59,134] As an inhibitor of SOCE, SKF-96365 prevented tumor cell metastasis in a mouse model of breast cancer.[79] In another study, the blockade of SOCE by SKF-96365 retarded the growth and angiogenesis of cervical cancer cells.[66] However, SKF-96365 is not selective for CRAC channels and could block other Ca^{2+} channels,[131] and thus more studies need to be performed to specifically delineate its mechanisms in various types of cancer cells.
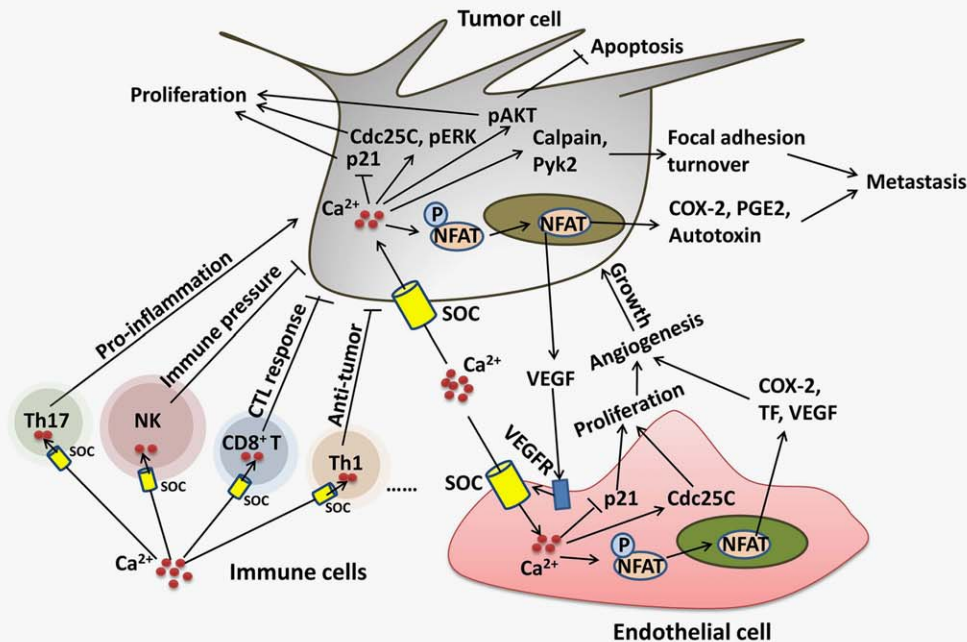
Figure 2. Proposed roles of SOCE in cancer. In tumor cells, SOCE promotes cancer cell proliferation *via* the upregulation of Cdc25C and the downregulation of p21. It also promotes cancer cell metastasis *via* the modulation of calpain- and Pyk2-mediated focal adhesion turnover or through upregulating the expression of COX-2, PGE2 and autotoxin. SOCE also contributes to drug resistance through an enhancement of AKT activity. In cancer cells, SOCE boosts the secretion of VEGF, which facilitates endothelial cell proliferation, angiogenesis and tumor growth. SOCE-mediated chronic inflammation through activation of Th17 cells is speculated to promote tumor growth. However, in NK, CD8^{+} T and Th1 cells, SOCE is required to inhibit tumor progression.

2-APB

Initial studies reported that 2-APB could inhibit IP3-induced Ca^{2+} release in rat cerebellar microsomes in a dose-dependent manner.[135] Later, it was found that its inhibitory effect was mainly due to a blockade of SOCE.[136] In addition, this inhibitory effect was IP3 receptor-independent and was more potent when it was applied extracellularly.[137] In native immune cells, 2-APB modulates SOCE activity in a paradoxical manner in that it exerts stimulatory effects at low concentrations and inhibitory effects at high concentrations.[137] This dual regulation is also observed in HEK293 cells.[138] It is speculated that low doses of 2-APB stimulate Ca^{2+} influx by promoting STIM1–ORAI1 interactions, while high doses inhibit SOCE in part due to its inhibition of STIM1 redistribution.[138] Interestingly, high doses of 2-APB could forcefully activate the ORAI3 channel and change its ion selectivity independently of STIM1 or Ca^{2+} store depletion.[56,139] It has been postulated that 2-APB directly binds to the ORAI3 channel, which results in an increase in channel conductance and limits selectivity. 2-APB was reported to inhibit the proliferation of hepatoma, cervical and gastric cancer cells[66,140,141] and the migration of cervical and colorectal cancer cells.[74,89]

Bistrifluoromethyl-pyrazole derivative, BTP2

First identified as a compound that blocks IL-2 production in lymphocytes,[142] BTP2 could potently and specifically inhibit SOCE in T lymphocytes without interference with other important Ca^{2+} influx pathways.[143] Additionally, BTP2 does not appear to affect STIM1 redistribution or STIM1–ORAI1 coupling.[144] Preincubation of Jurkat T-cells with BTP2 inhibits SOCE at an IC$_{50}$ of approximately 10 nM.[143] As mentioned above, the blockade of SOCE by BTP2 prevented antigen-induced T-cell responses through the inhibition of the antihost CTL response, donor T-cell expansion and IFN-γ production in mouse models of GvHD.[111] BTP2 could also inhibit antigen-induced cytokine secretion in mast cells, which is important in inflammation and antitumor immunity.[145] Mercer *et al.* showed that the actin reorganizing protein Drebrin was the target of BTP2 because the knockdown of Drebrin in Jurkat T cells inhibited SOCE similar to what occurred after treatment with BTP2.[146]

