STIM and Orai proteins as novel targets for cancer therapy. 
A Review in the Theme: Cell and Molecular Processes in Cancer Metastasis

Ayushi Vashisht,1 Mohamed Trebak,2 and Rajender K. Motiani1
1Systems Biology Group, CSIR-Institute of Genomics and Integrative Biology, New Delhi, India; and 2Department of Cellular and Molecular Physiology, The Pennsylvania State University School of Medicine, Hershey, Pennsylvania

Vashisht A, Trebak M, Motiani RK. STIM and Orai proteins as novel targets for cancer therapy. A Review in the Theme: Cell and Molecular Processes in Cancer Metastasis. Am J Physiol Cell Physiol 309: C457–C469, 2015. doi:10.1152/ajpcell.00064.2015.—Calcium (Ca2+) regulates a plethora of cellular functions including hallmarks of cancer development such as cell cycle progression and cellular migration. Receptor-regulated calcium rise in nonexcitable cells occurs through store-dependent as well as store-independent Ca2+ entry pathways. Stromal interaction molecules (STIM) and Orai proteins have been identified as critical constituents of both these Ca2+ influx pathways. STIMs and Orais have emerged as targets for cancer therapeutics as their altered expression and function have been shown to contribute to tumorigenesis. Recent data demonstrate that they play a vital role in development and metastasis of a variety of tumor types including breast, prostate, cervical, colorectal, brain, and skin tumors. In this review, we will retrospect the data supporting a key role for STIM1, STIM2, Orai1, and Orai3 proteins in tumorigenesis and discuss the potential of targeting these proteins for cancer therapy.

STIM1; STIM2; Orai1; Orai3; cancer; angiogenesis

CHANGES IN THE CYTOSOLIC Ca2+ levels activate downstream signaling pathways that regulate an array of cellular functions including secretion, excitation, contraction, motility, metabolism, transcription, growth, and apoptosis (9, 19, 94). A variety of growth factors, receptor agonists, and hormones activate the intricate interplay between Ca2+ release from intracellular reserves of endoplasmic reticulum (ER) and mitochondria and Ca2+ influx across the plasma membrane. In response to these stimuli, the changes in intracellular Ca2+ levels in turn dictate the course of different physiological functions (54, 57). For example, Ca2+ activates proliferative signals through nuclear factor for activated T cells (NFAT) (53) and is required for cell cycle progression to S phase (100). Alterations in Ca2+ levels play a crucial role in tumorigenesis as well (16). For instance, induction of mitochondrial stress by depletion of mitochondrial DNA results in rise in cytosolic Ca2+ that in turn stimulates prosurvival and invasive pathways in human pulmonary carcinoma cells (A549) (8). Amuthan et al. (2) reported that increased cytosolic Ca2+ levels result in activation of Ca2+-dependent MAP kinases and calcineurin. Further, expression of antiapoptotic proteins like Bcl-2 and Bcl-xL was increased by the rise in cytosolic Ca2+ levels (2). Liao et al. (59) reported that elevated extracellular Ca2+ levels result in increased Ca2+ sensing receptor (CaSR) expression in prostate cancer cells. Further, CaSR knockdown decreases proliferation and bone metastasis of prostate cancer cells (59). In contrast to the aforementioned findings, other studies have reported a role for rise in cytosolic Ca2+ levels in cancer cell apoptosis (88). Sareen et al. (88) showed that resveratrol induces breast cancer cell apoptosis via Ca2+-dependent activation of calpain protease. Resveratrol initiates ER Ca2+ release, which activates the protease calpain, eventually leading to plasma membrane Ca2+-ATPase isoform I degradation (88). Similarly, Wertz et al. (111) reported that depletion of ER Ca2+ stores can induce prostate cancer cell apoptosis by activating caspase 9, 3, and 7. Therefore, Ca2+ can regulate both cell proliferation and apoptosis, most likely by its ability of activating varied signaling pathways in different cell types depending on the stimuli. Further, differences in spatio-temporal localization of Ca2+ microdomains and in downstream Ca2+ handling proteins activated by changes in Ca2+ levels can lead to diverse outcomes.

The spatio-temporal regulation of Ca2+ is further implicated in controlling cytoskeleton and focal adhesion dynamics that regulate cell migration (16). Metastasis is also reported to be closely associated with changes in the expression of Ca2+ channels and corresponding signaling cascades activated by changes in intracellular Ca2+ levels (67). As cells migrate, a Ca2+ gradient is required for rear-end retractions that involve Ca2+-dependent myosin phosphorylation (13, 37, 84). Moreover, higher Ca2+ levels at the leading edge of cells are required for focal adhesion turnover (115).

One of the most ubiquitous pathways involved in receptor-regulated Ca2+ influx is store-operated Ca2+ entry (SOCE). The key molecular players that mediate SOCE were discovered as stromal interaction molecules (STIM) and Orai proteins (32, 60, 82, 85, 105, 106, 116, 122, 123). SOCE is initiated upon agonist binding to either G protein-coupled receptors or receptor tyrosine kinases, resulting in breakdown of phosphatidyli-phosphatidylinositol-3,4,5-trisphosphate (PIP3) and diacylglycerol (DAG). This leads to the release of Ca2+ from both the ER and Golgi apparatus and subsequent calcium influx through Orai channels. STIM proteins form an intracellular Ca2+ store that is dependent on Ca2+ influx for proper function. STIMs and Orais are conserved across species and are present in various tissues, including the heart, brain, and immune system. They play a crucial role in the regulation of cellular Ca2+ homeostasis and are essential for the proper functioning of the cell. In conclusion, STIM and Orai proteins are promising targets for cancer therapy and further research is needed to fully understand their role in the development and progression of cancer.
Recent work from Zhang et al. (126) suggests that STIM1, a single transmembrane low-affinity Ca^{2+} binding protein, ER Ca^{2+} release results in loss of Ca^{2+} from EF hand domain of STIM1 and eventually causes oligomerization of STIM1. STIM1 oligomers move to regions of ER in close proximity of plasma membrane where they physically interact and activate Orai proteins (48, 72, 79, 118). STIM1 protein has another homolog, STIM2, and the Orai family has three homologs, Orai1, 2, and 3 wherein Orai3 is exclusively present in mammals (14). In most eukaryotic cells, SOCE is mediated via STIM1 and Orai1; however, recent studies have shown that in estrogen receptor-expressing (ER+) breast cancer cells (68) and in lung adenocarcinomas (6), Orai3 is the native pore-forming unit of SOCE. An essential role for STIM and Orai proteins in promoting tumorigenesis has emerged, with a number of studies supporting the idea that STIM and Orai proteins could be therapeutically targeted for better management of cancers.

