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The Store-Operated Calcium Channels in Cancer Metastasis: from Cell Migration, Invasion to Metastatic Colonization

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Abstract

Store-operated calcium entry (SOCE) is the predominant calcium entry mechanism in most cancer cells. SOCE is mediated by the endoplasmic reticulum calcium sensor STIMs (STIM1 and 2) and plasma membrane channel forming unit Orais (Orai 1–3). In recent years there is increasing evidence indicating that SOCE in cancer cells is dysregulated to promote cancer cell migration, invasion and metastasis. The overexpression of STIM and Orai proteins has been reported to correlate with the metastatic progression of various cancers. The hyperactive SOCE may promote metastatic dissemination and colonization by reorganizing the actin cytoskeleton, degrading the extracellular matrix and remodeling the tumor microenvironment. Here we discuss how these recent progresses provide novel insights to our understanding of tumor metastasis.

Keywords

Store-operated Calcium Entry; SOCE; Calcium signaling; Cancer metastasis; STIM1; Orai1; Review

2. INTRODUCTION

Tumor metastasis is a critical hallmark of cancer that represents the terminal stage of tumor progression. Conceptually, the metastatic cascade can be simplified into the physical translocation of metastatic cells from primary tumor to distant organs and the metastatic expansion of disseminated cancer cells to form macroscopic secondary tumor (1). To disseminate from primary tumor to distant sites, metastatic cancer cells need to invade surrounding tissues, infiltrate the lymph and blood circulation (intravasation), survive in in the transit to distant organs and escape from microvasculature to the distant organ parenchyma. The disseminated cancer cells may remain dormant for an extended period of time before colonizing the distant site and establishing secondary tumors (metastatic expansion) (2). Although metastasis is responsible for about 90% of cancer-related mortalities, the molecular mechanisms underlying metastatic progression remain the most poorly understood aspect of cancer biology.

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$Ca2^+$ is a remarkably versatile second messenger that regulates a diverse range of physiological and pathological processes (3). $Ca2^+$ regulates enzymatic activities, protein-protein interactions and the subcellular localization of signaling molecules through calmodulin and other calcium-binding proteins. The cytosolic $Ca2^+$ concentration is tightly controlled by an elaborate $Ca2^+$ signaling system consisting of $Ca2^+$ channels, pumps, exchangers etc. (3, 4). Many extracellular stimuli (e.g. growth factors, chemokines) induce $Ca2^+$ response by promoting $Ca2^+$ release from the internal $Ca2^+$ store and/or $Ca2^+$ influx from the extracellular environment (4). In non-excitable cells (including most cancer cells), store-operated $Ca2^+$ entry (SOCE) is the predominant $Ca2^+$ entry mechanism (5). The store-operated calcium (SOC) channels are activated upon the $Ca2^+$ release from endoplasmic reticulum (ER), which is essential for the cell to replenish the $Ca2^+$ content in the ER after $Ca2^+$ release (5). The concept of SOCE was first proposed by James Putney in 1986 (6), and the $Ca2^+$ release-activated $Ca2^+$ channels (CRAC) were determined in the early 1990s (7, 8); however, the molecular identities of SOC channels were not identified until more recently. In the wake of high throughput RNAi screening technology, the stromal interaction molecule 1 (STIM1) and Orai1 were recently identified to be the ER $Ca2^+$ sensor (9, 10) and the plasma membrane channel pore-forming unit of SOC channels (11–15), respectively. The essential roles of STIM1 and Orai1 in cancer cell migration, invasion and metastasis were first reported in breast cancer in 2009 (16). Since then, there is rapidly accumulating evidence supporting a role for SOC channels in tumor metastasis and progression. It is becoming clear now that the SOCE is frequently activated in a variety of cancers to promote cell motility, invasiveness and metastatic progression. The overexpression of STIM and/or Orai proteins has been correlated with metastatic diseases and shorter survival in several cancers. Genetic or pharmacological blockade of SOC channels is able to inhibit cancer cell proliferation, migration, invasion, and the self-renewal of cancer stem-like cells *in vitro*, and tumor metastasis and tumorigenesis in various xenograft mouse models.

