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STIMs and ORAIs proteins: crucial roles in hallmarks of cancer

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Abstract

Intracellular Ca^{2+} signals play a central role in several cellular processes; therefore it is not surprising that altered Ca^{2+} 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 Ca^{2+} entry both in store-dependent (SOCE mechanism) or independent by intracellular store depletion and have been implicated in several cellular functions. In the last years both STIMs and ORAIs have emerged as possible molecular targets for cancer therapeutics. In this review we focus on the role of STIMs and ORAIs protein in cancer progression. In particular we analyse their role in the different hallmarks of cancer, which represent the organizing principle that describe the complex multistep process of neoplastic diseases.

Introduction

Changes in the cytosolic free Ca^{2+} 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 Ca^{2+} controls so many vital processes, disturbance of the Ca^{2+} homeostasis regulatory mechanisms leads to a vast variety of severe pathologies, including cancer. Indeed, the role of Ca^{2+} is well-established in many cell signalling pathways involved in carcinogenesis (65, 66, 81) .

The most common mechanism of Ca^{2+} signal generation results from the activation of plasma membrane G protein-coupled receptors (GPCRs) or tyrosine kinase receptors followed by PLC-mediated cleavage of phosphatidylinositol 4,5-bisphosphate (PIP2) into DAG and IP3 (90). DAG is a known PKC activator, but can also play a PKC-independent role in regulating Ca^{2+} signal generation. IP3 production results in cytoplasmic Ca^{2+} elevation that can be separated into two distinct phases. At first, IP3 activates ER-localized IP3Rs, thus releasing calcium from the ER into the cytosol. Second, the decrease in ER Ca^{2+} content (following IP3R activation) stimulates influx of extracellular Ca^{2+} via opening of plasma membrane Ca^{2+} channels in a process known as capacitative or store-operated Ca^{2+} 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 (3, 77) as well as in a number of pathological processes typical for cancer (for a review see also (9, 79).

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

STIMs and ORAIs proteins

Store-operated calcium channels (SOCs) represent one of the major calcium-entry pathways in non-excitable cells and are widely distributed in various cell types. SOCs are plasma membrane ion channels activated in response to ER Ca^{2+} store depletion and thereby provide Ca^{2+} for ER store refilling as well as for signalling purposes (71, 90). The major molecular components of SOC are stromal interaction molecule 1 (STIM1) and ORAI1 proteins, where ORAI1 constitutes plasma

membrane calcium channel and STIM1 represents mostly ER-localized single-transmembrane domain protein, functioning as a sensor of ER calcium. Following ER Ca^{2+}-depletion STIM1 translocate 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 N-terminal domain in the ER lumen, a single transmembrane segment, and a C-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 Kd of STIM2 for Ca^{2+} (~400 μM) is 2-fold higher than that of STIM1 (Kd ~200 μM) (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 has 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-PM junctions at low stimulus intensities, when ER Ca^{2+} stores are mildly depleted, thus increasing the sensitivity of Ca^{2+} signalling to agonists (70).

ORAI1 is the founding member of 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 N- and C- 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 activate 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 HEK293 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 (called Leukotriene C4-regulated calcium (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's not clear which STIM1 pool (ER or PM) 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 agonists/pathways and are important players in store-independent calcium entry (112).

Sustaining Proliferative Signalling / 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 have the ability to carefully regulate growth signals in order to maintain the proper cellular homeostasis required to organize the correct function of distinct organs. However, cancer cells dysregulate these mechanisms in order to sustain uncontrolled proliferation.

In this regard, it is known that regulated Ca^{2+} toolkit undergoes profound remodelling in cancer cells to favour activation of Ca^{2+}-dependent transcription factors, such as the nuclear factor of activated T cells (NFAT), c-Myc, c-Jun, 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 STIM1-ORAI1 has been extensively implicated in cancer progression in both native cancer cells as well in cells extracted from different tumours such as hepatoma, breast, prostate, glioblastoma, cervical cancer, colorectal cancer or renal cell carcinoma (RCC) (48, 102). Several groups have reported that STIM1-ORAI1 function as positive regulators of cell proliferation. STIM1 and ORAI1 knockdown, together with TRPC6, decrease indeed the protein levels of cyclin D1 in a hepatoma cell line (12). Chen et al. investigated in details the role of STIM1 and SOCE in cervical cancer, using both in vitro as well as in vivo models (18). 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 analysed the proliferative role of STIM1 in vitro. In this regard, STIM1 silencing in cervical cancer cell lines significantly inhibited cell proliferation by arresting 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 dependent on Ca^{2+} homeostasis. p21 upregulation was probably due to STIM1-dependent post translational regulation, implicating the control of the proteosome-dependent pathway responsible of p21 degradation process. In contrast, STIM1-mediated Cdc25C expression was regulated at 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. reported an increase expression level of ORAI1, but not STIM1 or ORAI3 in RCC: pharmacological inhibition of SOCE or ORAI1 or STIM1 downregulation, significantly impaired cell proliferation as well as cell migration of RCC Caki1 cell lines (50). However this data are a bit controversial and was not confirmed by Dragoni and colleagues who could not find any role for SOCE in cell proliferation of freshly isolated RCC metastatic cells from patients (22).

On the other hand a number of important "non classical" events involving other members of STIM and ORAI family has been proposed as key mediators of cancer cell proliferation. Indeed Feng et al. demonstrated that a store-independent ORAI1-mediated Ca^{2+} influx is critical for breast cancer cell proliferation which was dependent on an isoform of Secretory Pathway Ca^{2+}-ATPase, SPCA2 pump, upregulated in breast cancer-derived cells and human breast tumours. Based on the function of a series of chimeras and mutant proteins, the authors proposed a model in which cooperation of N- and C-termini of SPCA2 is required for ORAI1-mediated Ca^{2+} signalling. Whereas the N-terminus of SPCA2 binds strongly to ORAI1, the C-terminus elicits activation of Ca^{2+} influx, which is completely independent from SPCA2 Ca^{2+} pump activity as well as from STIM1 (29).

