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STIM and Orai in cellular proliferation and division

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1. ABSTRACT

Cellular proliferation and division are central processes in the development, survival and evolution of living systems. Transitioning into the cell division phase of the cell cycle encompasses dramatic remodeling of cellular organelles and signaling modules including Ca^{2+} signaling. As well, Ca^{2+} signals play important roles during progression through various stages of the cell cycle. A ubiquitous Ca^{2+} influx pathway that is activated based on intracellular Ca^{2+} store content is store-operated Ca^{2+} entry (SOCE). SOCE is activated through a complex interplay between a Ca^{2+} channel at the cell membrane, Orai1, and a Ca^{2+} sensor that localizes to the endoplasmic reticulum, STIM1. Herein, we discuss potential roles and regulation of STIM and Orai proteins during cellular proliferation.

2. CA^{2+} SIGNALING AND CELLULAR PROLIFERATION

Cellular proliferation depends on progression through the growth and division phases of the cell cycle. Typically, the cell cycle is divided into four phases starting with the first growth phase (G1) during which cells grow and accumulate components required for the ensuing DNA duplication phase (S-phase). Terminally differentiated cells exit the G1-phase to arrest in a so called G0 phase. Following the DNA duplication phase, cells go through a second growth phase, G2, before entering the short but dramatic division phase, M-phase. This results in the production of two daughter cells with an equivalent cellular organelle and DNA complement allowing the continuation of cellular growth and division in the next generation (1).

For the cell cycle to successfully support cellular proliferation it has to be sequential G1-S-G2-M, that is cells cannot undergo cellular division before having duplicated their DNA as this will result in cellular demise. In addition the cycle is unidirectional to prevent DNA endoreplication for example and ensure cellular survival. To guarantee the sequential and unidirectional nature of the cell cycle critical checkpoints and feedback loops are incorporated in the signaling cascades regulating cell cycle progression. Early embryonic cell cycles in the frog for example deviate from the typical cycle as they lack the growth phases and encompassed only the DNA synthesis and division phase. This is due to the need for rapid cell division at this stage of development, and the fact that macromolecular components required for cell cycle progression are stored in the large egg before fertilization.

Ca^{2+} signals have been implicated at several stages during cell cycle progression (2). Ca^{2+} is a fitting second messenger in that context given the versatility and specificity it affords based on its spatial, temporal and amplitudes dynamics (3; 4). Ca^{2+} signals have been shown to be important for nuclear envelope breakdown during prophase, DNA condensation, anaphase onset, and cytokinesis (5-12). Notably some interesting differences were documented in terms of the role of Ca^{2+} signals between the mitotic and meiotic cell cycles, including the requirement for Ca^{2+} signals during the breakdown of the nuclear envelope. In contrast to mitosis, Ca^{2+} signals are dispensable for nuclear envelope breakdown during meiosis (13; 14).

These dependencies on Ca^{2+} signals at various stages of the cell cycle have been translated to downstream Ca^{2+} signaling modules including calmodulin (CaM), Ca^{2+}-CaM dependent protein kinase II (CaMKII) and calcineurin among others. Progression through the cell cycle is associated with alterations in the levels of CaM expression with an increase during the G1-S transition (15; 16). Furthermore experimental manipulation of CaM levels affects progression through the G1 and M-phases, interferes with DNA replication and influences cellular growth and proliferation (17-20). CaM and extracellular Ca^{2+} levels have been linked to the cell cycle machinery through the phosphorylation state of Rb (21). Rb is a tumor suppressor that is phosphorylated by cyclin-dependent kinases Cdk4 and Cdk2 leading to its dissociation from E2F and the induction of genes required for the G1-S transition (22). Consistent with the effects of CaM on the cell cycle, inhibition of CaMKII results in defects in the G2-M and G1 phases (23). Furthermore, expression of a constitutively active CaMKII arrests the cell cycle in G2 (24). In yeast mutants defective in CaM exhibit defects in progression through mitosis (25-28).

The most clearly defined function for Ca^{2+} signal during cell cycle transitions is following fertilization of vertebrate eggs. Following maturation vertebrate eggs arrest at metaphase of meiosis II until fertilization. This arrest is mediated by cytostatic factor (CSF) which maintains Cdk1 activity at high levels and thus prevents the activation of the anaphase-promoting complex (APC/C) and the transition to anaphase (29). Fertilization leads to dramatic Ca^{2+} transient that activates CaMKII (30). CaMKII in turn phosphorylates the APC/C inhibitor Emi2 (31-33). Emi2 phosphorylated by CaMKII becomes a substrate for phosphorylation by polo-like kinase. Dual phosphorylation of Emi2 targets it for degradation by the proteasome thus releasing APC/C inhibition and releasing the metaphase II arrest (34).

The Ca^{2+}-CaM-dependent phosphatase calcineurin has also been implicated in the release of the metaphase II arrest and the activation of the anaphase promoting complex/cyclosome (APC/C) during the meiotic cell cycle (35; 36). Calcineurin inhibition has also been reported to arrest the cell cycle in G1 (37). Furthermore, calcineurin play a central role in the resumption of the cell cycle following antigen stimulation in T-cells. Antigen stimulation activates Ca^{2+} influx through SOCE leading to a sustained transient which activates the phosphatase calcineurin (38). Calcineurin dephosphorylate the transcription factor NFAT leading to its translocation to the nucleus and the transcription of genes required for T-cell clonal expansion.

Together these effects show that Ca^{2+} signaling is pervasive during the cell cycle, through downstream Ca^{2+}-dependent signaling modules that regulate various aspects of cell cycle transition and cellular proliferation. This raises interesting questions about how specificity is achieved to modulate different aspects of the cell cycle through Ca^{2+} signals. Are specific Ca^{2+} signaling pathways implicated differentially during different stages of the cycle? Is SOCE involved and does it play defined roles in cellular proliferation?