3. THE STORE-OPERATED CALCIUM CHANNELS

3.1. STIM1 and STIM2

STIM proteins are type 1 single-pass transmembrane proteins predominantly expressed in the ER membrane with N-terminal facing the ER lumen (17). In vertebrates, there are two STIM proteins, STIM1 and STIM2, that ubiquitously express in all cell types (18). STIM1 and STIM2 have distinctly different roles in store-operated $Ca2^+$ signaling (18). STIM1 is a much stronger activator of Orai1 than STIM2 and is mainly responsible for stimulated SOCE. On the other hand, STIM2 is a more sensitive detector for changes in luminal ER $Ca2^+$, and is responsible of maintaining the homeostasis of cytosolic and luminal ER $Ca2^+$ (19). The N-terminal of STIM proteins includes a $Ca2^+$ binding canonical EF-hand motif, non-$Ca2^+$ binding hidden EF-hand and a Sterile α-motif (SAM) mediating STIM protein oligomerization (20, 21). The cytosolic C terminal is composed of two coiled-coil domains(C-C), a CRAC channel activation domain (CAD) or STIM-ORAI activating region (SOAR), Pro/Ser/(P/S)-rich segments, microtubule interacting Ser/Thr-x-Ile-Pro(S/TxIP) sequences and lysine-rich clusters(22, 23). The N-terminal domains of STIMs operate together to form an elegant sensor of luminal ER $Ca2^+$ (18, 20, 21). Upon $Ca2^+$ release from the ER, the canonical EF hand loses $Ca2^+$ binding, which induces conformation changes that

lead to STIM oligomerization and translocation to the junctions between ER and plasma membrane (ER-PM junction). The activated STIM proteins adopt an elongated conformation, which allows the lysine-rich motif in the C-terminal to interact with negatively charged phospholipids on the plasma membrane. The conformation changes also release the intra-molecule inhibition of CAD/SOAR domain in the C-terminal and allow the CAD/SOAR to interact with and to activate Orai proteins on the plasma membrane to activate the opening of the channels.

3.2. Orai1, Orai2 and Orai3

The Orai proteins are highly conserved plasma membrane pore forming units of SOC channels (14, 15, 24). There are three closely related Orai isoforms (Orai1-3) in mammals. It is believed that Orai1 is the predominant channel protein responsible for SOCE, although all three isoforms of mammalian Orais are able to conduct highly selective CRAC currents when overexpressed (25). The Orai consist of four transmembrane domains (M1-4), with both N- and C-termini facing the cytosol (26). Although it is well accepted that Orai protein oligomers form the SOC channels with M1 transmembrane domain lining the ion selective pore (15, 24, 26), the stoichiometry of functional channels has been contentious. Earlier data indicate that active Orai channels are tetrameric (27–30). More recent structural and biochemical evidence strongly supports a hexameric model (26, 31). There is also evidence indicating that both the N- and C-termini of Orai1 interacts with the CAD/SOAR domain of STIM proteins (22), although it is still not completely understood how these interactions activate the channel.

4. SOCE In Cancer Metastasis

The hallmarks that distinguish malignant cancer from benign tumor growth are the abilities to invade surrounding tissue and to disseminate distant organ. The hyperactive cell motility and invasiveness are essential for the metastatic dissemination from primary tumor to distant organ. It was reported that blockade of SOCE, by STIM1 and Orai1 shRNAs or by pharmacological inhibitors, remarkably impaired the breast cancer cell mobility and invasiveness *in vitro,* and abrogated the lung metastasis of breast cancer cells in mouse models(16). Strikingly, stimulation of SOCE by ectopic expression of STIM1 together with Orai1 was able to confer human mammary epithelial cells MCF-10A with ability to invade Matrigel, suggesting that activation of SOCE in non-invasive epithelial cells with invasiveness (16). In the last several years, there is rapidly accumulating evidence supporting the pro-migration and pro-invasion activity of SOCE in a variety of cancers, including breast cancer (32–39), cervical cancer(40–42), hepatocellular carcinoma (43–45), renal carcinoma(46, 47), nasopharyngeal carcinoma (48), glioblastoma (49, 50), colorectal cancer (51–55) and melanoma(56–60). In this section we will discuss the molecular mechanisms by which SOCE promotes cancer cell migration and invasion.

4.1. A brief overview of cancer cell migration and invasion

The motility of most cancer cell is driven by protrusive forces in the front and contractile forces in the back (61). The protrusive forces in lamellipodia of migration cells are generated by a dendritic actin cytoskeleton network (62). The assembly and polymerization

of the dendritic F-actin in lamellipodia are catalyzed by actin nucleation complexes consisting of small GTPase Rac, WAVE, N-WASP and Arp2/3 complex (61). The pro-migration and invasion cues in the tumor microenvironment activate the phosphoinositide-3-kinase, which in turn recruits and activates actin nucleation complexes to generate membrane protrusion. In the rear end of the migrating cell, the retraction of the trailing tail and the net movement of nucleus and cell body is dependent on the contractile forces generated by the actomyosin fibers (61). The actomyosin contraction is regulated by small GTPase Rho, Rho-kinase, or by $Ca2^{+}$/calmodulin-activated myosin-light chain kinase (61, 63). The actin cytoskeleton in migration cell is anchored to the extracellular matrix (ECM) by a type of adhesive protein complex known as focal adhesion. The assembly of nascent focal adhesion in the front and the disassembly of mature focal adhesion in the back coordinate with the actin cytoskeleton dynamics in migrating cells to drive cell motility.