In regards to other ORAI proteins, another important role in cell proliferation is also mediated by ORAI3, which is overexpressed in different cancers such as breast and prostate cancers (23, 24). As far as breast cancer is concerned, several groups established that G1 progression and G1/S transition phases are dependent on ORAI3- 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, p21 and p53 through regulating the expression and the 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 ORAI3-dependent SOCE-activation in the MCF7 cell line (69). Interestingly no effect of ORAI3 was observed in the normal cancer cell lines MCF10-A or ER- MDA-MB231 or in ER silenced MCF7 cells, while ORAI3 introduction into MCF7 cells depleted of ER rescued SOCE activated by thapsigargin. The ORAI3 role has been also demonstrated in vivo in xenograft scid mice (24, 69). Similarly to breast cancer, recently Ay et al. Reported recently that ORAI3 expression was up-regulated in lung cancer tissues, correlating with high tumour grade, and the ORAI3- mediated Ca^{2+} entry was crucial to lung cancer cell proliferation (6). As far as prostate cancer, we recently demonstrated the role of ORAI3 in prostate cancer cells proliferation in vitro. We showed that enhanced ORAI3 expression favours heteromerization with ORAI1 to form a novel channel. The ORAI3-ORAI1 heterotetramer supports store-independent Ca^{2+} entry, driven by arachidonic acid (AA) thereby promoting cell proliferation and changing the equilibrium from functional homomeric

ORAI1-based store-operated channels, which are important in supporting susceptibility to apoptosis (23). It has to be noticed that in this particular cellular context, AA-mediated activation ORAI3-ORAI1 heterotetramer has similar properties to those described previously for ARC (61, 64). However, in PCa our results clearly show that STIM1 is not implicated in AA-mediated Ca^{2+} signalling. For these reasons, we maintained the term ‘‘heteromeric association of ORAI1/ORAI3’’ instead of ARC. Moreover our results show that the signalling pathway activated downstream of AA-activated ORAI3 channels involves a Ca^{2+}/calcineurin-dependent transcription factor, NFAT, followed by the stimulation of the expression of the key rate-limiting controller of G1/S phase transition, cyclin D1 (23). Finally by means of xenograft tumour models we identified a key role of ORAI3 in prostate cancer. Indeed treatment of xenograft mice with siORAI3 significantly reduced tumour growth while on the contrary ovexpression of ORAI3 promoted an increase of tumour volume *in vivo (23)*. We can therefore speculate that manipulating ORAI3 could simultaneously act on two different hallmarks of cancer in prostate cancer by shifting the ORAI1 channels composition from homo to ORAI1-ORA3 heteromutlimer 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 anti-proliferative protein both in melanomas as well as colorectal tumours (7, 93, 94). Interestingly in humans STIM2 is located at the short arm of chromosome 4, in 4p15.2 where loss of heterozygosis 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 it has bee described an intriguing role for STIM2-ORAI1 expression in the switch from a more proliferative to a more migratory phenotype and vice versa (94). While higher expression levels of both Orai1 and STIM2 lead to increased basal $[Ca^{2+}]i$ and consequently higher invasive potential, reduction in their expression levels decreases the basal $[Ca^{2+}]i$ and causes enhanced melanoma growth. The data nicely correlates with *in vivo* data on paraffin-embedded human melanoma samples: double MITF (proliferative marker for melanoma) and Orai1 or STIM2 IHC staining depict higher expression of MITF in central tumour areas, while Orai1 and STIM2 were more prominent in the invasive rims (94).

Taking together all the data presented above it is clear that STIM1, ORAI1 and ORAI3-mediated Ca^{2+} signals represent proliferative stimuli for different cancer types. These proteins act act via different mechanisms that are represented by classical STIM1-ORAI1-mediated SOCE to store independent unconventional ORAI1 to AA-activated ORAI3. In contrast STIM2 seems to have an anti proliferative role, at least for melanoma and colorectal cancer.

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. Also, it has become evident that several tumours 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 anti-apoptotic functions depending on several factors such as the cancer type, apoptotic stimuli or intracellular signalling (52). Indeed, ORAI1 contribute 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 TG- or oxaliplatin/cisplatin-induced apoptosis (36). In the 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 the recent data by us showing that PC3 with reduced SOCE demonstrated lower sensitivity to the TG-induced apoptosis (23). Consistent with this, pharmacological SOCE inhibition or STIM1 knockdown have 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).

Beside the role in apoptosis, Xu et al. recently proposed a role for of STIM1 and ORAI1 in the mechanism of senescence in prostate cancer cells (107), 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 pro-apoptotic role in prostate cancer just summarized, pharmacological inhibition of SOCE or downregulation of STIM1 have 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 anti-apoptotic role in these cells (87). A complex interplay of mitochondria and STIM1 dependent SOCE-mediated Ca^{2+} signalling 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 a anti apoptotic role for ORAI1, and with less extent STIM1, in pancreatic adenocarcinoma cell line following chemotherapic 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 pro-apoptotic in colorectal cancer. As previously stated, Sobradillo et al. described a down regulation of STIM2 in colon carcinoma cancer cell as compared with normal epithelial cells. In addition, in colorectal carcinoma STIM2 downregulation confers an apoptotic-resistance to normal cells (93). These data are in agreement with previously reported role for STIM2 and tumour 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, STIMs and ORAIs protein may interact with other channels to generate different responses. As an example, we recently demonstrated that overexpression of 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 if their modulators could be effective in cancer treatment in each particular case.

Inducing Angiogenesis

Blood vessels supply oxygen and nutrients to tumours and provide gateways for immune surveillance needed for solid tumour 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 (EC) line the inner surface of vessels to support tissue growth and repair. Beside providing metabolic support, vessels are also used as routes for tumour cells to metastasize (16, 75).

Although the induction of angiogenesis may initially provide the tumour 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, tumour vasculature is highly disorganized; vessels are tortuous and dilated, with uneven diameter, excessive branching and shunts. Consequently, tumour blood flow is chaotic and variable and leads to hypoxic and acidic regions in tumours (15, 105). It is now well established that tumour-derived endothelial cells (TECs) of different origins, and normal ECs are highly heterogeneous at genetic, epigenetic and functional levels (14). Moreover, TEC-mediated intracellular signalling is quite different from that observed in normal human microvascular EC. Interestingly, proangiogenic Ca^{2+} signals and their related pathways are significantly altered in TEC compared with normal EC (33–35, 82).