Interestingly, in addition to their canonical role in SOCE, STIM and Orai proteins can also contribute to store-independent Ca^{2+} influx pathways (31, 35, 91). For instance, STIM1 is required for the activation of store-independent arachidonic acid-regulated Ca^{2+} (ARC) channels as well as leukotriene C_4-stimulated Ca^{2+} (LRC) channels. Both the ARC and LRC channels are constituted by Orai1/Orai3 heteromultimers (35, 91, 125). Recent work from Zhang et al. (126) suggests that these two store-independent Ca^{2+} entry pathways are likely mediated by the same channel entity and probably LTRC is the common physiological trigger activating these pathways. Both ARC and LRC channel activities play an important role in cellular proliferation and migration (25, 35, 124). In vivo mice studies demonstrated that both Orai3 and LTCa synthase (rate-limiting enzyme in the activation of LRC channels) play critical roles in neointimal hyperplasia (35, 124). Interestingly, ARC-mediated Ca^{2+} entry was recently shown to play a vital role in prostate cancer cell proliferation and tumorigenesis (25). Furthermore, STIM1-independent activation of Orai1 by secretory pathway calcium ATPase 2 (SPCA2) has been reported in MCF-7 breast cancer cells (31). Feng et al. (31) showed that SPCA2 physically interacts and activates Orai1-mediated constitutive Ca^{2+} influx responsible for breast cancer tumorigenesis independently of store depletion, STIM1, and STIM2. Collectively, STIM and Orai proteins contribute to cancers not only via canonical SOCE pathways but also via Ca^{2+} store-independent signaling mechanisms. In the following sections, we will discuss the role of STIM and Orai proteins in different cancer types.

BREAST CANCER

Breast cancer is one of the most prevalent forms of cancers (92). Although improvement in treatment strategies has increased the mean survival time of affected patients, quality of life is severely hampered by current chemotherapy regimens (45). Therefore, there is constant need for identification of novel targets with minimal side effects for better management of breast cancer. Recent data suggest that targeting of STIM and Orai proteins in breast cancer could be one of the ways for achieving this goal.

Yang et al. (115) were the first to demonstrate a critical role for STIM1- and Orai1-mediated SOCE in breast cancer migration and metastasis. Yang and colleagues (115) reported that SOCE-mediated Ca^{2+} influx is essential for breast cancer cell migration. Using knockdown and rescue strategies, the authors showed that STIM1 and Orai1 mediate SOCE in triple negative MDA-MB231 cells and regulate their migration but not proliferation (115). STIM1 and Orai1 knockdown decreased the rate of focal adhesion turnover, resulting in reduced migration (115). It was further demonstrated that metastasis of MDA-MB231 is drastically reduced upon STIM1 or Orai1 knockdown (115).

Interestingly, work from our group has established an essential role for Orai3 in ER+ breast tumorigenesis. We identified a novel functional Orai3 channel in ER+ breast cancer cells (68, 70) and demonstrated that higher Orai3 expression in ER+ breast cancer cells results in its functional recruitment for mediating SOCE (68). This work suggested a possible involvement of estrogen receptor signaling in regulating Orai3 function. In a follow-up study, we revealed a selective regulation of Orai3 by estrogen receptor-α (ERα) (71). We further showed that Orai3 plays a crucial role in proliferation and invasion of ER+ MCF-7 cells (71). Most importantly, in vivo orthotopic xenograft studies in mice established a vital role for Orai3 in ER+ breast tumorigenesis (71).

Subsequent studies by Faouzi et al. (26) confirmed the upregulation of Orai3 in around 70% of breast cancer specimens. Using ER+ MCF-7 and T47D cell lines, Faouzi et al. (26) reported that Orai3 is crucial for cell cycle progression and cell survival. Interestingly, Orai3 was shown to regulate cell survival and cell cycle progression only in case of cancerous cells but not in normal breast epithelial cells (26). The authors attributed this selective role of Orai3 in cancerous cells to its higher expression in these cells. In an independent study,
Faouzi et al. reported strong positive correlation between the oncogene c-myc and Orai3 expression in human breast cancer samples (27). Interestingly, they showed that Orai3 knockdown significantly reduced c-myc expression and MCF-7 cell survival (27). The authors suggested that Orai3 silencing results in inactivation of MAP kinase pathway, leading to c-myc down-regulation (27).