The motility of cancer cells *in vivo* is often blocked by the ECM barriers. Therefore, gaining invasiveness is a critical step in cancer metastasis (64, 65). Cancer cells are able to invade the surrounding tissue by proteolytic degradation of extracellular matrix, or by squeezing through loosely crosslinked extracellular matrix in the interstitial spaces in a protease-independent manner (66–69). Invadopodia are adhesive membrane protrusions coordinating focalized ECM degradation in malignant tumor cells (70, 71). The actin rich core of invadopodium is regulated by non-receptor tyrosin kinase Src, scaffolding proteins cortactin, Tsk5, Nck and actin nucleating proteins N-WASP and Arp2/3 (71). The matured invadopodia are able to recruit or secret matrix metalloproteases to degrade extracellular matrix (71). Invadopodia have been shown to be critical for metastasis in mouse models and cancer patients (71–73). Although amoeboid cancer cells may pass through reconstituted ECM in a protease-independent manner (68, 69, 74), focalized proteolysis is essential for cancer cells to breach high density ECM *in vivo* (66, 75).

4.2. SOCE and focal adhesion turnover

The dynamic turnover of focal adhesions in migrating cells is required for both the leading edge protrusion and trailing tail retraction. The formation of nascent focal complex in the leading edge is essential for the dendritic actin network in the lamellipodia to provide protrusive forces. On the other hand, disassembly of focal adhesion of in the trailing tail is prerequisite for the retraction of trailing tail and the forward movement of the cell body. It is believed that SOCE regulates cancer cell migration at least partially through regulation of focal adhesion turnover (16, 40, 76). Blockade of SOCE in breast and cervical cancer cells lead to large peripheral focal adhesions that have slower assembly and disassembly rates, suggesting impaired focal adhesion turnover in these cells (16, 40). Another interesting observation is that new protrusions in the lamellipodia of migrating cells fail to assemble nascent focal complex and quickly retract (16). Therefore, by regulating focal adhesion assembly and disassembly, SOCE is likely to control both the leading edge protrusion and trailing tail retraction during cancer cell migration. Mechanistically, SOCE regulates focal adhesion turnover through small GTPase, Ras and Rac (Figure 1). Increase in cytosolic $Ca2^{+}$ activates Ras/Rac, and ectopic expression of constitutively active Ras and Rac is able to rescue focal adhesion turnover and cell migration defects after SOCE inhibition (16, 55). Several Ras and Rac GEFs (e.g. RasGRF1, RasGFR2, RasGRP) are activated by binding to

$Ca2^+$ or calmodulin, and thus could potentially mediate the activation of small GTPase downstream of SOCE (Figure 1). SOCE may also control focal adhesion turnover through focal adhesion kinases FAK and Pyk2, since SOCE inhibition also decreases the levels of active FAK and Pyk2 (36, 41, 77). FAK has no $Ca2^+$ or calmodulin binding motif, and therefore FAK is likely to be regulated by SOCE indirectly. Pyk2 has a calmodulin binding IQ motif in its auto-inhibitory IQ motif (78), and could be a direct target mediating SOCE regulation of focal adhesion turnover (Figure 1). Finally, the $Ca2^+$-binding protease calpain may play important role in SOCE-mediated focal adhesion turnover (Figure 1). Calpain contains several $Ca2^+$-binding motifs in its catalytic and regulatory subunits. $Ca2^+$ binding is required for calpain activation (79), and SOCE blockade inhibits its activity in cervical and colon cancer cells (40, 55). The proteolytic processing of some focal adhesion proteins, such as talin, paxillin, FAK, is a rate-limiting step in focal adhesion disassembly (79–82). Interestingly, inhibition of calpain also results in large peripheral focal adhesion and defective focal adhesion disassembly (80), a phenotype that is reminiscent of focal adhesions in SOCE inhibited cancer cells (16).

In addition to inhibiting focal adhesion disassembly, SOCE blockade also impaired the assembly of nascent focal adhesion in the leading edge of migrating cells (16). The assembly defects could be due to the trapping of focal adhesion structural proteins in mature focal adhesions. Indeed, it has been previously demonstrated that the recycling of the $\alpha_v\beta_3$ integrin from the trailing-tail to the leading edge is dependent on $Ca2^+$ and calcineurin (83). In addition, $Ca2^+$ may regulate the initiation of focal adhesion assembly through integrin inside-out signaling(84), and the maturation of focal adhesion through myosin-mediated contractility (85, 86).