Intracellular Ca^{2+} signals are involved at different critical phases in the regulation of the complex process of vascularization and tumour 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 intracellular Ca^{2+} ($[Ca^{2+}]i$) increase in ECs and trigger complex intracellular cascades (32, 34). Abdulaev et al. 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 HUVEC (human umbilical vein endothelial cells) (1). 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. 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 tubule 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 EC has been questioned by Antigny et al.; although they described STIM1 as a key player (together with TRPC1 and TRPC4) in tube formation in both HUVEC and EA.hy926 cells (4). Recently, a very detailed and elegant study by Tsai and co-workers proposed an integrated model of the spatial organization of the STIM1 signalling complex, which generate a

coordinated Ca^{2+} control system that dynamically controls the polarization and persistence of migration as well as local adhesion and turning of EC, together with the actin regulators Rac, Cdc42, RhoA and PtdIns (3;4;5)P3 (101). 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) signalling. The resulting polarized SOC signalling provides a key mechanism to maintain the spatial and temporal dynamics of the Ca^{2+} signalling system (101).

Beside ORAI1-STIM1, a study from the Beech group recently suggested a role for ORAI3 in VEGF-induces EC remodelling with a consequent role for *in vitro* and *in vivo* tubulogenesis (55). Although the mechanism was not thoroughly analysed, they propose a Ca^{2+} release-dependent mechanism for activation of this ORAI3 system in which VEGF-mediated Ca^{2+} release activate cPLA2α to catalyse the production of AA which in turn activate ORAI3 by exposing it to the surface membrane (55). Further studies will be needed to better understand the reciprocal regulation between the "classical" ORA1-STIM1 SOCE activation and ORAI3 as previously showed 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 is unrelated to Orai1 and Ca^{2+} entry (88). At the same time Sundivakkam et al reported a novel phosphorylation mechanism of STIM1 involving AMPK/p38 MAPK pathway responsible of inhibiting SOCE in lung microvascular EC. This effect is responsible for regulating the thrombin-mediated permeability responses which are SOCE mediated (96).

These data strongly suggest the role for the "classical" STIM-ORAI1-mediated SOCE in EC 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 signalling 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 represent 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 STIMs and ORAIs proteins in this particular hallmark of cancer has been extensively studied and important progress in understanding the molecular mechanisms has been achieved in the last 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 migration rate of MDA-MB-231 cells while silencing reverted the effect (109). The authors of this study dissected partially the molecular 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, similar pro-invasive 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 tumour biopsies as well as in commercially available human glioma cell lines (68, 114). In particular, Zhu et al. showed ORAI1-mediated regulation of Pyk2 phosphorylation by the Ca^{2+} dependent calpain, which is considered essential for focal adhesion turnover and epithelial to mesenchimal transition of cancer cells (114). These data confirm therefore the role of SOCE in tumour 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 be also 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 remodelling of endoplasmic reticulum morphology, which is in turn mediating intracellular processes such as cell polarization and migration (2, 38). Moreover, Chen et al recently showed that microtubule associated Histone Deacetylase6 (HDAC6) is required for SOCE activation by optimizing the localization of the endoplasmic reticulum Ca^{2+} sensor STIM1 toward plasma membrane (19). Interestingly, the pro-migratory 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 important role in melanoma cell migration. In particular as reported above, Staniz et al. propose that STIM2-ORAI1-mediated SOCE expression switches the phenotype of melanocytes from proliferative to migratory (94). The data therefore show that STIM2 can act both as a tumour suppressor in highly proliferative cells (by increasing basal Ca^{2+} to a level where it inhibits proliferation) as well as a tumour promoter in invasive cancers where the increased basal $[Ca^{2+}]i$ results in a more invasive phenotype (94).

Beside the classical STIM1-ORAI1 mediated SOCE activation, several groups reported recently the role of K^+ channels-ORAI1 interaction to sustain breast cancer cells migration. A first study correlated Ether a` go-go (hEag1) K^+ Channels as relevant player in controlling Ca^{2+} entry through ORAI1 channels and consequent cell migration (42). Subsequently, Chantome et al. revealed a novel signalling 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+}-signalling that promoted breast cancer cell migration and bone metastasis formation. The functional complex is localized in the lipid rafts 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 pro-invasive 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 STIMs and ORAIs proteins in the immune system in the context of cancer development and progression since this has been classified as emerging hallmarks of cancer (44).

Infiltration of immune cells in tumour tissues has been long recognized by pathologists. The presence of this infiltration has been explained by the attempt of the immune system to eradicate tumours. Indeed, increasing evidence in the past few years show 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 tumour incidence (99). In addition it is well established that STIM1-ORAI1-mediated CRAC represent the predominant Ca^{2+} influx mechanism in lymphocytes (76). Moreover mutations and loss of function or mutations in STIM1 in T cells gene inhibits 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 tumour immunosurvaillance 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 tumour melanoma cells allografts while STIM1 and STIM2 deficient CTLs fail to prevent tumour 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-g and TNF-a, to release perforin-containing cytolytic granules, induce FasL and kill tumour cells (104).

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

Conclusions

The data presented in the present review summarized the current literature on STIMs and ORAIs role in the different hallmarks of cancer. In the latest version, Hanhan and Weinberg presented ten different hallmarks (despite the six considered in the first review) including new ones emerging in the last years (44). As shown in Fig1, 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 ORAIs and STIM in the remaining hallmarks ("Tumour promoting inflammation", "Genome Instability" and "Deregulating cellular energetics", marked as "?" in Fig 1).