An intriguing study by Feng et al. (31) identified a STIM-independent mechanism of Orai1 activation in MCF-7 cells. They demonstrated that in breast cancer cells, SPCA2, located in the Golgi, constitutively activates Orai1 channels independently of STIM1 and store depletion (31). Interestingly, Orai1 activation by SPCA2 was also independent of its Ca\(^{2+}\) ATPase activity and was mediated via direct physical interaction of these proteins (31). By performing mice xenograft studies, Feng et al. further showed that this constitutive activity of Orai1 plays a critical role in breast cancer development in vivo. Recent work from this group further suggests that SPCA2 is also involved in plasma membrane trafficking of Orai1 (20). In addition to pathological contribution to breast tumorigenesis, Orai1 plays an essential role in mediating lactation (21, 64). The Putney lab recently demonstrated that under physiological conditions, functional Orai1 is required in mammary epithelial cells for concentrating milk with Ca\(^{2+}\) and for milk expulsion through alveolar unit contraction (64). Therefore, under resting conditions, Orai1 mediates physiological functions of mammary glands whereas during mammary tumorigenesis it promotes cellular proliferation and migration.

Studies have also implicated a role for STIM1 and Orai1 in epithelial to mesenchymal transition (EMT) of breast cancer cells, an essential step involved in cancer metastasis (22, 42). It was demonstrated that transforming growth factor β (TGF-β) induces EMT by enhancing STIM1-Orai1 expression and SOCE amplitude (42). Recently, Montechi’s group reported that in breast cancer cells, epidermal growth factor (EGF)-induced EMT is associated with perturbations in Ca\(^{2+}\) homeostasis and the expression and function of Orai1 (22). Taken together, studies discussed in this section establish a vital role for STIM1, Orai1, and Orai3 in different types of breast cancers. It appears that Orai1-mediated SOCE mainly contributes to metastasis whereas Orai3 contributes to cell survival and proliferation. This could be due to the differences in the signaling cascades initiated upon activation of different Orai homologs and/or due to their diverse spatio-temporal location. Orai1 has been reported to localize to leading edges of the migratory cells, and Ca\(^{2+}\) influx at these sites plays an important role in cellular motility (11, 115). Further studies aimed at delineating the molecular mechanisms leading to switching of the functional homologs of STIM/Orai proteins and the downstream signaling cascades activated by this switch will help in better understanding the pathophysiological relevance of such switching.

Prostate Cancer

SOCE has been frequently associated with cellular apoptosis in prostate cancer. In androgen-independent prostate cancer cells, reduced SOCE is credited for apoptotic resistance. Prostate cancer remodeling to an androgen-independent state brings out a more aggressive and apoptosis-resistant phenotype of this cancer (23, 103, 104). Understandably, decreased SOCE could be an adaptive mechanism by which prostate cancer cells escape apoptosis. Flourakis et al. (33) reported that Orai1 is associated with apoptosis resistance in androgen-independent human prostate cancer cells. The authors showed that Orai1 levels and SOCE are decreased upon androgen deprivation. Moreover, overexpression of Orai1 in these cells restored SOCE and the apoptotic rate that was similar to that in androgen-dependent cells (33). The authors suggested that androgen regulates Orai1 expression and also identified androgen response sites on the Orai1 promoter (33). Further, it was shown that a functional Orai1 channel is required for thapsigargin-, Cisplatin-, and TNF-α-evoked apoptosis. This work suggested that in androgen-independent prostate cancer cells, apoptotic resistance is coupled to abrogated SOCE and decreased Orai1 expression (33). It is not very clear how rise in Ca\(^{2+}\) levels in one case (e.g., breast cancer) increases cell survival and migration whereas in another case (e.g., prostate cancer) induces apoptosis. One of these could be differential activation of downstream signaling cascades by Ca\(^{2+}\) in these cells. Another possibility could be differential spatial localization of Orai channels in these cells that could in turn lead to cell-specific Ca\(^{2+}\) microdomains regulating contrasting physiological functions. Further, the extent of rise in Ca\(^{2+}\) levels may also dictate the cellular outcomes in different cancer types. In any case, just like other signaling pathways the effect of Orai-mediated Ca\(^{2+}\) entry is context and cell type specific.

Recently, an intriguing study from the Prevarskaya group showed that remodeling of functional Orai homologs plays a critical role in the progression of prostate cancer (25). Dubois et al. (25) reported that Orai3 levels are elevated in prostate cancer biopsies while that of other Orai isoforms and STIM1 remained largely unchanged. Unlike ER\(^{+}\) breast cancer cells (68), upregulation of Orai3 in prostate did not result in Orai3-mediated SOCE entry, rather STIM1 and Orai1 were the mediators of the SOCE pathway in these cells (25). Although only Orai1 mediates SOCE, all three Orai homologs contribute to prostate cancer cell proliferation and cell cycle progression (25). The authors showed that all Orai homologs regulate resting cytosolic and ER Ca\(^{2+}\) levels (25) and suggested that Orai homologs probably regulate cell cycle progression through control of cytosolic Ca\(^{2+}\) levels. Dubois et al. (25) further showed that just like in HEK293 cells (65), Orai1 and Orai3 contribute to arachidonic acid (AA)-activated ARC channels and that AA instigated prostate cancer cell proliferation (25). Interestingly, Dubois and colleagues (25) reported that Orai3 overexpression in prostate cancer decreases the amplitude of Orai1-mediated SOCE but increases AA-activated store-independent Ca\(^{2+}\) influx via ARC channels. Therefore, the tilt in balance in favor of AA-activated Ca\(^{2+}\) influx in prostate cancer enhances proliferative capabilities while the concomitant decrease in SOCE imparts protection from apoptosis. Using mice xenograft models, Dubois and coworkers (25) showed that Orai3 plays a critical role in prostate cancer development in vivo. In stark contrast to this study, Holzmann et al. (39) reported a decrease in Orai3 expression in prostate cancer samples compared with normal healthy tissues. Interestingly, these authors also proposed that in prostate cancer cells, Orai1 and Orai3 form heteromultimeric channels, which they referred to as altered calcium release-activated calcium current (\(i_{\text{CRAC}}\)) channels (39). The authors measured \(i_{\text{CRAC}}\) in normal prostate epithelial as well as in prostate cancer cell line
and reported intriguing effects of 2-APB application on \( I_{\text{CRAC}} \). They showed that in the control prostate epithelial cell line (hPEC), both 30 \( \mu \)M and 50 \( \mu \)M 2-APB application augments \( I_{\text{CRAC}} \). However, in the prostate cancer cell line (LNCaP), 30 \( \mu \)M 2-APB enhanced \( I_{\text{CRAC}} \) while 50 \( \mu \)M 2-APB caused an incomplete block (39). Further, knockdown of either Orai1 alone or Orai1 and Orai3 together almost completely abrogated IP3-activated \( I_{\text{CRAC}} \) in LNCaP cells, while knockdown of Orai3 alone increased IP3-activated \( I_{\text{CRAC}} \), suggesting that Orai3 is altering \( I_{\text{CRAC}} \) in LNCaP cells through interaction with Orai1 (39). Based on 2-APB pharmacology and molecular knockdown in LNCaP cells, the authors concluded that in normal prostate cells, Orai3 is the major contributor to \( I_{\text{CRAC}} \) while the decrease in Orai3 expression in prostate cancer cells favors the formation of Orai1-Orai3 heteromultimeric channels (39). Certainly, more studies from different groups are required for better understanding of specific Orai homolog contribution to prostate cancer.

