Calcium transport and signalling in breast cancer: functional and prognostic significance

AUTHOR(S)

Shane O'Grady, Maria Morgan

Calcium transport and signalling in breast cancer: functional and prognostic significance

Shane O'Grady[1,2], Maria P. Morgan[1*]

[1.] *Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, 123 St Stephen's Green, Dublin 2, Ireland*
[2.] *Present address: UCD Education & Research Centre, St. Vincent's University Hospital, Dublin, Ireland*

* *Corresponding author: Maria P. Morgan. Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, 123 St Stephen's Green, Dublin 2, Ireland (mmorgan@rcsi.ie)*

Abstract

Comprised of a complex network of numerous intertwining pathways, the Ca^{2+} signalling nexus is an essential mediator of many normal cellular activities. Like many other such functions, the normal physiological activity of Ca^{2+} signalling is frequently co-opted and reshaped in cases of breast cancer, creating a potent oncogenic drive within the affected cell population. Such modifications can occur within pathways mediating either Ca^{2+} import (e.g. TRP channels, ORAI-STIM1) or Ca^{2+} export (e.g. PMCA), indicating that both increases and decreases within cellular Ca^{2+} levels have the potential to increase the malignant potential of a cell. Increased understanding of these pathways may offer clinical benefit in terms of both prognosis and treatment; patient survival has been linked to expression levels of certain Ca^{2+} transport proteins, whilst selective targeting of these factors with novel anti-cancer agents has demonstrated a variety of anti-tumour effects in *in vitro* studies. In addition, the activity of several Ca^{2+} signalling pathways has been shown to influence chemotherapy response, suggesting that a synergistic approach coupling traditional chemotherapy with Ca^{2+} targeting agents may also improve patient outcome. As such, targeted modulation of these pathways represents a novel approach in precision medicine and breast cancer therapy.

Keywords

Breast cancer, calcium, calcium channels, store-operated calcium entry, targeted therapy

Introduction

Ca^{2+} ions represent a highly versatile and widespread secondary messenger within multiple intracellular signalling pathways and are involved in the regulation of a multitude of important cellular activities including proliferation, motility and apoptosis [1]. Due to its status as an element, Ca^{2+} can neither be synthesised nor degraded within a cell, unlike most other signalling molecules. Its activity as a secondary messenger is therefore dependent on the ability of a cell to tightly regulate both the temporal and spatial distribution of Ca^{2+} ions. This is accomplished through a careful balance of multiple interlinked pathways of Ca^{2+} influx, efflux and sequestration.

Many of the pathways regulated by Ca^{2+} are of significance to the development and spread of several forms of cancer[2]., including tumours of the breast [3]. Considering that the

physiological function of breast tissue includes concentration of Ca^{2+} during lactation, it is of little surprise that many Ca^{2+} transport proteins are intimately involved in the function of both normal and cancerous breast tissue [1]. Breast tissue undergoes significant remodelling in preparation for lactation and these changes include upregulation of many Ca^{2+} handling proteins to facilitate transfer of Ca^{2+} first across the basolateral membrane and then through the apical membrane into the alveolar lumen [4-10].

Similar changes in several of these Ca^{2+} transport proteins have been observed in breast tumours, with many studies also demonstrating mechanistic links between the alteration of a breast cells Ca^{2+} homeostatic system and increases in important tumour-associated properties such as proliferation, invasion and apoptotic resistance [11-14]. The ability of Ca^{2+} ions to influence development of a tumour is highly flexible as oncogenesis can be associated with increases in either Ca^{2+} influx or efflux, which typically lead to changes in proliferative and apoptotic signalling, respectively.

Such alterations have a direct, translational relevance to patient care. Knowledge of a patient's expression levels of key Ca^{2+} transport proteins has significant clinical value in prediction of both survival and response to treatment [15-17]. Furthermore, the presence of calcium hydroxyapatite microcalcifications, which we and other groups have suggested form as a consequence of dysregulation of Ca^{2+} pathways [18-20], are a common mammographic finding leading to the early detection of many breast tumours.

Alterations in Ca^{2+} transport and signalling are involved in all stages of a breast tumours initiation and progression. This review will summarise key findings regarding oncogenic modification of a select set of Ca^{2+} pathways within breast tumours and their influence on cellular behaviour and explore their prognostic relevance and emerging clinical potential as future targets in precision medicine.

Ca^{2+} signalling patterns

An important feature of Ca^{2+} signalling is that both the temporal and spatial influx of Ca^{2+} can be modulated, allowing for the generation of a diverse range of signals of far greater complexity than if Ca^{2+} signalling operated as a simple "ON-OFF" switch. Activation of Ca^{2+} influx does not necessarily generate a uniform increase in concentration throughout all regions of the cell. Instead, Ca^{2+} signals may occur in spatially and temporally organised blips, sparks, puffs, flickers, waves and oscillations (Fig. 1), each of which may be decoded and interpreted differently by the cell [21].

As an example, activation of certain signalling pathways can lead to a regular oscillation pattern in intracellular Ca^{2+}. Information can be encoded into both the frequency or amplitude of these oscillations, allowing for downstream Ca^{2+} binding proteins with different binding kinetics to be specifically activated by distinct patterns of Ca^{2+} oscillations [22]. Highly metastatic MDA-MB-231 cells were recently observed to display spontaneous oscillations in intracellular Ca^{2+}, which were not found in weakly metastatic MCF-7 cells [23], suggesting that these oscillations were associated with the metastatic potential of breast cancer cells.