3. DIVERSITY OF Ca^{2+} SIGNALING

As discussed above, Ca^{2+} signals and downstream Ca^{2+}-dependent effectors play important roles during cell proliferation, however it seems that the dependency on Ca^{2+} signals is defined in a cell type and developmental stage specific fashion (2; 39). Ca^{2+} signals maintain specificity despite their ubiquitous nature and involvement in disparate cellular behaviors often in the same cell. The attribute of Ca^{2+} signals that endow them with such versatility is the specific signature encoded in their spatial, temporal and amplitude dynamics (3; 40). This Ca^{2+} code, coupled to the affinity and association and dissociation constants of downstream Ca^{2+} binding proteins allows Ca^{2+} signals to activate distinct signal transduction pathways based on the need of the cell. Spatially, Ca^{2+} signals can localize to within a few nanometer at the mouth of a channel, and as such activate Ca^{2+}-binding proteins in the immediate vicinity of the channel (41). Alternatively Ca^{2+} waves can sweep through the entire cell or groups of coupled cells (42). Similarly, temporally Ca^{2+} signals can be very short lived on the order of μseconds during vesicular exocytosis, or they can persist for quite a long time as illustrated by the Ca^{2+} oscillations that last up to several hours after fertilization in mammals (43).

The regulation of Ca^{2+} signaling depends on different channels, transporters and pumps that localize to

the cell, mitochondria and ER membranes. The ER constitutes the primary intracellular Ca^{2+} storage organelle. Free Ca^{2+} is highest in the extracellular space (1-2mM) and ranges between 250-600 μM in the lumen of the ER (44). In the cytoplasm Ca^{2+} is maintained at low levels ~100nM to allow signaling to occur. In effect, the extracellular and intracellular Ca^{2+} pools are functionally linked through the store-operated Ca^{2+} entry (SOCE) pathway. Depletion of Ca^{2+} stores following agonist-induced Ca^{2+} release, results in Ca^{2+} influx at the cell membrane. Several of these Ca^{2+} signaling pathway could be differentially involved in generating the Ca^{2+} signals required for progression through different stages of the cell cycle. For example, Ca^{2+} release through the IP_3 receptor has been exclusively implicated in the resumption of the meiotic cell cycle during fertilization of the frog egg (45). In the following sections we discuss what is currently known about the role of SOCE during cellular development and proliferation.

4. STORE-OPERATED CA^{2+} ENTRY (SOCE)

SOCE represents a ubiquitous Ca^{2+} influx pathway that is pronounced in non-excitable but also present in excitable cells such as skeletal muscle (46; 47). SOCE is activated following store depletion in response to activation of G-protein or tyrosine kinase coupled receptors, which activate PLC resulting in inositol 1,4,5 trisphosphate ($InsP_3$) production leading to ER Ca^{2+} release. SOCE is not due to Ca^{2+} release *per se* or other PLC-dependent downstream messengers, since it can be induced by store depletion mechanisms that are PLC-independent such as inhibition of endoplasmic reticulum Ca^{2+}-ATPase using thapsigargin (46). SOCE is critical for several physiological functions including activation of immune cells and skeletal muscle development (48). As discussed above, following antigen stimulation of T-cells, Ca^{2+} influx through SOCE is important for T-cell clonal expansion by initiating re-entry into the cell cycle. Antigens crosslink T-cell receptors leading to PLCγ activation and the production of $InsP_3$. $InsP_3$ gates the $InsP_3$-receptor Ca^{2+} channel on the ER membrane, thus releasing Ca^{2+} and inducing store depletion, which stimulate Ca^{2+} influx through SOCE. This produces a sustained Ca^{2+} transient, which is required for calcineurin activation and dephosphorylation of NFAT (nuclear factor of activated T cells) (38; 49).

The molecular players mediating SOCE have been elucidated and studied extensively over the past five years. Large scale RNAi screens identified stromal interaction molecule 1 (STIM1) as the ER Ca^{2+} sensor (50; 51), and Orai1 as the SOCE Ca^{2+} channel at the cell membrane (52-54). STIM1 is a single pass trans-membrane domain protein with a luminal EF-hand allowing it to detect ER Ca^{2+} content. Orai1 is an integral membrane protein with four trans-membrane domains and cytoplasmic N- and C-termini. Co-expression of STIM1 and Orai1 replicates the biophysical properties of Ca^{2+}-release activated Ca^{2+} current (I_{CRAC}), which is the best characterized SOCE current biophysically (54-56). Furthermore, mutations of glutamate residues in the first and second trans-membrane domains confirm that Orai1 lines the SOCE channel pore as they alter SOCE current selectivity and permeation (57-59). Finally, mutations in STIM1 and Orai1 in human patients and knock-out strains of either protein in mice abrogate SOCE in cells of the immune system and other cells in the body (48; 52). Besides STIM1 and Orai1, mammalian genomes encode an additional STIM homologue, STIM2, and two additional Orai genes, Orai2 and 3 (60; 61). In addition to their roles in SOCE, STIM and Orai proteins are involved in other Ca^{2+} signaling pathways such as the arachidonate-regulated Ca^{2+} channel (ARC) (62; 63). STIM1 has also been implicated in the regulation of TRP channels (64).

Structure-function studies on STIM1 and Orai1 have greatly improved our understanding of the coupling mechanism between Ca^{2+} store depletion and Ca^{2+} influx at the cell membrane. We will briefly summarize STIM1-Orai1 coupling here since this topic is addressed in significant details in other reviews in this issue. When lumenal Ca^{2+} levels in the ER fall below a certain threshold Ca^{2+} dissociates from the STIM1 EF-hand inducing a conformational change in the protein that results in its clustering. Large STIM1 clusters, referred to as puncta, are stabilized in a cortical ER domain within a few nanometers of the cell membrane where they directly interact with Orai1 leading to its co-clustering and gating (53; 57; 59; 65-69). Therefore direct physical coupling between STIM1 -the ER Ca^{2+} sensor- and Orai1 -the Ca^{2+} channel at the cell membrane- results in functional coupling between Ca^{2+} levels in the ER lumen and Ca^{2+} influx. In addition to its coupling to Orai1, STIM1 also interacts with and gates members of the TRPC channel family (64; 70; 71), which may play a role in cellular proliferation.

