STIM and ORAI proteins: crucial roles in hallmarks of cancer

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1Université des Sciences et Technologies de Lille, Inserm, U1003 - PHYCELL - Physiologie Cellulaire, Lille, France; and 2Department of Life Science and Systems Biology, and Nanostructured Interfaces and Surfaces Centre of Excellence, University of Turin, Torino, Italy

Fiorio Pla A, Kondratska K, Prevarskaya N. STIM and ORAI proteins: crucial roles in hallmarks of cancer. Am J Physiol Cell Physiol 310: C509–C519, 2016; doi:10.1152/ajpcell.00364.2015.—Intracellular Ca2+ signals play a central role in several cellular processes; therefore it is not surprising that altered Ca2+ homeostasis regulatory mechanisms lead to a variety of severe pathologies, including cancer. Stromal interaction molecules (STIM) and ORAI proteins have been identified as critical components of Ca2+ entry in both store-dependent (SOCE mechanism) and independent by intracellular store depletion and have been implicated in several cellular functions. In recent years, both STIMs and ORAIs have emerged as possible molecular targets for cancer therapeutics. In this review we focus on the role of STIM and ORAI proteins in cancer progression. In particular we analyze their role in the different hallmarks of cancer, which represent the organizing principle that describes the complex multistep process of neoplastic diseases.

STIM; ORAI; Ca2+; cancer

CHANGES IN THE CYTOSOLIC FREE Ca2+ concentration are key players in many fundamental cellular processes including muscle contraction, transmitter release, cell proliferation, differentiation, gene transcription, and cell death (10). Given that Ca2+ controls so many vital processes, disturbance of the Ca2+ homeostasis regulatory mechanisms leads to a vast variety of severe pathologies, including cancer. Indeed, the role of Ca2+ is well established in many cell signaling pathways involved in carcinogenesis (65, 66, 81).

The most common mechanism of Ca2+ signal generation results from the activation of plasma membrane G protein-coupled receptors or tyrosine kinase receptors followed by PLC-mediated cleavage of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) (90). DAG is a known PKC activator, but it can also play a PKC-independent role in regulating Ca2+ signal generation. IP3 production results in cytoplasmic Ca2+ elevation that can be separated into two distinct phases. At first, IP3 activates ER-localized IP3 receptors (IP3Rs), thus releasing calcium from the endoplasmic reticulum (ER) into the cytosol. Second, the decrease in ER Ca2+ content (following IP3R activation) stimulates influx of extracellular Ca2+ via opening of plasma membrane Ca2+ channels in a process known as capacitative or store-operated Ca2+ entry (SOCE) (71). Numerous studies have demonstrated the important role of SOCE in a plethora of cellular processes and functions in different cell types (for reviews, see Refs. 3, 77) as well as in a number of pathological processes typical for cancer (for a review see also Refs. 9, 80).

The main molecular players of SOCE are STIMs, ER-localized single-transmembrane domain protein, and ORAI proteins, which are plasma membrane calcium channels. The present review is focused on the role of STIM and ORAI proteins in cancer progression. In particular we will describe their role in the different hallmarks of cancer, which represent the organizing principle that provides a logical framework with which to understand the remarkable complexity of neoplastic diseases (43). Besides the six initial hallmarks described by Hanahan and Weinberg (43), we will consider the newest aspect recently included by the same authors in 2011 (44).

STIM and ORAI Proteins

Store-operated calcium channels (SOCs) represent one of the major calcium-entry pathways in nonexcitable cells and are widely distributed in various cell types. SOCs are plasma membrane ion channels activated in response to ER Ca2+ store depletion and thereby provide Ca2+ for ER store refilling as well as for signaling purposes (71, 90). The major molecular components of SOC are stromal interaction molecule 1 (STIM1) and ORAI1 proteins, where ORAI1 constitutes the plasma membrane calcium channel and STIM1 represents mostly the ER-localized single-transmembrane domain protein, functioning as a sensor of ER calcium. Following ER Ca2+-depletion, STIM1 translocates to the plasma membrane, where it interacts with and activates ORAI1 channels, thereby mediating store-operated calcium entry (SOCE) (76, 111).