4.3. The regulation of actomyosin contractility by SOCE

The contractility of the actomyosin network in migrating cell is crucial for the retraction of the trailing tail and the forward movement of the nucleus. Cancer cells are also able to adopt actomyosin contractility-dependent amoeboid cell migration to invade the interstitial extracellular matrix in a protease-independent manner (69). In polarized migrating cells, the $Ca2^+$ concentration is high in the trailing tail and low in the leading edge, forming a front-to-back $Ca2^+$-concentration gradient (87, 88). The high $Ca2^+$ concentration in the trailing tail activates the actomyosin contraction and tail retraction through MLCK (63, 88). Although $Ca2^+$ is generally low in the leading edge, it is rich in transient $Ca2^+$ pulses termed "$Ca2^+$ flicker" (85, 86, 89). These $Ca2^+$ flickers in the front control focal adhesion formation and local contractility through activation of MLCK (85, 86). The local $Ca2^+$ pulse in the leading edge is supported by polarized activation of local $Ca2^+$ release from the ER and local activation of STIM1, which is transported to the front of migrating cells by binding to the microtubule plus ends (85).

4.4. SOCE and cancer cell invasion

Recently, STIM1 and Orai1-mediated SOCE signaling has been shown to be critical for invadopodial assembly and extracellular matrix degradation (57). SOCE promotes invadopodium assembly by activating the non-receptor tyrosine kinase Src (Figure 1), which activate actin-nucleating factors Arp2/3 complexes to promote the assembly of invadopodial

precursor (90–93). Consistent with this notion, SOCE blockade inhibits the assembly of invadopodial-precursor, without affecting invadopodial stability (57).

SOCE is critical for not only the assembly initiation, but also the maturation of invadopodia. SOCE blockade with specific shRNA or inhibitors decreases the numbers of invadopodia per cell, as well as the proteolytic activity of remaining invadopodia (57). The defective proteolytic activity of these invadopodia is due to the inhibition of the recycling of MT1-MMP (membrane type 1 matrix metalloprotease) from the endocytic compartment to the plasma membrane (57). MT1-MMP is a matrix metalloprotease with a single transmembrane domain, and one of the predominant protease responsible for cancer invasion (66). The plasma membrane MT1-MMP is constantly endocytosed and trafficked to invadopodia through VAMP7, a v-SNARE that is involved in $Ca2^{+}$-dependent exocytosis (94, 95). SOCE blockade has no effect on the endocytosis of MT1-MMP, but remarkably slows down the recycling of MT1-MMP from the endocytic compartment to the plasma membrane (57). It is possible that SOCE might regulate

4.5. The regulation of epithelial-to-mesenchymal transition (EMT) by SOCE

The epithelial-to-mesenchymal transition (EMT) is a biological process that allow epithelial cells to adopt many mesenchymal cell phenotypes, such as elevated motility, invasiveness and increased production of extracellular matrix proteins (96). There is emerging data suggesting that SOCE may play a role in the EMT of serval cancers (35, 54, 97, 98). In MCF7 breast cancer cells induction of EMT with transforming growth factor β (TGFβ) increased SOCE and the expression levels of STIM1 and Orai1 (35). The TGFβ-induced EMT was abrogated when STIM1 expression was suppressed with shRNA, suggesting that SOCE is required for TGFβ-mediated EMT (35). The role of SOCE in EMT has also been reported in prostate cancer (99), colon cancer (54) and gastric cancer (97), suggesting that SOCE may regulate cancer metastasis by triggering EMT.

4.6. The role of soce beyond metastatic dissemination

There is now convincing evidence from animal models and human patient specimens suggesting that dysregulation of STIM and Orai proteins promotes the tumor metastasis and progression. Blockade of SOCE with pharmacological inhibitors or RNA interference inhibits the metastasis of breast cancer (16, 39), melanoma (57), colorectal cancer (54), gastric cancer (97) in xenograft models; on the other hand, ectopic expression of STIM1 in SW480 colorectal cancer cells promotes lung metastasis (54). Notably, blockade of SOCE not only inhibits spontaneous metastasis from primary tumor (16, 39), but also reduces metastatic burden from intravenously injected metastatic cancer cells, suggesting that SOCE is critical for disseminated cancer cells to establish secondary tumor.

The metastatic colonization at distant site is an extremely inefficient process. Only a small fraction of disseminated cancer cells are able to successfully establish distant metastases (1). The microenvironment in distant organ is hostile to disseminated cancer cells, and the majority of them undergo apoptosis within 24 hours after extravasation (1). It has been suggested that secreted factors (e.g. VEGF, lysyl oxidase, exosomes) derived from primary tumor are able to remodel the distant sites to facilitate metastatic expansion (100–102).