As shown in table 1 and Fig 1, a clear role can be proposed for STIM1-ORAI1 and ORAI3 as promoters of sustaining proliferative signals while STIM2 seems to account for anti-proliferative signals. In addition, the "classical" STIM1-ORAI1-mediated SOCE plays different roles in apoptosis depending on the cancer cell types and apoptotic stimuli. While STIM1-ORAI1 mediated SOCE is sustaining apoptosis resistance in several cancer models such as lung cancer, melanoma, PDAC 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-ORA3 heteromutlimer, prevents apoptosis on one hand, and promotes uncontrolled cell migration on the other hand (23). Regarding tumour invasion and migration, STIM1-ORAI1 has been shown to mediate a pro-invasive migratory phenotype in all the cancer types analysed till know. Furthermore, increasing evidence unanimously point to a pro-

angiogenic role of "classical" STIM1-ORAI1 SOCE. It is clear that more data is necessary more data would be necessary to better integrate the molecular mechanism of the proangiogenic effect in the different types of cancers. Most of the data presented are infact 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 contribution. These data are very important since STIM1 and ORAI1 proteins constitute possible targets to increase inflammatory responses and cancer immunosurvaillance.

When analysing the overall role of STIMs and ORAIs protein in cancer, we finally need to consider their emerging interaction with other transportome proteins such as Ca^{2+} pumps, K^{+} channels or TRP channels. These interactions clearly affect the role of STIMs and ORAIs proteins towards the different hallmarks of cancer.

In conclusions there is increasing evidence indicating that STIM and ORAI proteins could be useful targets for different types of cancers. The specific types of Ca^{2+} 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.

Figure legend

Table 1 | STIMs and ORAIs functions in hallmarks of cancer.

Green arrows represent increasing hallmarks effect while red arrows represent decreasing hallmarks effect. "–" sign represent no role assigned to the studied proteins in particular hallmarks

Figure 1 | STIMs and ORAIs 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 corresponding mechanism involved are marked in the cartoons. All the ten hallmarks as described by Hanahan and Weinberg (44) are reported. "?" represent specific hallmark for which the role for ORAI1 and STIM1 has not yet been described.

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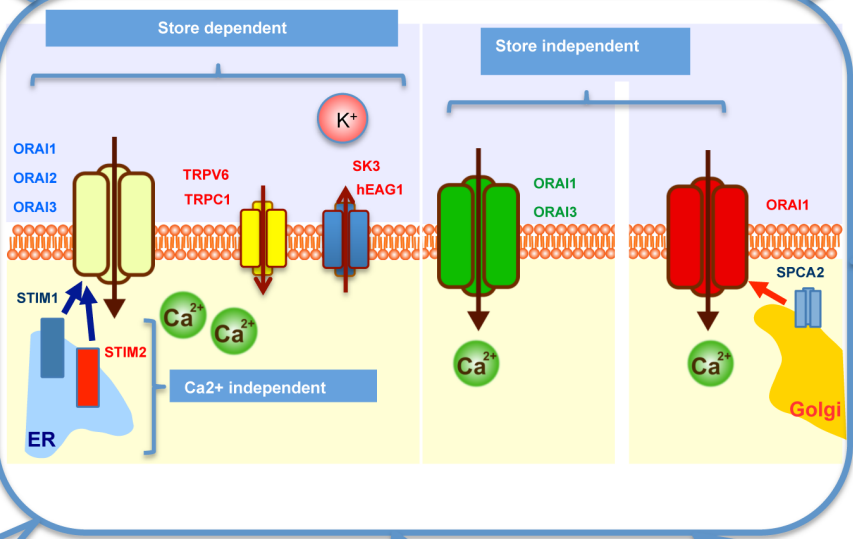
Sustaining proliferative signalling/ Evading growth suppressor
- Store dependent
- Store independent

Avoiding immune destruction
- Store dependent

Enabling replicative immortality
- Store dependent

Deregulating cellular energetics
- ?



Tumor Promoting Inflammation
- ?

Resisting cell death
- Store dependent

Genome instability
- ?

Inducing angiogenesis
- Store dependent
- Ca2+ independent
- Store independent ?

Activating invasion and metastasis
- Store dependent

	Sustaining proliferative signals	Resisting Cell Death/ Enabling Replicative Immortality	Inducing Angiogenesis	Activating Invasion and Metastasis	Evading Immune Destruction
Cervical cancer	STIM1/ORAI1 (18) ↑		STIM1/ORAI1 (18) ↑	STIM1/ORAI1 (18) ↑	
Glioblastoma	STIM1/ORAI1 (54, 58) ↑			STIM1/ORAI1 (68, 114) ↑	
Hypopharingeal canrcinoma	STIM1/ORAI1 (95) ↑				
Nasopharingeal canrcinoma				STIM1/ORAI1 (110) ↑	
Renal Cell Carcinoma (RCC)	STIM1/ORAI1 (50) ↑ STIM1/ORAI1 (22) –			STIM1/ORAI1 (50) ↑	
Breast cancer	ORAI1/SPCA2 (29) ↑ ORAI3 (24,25, 69) ↑			STIM1/ORAI1 (109, 21) ↑ STIM1/ORAI1/ hEag1 (42) ↑ STIM1/ORAI1/ SK3 (17) ↑	
Hepatoma cells	STIM1/ORAI1 (12) ↑			STIM1/ORAI1 (108) ↑	
Lung cancer	ORAI3 (6) ↑	STIM1/ORAI1 (54) ↑			
Prostate cancer	ORAI3 (23) ↑	STIM1/ORAI1 (36, 23, 84) ↓ enabling replic. immortality STIM1/ORAI1 (107) ↑			
Melanoma	STIM2 (94) ↓	STIM1/ORAI1 (27, 28) ↑		STIM2/ORAI1 (94) ↑	STIM1/STIM2 (104) ↓
Colorectal carcinoma	STIM2 (93) ↓	STIM2 (93) ↓			
Ovary carcinoma		STIM1/ORAI1 (87) ↑			
Pancreas Adenocarcinoma (PDAC)		STIM1/ORAI1 (51) ↑			
Leukemic T-cells		ORAI1 (49) ↑			
Endothelial Cells/ Endothelial Cell Precursosrs			STIM1/ORAI1 (1,56, 22, 59, 101) ↑ STIM1 (4, 88) ↑ ORAI3 (55) ↑		

Table 1 | STIMs and ORAIs functions in hallmarks of cancer.
Green arrows represent increasing hallmarks effect while red arrows represent decreasing hallmarks effect. – sign represent no role assigned to the studied proteins in particular hallmarks

STIMs and ORAIs proteins: crucial roles in hallmarks of cancer

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Running title: STIM and ORAI in cancer
Keywords: STIM, ORAI, Ca2+, cancer

Abstract

Intracellular Ca^{2+} signals play a central role in several cellular processes; therefore it is not surprising that altered Ca^{2+} 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 Ca^{2+} entry both in store-dependent (SOCE mechanism) or independent by intracellular store depletion and have been implicated in several cellular functions. In the last years both STIMs and ORAIs have emerged as possible molecular targets for cancer therapeutics. In this review we focus on the role of STIMs and ORAIs protein in cancer progression. In particular we analyse their role in the different hallmarks of cancer, which represent the organizing principle that describe the complex multistep process of neoplastic diseases.