### Colorectal Cancer

Colorectal cancer is one of the three leading causes of cancer-related deaths (43). The distinct pathological relevance of STIM1 and SOCE in colorectal cancers came from work recently published by Wang and colleagues (108). These authors compared STIM1 expression in colorectal cancer biopsies with healthy colon tissue and found increased STIM1 expression in cancerous tissue (108). Higher STIM1 expression correlated with the increased tumor size, tumor invasion, and metastasis (108). Interestingly, the same group had previously reported that EGF-mediated activation of proinflammatory and prometastatic gene cyclooxygenase2 (COX-2) requires STIM1-Orai1-mediated SOCE (107). Further, the authors demonstrated that STIM1 regulates colon cancer cell migration via selectively regulating COX-2 but not cyclooxygenase1 (COX-1) expression at the transcriptional level (108). Increase in COX-2 levels upon STIM1 overexpression was shown to be coupled with increased prostaglandin E2 (PGE2) secretion by colon cancer cells. Moreover, involvement of COX-2 and PGE2 in STIM1-regulated cancer cell migration was suggested as STIM1 knockdown-mediated decrease in migration was rescued upon exogenous COX-2 and/or PGE2 addition (108). Increased expression of proinflammatory COX-2 and PGE2 secretion by STIM1-overexpressing colon cancer cells prompted the authors to test the efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) on STIM1-mediated migration of colon cancer cells. Two NSAIDs, ibuprofen and indomethacin, used in micromolar concentrations almost completely abrogated STIM1-mediated migration (108).

Interestingly, Sobradillo et al. (96) recently reported a switch in molecular machinery of SOCE in human colorectal carcinoma cells. These cells displayed higher SOCE, Orai1-mediated highly Ca\(^{2+}\)-selective \( I_{\text{CRAC}} \), and TRPC1-mediated nonselective \( I_{\text{SOC}} \) in comparison to noncancerous cells. Further increase in SOCE was suggested to be associated with increased proliferation, invasion, and cell survival of these cells (96). It was demonstrated that expression of TRPC1, Orai1, Orai2, Orai3, and STIM1 increases whereas that of STIM2 decreases in colon cancer cells (96). Additionally, silencing of STIM2 in healthy colon cells not only diminished SOCE but also made the cells resistant to oxidative stress-mediated apoptosis (96). Collectively, this study showed that increased Orai1 and decreased STIM2 expression are critical to colon tumorigenesis (96). However, whether this remodeling is cell line specific or is consistent across colon cancer cells and tumor samples needs to be verified. Future studies aimed at understanding the exact role of STIM2 in colorectal cancer as well as molecular mechanisms connecting apoptosis to STIM2 expression are needed. Taken together, these studies suggest that STIM1 and Orai1 could be attractive therapeutic targets for colorectal cancer treatment and management.

### Brain Tumors: Glioblastomas and Neuroblastomas

Glioblastoma or glioblastoma multiform (GBM) is a highly malignant tumor of the brain arising from glial cells and/or astrocytes. In most cases, death is associated with secondary tumors due to the highly invasive and metastatic nature of GBM (47, 76). On the other hand, neuroblastomas are extracranial tumors of neurons that are comparatively benign in nature (38, 41). SOCE remodeling is reported during phenotypic switching of neurons (7). The extent of SOCE as well as STIM1 and Orai1 expression is higher in proliferative neuroblastomas in comparison to differentiated neurons (7). SOCE and STIM1-Orai1 expression was decreased upon retinoic acid-mediated induction of neuronal differentiation (7). This study presents evidence for possible targeting of STIM1 and Orai1 for inducing neuronal differentiation.