Two human STIM proteins exist, STIM1 and STIM2. Both are predominantly located in the ER, though a minor amount of STIM1 is expressed at the plasma membrane (91). Both STIMs have similar architecture, with an NH2-terminal domain in the ER lumen, a single-transmembrane segment, and a COOH-terminal cytoplasmic domain (46). In vertebrates, STIM1 and STIM2 are expressed ubiquitously throughout cell types and thought to function as ER calcium sensors (106). In contrast to
STIM1, STIM2 exclusively localizes in the ER. STIM2 has been reported to be a considerably weaker activator of ORAI1 than STIM1 while representing a more sensitive sensor of ER luminal Ca\(^{2+}\). The \(K_d\) of STIM2 for Ca\(^{2+}\) (~400 \(\mu M\)) is twofold higher than that of STIM1 (\(K_d\) ~200 \(\mu M\)) (91). Thus, it is assumed that the physiological role of STIM2 consists in stabilization of basal cytosolic and ER calcium levels (13). The role of STIM2 in the regulation of SOCE is complex. It has been reported that STIM2 protein mediates distinct store-dependent and store-independent modes of SOC channel activation (73). However, overexpression of STIM2 inhibited STIM1-mediated SOCE (92). Moreover, different splice variants of STIM2 have been shown to differentially regulate SOCE (60, 83). Recently, it has been proposed that STIM2 enhances agonist-mediated activation of SOCE by promoting STIM1 clustering in ER-plasma membrane junctions at low stimulus intensities, when ER Ca\(^{2+}\) stores are mildly depleted, thus increasing the sensitivity of Ca\(^{2+}\) signaling to agonists (70).

ORAI1 is the founding member of the ORAI family of Ca\(^{2+}\) channels that are phylogenetically distinct from other calcium-permeable channels. The ORAI family includes three members (ORAI1, 2, and 3) consisting of four transmembrane domains with cytosolic NH\(_2\) and COOH termini (45, 78). Although the first recordings of calcium release-activated calcium (CRAC) currents have been reported in 1980s, ORAI1 has been linked to these currents just in 2006 (30, 53, 76). ORAI1 is a widely expressed 33-kDa cell surface protein, the missense mutation of which has been associated with abrogated CRAC channel activity and human severe combined immune deficiency (SCID) syndrome (30). ORAI1 is localized on the plasma membrane and forms the Ca\(^{2+}\)-selective pore of the CRAC channel. The functional CRAC channel is believed to be a tetramer of four ORAI1 subunits; however, several studies suggested higher order of assembly (i.e., hexameric) (47, 63, 74, 113).

In a classical model of SOCE, activation of ORAI1 involves direct binding of STIM1 and ORAI1 (72). Ca\(^{2+}\) store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane, where it binds to and activates ORAI1 (45).

ORAI2 and ORAI3 represent two highly conserved paralogues of ORAI1. Like ORAI1, both ORAI2 and ORAI3 are highly calcium selective in physiological conditions, and both channels have been reported to be activated by calcium store depletion (20, 57). Similarly to ORAI1, ORAI2 and ORAI3 appear to have broad expression pattern (39, 40, 97). At the moment, functional implications for ORAI2 are sparse. Several publications suggest that ORAI2 mediates SOCE in immune cells with silenced ORAI1 (41, 103). However, no effect of siRNA against ORAI2 on SOCE has been reported by other groups (8, 97).

In contrast to ORAI1 and ORAI2, ORAI3 is an exclusively mammalian protein. In estrogen receptor-positive breast cancer cells, ORAI3 (but not ORAI1) has been shown to mediate SOCE (67). However, in HEK-293 and human fibroblasts silenced for ORAI1, ectopically expressed ORAI3 only partially restored SOCE, suggesting the primary role for ORAI1 in this process (40). ORAI3 was also reported to mediate decreased SOCE sensitivity to reactive oxygen species (ROS) in human T helper lymphocytes (11).

Along with ORAI1, ORAI3 contributes to store-independent calcium entry. Thus, ORAI3 has been reported to be an important component of store-independent arachidonate-regulated Ca\(^{2+}\) (ARC) entry in HEK-293 cells, as well as of a store-independent leukotriene C4-regulated Ca\(^{2+}\) (LRC) entry in vascular smooth muscle cells (37, 89).

ARC channels are activated in response to receptor-mediated derivation of arachidonic acid (AA), a polyunsaturated fatty acid that has multiple actions on living cells. The ARC channel is a small conductance, highly Ca\(^{2+}\)-selective ion channel whose activation is specifically dependent on low concentrations of AA acting at an intracellular site. Like the SOC channels, the ARC channels are expressed in a variety of different cell types (89). These channels are primarily involved in the generation and modulation of agonist-induced oscillatory calcium signals (61). Recent findings suggest that the same proteins that form SOC channels are also integral components of ARC channels; however, there are mechanistic differences between these channels. Activation of ARC channels depends on a pool of STIM1 that is constitutively present in the plasma membrane (but not ER-localized STIM1) (62, 100), whereas the pore of the ARC channels is thought to be formed by the heteromeric assembly (pentameric) of ORAI1 with its homologue ORAI3 (64).

Recently, it has been reported that a store-independent ARC-like channel (LRC channel) could be activated by leukotriene C4 (LTC4) in primary vascular smooth muscle cells (37). LTC4 is produced through the catalytic activity of leukotriene C4 synthase, which is the key enzyme responsible for the synthesis of cysteinyl leukotrienes through metabolism of arachidonic acid downstream of the 5-lipoxygenase pathways. LRC channel requires STIM1 for its activation. However, it is not clear which STIM1 pool (ER or plasma membrane) is important for this effect. Both ORAI1 and ORAI3 have been shown to contribute to LRC channel-mediated calcium entry, thus confirming that ORAI proteins could be activated by various agonist/pathways and are important players in store-independent calcium entry (112).