5. STIM1 AND ORAI1 DURING THE CELL CYCLE

It has been know for many years that soce inactivates during the division phase of the cell cycle (72). That is in both mitosis of mammalian cells and meiosis of frog oocytes, store depletion does not activate Ca^{2+} influx (73-76). Recent studies have shown that uncoupling of store depletion from Ca^{2+} influx during M-phase is due to the inability of STIM1 to cluster in response to depletion of Ca^{2+} stores (77; 78), and to internalization of Orai1 (77; 79). Orai1 is removed from the cell membrane and becomes enriched in endosomes during *Xenopus* oocyte meiosis. This occurs by targeting Orai1 for internalization through a caveolin and Rab5-dependent endocytic pathway (79). Combined STIM1 clustering inhibition and Orai1 internalization uncouple Ca^{2+} store depletion from Orai1 gating, thus inactivating soce. This is the only known physiological situation where soce is inhibited arguing for an important functional role. soce inactivation during cell division may reflect the tight regulation of Ca^{2+} signaling necessary to ensure proper transition through M-phase. Cell division encompasses dramatic changes to the cell, including the breakdown of the nuclear envelope, chromosome condensation, fragmentation of the Golgi apparatus and remodeling of the cell's cytoskeleton. As discussed above Ca^{2+} signals have been implicated during various stages of cell division. Hence, soce inactivation may be a mechanism to prevent unwanted Ca^{2+} influx,

which could derail Ca^{2+}-dependent processes during critical stages of M-phase. For the *Xenopus* egg sporadic Ca^{2+} influx through soce is likely to lead to egg activation prior to fertilization, which will result in its demise. The fully mature *Xenopus* egg is arrested at metaphase of meiosis II and is activated by a sweeping Ca^{2+} wave following sperm fusion (45; 80). A localized Ca^{2+} transient, as would be mediated by soce activation, is prone to produce a sweeping Ca^{2+} wave that will activate the egg, including the completion of meiosis in the absence of sperm (81). In fact simply pricking the egg in Ca^{2+} containing medium is sufficient to activate it (82).

In addition to soce other Ca^{2+} signaling pathways are also remodeled during cell division, which reflects specific signaling requirements during M-phase. Inositol (1,4,5)-trisphosphate ($InsP_3$)-dependent Ca^{2+} release is sensitized during both meiosis (83-85) and mitosis (86); and the plasma-membrane Ca^{2+}-ATPase is internalized during meiosis (87; 88).

6. STIM AND ORAI LOSS OF FUNCTION IN ANIMAL MODELS AND HUMANS

The identification of STIM1 and Orai1 as the molecular mediators of SOCE allows the analysis of the global function of these proteins. Indeed several whole animal and tissue specific knockout strains have been generated and their phenotypes characterized. The generation of STIM1-KO mice produces animals that mostly die either *in utero* or in the first hours/days of life (89-92), and the few surviving animals have a growth retardation phenotype (89; 93). The cause of the premature death of the animals is not clearly understood yet, with the most obvious phenotype being a respiratory failure in STIM1-KO mice due to severe skeletal muscle dysfunction (89; 91). STIM2-KO mice had a better survival rate (death occurring after 4 to 8 weeks), though they did also exhibit a growth retardation phenotype (90; 93; 94). Whether these growth phenotypes are linked to defects in cellular proliferation is presently not known. Knocking down STIM1 does not significantly affect immune system development, although splenomegaly was reported as well as a lymphoproliferative disease and infiltration of lymphocytes in non lymphoid tissue such as lung and liver. This phenotype is also observed in conditional T-cell restricted STIM1/2 double KO but is not yet fully understood, although it most likely involves the density/function of T_{reg} cells (90; 93).

In the case of Orai1, although Orai1 knockout leads to perinatal death, some mice lacking the channel protein were able to grow providing special breeding or backcrossing conditions but show reduced size, a hair loss phenotype and a deficit in lymphocytic function (95; 96). The proliferation of B-cells following stimulation with an anti-IgM was impaired in $Orai1^{-/-}$ (95). In the case of T-cells the effect of Orai1 deletion does not affect the development of naïve T-cell (96). The phenotype of these knockout lines may not be representative of acute knockdown of Orai1 as expression of other Orai proteins, Orai2 and 3, may complement part of the Orai1 function in lymphocytes and other tissues (95; 96).

The consequences of Orai1 or STIM1 deficiency in humans has been recently reviewed (48; 97). Briefly, patients lacking STIM1 or Orai1 functional genes display a severe immunodeficiency linked, but not restricted, to a reduced T-cell ability to proliferate and to release cytokines. The vulnerability to infections associated with a congenital myopathy strongly limits the survival of patients. In addition, some patients show lymphoproliferative symptoms, a phenotype also observed in STIM1 and STIM1/2 KO mice. Surprisingly, although STIM1 and Orai1 are quite ubiquitously expressed, their absence does not induce a total loss of function in many cell types, suggesting a compensation mechanism involving for instance STIM2 and Orai2 and 3.

Loss of function phenotypes of STIM1 and Orai1 in mice and humans confirm the central role of these proteins in the context of SOCE in the proliferation of immune cells in response to antigen stimulation. They also show that SOCE is not essential for the development and differentiation of immune cells. More detailed analyses of different cell types in these knockout models is warranted to better define the role of STIM and Orai1 in cell cycle progression and cell proliferation.

7. VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS

In vascular smooth muscle cells (VSMC), although SOCE has been recorded for quite some time, the molecular partners contributing to the generation of the calcium current are still a matter of debate. Recently, different strategies aimed at inactivating Orai1 and STIM1 in VSMCs provided new clues to functionally define SOCE in these cells. Knocking down Orai1 but not Orai2 or 3 reduces store-operated calcium influx and current. Moreover, silencing of Orai1 reduces cell proliferation but also cell migration during wound healing in culture (98; 99). Similar results were obtained in cultured VSMCs derived from pulmonary or coronary artery and aorta following the knock-down of STIM1, where both SOCE and VSMCs proliferation were reduced (99-102). The existence of a link between STIM1/Orai1 and cell proliferation is also supported by the finding that proliferating VSMCs have higher expression levels of both proteins (99; 102). Furthermore, siRNA mediated knockdown of STIM1 in VSMC results in G0-G1 arrest (101). This was coupled to increased expression of the CDK inhibitor p21 and an accumulation of the hypo-phosphorylated form of Rb. This would explain the G1 arrest and inability of the cells to progress to S-phase.

Two nuclear targets have been proposed so far to explain the regulation of cellular proliferation by SOCE. First, activation of SOCE by thapsigargin has been shown to induce the phosphorylation of CREB, while knocking down STIM1 reduces the amount of pCREB (100). Second, thapsigargin induced activation of NFAT was strongly reduced by the knockdown of STIM1 as well as production of the mRNA coding for the modulatory calcineurin protein 1 (MCPI1), an NFAT-driven gene (102). Finally, and in contradiction with the previously cited works, it was

reported that in VSMCs isolated from the human saphenous vein, the knockdown of STIM1 reduces SOCE without affecting cell proliferation. Conversely, in those cells, the inactivation of TRPC1 reduced cell migration and proliferation (103).