Our group provided one of the earliest evidence demonstrating a crucial role for STIM1 and Orai1 in human GBMs (69). We isolated and established glial cell lines from tumor biopsies and found that Orai1 expression was consistently higher in primary GBM compared with human primary astrocyte (HPA) cells whereas STIM1 expression remained largely unaltered (69). Further, higher Orai1 expression corresponded to higher SOCE and \( I_{\text{CRAC}} \) in GBMs. Interestingly, knockdown of either STIM1 or Orai1 drastically decreased the invasive capabilities of GBMs without any evident effect on HPA cells, suggesting that these proteins could selectively regulate GBM invasion. We also observed a small but significant decrease in GBMs proliferation upon Orai1 knockdown (69). On the other hand, Liu et al. (61) reported that in established human (U251) and rat (C6) glioblastoma cell lines, both STIM1 and Orai1 are essential for cell proliferation and cell survival. In a recent study, STIM1 knockdown significantly decreased U251 human glioblastoma proliferation by causing cell cycle arrest in G0/G1 phase (55). Excitingly, in vivo STIM1 silencing resulted in evident decrease in glioma formation in a mice xenograft model thereby highlighting the key oncogenic role of STIM1 in glioblastoma (55). Further implication of Orai1 in mediating glioblastoma invasion came from a clinico-pathological study where authors compared Orai1 expression between 61 glial tumors and 8 noncancerous brain biopsies (128). Zhu et al. (128) showed that Orai1 expression increases with enhanced invasive potential of human glioblastoma. In accordance with our earlier work, these authors reported that functional Orai1 is required for glioma cell migration and invasion (128). Further, they demonstrated that Orai1 regulates focal adhesion turnover and nonreceptor protein tyrosine kinase 2 (Pyk2) activation required for EMT. These data at least partially delineated the molecular mechanism driving Orai1 regulated invasion (128).
Collectively, these studies highlight a key role of STIM1 and Orai1 in GBM proliferation and invasion.

Melanoma

Melanoma is a type of skin cancer that originates from pigment-producing skin cells, melanocytes. UV exposure and less pigmented skin are considered to be key determinants contributing to melanoma in Caucasian populations (44). If early diagnosis of melanoma is not achieved, it leads to metastasis. Indeed, over 60% of skin cancer-related deaths are outcomes of metastatic melanomas (46). Interestingly, higher SOCE (28) and Akt expression (5) have been reported in malignant melanoma cells compared with nonmalignant melanoma cells. Fedida-Metula et al. (28) revealed that Akt renders malignant melanoma cells resistant to apoptosis in a Ca \(^{2+}\) and SOCE-dependent manner. A complex interplay of mitochondria and SOCE-mediated Ca \(^{2+}\) signaling is required for Akt activation and survival of melanoma cells (30). Fedida-Metula and colleagues (29) subsequently demonstrated that STIM1 silencing could decrease Akt activation in malignant melanoma cells.

Recently, Stanisz et al. (97) reported high Orai1 and STIM2 expression in three different melanoma cell lines. The functional significance of Orai1 and STIM2 was established in SK-Mel-5, a metastatic melanoma cell line wherein Orai1 or STIM2 silencing resulted in significant decrease in SOCE (97). Intriguingly, combined knockdown of STIM1 and STIM2 further abrogated SOCE, suggesting the possibility of a small but significant role of STIM1 in activating Orai1-mediated SOCE (97). Surprisingly, silencing of Orai1 and STIM2 in melanoma cells increased their proliferation but at the same time reduced their migratory and invasive capabilities (97). Further, the evaluation of melanoma biopsies demonstrated that Orai1 and STIM2 expression were highest at the rim of the invading tumors, implicating their potential role in tumor invasion and/or metastasis in vivo (97). Interestingly, a similar localization of Orai1 to the leading edges of migratory cells has been reported earlier for vascular smooth muscle cells (11), suggesting Ca \(^{2+}\) requirement at these sites during cell migration. The authors proposed that higher Orai1-STIM2-mediated SOCE is required for melanoma to switch from a proliferative to a more migratory state (97). In contrast to the work discussed above, a recent study demonstrated that STIM1 expression is higher in metastatic melanoma biopsies as well as in metastatic melanoma cell lines in comparison to control biopsies and primary melanoma cell line (WM3248), respectively (102). Umemura and colleagues (102) reported that STIM1-Orai1 knockdown abrogated SOCE, reduced melanoma cell proliferation and migration (102). Additionally, using an in vivo mice model, the authors demonstrated that either STIM1 or Orai1 silencing could significantly suppress melanoma cell metastasis to the lungs (102). Surprisingly, Umemura et al. (102) failed to detect significant STIM2 expression in melanomas and metastatic cell lines.

Mechanistic details on role of SOCE in melanoma invasion were provided by an independent study (98). Sun et al. (98) reported that Ca \(^{2+}\) oscillations as a consequence of SOCE are required for invadopodium formation and extracellular matrix (ECM) degradation. Using knockdown strategies, the authors demonstrated that both Orai1 and STIM1 are critical for mediating invadopodium formation and its proteolytic activity (98). Suppression of SOCE abrogated recruitment of matrix metalloproteinase (MT1-MMP) to the plasma membrane (98). Further, microarray analysis on melanoma tissues showed higher STIM1 and Orai1 expression in metastatic tissues compared with benign samples (98). Interestingly, STIM1 knockdown reduced lung metastasis of highly metastatic melanoma cell lines (98). These evidences from various reports clearly reinstate the role of STIM1 and Orai1 in migration; however, the role of STIM2 remains incompletely understood. It is difficult to explain the differences observed by these groups in terms of the STIM homolog involved in regulating melanoma metastasis. One possibility could be different sources of melanomas, reflecting variation between distinct populations. Certainly, more efforts are required to completely understand this STIM homolog switch during melanomas.

Cervical Cancer

Chen et al. (17) reported STIM1 upregulation in cervical tumors in comparison to normal cervical tissues. Further, it was shown that increased STIM1 expression correlated with increased metastasis and lower survival. Mechanistically, the authors demonstrated that EGF activated STIM1-dependent SOCE, which in turn regulated cervical cancer cell lines’ migration in vitro (17). STIM1 knockdown reduced cell motility by decreasing the activation of proline-rich tyrosine kinase (PyK) and by reducing focal adhesion turnover (17).