**Sustaining Proliferative Signaling/Evading Growth Suppressors**

The most evident and distinctive characteristic of cancer is surely its ability to sustain proliferative signals and at the same time to evade growth suppressors. Normal tissue in fact has the ability to carefully regulate growth signals to maintain the proper cellular homeostasis required to organize the correct function of distinct organs. However, cancer cells dysregulate these mechanisms to sustain uncontrolled proliferation.

In this regard, it is known that the regulated Ca\(^{2+}\) toolkit undergoes profound remodeling in cancer cells to favor activation of Ca\(^{2+}\)-dependent transcription factors, such as the nuclear factor of activated T cells (NFAT), c-Myc, c-Jun, and c-Fos, that promote cell growth controlling expression of the G1 and G1/S phase transition cyclins (D and E) and associated cyclin-dependent kinases (CDK4 and CDK2) (80, 86).

SOCE derived from “classical” interaction of STIM1-ORAI1 has been extensively implicated in cancer progression in both native cancer cells as well as in cells extracted from different tumors such as hepatoma, breast, prostate, glioblastoma, cervical cancer, colorectal cancer, and renal cell carci-
noma (RCC) (48, 102). Several groups have reported that STIM1-ORA11 function as positive regulators of cell proliferation. STIM1 and ORA11 knockdown, together with transient receptor potential canonical 6 (TRPC6), decrease indeed the protein levels of cyclin D1 in a hepatoma cell line (12). Chen et al. (18) investigated in detail the role of STIM1 and SOCE in cervical cancer, using both in vitro as well as in vivo models. They showed that increased STIM1 expression correlated with increased metastasis and lower survival. Although the authors describe a major role for STIM1 in cell migration due to focal adhesion turnover (see next paragraphs), they also analyzed the proliferative role of STIM1 in vitro. In this regard, STIM1 silencing in cervical cancer cell lines significantly inhibited cell proliferation by arresting the cell cycle in S and G2/M phases. STIM1 downregulation correlated with increase of p21 protein level and a decrease of Cdc25C protein, whose turnover is responsible of p21 degradation process. In contrast, STIM1-mediated Cdc25C expression was regulated at the transcriptional level in cervical cancer cell lines (18). Similar results were observed in glioblastoma cell lines and hypopharyngeal carcinoma where STIM1 silencing inhibited cell proliferation by inducing a cell cycle arrest in G0-G1 with accumulation of p21 and downregulation of cyclin D1 (54, 58, 95). Moreover, Kim et al. (50) reported an increase expression level of ORA11, but not STIM1 or ORA13, in RCC: pharmacological inhibition of SOCE or ORA1 or STIM1 downregulation significantly impaired cell proliferation as well as cell migration of RCC Caki-1 cell lines. However, these data are a bit controversial and were not confirmed by Dragoni and colleagues (22), who could not find any role for SOCE in cell proliferation of freshly isolated RCC metastatic cells from patients.

On the other hand, a number of important “nonclassical” events involving other members of the STIM and ORAI family have been proposed as key mediators of cancer cell proliferation. Indeed, Feng et al. (29) demonstrated that a store-independent ORA11-mediated Ca\(^{2+}\) influx is critical for breast cancer cell proliferation, which was dependent on an isofrom of secretory pathway Ca\(^{2+}\)-ATPase SPCA2 pump, upregulated in breast cancer-derived cells and human breast tumors. Based on the function of a series of chimeras and mutant proteins, the authors proposed a model in which cooperation of NH\(_2\) and COOH termini of SPCA2 is required for ORA11-mediated Ca\(^{2+}\) signaling. Whereas the NH\(_2\) terminus of SPCA2 binds strongly to ORA11, the COOH terminus elicits activation of Ca\(^{2+}\) influx, which is completely independent from SPCA2 Ca\(^{2+}\) pump activity as well as from STIM1 (29).