These factors mobilize angiogenesis at distant sites to form pre-metastasis niche, which supports the metastatic colonization of disseminated cancer cells. The STIM1 and Orai1-mediated SOCE promotes the secretion of VEGF and prostaglandins E2 in cervical cancer and colorectal cancer (40, 53), which may contribute to mobilizing endothelial cells at pre-metastasis niche. Furthermore, SOCE could promote metastatic colonization through proteolytic remodeling of the ECM in distant organ, which is essential for cancer cells growth in 3D collagen I matrix (103). The proteolytic remodeling of the ECM is also essential for cancer cells to activate latent growth factors (e. g. TGFβ, VEGF) deposited in the ECM (71). The growth factors released from the ECM may further modify the tumor environment by recruiting endothelial cells, inflammatory immune cells and other stromal cells to create a niche conducive to the survival and expansion of disseminated cancer cells.

5. THE DYSREGULATION OF SOCE IN CANCER

The expression levels of STIM1 and Orai1 have been examined in cervical cancer, glioma, melanoma, hepatocellular carcinoma, non-small cell lung cancer, colorectal cancer, esophageal squamous cell carcinoma, prostate cancer and breast cancer, and these findings have been summarized in two excellent recent reviews (104, 105). The expression levels of STIM1 and/or Orai1 in cervical cancer (40, 42), colorectal cancer (53, 54), gastric cancer (97), non-small cell lung cancer (106) and esophageal cancer (107) are found to be correlated with metastatic progression, poor prognosis and shorter survival. These correlative studies using patient specimens are consistent with findings from cultured cells and xenograft mouse models.

In breast cancer, the highly aggressive basal-like subtype is characterized by an elevated ratio of STIM1/STIM2 ratio (108). Correspondingly, the patients with high STIM1/STIM2 ratio and high STIM1 expression levels have an increased metastatic potential and a decreased survival rate, indicating that STIM proteins are potential regulators of breast cancer progression. The single-nucleotide polymorphisms of ORAI1 gene in breast cancer are strongly associated with lymph node involvement and estrogen receptor status, suggesting the genetic variant of SOCE genes might predispose patients to breast cancer (109). Orai1 isoform is not the only Orai protein that associates with cancer. In estrogen receptor-positive breast cancer cell lines, native SOCE and I_{CRAC} are mediated by STIM1/2 and Orai3, while estrogen receptor-negative breast cancer cells mainly rely on Orai1 (34). The transcription of Orai3 is regulated by estrogen receptor, which may explain the differential expression of Orai3 in different subtypes of breast cancer (34). The overexpression of Orai3 has also been reported in lung adenocarcinoma (110). In a cohort of 200 lung cancer patients, Orai3 protein and mRNA expression was increased in tumor tissues than in matched normal tissues, and Orai3 overexpression independently correlated with overall survival and metastasis free survival (110).

In addition to the canonical regulation of Orai channels by STIM1/2, there are reports of store-independent activation mechanism. It has been reported in luminal breast cancer that SPCA2 is able to activate Orai1 in a STIM1 and ER $Ca2^{+}$ store-independent manner to promote tumor growth (32). Another example of store-independent activation of Orai1 is the SK3-Orai1 complex in breast cancer and colon cancer models (39, 55). It is reported that

SK3 potassium channel forms complex with Orai1 in the plasma membrane lipid rafts to activate Orai1 through plasma membrane hyperpolarization (39). Disruption of lipid rafts with the alklyl-lipid Ohmline interfered with the formation of SK3-Orai1 complex formation and inhibited the breast cancer cell migration (39). There is also evidence that prostate cancer may switch from the canonical SOCE to store-independent arachidonic acid regulated channels (consisting of Orai1/Orai3 heteromer) to support tumor cell proliferation (111). The non-canonical regulation of Orai1 channels is an interesting concept that implicates the complexity of the $Ca2^+$ signaling dysregulation in cancers. However, the biochemistry and electrophysiology of these novel regulatory mechanisms are still not well-defined and future studies in these areas are needed.