In their subsequent study, Chen and colleagues (18) reported an upregulation of Orai1, STIM1, and histone deacetylase 6 (HDAC6) levels in cervical cancer cells compared with normal cervical cells. Chen et al. (18) further showed that in cervical cancer cells, but not in normal cervical cells, HDAC6 is required for STIM1 translocation on microtubules and resulting activation of Orai1-mediated SOCE. HDAC6 inactivation using pharmacological inhibitors or molecular knockdown resulted in hypo-acetylated \(\alpha\)-tubulin and SOCE abrogation. Interestingly, in most of the cervical cancer specimens tested, expression of STIM1 and Orai1 was increased whereas that of acetylated \(\alpha\)-tubulin was decreased (18), suggesting that specific targeting of HDAC6 in cervical cancers can lead to inhibition of STIM1-Orai1-mediated cervical tumorigenesis. However, it remains to be determined whether this HDAC6-mediated regulation of STIM1 movement is a generalized phenomenon or whether it is specific to cervical cancer.

Esophageal Squamous Cell Carcinoma

Esophageal squamous cell carcinoma is highly prevalent with poor prognosis and high mortality rate (74, 127). A recent study on esophageal squamous cell carcinoma (ESCC) attributes tumorigenesis to higher Orai1-mediated intracellular Ca \(^{2+}\) oscillations (127). Expression analysis of clinical tumor samples from ESCC patients and established ESCC cells showed elevated expression and activity of Orai1 compared with normal esophageal epithelial tissues (127). However, no significant change was observed in STIM1 protein levels in both cell lines as well as human tumor biopsies. On the other hand, STIM2 levels were higher in ESCC cell lines (127), bringing up the possibility of Orai1 coupling with STIM2 for regulating basal resting Ca \(^{2+}\) levels in ESCC cell lines. The authors observed higher frequency of cytosolic Ca \(^{2+}\) oscillations...
tions in ESCC cells in comparison to nontumorous esophageal cells and attributed this phenomenon to higher Orai1-mediated SOCE (127). Indeed, both pharmacological inhibition and molecular knockdown of Orai1 decreased Ca\(^{2+}\) oscillations. Further Orai1 silencing decreased proliferation, caused cell cycle arrest and severely deteriorated the migratory and invasive capabilities of ESCC cells (127). Most importantly, exploiting in vivo mouse xenograft model, the authors showed that both in vivo pharmacological blockade of SOCE and Orai1 silencing could reduce tumor volume and increase tumor free survival (127). Evaluation of clinico-pathological data also corroborated that higher Orai1 expression was associated with higher reoccurrence of esophageal carcinomas and lower patient survival (127).

**Lung Adenocarcinoma**

Li et al. (58) performed histopathological analysis on non-small cell lung carcinoma (NSCLC) samples and reported higher STIM1 expression in cancerous biopsies in comparison to normal lung tissue. Interestingly, the chemotherapeutic drug Cisplatin decreased STIM1 expression and basal intracellular Ca\(^{2+}\) levels in NSCLC cells (58). The authors further reported that Cisplatin-induced NSCLC cell apoptosis was increased upon either pharmacological inhibition of SOCE or STIM1 knockdown. Therefore, Li and colleagues concluded that Cisplatin induces apoptosis and tumor arrest at least partially by regulating STIM1 expression and SOCE (58). These authors did not study the specific role of Orai isoforms in Cisplatin-induced alterations in Ca\(^{2+}\) homeostasis. A recent study reported that overexpression of Orai1 in a NSCLC cell line results in SOCE abrogation and cell cycle arrest (40). However, the significance of these findings is uncertain as it is well appreciated that overexpression of Orai1 without coexpression with STIM1 can result in dominant negative effects on SOCE (95).

Interestingly, work from Ay and colleagues (6) implicated an essential role for Orai3 in NSCLC cell proliferation. Ay et al. (6) demonstrated upregulation of Orai3 expression in nearly 67% of lung adenocarcinoma tissue samples evaluated. Further, higher Orai3 levels correlated with advanced tumor grade (6). In characterized NSCLC cell lines, NCI-H23 and NCI-H460, Orai3 suppression abrogated thapsigargin-evoked SOCE whereas Orai1 and Orai2 knockdown had no effect on SOCE (6). Intriguingly, the authors reported an almost 80% decrease in protein levels with Orai3 siRNA, but it resulted in only 40% SOCE abrogation (6). This suggests that, along with Orai3, other channel proteins might be contributing to SOCE in these cells. Current measurements would have assisted in drawing concrete conclusions regarding the identities of Ca\(^{2+}\) channels contributing to SOCE in these cells. Further, Orai3 knockdown resulted in impairment of cellular proliferation and cell cycle arrest at G\(_0\)/G\(_1\) phase, but no effect on apoptosis was observed (6). Mechanistically, Orai3 knockdown in these cells inhibited thapsigargin- and serum-induced Akt phosphorylation (6). Taken together, these data suggest that STIM1 and Orai3 play an important role in lung cancers.