Introduction

Changes in the cytosolic free Ca^{2+} 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 Ca^{2+} controls so many vital processes, disturbance of the Ca^{2+} homeostasis regulatory mechanisms leads to a vast variety of severe pathologies, including cancer. Indeed, the role of Ca^{2+} is well-established in many cell signalling pathways involved in carcinogenesis (65, 66, 81) .

The most common mechanism of Ca^{2+} signal generation results from the activation of plasma membrane G protein-coupled receptors (GPCRs) or tyrosine kinase receptors followed by PLC-mediated cleavage of phosphatidylinositol 4,5-bisphosphate (PIP2) into DAG and IP3 (90). DAG is a known PKC activator, but can also play a PKC-independent role in regulating Ca^{2+} signal generation. IP3 production results in cytoplasmic Ca^{2+} elevation that can be separated into two distinct phases. At first, IP3 activates ER-localized IP3Rs, thus releasing calcium from the ER into the cytosol. Second, the decrease in ER Ca^{2+} content (following IP3R activation) stimulates influx of extracellular Ca^{2+} via opening of plasma membrane Ca^{2+} channels in a process known as capacitative or store-operated Ca^{2+} 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 (3, 77) as well as in a number of pathological processes typical for cancer (for a review see also (9, 79).

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

STIMs and ORAIs proteins

Store-operated calcium channels (SOCs) represent one of the major calcium-entry pathways in non-excitable cells and are widely distributed in various cell types. SOCs are plasma membrane ion channels activated in response to ER Ca^{2+} store depletion and thereby provide Ca^{2+} for ER store refilling as well as for signalling purposes (71, 90). The major molecular components of SOC are stromal interaction molecule 1 (STIM1) and ORAI1 proteins, where ORAI1 constitutes plasma

membrane calcium channel and STIM1 represents mostly ER-localized single-transmembrane domain protein, functioning as a sensor of ER calcium. Following ER Ca^{2+}-depletion STIM1 translocate 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 N-terminal domain in the ER lumen, a single transmembrane segment, and a C-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 Kd of STIM2 for Ca^{2+} (~400 μM) is 2-fold higher than that of STIM1 (Kd ~200 μM) (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 has 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-PM junctions at low stimulus intensities, when ER Ca^{2+} stores are mildly depleted, thus increasing the sensitivity of Ca^{2+} signalling to agonists (70).

ORAI1 is the founding member of 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 N- and C- 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 activate 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 HEK293 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 (called Leukotriene C4-regulated calcium (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's not clear which STIM1 pool (ER or PM) 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 agonists/pathways and are important players in store-independent calcium entry (112).

Sustaining Proliferative Signalling / 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 have the ability to carefully regulate growth signals in order to maintain the proper cellular homeostasis required to organize the correct function of distinct organs. However, cancer cells dysregulate these mechanisms in order to sustain uncontrolled proliferation.

In this regard, it is known that regulated Ca^{2+} toolkit undergoes profound remodelling in cancer cells to favour activation of Ca^{2+}-dependent transcription factors, such as the nuclear factor of activated T cells (NFAT), c-Myc, c-Jun, 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 STIM1-ORAI1 has been extensively implicated in cancer progression in both native cancer cells as well in cells extracted from different tumours such as hepatoma, breast, prostate, glioblastoma, cervical cancer, colorectal cancer or renal cell carcinoma (RCC) (48, 102). Several groups have reported that STIM1-ORAI1 function as positive regulators of cell proliferation. STIM1 and ORAI1 knockdown, together with TRPC6, decrease indeed the protein levels of cyclin D1 in a hepatoma cell line (12). Chen et al. investigated in details the role of STIM1 and SOCE in cervical cancer, using both in vitro as well as in vivo models (18). 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 analysed the proliferative role of STIM1 in vitro. In this regard, STIM1 silencing in cervical cancer cell lines significantly inhibited cell proliferation by arresting 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 dependent on Ca^{2+} homeostasis. p21 upregulation was probably due to STIM1-dependent post translational regulation, implicating the control of the proteosome-dependent pathway responsible of p21 degradation process. In contrast, STIM1-mediated Cdc25C expression was regulated at 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. reported an increase expression level of ORAI1, but not STIM1 or ORAI3 in RCC: pharmacological inhibition of SOCE or ORAI1 or STIM1 downregulation, significantly impaired cell proliferation as well as cell migration of RCC Caki1 cell lines (50). However this data are a bit controversial and was not confirmed by Dragoni and colleagues who could not find any role for SOCE in cell proliferation of freshly isolated RCC metastatic cells from patients (22).

On the other hand a number of important "non classical" events involving other members of STIM and ORAI family has been proposed as key mediators of cancer cell proliferation. Indeed Feng et al. demonstrated that a store-independent ORAI1-mediated Ca^{2+} influx is critical for breast cancer cell proliferation which was dependent on an isoform of Secretory Pathway Ca^{2+}-ATPase, SPCA2 pump, upregulated in breast cancer-derived cells and human breast tumours. Based on the function of a series of chimeras and mutant proteins, the authors proposed a model in which cooperation of N- and C-termini of SPCA2 is required for ORAI1-mediated Ca^{2+} signalling. Whereas the N-terminus of SPCA2 binds strongly to ORAI1, the C-terminus elicits activation of Ca^{2+} influx, which is completely independent from SPCA2 Ca^{2+} pump activity as well as from STIM1 (29).