The importance of SOCE in cell proliferation within the vascular system is not restricted to VSMCs. Although the players underlying SOCE in endothelial cells remain controversial (104; 105), STIM and Orai1 proteins have been shown to regulate endothelial cell proliferation. In human umbilical vein endothelial cells in culture, silencing Orai1 increased the proportion of cells in phase S and G2-M. A similar but much lower effect was observed with STIM1 or 2 knockdown, suggesting that Orai1 might act, at least partly, independently of STIM proteins (104). These results also argue that the role of soce in modulating the cell cycle of endothelial cells differ from that of VSMCs. In endothelial precursor cells derived from rat bone marrow, STIM1 levels have been shown to increase during cell proliferation induced by hepatocyte growth factor (106), while STIM1 knockdown limited the proliferation of naïve and stimulated cells (106; 107). These observations are supported in a vascular injury model using balloon angioplasty in rat carotid arteries. After arterial injury, STIM1 expression increases in smooth muscle cells in the media of the injured vessel but also in the neointima, confirming the increased expression of STIM1 in proliferating cells (101). Consistently, knockdown of STIM1 using adenoviral delivery of shRNA and siRNA prevents neointima formation and restores normal lumen diameter (101; 102).

In contrast, STIM1 knockdown in HEK cells in culture has been reported to have no effect on cellular proliferation. siRNA-mediated Orai1 knockdown in HEKs decreases cell proliferation, as does STIM2 knockdown but to lowers level than Orai1 (108). Serum starvation of HEK cells or pharmacological inhibition of cdk1 results in decreased Orai1 protein without affecting RNA levels arguing for translational control of Orai1 expression under these conditions. Therefore the effects of STIM1 knockdown appear to display a cell-type specific effect on cellular proliferation.

8. CANCER CELLS

STIM1 was originally isolated as a tumor suppressor and was termed GOK (109), linking it to cell proliferation and cancer. STIM1 was shown to be expressed in normal skeletal muscle but not in the muscle-derived rhabdomyosarcoma and rhadbdoid tumor cell lines. Moreover, in those cell lines, restoration of STIM1 induces cell death. This tumor suppressive phenotype of STIM1 was shown to be cell-type specific since it was not observed in the breast cancer cell line HBL100 (109). More recently, increased understanding of the structure and function of STIM and Orai proteins, has rekindled interest in the role of SOCE in various cancers, particularly in the case of breast cancer.

Orai1 expression is increased in breast cancer tissue and in the cancer cell line MCF-7 as compared to normal human mammary epithelial cells and to the non-cancerous cell line MCF-10A (110; 111). Knockdown of Orai3 inhibits the proliferation of MCF-7 cells and produces an arrest in G1 phase. A detailed analysis of the key proteins regulating the G1 and G1-S transition revealed that the expression of cyclin D1 and E was decreased as well as their corresponding Cdks (Cdk4/2). At the same time, over-expression of the CDK inhibitor $p21^{Waf1/Cip1}$ and of the tumor suppressor p53 were observed (110). However it appears that breast cancer cell lines are not homogenous regarding STIM/Orai expression, and can be divided into al least two distinct pools: estrogen receptor-positive cells that express STIM1/2 and Orai3, and estrogen receptor-negative cells that express STIM1 and Orai1 (111).

The knockdown of Orai1 has been shown to reduce the proliferation of MCF-7 but also to reduce tumor generation and inhibit metastasis in mice (112; 113). Conversely, the knockdown of STIM1 did not affect the proliferation of the MCF-7 (112) or of the cell line MDA-MB-231 while reducing serum induced cell migration (114). Adding to the complexity of the pattern, it was also found that Orai1 knockdown can reduce tumor generation and proliferation of the MCF-7 cell line, but that this was independent of STIM1/2 expression and activation. Orai1 has been shown in that case to form a complex with the Secretory Pathway Ca^{2+}-ATPase (SPCA2) to trigger a store-independent calcium influx promoting tumorogenesis (112).

The implication of modulating STIM and Orai protein levels in other types of cancerous cell lines has also been reported although the data are limited to date. In human hepatoma cells, the knockdown of either STIM1, Orai1 or TRPC6 decreases SOCE and cyclin D1 levels (115). In human prostate cancer cells, STIM1/Orai1-dependent SOCE is a major contributor to calcium-induced apoptosis and the expression of Orai1 depends on expression of the androgen receptor, making androgen-independent cells more resistant to apoptosis (116). Although much more remains to be learned about the role of STIM and Orai in cancers it is clear that they are involved in cancer development and metastasis, with the added complexity that their specific role in different types of cancer appears to be cell-type specific.

9. PERSPECTIVES

Over the past few years we have learned significantly about the basic molecular mechanisms of STIM1-Orai1 coupling in the context of soce, however our understanding of the contribution of STIM and Orai proteins to cell cycle progression, cellular proliferation and to cell physiology in general remains in its infancy.

Many questions remain. What are the mechanisms underlying the dramatic inhibition of SOCE during the division phase of the cell cycle? Are the effects of Orai and STIM modulation during cellular proliferation linked to SOCE and if not what are the specific pathways involved? The interaction of Orai1 with SPCA2 illustrates one example where Orai1 functions in an soce independent fashion. Are there other physiological or pathological situations where this is the case, for either STIM or Orai proteins? What determines the cell-type specific differential roles

of STIMs and Orais that is already emerging in the literature? It is fair to speculate that additional molecular partners of STIMs and Orais remain to be discovered and that they may modulate the function of these proteins in a cell-type and developmental specific fashion. Recent reports pointing to STIM-independent, Orai-dependent processes suggests that the cellular physiology of these molecules deviates from a simple model of STIM/Orai interaction. This would explain the differential phenotype observed in distinct cell types following modulation of STIM or Orai expression levels. Moreover, the relative viability of STIM1 and Orai1 knockouts tends to suggests that STIM1 and Orai1 are not key players in the normal process of cell division, or that other isoforms of the proteins can fulfill their roles when they are inactivated. In summary, the future promises exciting discoveries regarding the roles of STIMs and Orais in cellular proliferation and their regulation during the cell cycle.