In regard to other ORAI proteins, another important role in cell proliferation is also mediated by ORA13, which is over-expressed in different cancers such as breast and prostate cancers (23, 24). As far as breast cancer is concerned, several groups established that G\(_1\) progression and G\(_1\)/S transition phases are dependent on ORA13-mediated SOCE in estrogen expressing (ER+) cell lines (MCF7 cell line) (24, 25, 69), by positively regulating the expression of cyclins (D1, E), CDK4 and 2, and suppressing cyclin-dependent kinase inhibitors (CDKIs), such as p21 and p53, through regulating the expression and activity of c-myc (24, 25). In addition, the downstream Ca\(^{2+}\) effectors have been identified, as both NFAT activity and ERK1/2 phosphorylation were increased by ORA13-dependent SOCE-activation in the MCF7 cell line (69). Interestingly, no effect of ORA13 was observed in the normal cancer cell lines MCF10-A or ER~ MD-MB231 or in ER-silenced MCF7 cells, while ORA13 introduction into MCF7 cells depleted of ER rescued SOCE activated by thapsigargin. The ORA13 role has been also demonstrated in vivo in xenograft SCID mice (24, 69). Similarly to breast cancer, Ay et al. (6) reported recently that ORA13 expression was upregulated in lung cancer tissues, correlating with high tumor grade, and the ORA13-mediated Ca\(^{2+}\) entry was crucial to lung cancer cell proliferation (6). As far as prostate cancer, we recently demonstrated the role of ORA13 in prostate cancer cell proliferation in vitro. We showed that enhanced ORA13 expression favors heteromerization with ORA11 to form a novel channel. The ORA13-ORA11 heterotetramer supports store-independent Ca\(^{2+}\) entry, driven by arachidonic acid (AA) thereby promoting cell proliferation and changing the equilibrium from functional homomeric ORA11-based store-operated channels, which are important in supporting susceptibility to apoptosis (23). It has to be noted that in this particular cellular context, AA-mediated activation ORA13-ORA11 heterotetramer has similar properties to those described previously for ARC (61, 64). However, in prostate cancer our results clearly show that STIM1 is not implicated in AA-mediated Ca\(^{2+}\) signaling. For these reasons, we maintained the term “heteromeric association of ORA11/ORA13” instead of ARC. Moreover, our results show that the signaling pathway activated downstream of AA-activated ORA13 channels involves a Ca\(^{2+}\)/calcineurin-dependent transcription factor, NFAT, followed by the stimulation of the expression of the key rate-limiting controller of G/S phase transition, cyclin D1 (23). Finally, by means of xenograft tumor models we identified a key role of ORA13 in prostate cancer. Indeed, treatment of xenograft mice with siORA13 significantly reduced tumor growth, while on the contrary, coexpression of ORA13 promoted an increase of tumor volume in vivo (23). We can therefore speculate that manipulating ORA13 could simultaneously act on two different hallmarks of cancer in prostate cancer: shifting the ORA11 channel composition from homo- to ORA11-ORA13 hetero-multimer prevents apoptosis (see also next paragraph) and promotes uncontrolled cell migration.

In addition to STIM1, there are now several pieces of evidence ascribing a role of STIM2 in hallmarks of cancer. In particular, STIM2 has been proposed as an antiproliferative protein both in melanomas as well as in colorectal tumors (7, 93, 94). Interestingly, in humans, STIM2 is located at the short arm of chromosome 4, in 4p15.2, where loss of heterozygosity at 4p15 of the D4S2397 microsatellite marker has been previously associated with diminished disease-free survival and a more aggressive phenotype (5, 7). In melanoma cells an intriguing role has been described for STIM2-ORA11 expression in the switch from a more proliferative to a more migratory phenotype and vice versa (94). While higher expression levels of both Oral1 and STIM2 lead to increased basal intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)) and consequently higher invasive potential, reduction in their expression levels decreases the basal [Ca\(^{2+}\)]\(_{i}\), and causes enhanced melanoma growth. The data nicely correlate with in vivo data on paraffin-embedded human melanoma samples: double MITF (proliferative marker for melanoma) and Oral1 or STIM2 immunohisto-
Themes

Resisting Cell Death/Enabling Replicative Immortality

The idea that apoptosis can represent a barrier for cancer progression has been well established by several functional studies. In addition, it has become evident that several tumors have the ability to evade apoptosis and therefore break the balance between cell death and cell proliferation, leading to accumulation of “undead” cells with the final effect of promoting cancer progression. For this reason, resisting cell death by “evading apoptosis” has been defined by Hanahan and Weinberg as one of cancer’s hallmarks (43, 44).

The role of “classical” SOCE mediated by ORAI1 and STIM1 in apoptosis is complex. SOCE has been described to exert pro- or antiapoptotic functions depending on several factors such as the cancer type, apoptotic stimuli, or intracellular signaling (52). Indeed, ORAI1 contributes to apoptosis induced by various stress stimuli in the prostate, contributing to the establishment of an apoptosis-resistant phenotype in prostate cancer cells, while ORAI1 knockdown protected LNCaP cells against thapsigargin (TG)- or oxaliplatin/cisplatin-induced apoptosis (36). In the above mentioned study we proposed that ORAI1 constitutes the principal source of Ca\(^{2+}\) influx used by prostate cancer cells to trigger apoptosis via mitochondrial and cytosolic mechanisms (36). These data are in agreement with our recent data showing that PC3 with reduced SOCE demonstrated lower sensitivity to TG-induced apoptosis (23). Consistent with this, pharmacological SOCE inhibition or STIM1 knockdown has been shown to inhibit hydrogen peroxide-induced apoptosis in HT22 cells via alleviation of intracellular Ca\(^{2+}\) overload, restoration of the mitochondrial membrane potential, and decrease of cytochrome c release (84).