Although the overexpression of STIM and Orai proteins has been widely investigated in a variety of cancers, the molecular mechanisms underlying their overexpression are only beginning to be revealed. In glioblastoma and colorectal cancer, the overexpression of STIM2 appeared to be due to amplification of the 4p15 locus, which increases STIM2 gene copy numbers (112, 113). Paradoxically, overexpression of STIM2 promotes cell migration but inhibits cell proliferation (60, 112), implicating complexity of SOCE signaling at different stages of tumor progression. The inflammatory and hypoxic tumor microenvironment may be responsible for overexpression of STIM and Orai in some tumors. The overexpression of STIM1 in hepatocarcinoma is driven by the hypoxic tumor microenvironment and hypoxia-inducible factor-1 (HIF-1) (114). HIF1-α induces the expression of STIM1 by binding to hypoxia response elements in STIM1 promoter (114). In contrast, the expression of STIM2 is not regulated by hypoxia, since its promoter contains no hypoxia response elements (114). Hypoxia, a common feature of the microenvironment of most solid tumors, increases HIF-1α levels by stabilizing the protein. The expression levels of HIF-1α in cancer could also be increased by non-hypoxic regulations (such as mutations of von Hippel-Lindau tumor suppressor) (115). Therefore, it is likely that the hypoxic tumor microenvironment and HIF-1α also contribute to STIM1 overexpression in other solid tumors. In addition to transcriptional regulation, microRNA may regulate STIM1 at the post-transcriptional level. In colorectal cancer, STIM1 was found to be a direct target of microRNA-185, a microRNA that is downregulated in metastatic colorectal cancer cells (54).

6. THE SPATIAL AND TEMPORAL REGULATION OF SOCE IN CANCER CELL

The cytosolic $Ca2^+$ concentration is organized in intricate spatial and temporal patterns. The spatial and temporal coding of is essential for the specificity and versatility of $Ca2^+$ signaling (3, 64, 116). It is estimated that hundreds of proteins encoded by the human genome contain $Ca2^+$ motifs, and many more are indirectly regulated by $Ca2^+$ through calmodulin (116). The spatiotemporal organization of $Ca2^+$ signals allows cells to selectively activate their target proteins at the defined time and subcellular compartments (3). During prolonged stimulation, the cytosolic $Ca2^+$ signals are organized as repeated $Ca2^+$ pulse known as $Ca2^+$ oscillations (3). Each oscillation is coordination between $Ca2^+$ release from the ER and $Ca2^+$ influx from extracellular $Ca2^+$. The oscillating $Ca2^+$ transients

provides extended period of $Ca2^+$ stimulation while avoiding toxic side effects associated with $Ca2^+$ overload (117). Such extended $Ca2^+$ signal is essential for many biological processes such as gene transcription and cell migration.

The frequency and amplitude of oscillatory $Ca2^+$ signal may serve as digital signals that control downstream signaling, and STIM1 is believed to be a $Ca2^+$ sensor specialized for coding such digital signaling (118). STIM1 is able to detect the transient decrease in the ER $Ca2^+$ reservoir following each oscillation, and thus activates Orai1 channel to replenish the ER $Ca2^+$ (118). STIM1- and Orai1-mediated SOCE is obligatory for $Ca2^+$ oscillation in human embryonic kidney cells and melanoma cells (57, 119). The assembly of invadopodial precursors in invasive melanoma cells is accompanied by oscillatory $Ca2^+$ signals (57). Depletion of STIM1 and Orai1 decreased oscillation frequency and impaired the assembly of invadopodia, suggesting that oscillatory $Ca2^+$ signal is required for the assembly of invadopodia (57). The hyperactive $Ca2^+$ oscillation has also been reported in esophageal squamous cell carcinoma cells (107). Intriguingly, in esophageal cancer the hyperactive SOCE appeared to be due to Orai1 overexpression alone. shRNA depletion of up to two thirds of STIM1 protein had minimal effect on SOCE in these cells (107).

The spatial organization of $Ca2^+$ channels and $Ca2^+$ effectors into super complexes is another way for the cell to control the robustness and specificity of $Ca2^+$ signaling (3). Under normal physiological condition, the cytosolic $Ca2^+$ from $Ca2^+$ influx/release is quickly sequestered by the ER, mitochondria or pumped out of the cells by plasma membrane $Ca2^+$-ATPase. As a result, diffusion of $Ca2^+$ through opened channels creates local $Ca2^+$ microdomains (120), which allows selective activation of $Ca2^+$ effectors localized to the vicinity of active $Ca2^+$ channels. The local $Ca2^+$ pulses in the front of migrating cells control focal adhesion maturation and lamellipdoium retraction by local activation of myosin contraction (85, 86, 89). It is known that $Ca2^+$ effectors such as calcineurin, myosin and S100 proteins are enriched in proteolytic invadopodia and podosomes (71). The presence of STIM1 and Orai1 in the podosomes of microglia has been recently reported (121). By bringing together SOC channels and $Ca2^+$ effectors, invadopodia may serve as signaling hubs coordinating ECM remodeling and cancer cell invasion.