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11. REFENRENCES

1. Murray,A. & Hunt,T. The Cell Cycle. Oxford University Press, New York (1993).

2. K. Machaca: Ca2+ signaling, genes and the cell cycle. Cell Calcium In Press, (2010)

3. M. J. Berridge, P. Lipp and M. D. Bootman: The versatility and universality of calcium signalling. Nat. Rev. Mol. Cell Biol. 1, 11-21 (2000)

4. D. E. Clapham: Calcium signaling. Cell 131, 1047-1058 (2007)

5. S. M. Wolniak, P. K. Hepler and W. T. Jackson: Ionic changes in the mitotic apparatus at the metaphase/anaphase transition. J Cell Biol 96, 598-605 (1983)

6. C. H. Keith, R. R. Ratan, F. R. Maxfield, A. Bajer and M. L. Shelanski: Local cytoplasmic calcium gradients in living mitotic cells. Nature 316, 848-850 (1985)

7. M. Poenie, J. M. Alderton, R. Y. Tsien and R. A. Steinhardt: Changes of free calcium levels with stages of the cell division cycle. Nature 315, 147-149 (1985)

8. M. Poenie, J. Alderton, R. A. Steinhardt and R. Y. Tsien: Calcium rises abruptly and briefly throughout the cell at the onset of anaphase. Science 233, 886-889 (1986)

9. R. R. Ratan, F. R. Maxfield and M. L. Shelanski: Long-lasting and rapid calcium changes during mitosis. *J. Cell Biol.* 107, 993-999 (1988)

10. R. A. Steinhardt and J. M. Alderton: Intracellular free calcium rise triggers nuclear envelope breakdown in the sea urchin embryo. *Nature* 332, 364-366 (1988)

11. J. Twigg, R. Patel and M. Whitaker: Translational control of InsP3-induced chromatin condensation during the early cell cycles of sea urchin embryos. *Nature* 332, 366-369 (1988)

12. J. P. Kao, J. M. Alderton, R. Y. Tsien and R. A. Steinhardt: Active involvement of Ca2+ in mitotic progression of Swiss 3T3 fibroblasts. *J. Cell Biol.* 111, 183-196 (1990)

13. R. M. Tombes, C. Simerly, G. G. Borisy and G. Schatten: Meiosis, egg activation, and nuclear envelope breakdown are differentially reliant on Ca^{2+}, whereas germinal vesicle breakdown is Ca^{2+} independent in the mouse oocyte. *J. Cell Biol.* 117, 799-811 (1992)

14. L. Sun and K. Machaca: Ca^{2+}cyt negatively regulates the initiation of oocyte maturation. *J. Cell Biol.* 165, 63-75 (2004)

15. J. G. Chafouleas, W. E. Bolton, H. Hidaka, A. E. Boyd, III and A. R. Means: Calmodulin and the cell cycle: involvement in regulation of cell-cycle progression. *Cell* 28, 41-50 (1982)

16. J. G. Chafouleas, L. Lagace, W. E. Bolton, A. E. Boyd, III and A. R. Means: Changes in calmodulin and its mRNA accompany reentry of quiescent (G0) cells into the cell cycle. *Cell* 36, 73-81 (1984)

17. C. D. Rasmussen and A. R. Means: Calmodulin is required for cell-cycle progression during G1 and mitosis. *EMBO J.* 8, 73-82 (1989)

18. R. Schmalzigaug, Q. Ye and M. W. Berchtold: Calmodulin protects cells from death under normal growth conditions and mitogenic starvation but plays a mediating role in cell death upon B-cell receptor stimulation. *Immunology* 103, 332-342 (2001)

19. G. P. Reddy, W. C. Reed, E. Sheehan and D. B. Sacks: Calmodulin-specific monoclonal antibodies inhibit DNA replication in mammalian cells. *Biochem.* 31, 10426-10430 (1992)

20. H. Hidaka, Y. Sasaki, T. Tanaka, T. Endo, S. Ohno, Y. Fujii and T. Nagata: N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, a calmodulin antagonist, inhibits cell proliferation. *Proc. Natl. Acad. Sci. U. S. A* 78, 4354-4357 (1981)

21. N. Takuwa, W. Zhou, M. Kumada and Y. Takuwa: Ca(2+)-dependent stimulation of retinoblastoma gene product phosphorylation and p34cdc2 kinase activation in serum-stimulated human fibroblasts. *J. Biol Chem.* 268, 138-145 (1993)

22. C. J. Sherr: The ins and outs of Rb: coupling gene expression to the cell cycle clock. Trends Cell Biol. 4, 15-18 (1994)

23. C. R. Kahl and A. Means: Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. Endocrine Reviews 24, 719-736 (2003)

24. M. D. Planas-Silva and A. R. Means: Expression of a constitutive form of calcium/calmodulin dependent protein kinase II leads to arrest of the cell cycle in G2. EMBO J. 11, 507-517 (1992)

25. T. N. Davis: A temperature-sensitive calmodulin mutant loses viability during mitosis. J Cell Biol 118, 607-617 (1992)

26. M. J. Moser, M. R. Flory and T. N. Davis: Calmodulin localizes to the spindle pole body of Schizosaccharomyces pombe and performs an essential function in chromosome segregation. J Cell Sci 110 (Pt 15), 1805-1812 (1997)

27. Y. Ohya and Y. Anraku: A galactose-dependent cmd1 mutant of Saccharomyces cerevisiae: involvement of calmodulin in nuclear division. Curr Genet 15, 113-120 (1989)

28. Y. Ohya and D. Botstein: Diverse essential functions revealed by complementing yeast calmodulin mutants. Science 263, 963-966 (1994)

29. B. J. Tunquist and J. L. Maller: Under arrest: cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev.* 17, 683-710 (2003)

30. T. Lorca, F. H. Cruzalegui, D. Fesquet, J. C. Cavadore, J. Mery, A. Means and M. Doree: Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of Xenopus eggs. *Nature* 366, 270-273 (1993)

31. j. Liu and J. L. Maller: Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytostatic factor. *Curr. Biol.* 15, 1458-1468 (2005)

32. D. V. Hansen, J. J. Tung and P. K. Jackson: CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. *Proc. Natl. Acad. Sci. U. S. A* 103, 608-613 (2006)

33. N. R. Rauh, A. Schmidt, J. Bormann, E. A. Nigg and T. U. Mayer: Calcium triggers exit from meiosis II by targeting the APC/C inhibitor XErp1 for degradation. *Nature* 437, 1048-1052 (2005)

34. A. Schmidt, P. I. Duncan, N. R. Rauh, G. Sauer, A. M. Fry, E. A. Nigg and T. U. Mayer: Xenopus polo-like kinase Plx1 regulates XErp1, a novel inhibitor of APC/C activity. *Genes Dev.* 19, 502-513 (2005)