Besides the role in apoptosis, Xu et al. (107) recently proposed a role for STIM1 and ORAI1 in the mechanism of senescence in prostate cancer cells, which seems to provide a protective barrier for neoplastic expansion (26). Enhanced senescence properties were in fact observed in PC3 cells overexpressing STIM1 or ORAI1, while less senescent cells were detected when knocking down either STIM1 or ORAI1 (107).

In contrast to the proapoptotic role in prostate cancer just summarized, pharmacological inhibition of SOCE or down-regulation of STIM1 has been shown to enhance apoptosis induced by cisplatin in non-small cell lung cancer cells (54) as well as in ovary carcinoma where ORAI1/STIM1 play a protective antiapoptotic role in these cells (87). A complex interplay of mitochondria and STIM1-dependent SOCE-mediated Ca\(^{2+}\) signaling has been shown to be required for Akt activation, survival, and apoptosis resistance of melanoma cells (27, 28). In line with these observations we recently showed an antiapoptotic role for ORAI1, and to a lesser extent STIM1, in a pancreatic adenocarcinoma cell line following chemotherapy stimulation (51). Furthermore, it was reported that ORAI1-driven Ca\(^{2+}\) entry delays the induction of the CD95-mediated apoptotic signal in leukemic T-cell lines, thus driving a transient negative feedback loop, introducing a lag phase in the early steps of the CD95 signal (49). This prevented CD95-mediated caspase activation and delayed the delivery of the apoptotic signal (49).

Recently, STIM2 was implicated as proapoptotic in colorectal cancer. As previously stated, Sobradillo et al. (93) described a downregulation of STIM2 in a colon carcinoma cancer cell as compared with normal epithelial cells. In addition, in colorectal carcinoma, STIM2 downregulation confers an apoptotic resistance to normal cells. These data are in agreement with the previously reported role for STIM2 as a tumor suppressor in colorectal carcinoma (see also previous paragraph) (7).

The apparent discrepancies underlined by opposing function in different types of cancer could be explained by taking into account different ORAI and STIM combinations, considering the existence of three ORAI and two STIM proteins. All of these proteins participate in SOCE in different ways, and in addition they have SOCE-independent functions. Therefore, considering the particular cancer types is of vital importance to potentially target ORAI and STIM proteins. Moreover, as previously reported, STIM and ORAI proteins may interact with other channels to generate different responses. As an example, we recently demonstrated that overexpression of transient receptor potential vanilloid subfamily member 6 (TRPV6) in prostate cancer cells promotes cell survival by enhancing proliferation and conferring apoptosis resistance both in vitro as well as in vivo (85). We also showed that STIM1-ORAI1-TRPC1-mediated SOCE is required for TRPV6 translocation at the plasma membrane. However, the TRPV6 channel is not a SOC, although ORAI1 or TRPC1 or any other SOC is needed for its translocation to the plasma membrane. We propose that the interplay between calcium entering through the TRPV6 channel may produce a negative feedback loop thus creating Ca\(^{2+}\) transients engaged in cancer cell survival and apoptotic resistance (85). Finally, understanding the specific mechanisms of apoptosis regulation by SOCE will be of great importance to conclude whether their modulators could be effective in cancer treatment in each particular case.

Inducing Angiogenesis

Blood vessels supply oxygen and nutrients to tumors and provide gateways for immune surveillance needed for solid tumor sustenance. As this network nourishes all tissues, it is not surprising that structural or functional vessel abnormalities contribute to cancer progression and have been listed as hallmarks of cancer (44, 75). Endothelial cells (ECs) line the inner surface of vessels to support tissue growth and repair. Besides providing metabolic support, vessels are also used as routes for providing metabolic support, vessels are also used as routes for immune surveillance needed for solid tumor metastasis (16, 75).

Although the induction of angiogenesis may initially provide the tumor with more oxygen and nutrients, the ultimate response is poor and the perivascular coverage does not lead to
mature vessels with proper function. In contrast to normal vessels, tumor vasculature is highly disorganized; vessels are tortuous and dilated, with uneven diameter, excessive branching, and shunts. Consequently, tumor blood flow is chaotic and variable and leads to hypoxic and acidic regions in tumors (15, 105). It is now well established that tumor-derived endothelial cells (TECs) of different origins, and normal ECs are highly heterogeneous at genetic, epigenetic, and functional levels (14). Moreover, TEC-mediated intracellular signaling is quite different from that observed in normal human microvascular ECs. Interestingly, proangiogenic Ca\(^{2+}\) signals and their related pathways are significantly altered in TECs compared with normal ECs (33–35, 82).