Anti-ORAI1 monoclonal antibodies

Because the ORAI1 protein is the pore subunit of the calcium channel responsible of SOCE and may serve as an attractive therapeutic target, specific anti-human ORAI1 monoclonal antibodies (mAbs) have been generated.[147] Recently, Cox *et al.* described a newly generated anti-ORAI1 mAb with specificity for the second extracellular loop that can inhibit T-cell activation *in vitro* and T-cell-mediated GvHD *in vivo*. This indicates its therapeutic potential for the treatment of autoimmune diseases and prevention of xenograft rejection.[148]

Other small molecular inhibitors

In recent years, several other small molecules have been developed as specific inhibitors of SOCE.[120,149,150] For

example, the blockade of calcium influx by RO2959, which was developed by Roche, could inhibit proliferation and activation of human T cells as well as cytokine production in these cells.[150] Kim et al. reported that compound 5D prevented the development of experimental autoimmune encephalomyelitis (EAE) in mice, potentially via the inhibition of the differentiation of Th17 cells according to tests in animal models.[120]

Until now, no SOCE inhibitors have been approved to treat cancer patients. Carboxyamidotriazole (CAI), a novel synthetic compound that can inhibit stimulated calcium uptake, was reported to exert potent antitumor effects,[151,152] and has recently been investigated in preclinical studies and clinical trials. Several phase I/II/III clinical trials (http://clinicaltrials.gov/) that involve CAI are currently underway. A phase II trial of CAI in patients with relapsed epithelial ovarian cancer showed that CAI could promote disease stabilization in these patients. Given its limited toxicity profile, it may serve as a maintenance therapy for this disease.[153]

Conclusions and Perspectives

SOCE, an essential component involved in maintaining intracellular Ca^{2+} homeostasis, regulates several aspects of the malignant behavior of cancer cells, including tumor growth, angiogenesis and metastasis. SOCE represents a promising target for anticancer therapy. The molecular mechanisms and the consequences of aberrant SOCE signaling are context-dependent. As shown in Supporting Information Figure 1, AKT, ERK, NFAT and COX-2 are critical molecules that act downstream of SOCE. Because the molecular compositions of SOCE and their interactions are complicated, our understanding of the role of SOCE in cancer cells is still limited. STIM/ORAI-mediated SOCE appears to play a dual function during tumorigenesis. On one hand, augmented SOCE has been reported to promote tumor growth and metastasis in a number of cancer types. On the other hand, STIM1 causes growth arrest in the human rhabdomyosarcoma and rhabdoid tumor cell lines RD and G401.[70] In addition, ORAI1 has been reported to facilitate apoptosis of PCa cells, and the knockdown of ORAI1 leads to drug resistance.[71] Thus, the function of SOCE in tumors is inconsistent in different cell types and tumor stages. A careful analysis is necessary to determine the effects of SOCE on tumors.

More attention should be paid to the role of SOCE in immunosuppression. As discussed above, SOCE participates in cytotoxic T-cell differentiation and activation, and during the process of tumorigenesis, the host immune system plays an important role in immune surveillance. In the early stages of tumorigenesis, a small number of premalignant epithelial cells act independently of oncogenic pathways and angiogenesis. Hence, SOCE inhibitors may not exert anticancer effects, but rather, they may promote tumorigenesis because of their immunosuppressive function. After tumor formation, tumor cells acquire immune tolerance, and immune cells in the tumor tissue secrete large amounts of inflammatory cytokines that in turn promote the proliferation of tumor cells and angiogenesis. Based on the aforementioned discussion, there might be specific thresholds of SOCE signaling, so as a therapeutic window might exist, in which functions of cancer cells and proinflammatory Th17 cells can be targeted with moderate SOCE inhibition without affecting antineoplastic functions of NK, CTL and Th1 cells. The blockage of SOCE could suppress tumor growth through the following three mechanisms: (i) the inhibition of proliferation and metastasis of cancer cells; (ii) the inhibition of the activation of immune cells that secrete tumor-promoting inflammatory cytokines and (iii) the inhibition of vascular endothelial cell proliferation, migration, tube formation and angiogenesis.

Because the STIM and ORAI proteins are ubiquitously expressed, toxicity to normal cells should also be considered when SOCE inhibitors are applied systemically. An ideal solution is to develop a class of chemical modulators of CRAC channels that specifically targets tumor cells or tumor vascular endothelial cells. Alternatively, local drug administration could achieve enhanced antitumor effects while reducing toxicity. Furthermore, some known downstream signaling pathways that are regulated by SOCE such as AKT, ERK, COX-2 and NFAT are specifically overexpressed or activated in tumor tissues. These pathways can also be considered for cancer intervention. Since the discovery of the STIM and ORAI proteins, we have witnessed tremendous progress in the mechanistic dissection of SOCE and functional characterization of SOCE deficiency in murine models. Because SOCE plays paradoxical roles in tumorigenesis and tumor progression, the specific role of SOCE in different stages of and types of cancer warrants further investigation.

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