35. S. Mochida and T. Hunt: Calcineurin is required to release Xenopus egg extracts from meiotic M phase. *Nature* 449, 336-340 (2007)

36. T. Nishiyama, N. Yoshizaki, T. Kishimoto and K. Ohsumi: Transient activation of calcineurin is essential to initiate embryonic development in Xenopus laevis. *Nature* 449, 341-345 (2007)

37. G. Schneider, F. Oswald, C. Wahl, F. R. Greten, G. Adler and R. M. Schmid: Cyclosporine inhibits growth through the activating transcription factor/cAMP-responsive element-binding protein binding site in the cyclin D1 promoter. *J Biol Chem* 277, 43599-43607 (2002)

38. M. Oh-hora and A. Rao: Calcium signaling in lymphocytes. *Curr. Opin. Immunol.* 20, 250-258 (2008)

39. L. Lipskaia and A. M. Lompre: Alteration in temporal kinetics of Ca2+ signaling and control of growth and proliferation. *Biol. Cell* 96, 55-68 (2004)

40. D. E. Clapham: Calcium Signaling. *Cell* 80, 259-268 (1995)

41. A. B. Parekh: Ca2+ microdomains near plasma membrane Ca2+ channels: impact on cell function. *J. Physiol* 586, 3043-3054 (2008)

42. M. J. Berridge: Elementary and global aspects of calcium signalling. *J. Physiol.* 499, 291-306 (1997)

43. M. J. Berridge, M. D. Bootman and H. L. Roderick: Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol* 4, 517-529 (2003)

44. N. Demaurex and M. Frieden: Measurements of the free luminal ER Ca(2+) concentration with targeted "cameleon" fluorescent proteins. *Cell Calcium* 34, 109-119 (2003)

45. R. Nuccitelli, D. L. Yim and T. Smart: The sperm-induced Ca2+ wave following fertilization of the Xenopus egg requires the production of Ins(1, 4, 5)P3. *Dev. Biol.* 158, 200-212 (1993)

46. A. B. Parekh and J. W. Putney: Store-Operated Calcium Channels. *Physiol. Rev.* 85, 757-810 (2005)

47. B. S. Launikonis and E. Rios: Store-operated Ca2+ entry during intracellular Ca2+ release in mammalian skeletal muscle. *J Physiol* 583, 81-97 (2007)

48. S. Feske: ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca2+ entry in the immune system and beyond. *Immunol. Rev.* 231, 189-209 (2009)

49. R. S. Lewis: Calcium signaling mechanisms in T lymphocytes. *Annu. Rev. Immunol.* 19, 497-521 (2001)

50. J. Liou, M. L. Kim, W. D. Heo, J. T. Jones, J. W. Myers, J. E. Ferrell, Jr. and T. Meyer: STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr. Biol* 15, 1235-1241 (2005)

51. J. Roos, P. J. DiGregorio, A. V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J. A. Kozak, S. L. Wagner, M. D. Cahalan, G. Velicelebi and K. A. Stauderman: STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J. Cell Biol* 169, 435-445 (2005)

52. S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S. H. Puppel, B. Tanasa, P. G. Hogan, R. S. Lewis, M. Daly and A. Rao: A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441, 179-185 (2006)

53. M. Vig, C. Peinelt, A. Beck, D. L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner and J. P. Kinet: CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science* 312, 1220-1223 (2006)

54. S. L. Zhang, A. V. Yeromin, X. H. Zhang, Y. Yu, O. Safrina, A. Penna, J. Roos, K. A. Stauderman and M. D. Cahalan: Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. *Proc. Natl. Acad. Sci. U. S. A* 103, 9357-9362 (2006)

55. C. Peinelt, M. Vig, D. L. Koomoa, A. Beck, M. J. Nadler, M. Koblan-Huberson, A. Lis, A. Fleig, R. Penner and J. P. Kinet: Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol* 8, 771-773 (2006)

56. J. Soboloff, M. A. Spassova, X. D. Tang, T. Hewavitharana, W. Xu and D. L. Gill: Orai1 and STIM reconstitute store-operated calcium channel function. *J. Biol Chem.* 281, 20661-20665 (2006)

57. A. V. Yeromin, S. L. Zhang, W. Jiang, Y. Yu, O. Safrina and M. D. Cahalan: Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 443, 226-229 (2006)

58. M. Vig, A. Beck, J. M. Billingsley, A. Lis, S. Parvez, C. Peinelt, D. L. Koomoa, J. Soboloff, D. L. Gill, A. Fleig, J. P. Kinet and R. Penner: CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr. Biol* 16, 2073-2079 (2006)

59. M. Prakriya, S. Feske, Y. Gwack, S. Srikanth, A. Rao and P. G. Hogan: Orai1 is an essential pore subunit of the CRAC channel. *Nature* 443, 230-233 (2006)

60. M. D. Cahalan: STIMulating store-operated Ca(2+) entry. *Nat. Cell Biol* 11, 669-677 (2009)

61. M. D. Cahalan, S. L. Zhang, A. V. Yeromin, K. Ohlsen, J. Roos and K. A. Stauderman: Molecular basis of the CRAC channel. *Cell Calcium* (2007)

62. O. Mignen, J. L. Thompson and T. J. Shuttleworth: Both Orai1 and Orai3 are essential components of the arachidonate-regulated Ca2+-selective (ARC) channels. *J Physiol* 586, 185-195 (2008)

63. O. Mignen, J. L. Thompson and T. J. Shuttleworth: STIM1 regulates Ca2+ entry via arachidonate-regulated Ca2+-selective (ARC) channels without store depletion or translocation to the plasma membrane. *J Physiol* 579, 703-715 (2007)

64. W. Zeng, J. P. Yuan, M. S. Kim, Y. J. Choi, G. N. Huang, P. F. Worley and S. Muallem: STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. *Mol. Cell* 32, 439-448 (2008)

65. J. Liou, M. Fivaz, T. Inoue and T. Meyer: Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca2+ store depletion. *Proc. Natl. Acad. Sci. U. S. A* 104, 9301-9306 (2007)

66. P. B. Stathopulos, G. Y. Li, M. J. Plevin, J. B. Ames and M. Ikura: Stored Ca2+ depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: An initiation mechanism for capacitive Ca2+ entry. *J. Biol Chem.* 281, 35855-35862 (2006)

67. R. M. Luik, M. M. Wu, J. Buchanan and R. S. Lewis: The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J. Cell Biol* 174, 815-825 (2006)