In regards to other ORAI proteins, another important role in cell proliferation is also mediated by ORAI3, which is overexpressed in different cancers such as breast and prostate cancers (23, 24). As far as breast cancer is concerned, several groups established that G1 progression and G1/S transition phases are dependent on ORAI3- 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, p21 and p53 through regulating the expression and the 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 ORAI3-dependent SOCE-activation in the MCF7 cell line (69). Interestingly no effect of ORAI3 was observed in the normal cancer cell lines MCF10-A or ER- MDA-MB231 or in ER silenced MCF7 cells, while ORAI3 introduction into MCF7 cells depleted of ER rescued SOCE activated by thapsigargin. The ORAI3 role has been also demonstrated in vivo in xenograft scid mice (24, 69). Similarly to breast cancer, recently Ay et al. Reported recently that ORAI3 expression was up-regulated in lung cancer tissues, correlating with high tumour grade, and the ORAI3- mediated Ca^{2+} entry was crucial to lung cancer cell proliferation (6). As far as prostate cancer, we recently demonstrated the role of ORAI3 in prostate cancer cells proliferation in vitro. We showed that enhanced ORAI3 expression favours heteromerization with ORAI1 to form a novel channel. The ORAI3-ORAI1 heterotetramer supports store-independent Ca^{2+} entry, driven by arachidonic acid (AA) thereby promoting cell proliferation and changing the equilibrium from functional homomeric

ORAI1-based store-operated channels, which are important in supporting susceptibility to apoptosis (23). It has to be noticed that in this particular cellular context, AA-mediated activation ORAI3-ORAI1 heterotetramer has similar properties to those described previously for ARC (61, 64). However, in PCa our results clearly show that STIM1 is not implicated in AA-mediated Ca^{2+} signalling. For these reasons, we maintained the term ‘‘heteromeric association of ORAI1/ORAI3’’ instead of ARC. Moreover our results show that the signalling pathway activated downstream of AA-activated ORAI3 channels involves a Ca^{2+}/calcineurin-dependent transcription factor, NFAT, followed by the stimulation of the expression of the key rate-limiting controller of G1/S phase transition, cyclin D1 (23). Finally by means of xenograft tumour models we identified a key role of ORAI3 in prostate cancer. Indeed treatment of xenograft mice with siORAI3 significantly reduced tumour growth while on the contrary ovexpression of ORAI3 promoted an increase of tumour volume *in vivo (23)*. We can therefore speculate that manipulating ORAI3 could simultaneously act on two different hallmarks of cancer in prostate cancer by shifting the ORAI1 channels composition from homo to ORAI1-ORA3 heteromutlimer 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 anti-proliferative protein both in melanomas as well as colorectal tumours (7, 93, 94). Interestingly in humans STIM2 is located at the short arm of chromosome 4, in 4p15.2 where loss of heterozygosis 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 it has bee described an intriguing role for STIM2-ORAI1 expression in the switch from a more proliferative to a more migratory phenotype and vice versa (94). While higher expression levels of both Orai1 and STIM2 lead to increased basal $[Ca^{2+}]i$ and consequently higher invasive potential, reduction in their expression levels decreases the basal $[Ca^{2+}]i$ and causes enhanced melanoma growth. The data nicely correlates with *in vivo* data on paraffin-embedded human melanoma samples: double MITF (proliferative marker for melanoma) and Orai1 or STIM2 IHC staining depict higher expression of MITF in central tumour areas, while Orai1 and STIM2 were more prominent in the invasive rims (94).

Taking together all the data presented above it is clear that STIM1, ORAI1 and ORAI3-mediated Ca^{2+} signals represent proliferative stimuli for different cancer types. These proteins act act via different mechanisms that are represented by classical STIM1-ORAI1-mediated SOCE to store independent unconventional ORAI1 to AA-activated ORAI3. In contrast STIM2 seems to have an anti proliferative role, at least for melanoma and colorectal cancer.

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. Also, it has become evident that several tumours 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 anti-apoptotic functions depending on several factors such as the cancer type, apoptotic stimuli or intracellular signalling (52). Indeed, ORAI1 contribute 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 TG- or oxaliplatin/cisplatin-induced apoptosis (36). In the 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 the recent data by us showing that PC3 with reduced SOCE demonstrated lower sensitivity to the TG-induced apoptosis (23). Consistent with this, pharmacological SOCE inhibition or STIM1 knockdown have 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).

Beside the role in apoptosis, Xu et al. recently proposed a role for of STIM1 and ORAI1 in the mechanism of senescence in prostate cancer cells (107), 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 pro-apoptotic role in prostate cancer just summarized, pharmacological inhibition of SOCE or downregulation of STIM1 have 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 anti-apoptotic role in these cells (87). A complex interplay of mitochondria and STIM1 dependent SOCE-mediated Ca^{2+} signalling 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 a anti apoptotic role for ORAI1, and with less extent STIM1, in pancreatic adenocarcinoma cell line following chemotherapic 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 pro-apoptotic in colorectal cancer. As previously stated, Sobradillo et al. described a down regulation of STIM2 in colon carcinoma cancer cell as compared with normal epithelial cells. In addition, in colorectal carcinoma STIM2 downregulation confers an apoptotic-resistance to normal cells (93). These data are in agreement with previously reported role for STIM2 and tumour 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, STIMs and ORAIs protein may interact with other channels to generate different responses. As an example, we recently demonstrated that overexpression of 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 if their modulators could be effective in cancer treatment in each particular case.

Inducing Angiogenesis

Blood vessels supply oxygen and nutrients to tumours and provide gateways for immune surveillance needed for solid tumour 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 (EC) line the inner surface of vessels to support tissue growth and repair. Beside providing metabolic support, vessels are also used as routes for tumour cells to metastasize (16, 75).

Although the induction of angiogenesis may initially provide the tumour 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, tumour vasculature is highly disorganized; vessels are tortuous and dilated, with uneven diameter, excessive branching and shunts. Consequently, tumour blood flow is chaotic and variable and leads to hypoxic and acidic regions in tumours (15, 105). It is now well established that tumour-derived endothelial cells (TECs) of different origins, and normal ECs are highly heterogeneous at genetic, epigenetic and functional levels (14). Moreover, TEC-mediated intracellular signalling is quite different from that observed in normal human microvascular EC. Interestingly, proangiogenic Ca^{2+} signals and their related pathways are significantly altered in TEC compared with normal EC (33–35, 82).