Intracellular Ca\(^{2+}\) signals are involved at different critical phases in the regulation of the complex process of vascularization and tumor progression. Most importantly in this context is the large amount of data demonstrating that pro-angiogenic tyrosine kinase receptor-binding growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), stimulate [Ca\(^{2+}\)]\(_i\), increase in ECs, and trigger complex intracellular cascades (32, 34). Abdulaev et al. (1) were the first to demonstrate the functional role for the "classical" STIM1-ORAI1 activation, by showing that SOCE has an important role in cell proliferation and cycle progression in human umbilical vein endothelial cells (HUVEC). Interestingly, ORAI1 knockdown showed a stronger effect as compared with STIM1 or STIM2 knockdown. These data suggest that part of the role of ORAI1 on HUVEC proliferation could be independent from STIM1 or STIM2 clustering (1). On the other hand, STIM1 overexpression promoted in vivo angiogenesis (measured as total vessel number) as well as an increase in VEGF production in cervical cancer (18). The direct link between ORAI1-STIM1 in VEGF-mediated SOCE in HUVEC came from a subsequent research presented by Li et al. (56). In this research, the authors demonstrate the involvement of ORAI1 in VEGF activated in vitro tubulogenesis and in vivo angiogenesis using the chick chorioallantoic membrane model (56). Similarly to HUVEC, suppression of ORAI1 in endothelial progenitor cells (EPCs) prevents VEGF-mediated SOCE and tube formation (22, 56). Moreover, EPCs isolated from RCC patients (RCC-EPCs) display an increased SOCE, which correlates with ORAI1, STIM1, and TRPC1 overexpression when compared with EPCs from healthy patients: genetic suppression of STIM1, ORAI1, and TRPC1 affects SOCE in RCC-EPCs (59). It has to be noted that the role of ORAI1 in ECs has been questioned by Antigny et al. (4), although they described STIM1 as a key player (together with TRPC1 and TRPC4) in tube formation in both HUVEC and EA.hy926 cells. Recently, a very detailed and elegant study by Tsai and coworkers (101) proposed an integrated model of the spatial organization of the STIM1 signaling complex, which generates a coordinated Ca\(^{2+}\) control system that dynamically controls the polarization and persistence of migration as well as local adhesion and turning of ECs, together with the actin regulators Rac, Cdc42, RhoA, and phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P\(_3\)]. In particular, the authors show that STIM1 is activated locally in the front edge of migrating cells as a result of directed STIM1 transport to the front. This transport is mediated by microtubule plus-end, and lower ER Ca\(^{2+}\) levels in the front mediated by local tyrosine kinase receptor (RTK) signaling. The resulting polarized SOC signaling provides a key mechanism to maintain the spatial and temporal dynamics of the Ca\(^{2+}\) signaling system (101).

Besides ORAI1-STIM1, a study from the Beech group recently suggested a role for ORAI3 in VEGF-induced EC remodeling with a consequent role for in vitro and in vivo tubulogenesis (55). Although the mechanism was not thoroughly analyzed, they propose a Ca\(^{2+}\) release-dependent mechanism for activation of this ORAI3 system in which VEGF-mediated Ca\(^{2+}\) release activates cPLA2\(_\alpha\) to catalyze the production of AA, which in turn activates ORAI3 by exposing it to the surface membrane (55). Further studies will be needed to better understand the reciprocal regulation between the "classical" ORAI1-STIM1 SOCE activation and ORAI3 as previously shown for prostate cancer cells (23).

Recently, STIM1 has been described as a key player in EC permeability, which is an important step in sprouting angiogenesis and in new vessel maturation. Interestingly, thrombin-induced decrease in EC permeability requires STIM1, but it is unrelated to Orai1 and Ca\(^{2+}\) entry (88). At the same time, Sundivakkam et al. (96) reported a novel phosphorylation mechanism of STIM1 involving an AMPK/\p38 MAPK pathway responsible of inhibiting SOCE in lung microvascular ECs. This effect is responsible for regulating the thrombin-mediated permeability responses, which are SOCE mediated (96).

These data strongly suggest a role for "classical" STIM-ORAI1-mediated SOCE in ECs in the first steps of sprouting angiogenesis such as proliferation, migration, and formation in vitro of capillary-like structures, where STIM1 but not ORAI1 could be involved in EC permeability which is required for functional vessel formation. Moreover, whereas the interference with the bulk VEGF signaling alters the activity of a multitude of different cellular functions, targeting ORAI1 and ORAI3 may affect only EC migration and proliferation, whereas targeting STIM1 may selectively influence vascular permeability.

Activating Invasion and Metastasis

Tissue invasion or metastasis to distant organs represents one of the six initial cancer hallmarks, as proposed by Hanahan and Weinberg (43, 44). This multistep process is a consequence of several discrete steps that begin with local invasion, intravasation into blood and lymphatic vasculature and culminate with extravasation and colonization, resulting in formation of secondary metastatic lesions (98).