68. M. M. Wu, J. Buchanan, R. M. Luik and R. S. Lewis: Ca2+ store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J. Cell Biol* 174, 803-813 (2006)

69. C. Y. Park, P. J. Hoover, F. M. Mullins, P. Bachhawat, E. D. Covington, S. Raunser, T. Walz, K. C. Garcia, R. E. Dolmetsch and R. S. Lewis: STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 136, 876-890 (2009)

70. G. N. Huang, W. Zeng, J. Y. Kim, J. P. Yuan, L. Han, S. Muallem and P. F. Worley: STIM1 carboxyl-terminus activates native SOC, I(crac) and TRPC1 channels. *Nat. Cell Biol* 8, 1003-1010 (2006)

71. J. P. Yuan, W. Zeng, G. N. Huang, P. F. Worley and S. Muallem: STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. *Nat. Cell Biol.* 9, 636-645 (2007)

72. A. Arredouani, F. Yu, L. Sun and K. Machaca: Regulation of store-operated Ca2+ entry during the cell cycle. J. Cell Sci. 123, 2155-2162 (2010)

73. K. Machaca and S. Haun: Store-operated Calcium Entry Inactivates at the Germinal Vesicle Breakdown Stage of Xenopus Meiosis. J. Biol. Chem. 275, 38710-38715 (2000)

74. K. Machaca and S. Haun: Induction of maturation-promoting factor during Xenopus oocyte maturation uncouples Ca^{2+} store depletion from store-operated Ca^{2+} entry . *J. Cell Biol.* 156, 75-85 (2002)

75. S. F. Preston, R. I. Sha'afi and R. D. Berlin: Regulation of Ca^{2+} influx during mitosis: Ca^{2+} influx and depletion of intracellular Ca^{2+} stores are coupled in interphase but not mitosis. *Cell Regulation* 2, 915-925 (1991)

76. D. Tani, M. K. Monteilh-Zoller, A. Fleig and R. Penner: Cell cycle-dependent regulation of store-operated I(CRAC) and Mg2+-nucleotide-regulated MagNuM (TRPM7) currents. *Cell Calcium* 41, 249-260 (2007)

77. F. Yu, L. Sun and K. Machaca: Orai1 internalization and STIM1 clustering inhibition modulate SOCE inactivation during meiosis. *Proc. Natl. Acad. Sci. U. S. A* 106, 17401-17406 (2009)

78. J. T. Smyth, J. G. Petranka, R. R. Boyles, W. I. DeHaven, M. Fukushima, K. L. Johnson, J. G. Williams and J. W. Putney, Jr.: Phosphorylation of STIM1 underlies suppression of store-operated calcium entry during mitosis. *Nat Cell Biol* 11, 1465-1472 (2009)

79. F. Yu, L. Sun and K. Machaca: Constitutive recycling of the store-operated Ca^{2+} channel Orai1 and its internalization during meiosis. *J. Cell Biol.* In Press, (2010)

80. W. B. Busa and R. Nuccitelli: An Elevated Free Cytosolic Ca^{2+} Wave Follows Fertilization in Eggs of the Frog, *Xenopus laevis*. *J. Cell Biol.* 100, 1325-1329 (1985)

81. S. A. Stricker: Comparative biology of calcium signaling during fertilization and egg activation in animals. *Dev. Biol.* 211, 157-176 (1999)

82. M. Charbonneau and R. D. Grey: The onset of activation responsiveness during maturation coincides with the formation of the cortical endoplasmic reticulum in oocytes of Xenopus laevis. *Dev. Biol.* 102, 90-97 (1984)

83. T. Fujiwara, K. Nakada, H. Shirakawa and S. Miyazaki: Development of inositol trisphosphate-induced calcium release mechanism during maturation of hamster oocytes. *Dev. Biol.* 156, 69-79 (1993)

84. K. Machaca: Increased sensitivity and clustering of elementary Ca^{2+} release events during oocyte maturation. *Dev. Biol* 275, 170-182 (2004)

85. L. Sun, S. Haun, R. C. Jones, R. D. Edmondson and K. Machaca: Kinase-dependent regulation of IP3-dependent $_{Ca}{}^{2+}$ release during oocyte maturation. *J. Biol Chem.* 284, 20184-20196 (2009)

86. K. Malathi, S. Kohyama, M. Ho, D. Soghoian, X. Li, M. Silane, A. Berenstein and T. Jayaraman: Inositol 1,4,5-trisphosphate receptor (type 1) phosphorylation and modulation by Cdc2. *J. Cell Biochem.* 90, 1186-1196 (2003)

87. W. El Jouni, B. Jang, S. Haun and K. Machaca: Calcium signaling differentiation during Xenopus oocyte maturation. *Dev. Biol* 288, 514-525 (2005)

88. W. El Jouni, S. Haun and K. Machaca: Internalization of plasma membrane Ca2+-ATPase during Xenopus oocyte maturation. *Dev Biol* 324, 99-107 (2008)

89. Y. Baba, K. Nishida, Y. Fujii, T. Hirano, M. Hikida and T. Kurosaki: Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nat. Immunol.* 9, 81-88 (2008)

90. M. Oh-hora, M. Yamashita, P. G. Hogan, S. Sharma, E. Lamperti, W. Chung, M. Prakriya, S. Feske and A. Rao: Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat. Immunol.* 9, 432-443 (2008)

91. J. Stiber, A. Hawkins, Z. S. Zhang, S. Wang, J. Burch, V. Graham, C. C. Ward, M. Seth, E. Finch, N. Malouf, R. S. Williams, J. P. Eu and P. Rosenberg: STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle. *Nat Cell Biol* 10, 688-697 (2008)

92. D. Varga-Szabo, A. Braun, C. Kleinschnitz, M. Bender, I. Pleines, M. Pham, T. Renne, G. Stoll and B. Nieswandt: The calcium sensor STIM1 is an essential mediator of arterial thrombosis and ischemic brain infarction. *J Exp. Med.* 205, 1583-1591 (2008)

93. N. Beyersdorf, A. Braun, T. Vogtle, D. Varga-Szabo, R. R. Galdos, S. Kissler, T. Kerkau and B. Nieswandt: STIM1-independent T cell development and effector function in vivo. *J Immunol.* 182, 3390-3397 (2009)

94. A. Berna-Erro, A. Braun, R. Kraft, C. Kleinschnitz, M. K. Schuhmann, D. Stegner, T. Wultsch, J. Eilers, S. G. Meuth, G. Stoll and B. Nieswandt: STIM2 regulates capacitive Ca2+ entry in neurons and plays a key role in hypoxic neuronal cell death. *Sci. Signal.* 2, ra67 (2009)

95. Y. Gwack, S. Srikanth, M. Oh-hora, P. G. Hogan, E. D. Lamperti, M. Yamashita, C. Gelinas, D. S. Neems, Y. Sasaki, S. Feske, M. Prakriya, K. Rajewsky and A.