Intracellular Ca^{2+} signals are involved at different critical phases in the regulation of the complex process of vascularization and tumour 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 intracellular Ca^{2+} ($[Ca^{2+}]i$) increase in ECs and trigger complex intracellular cascades (32, 34). Abdulaev et al. 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 HUVEC (human umbilical vein endothelial cells) (1). 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. 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 tubule 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 EC has been questioned by Antigny et al.; although they described STIM1 as a key player (together with TRPC1 and TRPC4) in tube formation in both HUVEC and EA.hy926 cells (4). Recently, a very detailed and elegant study by Tsai and co-workers proposed an integrated model of the spatial organization of the STIM1 signalling complex, which generate a

coordinated Ca^{2+} control system that dynamically controls the polarization and persistence of migration as well as local adhesion and turning of EC, together with the actin regulators Rac, Cdc42, RhoA and PtdIns (3;4;5)P3 (101). 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) signalling. The resulting polarized SOC signalling provides a key mechanism to maintain the spatial and temporal dynamics of the Ca^{2+} signalling system (101).

Beside ORAI1-STIM1, a study from the Beech group recently suggested a role for ORAI3 in VEGF-induces EC remodelling with a consequent role for *in vitro* and *in vivo* tubulogenesis (55). Although the mechanism was not thoroughly analysed, they propose a Ca^{2+} release-dependent mechanism for activation of this ORAI3 system in which VEGF-mediated Ca^{2+} release activate cPLA2α to catalyse the production of AA which in turn activate ORAI3 by exposing it to the surface membrane (55). Further studies will be needed to better understand the reciprocal regulation between the "classical" ORA1-STIM1 SOCE activation and ORAI3 as previously showed 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 is unrelated to Orai1 and Ca^{2+} entry (88). At the same time Sundivakkam et al reported a novel phosphorylation mechanism of STIM1 involving AMPK/p38 MAPK pathway responsible of inhibiting SOCE in lung microvascular EC. This effect is responsible for regulating the thrombin-mediated permeability responses which are SOCE mediated (96).

These data strongly suggest the role for the "classical" STIM-ORAI1-mediated SOCE in EC 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 signalling 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 represent 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 STIMs and ORAIs proteins in this particular hallmark of cancer has been extensively studied and important progress in understanding the molecular mechanisms has been achieved in the last 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 migration rate of MDA-MB-231 cells while silencing reverted the effect (109). The authors of this study dissected partially the molecular 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, similar pro-invasive 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 tumour biopsies as well as in commercially available human glioma cell lines (68, 114). In particular, Zhu et al. showed ORAI1-mediated regulation of Pyk2 phosphorylation by the Ca^{2+} dependent calpain, which is considered essential for focal adhesion turnover and epithelial to mesenchimal transition of cancer cells (114). These data confirm therefore the role of SOCE in tumour 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 be also 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 remodelling of endoplasmic reticulum morphology, which is in turn mediating intracellular processes such as cell polarization and migration (2, 38). Moreover, Chen et al recently showed that microtubule associated Histone Deacetylase6 (HDAC6) is required for SOCE activation by optimizing the localization of the endoplasmic reticulum Ca^{2+} sensor STIM1 toward plasma membrane (19). Interestingly, the pro-migratory 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 important role in melanoma cell migration. In particular as reported above, Staniz et al. propose that STIM2-ORAI1-mediated SOCE expression switches the phenotype of melanocytes from proliferative to migratory (94). The data therefore show that STIM2 can act both as a tumour suppressor in highly proliferative cells (by increasing basal Ca^{2+} to a level where it inhibits proliferation) as well as a tumour promoter in invasive cancers where the increased basal $[Ca^{2+}]i$ results in a more invasive phenotype (94).

Beside the classical STIM1-ORAI1 mediated SOCE activation, several groups reported recently the role of K^+ channels-ORAI1 interaction to sustain breast cancer cells migration. A first study correlated Ether a` go-go (hEag1) K^+ Channels as relevant player in controlling Ca^{2+} entry through ORAI1 channels and consequent cell migration (42). Subsequently, Chantome et al. revealed a novel signalling 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+}-signalling that promoted breast cancer cell migration and bone metastasis formation. The functional complex is localized in the lipid rafts 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 pro-invasive 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 STIMs and ORAIs proteins in the immune system in the context of cancer development and progression since this has been classified as emerging hallmarks of cancer (44).

Infiltration of immune cells in tumour tissues has been long recognized by pathologists. The presence of this infiltration has been explained by the attempt of the immune system to eradicate tumours. Indeed, increasing evidence in the past few years show 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 tumour incidence (99). In addition it is well established that STIM1-ORAI1-mediated CRAC represent the predominant Ca^{2+} influx mechanism in lymphocytes (76). Moreover mutations and loss of function or mutations in STIM1 in T cells gene inhibits 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 tumour immunosurvaillance 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 tumour melanoma cells allografts while STIM1 and STIM2 deficient CTLs fail to prevent tumour 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-g and TNF-a, to release perforin-containing cytolytic granules, induce FasL and kill tumour cells (104).

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

Conclusions

The data presented in the present review summarized the current literature on STIMs and ORAIs role in the different hallmarks of cancer. In the latest version, Hanhan and Weinberg presented ten different hallmarks (despite the six considered in the first review) including new ones emerging in the last years (44). As shown in Fig1, 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 ORAIs and STIM in the remaining hallmarks ("Tumour promoting inflammation", "Genome Instability" and "Deregulating cellular energetics", marked as "?" in Fig 1).