7. TARGETING SOCE IN METASTATIC CANCER: OPPORTUNITIES AND CHALLENGES

STIM and Orai proteins are frequently overexpressed / activated in metastatic cancer, and the activation of these channels promotes tumor growth and metastatic progression. These exciting new findings suggest that STIM and Orai proteins are promising targets in combating metastatic cancer. Indeed, SOCE blockers SKF96365 and 2-APB have been used to inhibit tumor growth and metastasis in the xenograft mouse models for breast, cervical and esophageal cancers (16, 40, 107). The activity of the store-independent SK3-Orai1 channel could be inhibited by Ohmline, an alkyl-lipid that disrupt the SK3-Orai1 complex by interfering with lipid raft formation. Ohmline treatment inhibited the activity of SK3-Orai1 $Ca2^+$ influx *in vitro*, and the bone and lung metastasis of MDA-MB-435 breast cancer cells in a mouse model (39). There is also evidence that SOCE is required for

chemoresistance in 5-FU treated pancreatic and ovarian cancer cells, suggesting that SOCE blocker could be useful in combination with chemotherapies to treat refractory tumors (122, 123). These studies provide proof-of-principle for targeting STIMs and Orais in cancer treatment.

In addition to inhibit tumor growth and metastasis by directly acting on cancer cells, SOCE inhibitors may also be used to “normalize” the inflammatory tumor microenvironment. Cancers are considered “wounds that do not heal” and chronic inflammation is a common feature of the tumor microenvironment (124). Inflammatory cytokines secreted by tumor cells and infiltrating immune cells promote tumor cell proliferation, migration, invasion and therapy resistance (124). STIM1 promotes COX2 overexpression and prostaglandin production in colorectal cancer cells, implicating a role for SOCE in tumor chronic inflammation (52, 53). SOCE has also been implicating in the production of inflammatory cytokine by endothelial cell, T cell, and mast cells (125–128), while its function in macrophage is more controversial (129, 130). Non-steroidal anti-inflammatory drugs such as Aspirin have shown promising chemopreventive potential in various cancers (131). Notably, many NSAIDs, such as indomethacin, ibuprofen and sulindac, are also able to inhibit SOCE (132). Therefore, these over-the-counter pain killers could potentially be used to target SOCE and the inflammatory pathways downstream of SOCE in cancer. Other Selective SOCE blockers, such as BTP2, Synta-66 (133), might also be used to prevent tumor progression, although their anti-tumor efficacies have yet to be determined.

8. CONCLUSIONS AND FUTURE DIRECTIONS

Recent years have seen significant progress in our understanding of SOCE in cancer progression. There is now convincing evidence indicating that STIM and Orai proteins are overexpressed in solid tumors, and the overexpression of these proteins promotes tumor cell migration, invasion, proliferation, apoptosis resistance and chemoresistance. There is a high interest in developing selective SOCE blockers (133). These inhibitors could potentially be useful in preventing cancer metastasis.

Despite significant progress in this young field, the molecular mechanisms underlying SOCE-mediated cancer metastasis remain poorly understood. Pro-metastasis factors in the tumor microenvironment (e.g. hypoxia, acidic pH, reactive oxygen species, inflammatory cytokines etc) are likely to regulate the expression and activity of STIM and Orai proteins in cancer cells as well as stromal cells. The mechanistic role of SOCE in metastatic cancer cells is now beginning to be understood, while the functions of SOCE in tumor stromal cells remain uncharted territory. Future investigators in these areas will likely bring important insights.

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Abbreviation

SOCE	store operated calcium entry
ER	endoplasmic reticulum
CRAC	calcium release-activated calcium channels
STIM1/2	stromal interaction molecule 1/2
SAM	sterile α-motif
CAD	CRAC channel activation domain
SOAR	STIM-ORAI activating region
GEF	Guanine nucleotide exchange factor
FAK	focal adhesion kinase
Pyk2	protein tyrosine kinase 2 beta
MLCK	myosin-light chain kinase
MT1-MMP	membrane type 1 matrix metalloprotease
VAMP7	Vesicle Associated Membrane Protein 7
EMT	epithelial-to mesenchymal transition
TGFβ	transforming growth factor β
VEGF	vascular endothelial growth factor
HIF1-α	hypoxia-inducible factor-1