The emerging role of STIM and ORAI proteins in this particular hallmark of cancer has been extensively studied, and important progress in understanding the molecular mechanisms has been achieved in recent years.

Several cancer types rely on the "classical" SOCE mechanism due to STIM1 and ORAI1. One of the first studies demonstrating the role of the two proteins in breast cancer migration and metastasis progression showed that upregulation of ORAI1 or STIM1 significantly increased the migration rate of MDA-MB-231 cells whereas silencing reversed the effect (109). The authors of this study dissected partially the molec-
ular mechanism responsible for the migration impairment by showing that STIM1 and ORAI1-mediated SOCE affected the turnover of focal adhesion, which is a crucial step in the process of cell migration. This defect could be rescued by the small GTPases Ras and Rac. Finally, in vivo data obtained from a metastatic in vivo model showed that downregulation of STIM1 or ORAI1 significantly inhibited lung metastases (109). Subsequently, a similar proinvasive role for STIM1 and ORAI1-mediated SOCE was described for several cancers such as cervical cancer (18), hepatocellular carcinoma (108), renal cell carcinoma (50), nasopharyngeal carcinoma (110), and glioblastoma—both in a primary human cell line isolated from tumor biopsies as well as in commercially available human glioma cell lines (68, 114). In particular, Zhu et al. (114) showed ORAI1-mediated regulation of Pyk2 phosphorylation by the Ca^{2+}-dependent calpain, which is considered essential for focal adhesion turnover and epithelial-to-mesenchymal transition of cancer cells. These data confirm therefore the role of SOCE in tumor cell migration and invasion via modulation of focal adhesion turnover already described for breast cancer. The molecular mechanism of STIM1 in focal adhesion turnover regulation could also be explained by the interaction with microtubules as STIM1 binding to the microtubule plus-end-binding protein EB1 at the growing plus-end plays an important role in the remodeling of endoplasmic reticulum morphology, which, in turn, is mediating intracellular processes such as cell polarization and migration (2, 38). Moreover, Chen et al. (19) recently showed that microtubule-associated histone deacetylase 6 (HDAC6) is required for SOCE activation by optimizing the localization of the endoplasmic reticulum Ca^{2+} sensor STIM1 toward the plasma membrane (19). Interestingly, the promigratory effect of STIM1-ORAI1-mediated SOCE was recently correlated with enolase-1 (ENO-1), a glycolytic enzyme that can be translocated to the cell surface in breast cancer cells and thus regulate cell migration and invasion (21).

Consistent with the role of SOCE in cancer cell migration, STIM2 has also been shown to play an important role in melanoma cell migration. In particular as reported above, Staniz et al. (94) propose that STIM2-ORAI1-mediated SOCE expression switches the phenotype of melanocytes from proliferative to migratory. The data therefore show that STIM2 can act both as a tumor suppressor in highly proliferative cells (by increasing basal Ca^{2+}) to a level where it inhibits proliferation) as well as a tumor promoter in invasive cancers where the increased basal [Ca^{2+}]i results in a more invasive phenotype (94).

Besides the classical STIM1-ORAI1-mediated SOCE activation, several groups reported recently the role of K^+-channel-ORAI1 interaction to sustain breast cancer cell migration. A first study correlated ether à go-go (hEag1) K^+ channels as a relevant player in controlling Ca^{2+} entry through ORAI1 channels and consequent cell migration (42). Subsequently, Chantôme et al. (17) revealed a novel signaling pathway in which the interaction of ORAI1 with the SK3 channel, part of the family of Ca^{2+}-activated K^+ channels, elicited a constitutive and store-independent Ca^{2+} signaling that promoted breast cancer cell migration and bone metastasis formation. The functional complex is localized in the lipid raft microdomains, and this localization is mediated by SK3 expression since its downregulation completely delocalized ORAI1 outside of the raft components (17).

Taking all the data together, it is clear that STIM1-ORAI1 mediates a proinvasive migratory phenotype in several types of cancer types. On the other hand, the role of K^+ channels in ORAI1 interaction seems to be relevant for breast cancer migration and metastasis formation.

**Emerging Hallmarks: Immune System Contribution**

In this last section, we will discuss the role of STIM and ORAI proteins in the immune system in the context of cancer development and progression, since this has been classified as an emerging hallmark of cancer (44).