As shown in table 1 and Fig 1, a clear role can be proposed for STIM1-ORAI1 and ORAI3 as promoters of sustaining proliferative signals while STIM2 seems to account for anti-proliferative signals. In addition, the "classical" STIM1-ORAI1-mediated SOCE plays different roles in apoptosis depending on the cancer cell types and apoptotic stimuli. While STIM1-ORAI1 mediated SOCE is sustaining apoptosis resistance in several cancer models such as lung cancer, melanoma, PDAC 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-ORA3 heteromutlimer, prevents apoptosis on one hand, and promotes uncontrolled cell migration on the other hand (23). Regarding tumour invasion and migration, STIM1-ORAI1 has been shown to mediate a pro-invasive migratory phenotype in all the cancer types analysed till know. Furthermore, increasing evidence unanimously point to a pro-

angiogenic role of "classical" STIM1-ORAI1 SOCE. It is clear that more data is necessary more data would be necessary to better integrate the molecular mechanism of the proangiogenic effect in the different types of cancers. Most of the data presented are infact 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 contribution. These data are very important since STIM1 and ORAI1 proteins constitute possible targets to increase inflammatory responses and cancer immunosurvaillance.

When analysing the overall role of STIMs and ORAIs protein in cancer, we finally need to consider their emerging interaction with other transportome proteins such as Ca^{2+} pumps, K^{+} channels or TRP channels. These interactions clearly affect the role of STIMs and ORAIs proteins towards the different hallmarks of cancer.

In conclusions there is increasing evidence indicating that STIM and ORAI proteins could be useful targets for different types of cancers. The specific types of Ca^{2+} 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.

Figure legend

Table 1 | STIMs and ORAIs functions in hallmarks of cancer.

Green arrows represent increasing hallmarks effect while red arrows represent decreasing hallmarks effect. "–" sign represent no role assigned to the studied proteins in particular hallmarks

Figure 1 | STIMs and ORAIs 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 corresponding mechanism involved are marked in the cartoons. All the ten hallmarks as described by Hanahan and Weinberg (44) are reported. "?" represent specific hallmark for which the role for ORAI1 and STIM1 has not yet been described.

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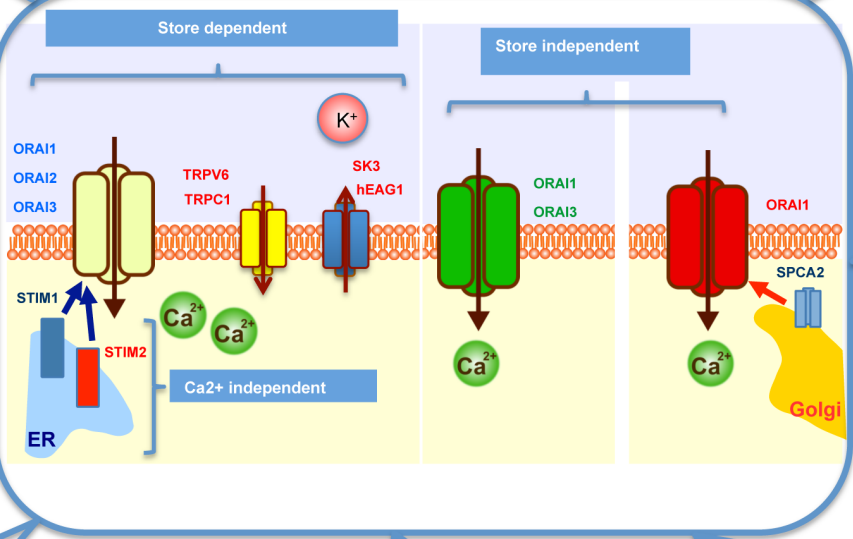
Sustaining proliferative signalling/ Evading growth suppressor
- Store dependent
- Store independent

Avoiding immune destruction
- Store dependent

Enabling replicative immortality
- Store dependent

Deregulating cellular energetics
- ?



Tumor Promoting Inflammation
- ?

Resisting cell death
- Store dependent

Genome instability
- ?

Inducing angiogenesis
- Store dependent
- Ca2+ independent
- Store independent ?

Activating invasion and metastasis
- Store dependent

	Sustaining proliferative signals	Resisting Cell Death/ Enabling Replicative Immortality	Inducing Angiogenesis	Activating Invasion and Metastasis	Evading Immune Destruction
Cervical cancer	STIM1/ORAI1 (18) ↑		STIM1/ORAI1 (18) ↑	STIM1/ORAI1 (18) ↑	
Glioblastoma	STIM1/ORAI1 (54, 58) ↑			STIM1/ORAI1 (68, 114) ↑	
Hypopharingeal canrcinoma	STIM1/ORAI1 (95) ↑				
Nasopharingeal canrcinoma				STIM1/ORAI1 (110) ↑	
Renal Cell Carcinoma (RCC)	STIM1/ORAI1 (50) ↑ STIM1/ORAI1 (22) –			STIM1/ORAI1 (50) ↑	
Breast cancer	ORAI1/SPCA2 (29) ↑ ORAI3 (24,25, 69) ↑			STIM1/ORAI1 (109, 21) ↑ STIM1/ORAI1/ hEag1 (42) ↑ STIM1/ORAI1/ SK3 (17) ↑	
Hepatoma cells	STIM1/ORAI1 (12) ↑			STIM1/ORAI1 (108) ↑	
Lung cancer	ORAI3 (6) ↑	STIM1/ORAI1 (54) ↑			
Prostate cancer	ORAI3 (23) ↑	STIM1/ORAI1 (36, 23, 84) ↓ enabling replic. immortality STIM1/ORAI1 (107) ↑			
Melanoma	STIM2 (94) ↓	STIM1/ORAI1 (27, 28) ↑		STIM2/ORAI1 (94) ↑	STIM1/STIM2 (104) ↓
Colorectal carcinoma	STIM2 (93) ↓	STIM2 (93) ↓			
Ovary carcinoma		STIM1/ORAI1 (87) ↑			
Pancreas Adenocarcinoma (PDAC)		STIM1/ORAI1 (51) ↑			
Leukemic T-cells		ORAI1 (49) ↑			
Endothelial Cells/ Endothelial Cell Precursosrs			STIM1/ORAI1 (1,56, 22, 59, 101) ↑ STIM1 (4, 88) ↑ ORAI3 (55) ↑		

Table 1 | STIMs and ORAIs functions in hallmarks of cancer.
Green arrows represent increasing hallmarks effect while red arrows represent decreasing hallmarks effect. – sign represent no role assigned to the studied proteins in particular hallmarks