**Other Cancers**

Along with the above discussed cancers, a role for STIM and Orai proteins is also emerging in other cancer types. For instance, Kondratska et al. (50) reported that STIM1 and Orai1 regulate cell survival in pancreatic adenocarcinomas. The authors showed that in pancreatic adenocarcinoma cell lines, STIM1 and Orai1 expression is higher than normal pancreatic ductal epithelial cells. Further, it was demonstrated that silencing of these proteins increases pancreatic adenocarcinoma cell sensitivity to chemotherapeutic drug-induced apoptosis (50). Similarly, Yoshida et al. (117) reported that STIM1 contributes to proliferation and tumorigenesis of epidermal carcinoids. The authors showed that stable knockdown of STIM1 in epidermal carcinoid cell line A431 results in decreased tumor growth in athymic mice. By performing immunohistochemistry and Western blotting, the authors reported that STIM1-mediated SOCE is required for EGF receptor phosphorylation and further suggested that STIM1 could be involved in EGF-induced proliferative cascade (117). Yanamandra and colleagues (113) implicated Orai3-mediated Ca\(^{2+}\) influx in regulating apoptosis in myeloid leukemia cells. The authors reported higher Orai3 expression in Tipifarnib-sensitive myeloid leukemia cells in comparison to Tipifarnib-insensitive leukemia cells. Further, using pharmacological tools, Yanamandra et al. (113) showed that Orai3 contributes to Tipifarnib-induced apoptosis in myeloid leukemia cell lines. In contrast to a large number of studies, Peng et al. (81) reported that increased STIM1 expression and SOCE negatively regulate benign cancerous conditions of tuberous sclerosis complex. The authors showed that STIM1 knockdown increased Akt phosphorylation and aggravated tumorigenesis in nude mice (81). Interestingly, STIM1 was discovered as a tumor suppressor gene in skeletal muscle tumors years before its Ca\(^{2+}\) handling role was established (86). Further, a role for STIM and Orai proteins is also emerging in ovarian (89), nasopharyngeal (121), hepatocellular (62, 112, 114), and renal carcinomas (49). Collectively, a large body of data has established a critical role for STIMs and Orais in mediating tumorigenesis (for summary, please refer to Table 1 and Fig. 1). Apart from regulating cancer cell survival, proliferation, invasion, and metastasis, STIM1 and Orai1 have also been implicated in mediating tumor angiogenesis as discussed below.

**Tumor Angiogenesis**

One of the characteristic features of cancer development and metastasis is the onset and aggressive surge in tumor vascularization. Several studies have reported that circulating endothelial progenitor cells (EPCs) contribute to tumor angiogenesis and metastasis (34, 75). It is well appreciated that endothelial cells require an increase in cytosolic Ca\(^{2+}\) levels for proliferation and tubulogenesis (24, 66). Our group was the first to establish a critical role for STIM1 and Orai1 in endothelial SOCE, proliferation, and cell cycle progression (1). We demonstrated that the key proangiogenic stimulus, vascular endothelial growth factor (VEGF), activates STIM1-Orai1-mediated SOCE in endothelial cells. In a follow-up work, Li et al. (56) described the involvement of Orai1 in VEGF-activated in vitro tubulogenesis and in vivo angiogenesis using the chick chorioallantoic membrane model. Interestingly, these authors also reported an essential requirement for Orai1 in EPC tubulogenesis, suggesting a pivotal role for Orai1 in tumor angiogenesis (56). Nevertheless, work from Antigny et al. (3) challenged the involvement of Orai1 in primary endothelial...
Surprisingly, the authors reported that although Orai1 regulates endothelial cell proliferation, it does not contribute to tubulogenesis. In any case, Antigny and colleagues (3) showed that STIM1 plays a critical role in tubulogenesis.

A study by Chen et al. (17) reported an essential role for STIM1 in cervical cancer angiogenesis. Using knockdown and overexpression tools, the authors showed that STIM1 regulates VEGF secretion as well as in vivo tumor angiogenesis in mice (17). Sanchez-Hernandez et al. (87) assessed SOCE in EPCs.
and later on characterized the SOCE players in EPCs derived from renal carcinoma (RCC-EPCs) blood samples (63). The authors further established that STIM1 and Orai1 regulate RCC-EPCs SOCE, proliferation, and tubulogenesis (63). Interestingly, STIM1 and Orai1 expression was higher in RCC-EPCs compared with normal EPCs, which in turn resulted in elevated SOCE and angiogenesis (63). Collectively, these studies highlight an important role for STIM1 and Orai1 in normal as well as tumor-aggravated angiogenesis. Moccia et al. (66) have further suggested that STIM1 and Orai1 could be attractive therapeutic targets for dismantling tumor vascularization and thereby arresting tumor growth.

**Future Perspectives**

The studies discussed emphasize a pivotal role for STIM and Orai proteins in mediating tumorigenesis. In most cases, cancer progression correlates with either increased expression or a molecular switch between the functional homologs of these proteins (Fig. 2). This cancer-specific scenario makes STIM and Orai proteins very attractive therapeutic targets for controlling tumor growth and metastasis. For instance, specific inhibitors of Orai3 could be exciting lead molecules for better management of ER$^+$ breast cancer and lung adenocarcinomas. Similarly, the drugs that can regulate STIM and Orai protein expression in tumors might assist in targeting several types of tumors. Unfortunately, there is a severe dearth of studies delineating the molecular mechanisms that drive the expression of these proteins in general and in cancers specifically. Therefore, more studies are warranted to understand the signaling cascades and molecular players that regulate STIM and Orai protein expression.

Although STIM proteins have emerged as attractive therapeutic targets, a recent study by Weidinger et al. (110) has appropriately questioned the systemic use of STIM1 and STIM2 blockers for cancer therapeutics because SOCE is an essential component of the human immune system and any systemic treatment regimen would likely result in severe side effects related to compromised immunity. Using conditional double-knockout mice lacking STIM1 and STIM2 in CD8$^+$ T cells, these authors demonstrated that SOCE mediated by STIM proteins is essential for antitumor immunity (110). SOCE in CD8$^+$ T cells is required for efficient tumor-specific cytolytic functions of these cells as well as for stalling engraftment of tumor cells in vivo (110). Therefore, perhaps more localized and tumor-specific approaches for targeting STIM and Orai proteins have to be considered. With the advancements in nanoparticle-mediated drug delivery, it might be possible to locally deliver therapeutic drugs with desirable release kinetics for stalling tumor growth (93).