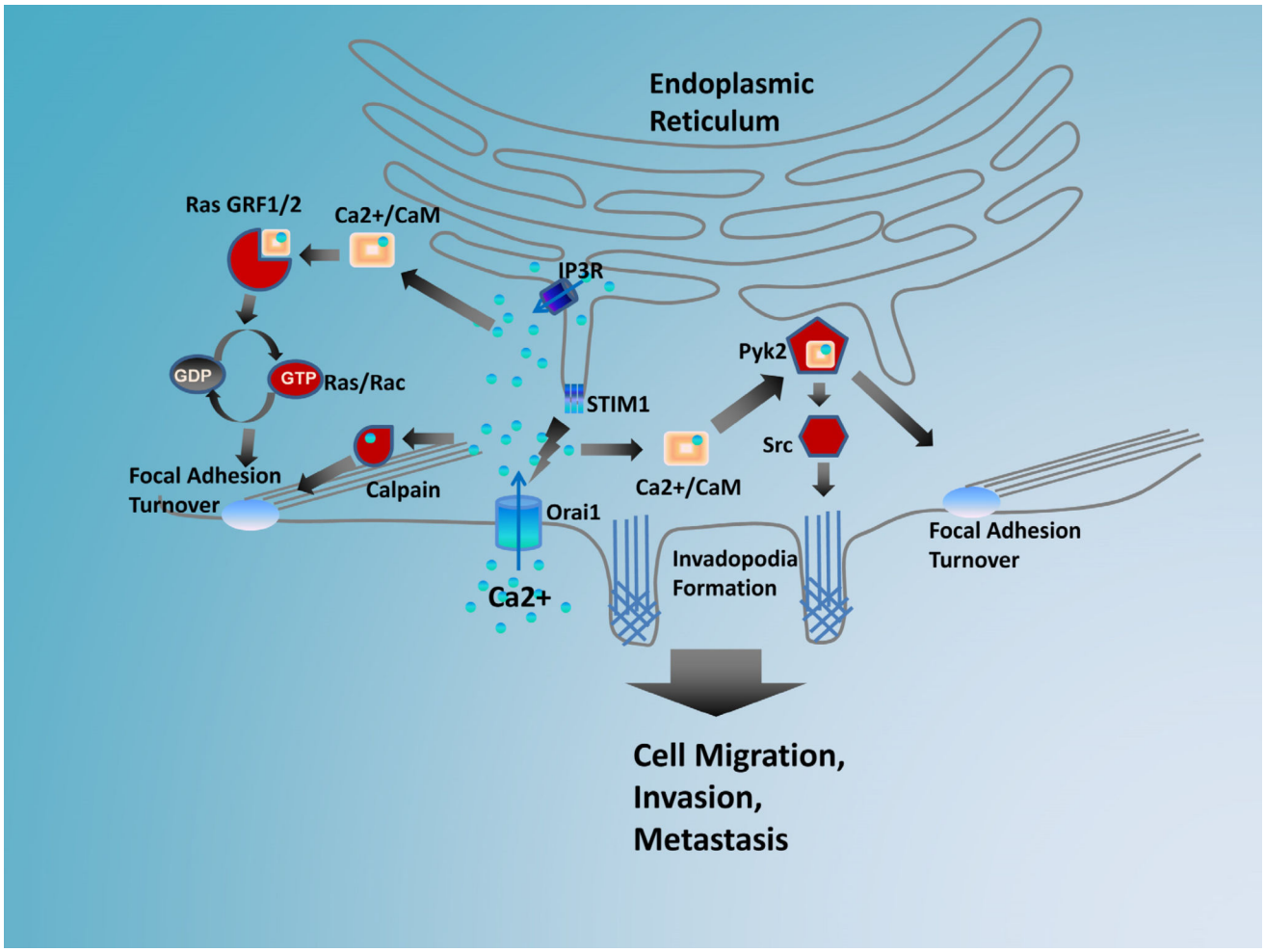

Figure 1.

Upon $Ca2^{+}$ depletion from the ER lumen, STIM1 molecules on the ER membrane oligomerize and translocate to the junctions between the ER and plasma membrane. At the ER-PM junction, STIM1 oligomers interact with Orai1 channels to activate SOCE. The hyperactive SOCE in cancer cells promotes metastasis by facilitating focal adhesion turnover and invodopodia formation. The elevation of cytosolic $Ca2^{+}$ may facilitate focal adhesion turnover through small GTPases Ras/Rac. The binding of $Ca2^{+}$/calmodulin to IQ-motif containing guanine nucleotide exchanges factors (e.g. RasGRF1 and 2) promotes the GTP binding and Ras/Rac activation. Cytosolic $Ca2^{+}$ may also facilitate focal adhesion turnover through $Ca2^{+}$-activated protease calpain, which cleave many focal adhesion proteins. The cytosolic $Ca2^{+}$ may also activate Pyk2 through $Ca2^{+}$/calmodulin, which binds to an IQ motif at the FERM domain of Pyk2. The binding of calmodulin to Pyk2 promotes the Pyk2 auto-phosphorylation at tyrosine 402. The phosphorylation of Pyk2 at Y402 provides docking site for the activation of Src kinase, which is able to further promote the assembly of invadopodia and remodeling of extracellular matrix. By regulating focal adhesion turnover and invadopodia formation, the hyperactive SOCE in cancer cells is able to promote cancer cell migration, invasion and metastasis.