Rao: Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol. Cell Biol.* 28, 5209-5222 (2008)

96. M. Vig, W. I. DeHaven, G. S. Bird, J. M. Billingsley, H. Wang, P. E. Rao, A. B. Hutchings, M. H. Jouvin, J. W. Putney and J. P. Kinet: Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat. Immunol.* 9, 89-96 (2008)

97. S. Feske: CRAC channelopathies. *Pflugers Arch.* 460, 417-435 (2010)

98. S. G. Baryshnikov, M. V. Pulina, A. Zulian, C. I. Linde and V. A. Golovina: Orai1, a critical component of store-operated Ca2+ entry, is functionally associated with Na+/Ca2+ exchanger and plasma membrane Ca2+ pump in proliferating human arterial myocytes. *Am. J Physiol Cell Physiol* 297, C1103-C1112 (2009)

99. M. Potier, J. C. Gonzalez, R. K. Motiani, I. F. Abdullaev, J. M. Bisaillon, H. A. Singer and M. Trebak: Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: role in proliferation and migration. *FASEB J* 23, 2425-2437 (2009)

100. Y. Takahashi, H. Watanabe, M. Murakami, K. Ono, Y. Munehisa, T. Koyama, K. Nobori, T. Iijima and H. Ito: Functional role of stromal interaction molecule 1 (STIM1) in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 361, 934-940 (2007)

101. R. W. Guo, H. Wang, P. Gao, M. Q. Li, C. Y. Zeng, Y. Yu, J. F. Chen, M. B. Song, Y. K. Shi and L. Huang: An essential role for stromal interaction molecule 1 in neointima formation following arterial injury. *Cardiovasc. Res.* 81, 660-668 (2009)

102. F. C. Aubart, Y. Sassi, A. Coulombe, N. Mougenot, C. Vrignaud, P. Leprince, P. Lechat, A. M. Lompre and J. S. Hulot: RNA interference targeting STIM1 suppresses vascular smooth muscle cell proliferation and neointima formation in the rat. *Mol. Ther.* 17, 455-462 (2009)

103. J. Li, P. Sukumar, C. J. Milligan, B. Kumar, Z. Y. Ma, C. M. Munsch, L. H. Jiang, K. E. Porter and D. J. Beech: Interactions, functions, and independence of plasma membrane STIM1 and TRPC1 in vascular smooth muscle cells. *Circ. Res.* 103, e97-104 (2008)

104. I. F. Abdullaev, J. M. Bisaillon, M. Potier, J. C. Gonzalez, R. K. Motiani and M. Trebak: Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ. Res.* 103, 1289-1299 (2008)

105. M. Freichel, S. H. Suh, A. Pfeifer, U. Schweig, C. Trost, P. Weissgerber, M. Biel, S. Philipp, D. Freise, G. Droogmans, F. Hofmann, V. Flockerzi and B. Nilius: Lack of an endothelial store-operated Ca2+ current impairs agonist- dependent vasorelaxation in TRP4-/- mice. *Nat. Cell Biol.* 3, 121-127 (2001)

106. Y. Shi, M. Song, R. Guo, H. Wang, P. Gao, W. Shi and L. Huang: Knockdown of stromal interaction molecule 1 attenuates hepatocyte growth factor-induced endothelial progenitor cell proliferation. *Exp. Biol Med.* 235, 317-325 (2010)

107. C. Y. Kuang, Y. Yu, R. W. Guo, D. H. Qian, K. Wang, M. Y. Den, Y. K. Shi and L. Huang: Silencing stromal interaction molecule 1 by RNA interference inhibits the proliferation and migration of endothelial progenitor cells. *Biochem. Biophys. Res. Commun.* 398, 315-320 (2010)

108. C. El Boustany, M. Katsogiannou, P. Delcourt, E. Dewailly, N. Prevarskaya, A. S. Borowiec and T. Capiod: Differential roles of STIM1, STIM2 and Orai1 in the control of cell proliferation and SOCE amplitude in HEK293 cells. *Cell Calcium* 47, 350-359 (2010)

109. S. Sabbioni, G. Barbanti-Brodano, C. M. Croce and M. Negrini: GOK: a gene at 11p15 involved in rhabdomyosarcoma and rhabdoid tumor development. *Cancer Res.* 57, 4493-4497 (1997)

110. M. Faouzi, F. Hague, M. Potier, A. Ahidouch, H. Sevestre and H. Ouadid-Ahidouch: Down-regulation of Orai3 arrests cell cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells. *J Cell Physio.* 226, 542-551 (2011)

111. R. K. Motiani, I. F. Abdullaev and M. Trebak: A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. *J Biol Chem.* 285, 19173-19183 (2010)

112. M. Feng, D. M. Grice, H. M. Faddy, N. Nguyen, S. Leitch, Y. Wang, S. Muend, P. A. Kenny, S. Sukumar, S. J. Roberts-Thomson, G. R. Monteith and R. Rao: Store-independent activation of Orai1 by SPCA2 in mammary tumors. *Cell* 143, 84-98 (2010)

113. S. Yang, J. J. Zhang and X. Y. Huang: Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 15, 124-134 (2009)

114. S. Yang, J. J. Zhang and X. Y. Huang: Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 15, 124-134 (2009)

115. C. El Boustany, G. Bidaux, A. Enfissi, P. Delcourt, N. Prevarskaya and T. Capiod: Capacitative calcium entry and transient receptor potential canonical 6 expression control human hepatoma cell proliferation. *Hepatology* 47, 2068-2077 (2008)

116. M. Flourakis, V. Lehen'kyi, B. Beck, M. Raphael, M. Vandenberghe, F. V. Abeele, M. Roudbaraki, G.

Lepage, B. Mauroy, C. Romanin, Y. Shuba, R. Skryma and N. Prevarskaya: Orai1 contributes to the establishment of an apoptosis-resistant phenotype in prostate cancer cells. *Cell Death Dis* 1, e75 (2010)

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