Infiltration of immune cells in tumor tissues has long been recognized by pathologists. The presence of this infiltration has

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**Fig. 1.** STIM and ORAI mechanisms in the different hallmarks of cancer. Schematic representation of the different mechanisms involved in ORAI and STIM activity described in the present review. Store-dependent, store-independent, and Ca^{2+}-independent mechanisms of action are represented in the figure; different hallmarks of cancer (marked in different colors) and the corresponding mechanisms involved are marked in the cartoons. All of the ten hallmarks as described by Hanahan and Weinberg (44) are reported. “?” represents a specific hallmark for which the role of ORAI1 and STIM1 has not yet been described.
been explained by the attempt of the immune system to eradicate tumors. Indeed, increasing evidence in the past few years shows that immune systems operate as a barrier against cancer progression and invasion. In particular, deficiencies in the development or function of CD8+ cytotoxic T lymphocytes (CTLs), CD4+ Th1 helper T cells, or natural killer (NK) cells each led to demonstrable increases in tumor incidence (99). In addition it is well established that STIM1-ORAI-mediated CRAC represents the predominant Ca\(^{2+}\) influx mechanism in lymphocytes (76). Moreover, mutations and loss of function or mutations in STIM1 in T cell genes inhibit Ca\(^{2+}\) influx and cause immunodeficiency in patients (30, 31). Interestingly, Feske’s group reported recently that CRAC channels activated by STIM1 and STIM2 proteins are essential for tumor immunosurveillance by CD8+ T cells (104). The authors used a mouse model with T cells carrying specific deletion of Stim1 and Stim2 genes that lack SOCE in CD4+ and CD8+ T cells and found that SOCE in T cells curtails the growth of tumor melanoma cell allografts while STIM1- and STIM2-deficient CTLs fail to prevent tumor cell engraftment. This effect is not due to an inhibition of priming, expansion, and homing of CTLs, but instead STIM1 and STIM2 are crucial for cytolytic effector functions of CTLs, especially their ability to produce IFN-γ and TNF-α, to release perforin-containing cytolytic granules, induce FasL and kill tumor cells (104).

In conclusion, although more data are needed to better study the role of ORAI and STIM proteins in cancer immunosurveillance, it is easy to imagine that these two proteins are very important players in this process emerging as new hallmarks of cancer.
Conclusion

The data presented in the present review summarize the current literature on STIMs and ORAIs role in the different hallmarks of cancer. In the latest version, Hanahan and Weinberg (44) presented ten different hallmarks (despite the six considered in the first review) including new ones emerging in the last few years. As shown in Fig. 1, ORAI and STIM proteins play relevant roles in seven out of ten considered hallmarks. However, we did not find any data regarding the role of ORAI and STIM in the remaining hallmarks (“tumor promoting inflammation,” “genome instability,” and “deregulating cellular energetics”, marked as “?” in Fig. 1).

As shown in Figs. 1 and 2, a clear role can be proposed for STIM1-ORAI1 and ORAI3 as promoters of sustaining proliferative signals, while STIM2 seems to account for antiproliferative signals. In addition, the “classical” STIM1-ORAI1-mediated SOCE plays different roles in apoptosis depending on the cancer cell type and apoptotic stimuli. While STIM1-ORAI1-mediated SOCE is sustaining apoptosis resistance in several cancer models such as lung cancer, melanoma, pancreatic ductal adenocarcinoma, and ovary cancer, it acts as pro-apoptotic in prostate cancer. Moreover, we clearly showed that prostate ORAI3 increased expression during cancer progression, switches the ORAI1 channels composition from homo- to ORAI1-ORAI3 heteromultimer, prevents apoptosis on one hand, and promotes uncontrolled cell migration on the other hand (23). Regarding tumor invasion and migration, STIM1-ORAI1 has been shown to mediate a proinvasive migratory phenotype in all the cancer types analyzed until now. Furthermore, increasing evidence unanimously points to a proangiogenic role of “classical” STIM1-ORAI1 SOCE. It is clear that more data are necessary to better integrate the molecular mechanism of the proangiogenic effect in the different types of cancers. Most of the data presented are in fact reflecting in vitro data on cultured endothelial cells.

Interestingly, STIM1-ORAI1 have been implicated in a newly emerging hallmark of cancer such as immune system control. These data are very important since STIM1 and ORAI1 proteins constitute possible targets to increase inflammatory responses and cancer immunosurveillance.

When analyzing the overall role of STIM and ORAI protein in cancer, we finally need to consider their emerging interaction with other transportome proteins such as Ca2+ pumps, K+ channels, and TRP channels. These interactions clearly affect the role of STIM and ORAI proteins in the different hallmarks of cancer.

In conclusion, there is increasing evidence indicating that STIM and ORAI proteins could be useful targets for different types of cancers. The specific types of Ca2+ sensing and transporting proteins expressed in cells as well as their specific microenvironment could affect which hallmark of cancer (e.g., apoptotic resistance) would likely be targeted.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.F.P. and N.P. conception and design of research; A.F.P. prepared figures; A.F.P. and K.K. drafted manuscript; A.F.P., K.K., and N.P. edited and revised manuscript; A.F.P.; K.K., and N.P. approved final version of manuscript.

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