An interesting theme that has started to emerge is the potential regulation of Orai proteins by sex hormones and the contribution of Orai isoforms to sexual dimorphism. Orai3 is regulated by estrogen in ER$^+$ breast cancer cells and contributes to ER$^+$ breast cancer development in vivo (68, 71). Similarly, Orai1 expression is controlled by androgen in prostate cancer cells and that in turn imparts apoptosis resistance (33). Further understanding is needed for the potential targeting of specific Orai isoforms in sex-specific cancers.

Intriguingly, data from several systems (35, 52, 90, 91) have implicated STIM and Orai proteins in Ca$^{2+}$ store-independent functions. For instance, Feng et al. (31) reported store-independent activation of Orai1 by SPCA2 in breast cancer and established a critical contribution of Orai1-mediated constitutive Ca$^{2+}$ influx to breast tumorigenesis. This suggests that STIM and Orai proteins could regulate cellular functions independent of their roles in SOCE. Indeed, Orai1 and Orai3...
were suggested to regulate proliferation and cell cycle progression in a battery of cell lines independent of their Ca\(^{2+}\) handling function (12). It is therefore important to identify the mechanisms by which Orai proteins control cell proliferation independently of Ca\(^{2+}\) influx. These findings highlight an important caveat that we should keep in mind going forward: that is we should not assume that STIM-regulated cell functions necessarily equate to Orai-regulated ones, nor should we assume that STIM-Orai-mediated cell functions are necessarily the result of their involvement in SOCE. Indeed, STIM1 can contribute to pathophysiological processes independent of Ca\(^{2+}\) entry (36, 90). Similarly, Orai1 can mediate cellular functions independently of STIM1 (31). Furthermore, in addition to activating Orais, STIM1 can regulate a number of ion channels such as transient receptor potential canonical (TRPC) channels, voltage-activated Ca\(^{2+}\) channels (CaV1.2), and store-independent ARC/LRC channels (35, 77, 80, 101, 109, 120, 125). Therefore, it is important to exercise caution in interpreting findings related to the role of any individual STIM-Orai isoform. Future in vivo findings from tissue-specific knockout mice lacking individual STIM and Orai isoforms are likely to shed light on the role of these molecules in different tissues. Clearly, comparative studies performed with these mice under control conditions and under different models of disease will assist in fulfilling the promise of targeting channels encoded by specific arrangements of STIM and Orai isoforms for therapy of cancer and other human diseases.

One of the major hurdles in translating bench work leads into bedside therapies is developing specific blockers/antagonists with minimal side effects. This could be potentially circumvented by testing the efficacy of clinically prescribed or FDA-approved drugs for targeting potential leads that are hitherto not tested with these drugs. Indeed, some recent studies underline the prospective of targeting STIM and Orai proteins with routinely used medications. For instance, NSAIDs were demonstrated to abrogate SOCE and STIM1-COX-2-mediated migration in colorectal cancer cell lines (108). Similarly, NSAIDs were also reported to block vascular smooth muscle cell proliferation by indirect inhibition of Orai1-mediated \(\text{I}_{\text{CRAC}}\) (73). However, the efficacy of such drugs in impeding cancer growth is likely marginal and in the absence of in vivo studies the potential of NSAIDs in controlling STIM-Orai-mediated tumor development is uncertain. Likewise, it was demonstrated that Sirolimus, a routinely used medication in drug-eluting stents for inhibiting restenosis, actually blocks arterial smooth muscle proliferation by abrogating STIM1-Orai1-mediated SOCE pathways (51). Although preliminary in nature, these studies present optimistic possibilities of targeting STIM and Orai protein-mediated tumorigenesis by localized tumor delivery of more efficacious drugs. This appears to hold promise, as downstream molecular mechanisms driving cellular proliferation in different cell types share many similarities. Future studies aimed at developing novel specific inhibitors of STIM/Orai proteins as well as strategies for tumor-specific release of these drugs will hopefully empower us with better therapeutics against cancer.

ACKNOWLEDGMENTS

The figures were adapted from Servier Medical Art (http://www.servier.com/content/servier-medical-art-now-licensed-under-creative-commons).

GRANTS

R. K. Motiani is funded by DST-INSPIRE Faculty Fellowship (IFA12, LSBM-038), Government of India. A. Vashisht is a Junior Research Fellow supported by IFA12. LSBM-038. M. Trebak is funded by National Heart, Lung, and Blood Institute Grants R01 HL-097111 and R01 HL-123364 from the National Institutes of Health and by American Heart Association Grant 14GRNT18880008.
AUTHOR CONTRIBUTIONS

A.V. and R.K.M. prepared figures; A.V., M.T., and R.K.M. drafted manuscript; A.V., M.T., and R.K.M. approved final version of manuscript; R.K.M. conceived and designed research.

REFERENCES

5. Assa-Kunik E, Fishman D, Killman-Pressman S, Tsory S, Elhyany M.
6. Assa-Kunik E, Fishman D, Killman-Pressman S, Tsory S, Elhyany M.
7. Assa-Kunik E, Fishman D, Killman-Pressman S, Tsory S, Elhyany M.
8. Assa-Kunik E, Fishman D, Killman-Pressman S, Tsory S, Elhyany M.


C468 STIMs AND ORAIS IN CANCERS

Themes

92. Siegel R, Naishadham D, Jemal A. Shuttleworth TJ.