A wide variety of modifications in Ca^{2+} signalling patterns are possible, each of which may yield a different outcome on cell properties. Increased Ca^{2+} influx can lead to a higher peak Ca^{2+} following stimulation or sustained intracellular levels over a more prolonged period, both of which may activate proliferative pathways [24, 25]. Conversely, an increase in Ca^{2+} efflux

activity will rapidly counteract any stimulation and can suppress apoptotic signalling, promoting cell survival. These changes occur as the result of altered expression levels of the various transporters and Ca^{2+}-binding proteins responsible for intracellular Ca^{2+} homeostasis. In order to understand the mechanistic link between altered Ca^{2+} signalling factors and tumourigenic properties, we will examine a select few of these factors and their possible clinical utility as prognostic markers and targets for personalised therapy.

Intracellular Ca^{2+} storage and release

Intracellular Ca^{2+} is typically maintained below 100 nM, significantly lower than the 1-2 mM found in the extracellular environment [26]. In order to maintain low intracellular Ca^{2+}, cells utilise a combination of active ATPase pumps and exchange transporters to both export Ca^{2+} from the cell and sequester it within cytoplasmic organelles including mitochondria and the endoplasmic reticulum (ER). In addition to forming the main site for intracellular Ca^{2+} storage, with an intra-organelle Ca^{2+} concentration between 100 and 800 µM [27], the ER also contains large amounts of Ca^{2+} binding proteins including calreticulin and calnexin, which help to buffer Ca^{2+} as well as acting as molecular chaperones to assist in the folding of newly synthesised proteins [28]. The buffering function of these proteins is essential as both excess and inadequate Ca^{2+} is detrimental to ER function [29]. The effect of persistent Ca^{2+} depletion within the ER has been investigated experimentally using the inhibitor thapsigargin and has been shown to lead to an accumulation of misfolded proteins due to loss of calcium-dependent chaperone activity and subsequent activation of the unfolded protein response (UPR) [30]. UPR signalling in breast cancer has been linked to increased resistance to multiple forms of therapy [31, 32].

Prolonged rises in intracellular Ca^{2+} can trigger apoptosis [33], necessitating a swift return to physiological Ca^{2} levels to avoid unwanted cell death. This is accomplished through active export via Ca^{2+} efflux pumps, such as the plasma membrane Ca^{2+} ATPase (PMCA) family, as well as re-uptake into the ER. Ca^{2+} is actively pumped into the ER by sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps. Vertebrates possess three paralogous SERCA genes, each capable of producing multiple isoforms via alternative-splicing [34]. The high-affinity SERCA2b isoform is ubiquitously expressed and operates in a “house-keeping“ role to maintain a sufficient Ca^{2+} supply within the ER and buffer cytoplasmic Ca^{2+} to within the nanomolar range [35]. SERCA2b has been linked to acquisition of aromatase inhibitor resistance by reducing ER stress and retaining ERα expression [36]. SERCA3, which is expressed in luminal and HER2+ but not triple negative breast cancers [37, 38], is upregulated during TGF-β -induced epithelial-mesenchymal transition (EMT) [39] and has recently been shown to also undergo upregulation in response to progesterone, leading to alterations in ER Ca^{2+} efflux and chromatin condensation [40]. Because of these pro-tumourigenic effects, inhibition of SERCA activity has been proposed as a therapeutic strategy and several SERCA inhibitors have now been identified that show efficacy against breast cancer cell lines in *in vitro* studies [41, 42].

The ER maintains an internal Ca^{2+} storage pool that can be selectively released in response to activation of pathways in order to propagate or amplify a Ca^{2+} signal. Ca^{2+} release from the ER is mediated by two families of intracellular channels. Inositol 1,4,5-trisphosphate receptors (IP_3R), are activated by binding of the IP_3 ligand, which is produced by phospholipase C (PLC) in response to triggering of an associated G-protein-coupled receptors (GPCR), receptor tyrosine kinase (RTK) or other associated receptor [43] Ryanodine receptors (RyR), on the other hand, are inactive at low levels of cytoplasmic Ca^{2+} but become activated by rises in intracellular Ca^{2+} , causing a positive feedback loop [44].

Both these families of intracellular Ca^{2+} channels have been implicated in the development of mammary tumours. RyR expression correlates with tumour grade [45] and knockdown of the RyR3 isoform significantly reduced proliferation and migration in the MCF-7 and MDA-MB-231 cell lines [46]. RyR channels were also recently shown to contribute to enrichment of breast cancer stem cells and development of resistance following chemotherapeutic treatment via cooperative interaction with glutathione S-transferase omega 1 (GSTO1) and activation of the STAT3/IL-6 pathway [47].

Many studies on the role of IP_3R in breast cancer have focused on the IP_3R3 isoform. Estrogen regulates both the expression and activity of the IP_3R3 isoform in MCF-7 cells, which contributes to the proliferative effect of estrogen signalling [48]. IP_3R also contributes to migration, with higher expression levels correlating with increased migratory rates in a panel of breast cancer cell lines and siRNA silencing markedly reducing migration through a Boyden chamber [49]. Both RyR and IP_3R may contribute to EMT as induction of EMT in triple-negative MDA-MB-468 cells by epidermal growth factor (EGF) stimulation upregulates expression of the IP_3R1, IP_3R3 and RYR2 channels [50].

In addition to the ER, cytosolic Ca^{2+} is also regulated by the mitochondria. Ca^{2+} entering the mitochondria passes first through the outer membrane via the high-conductance voltage-dependent anion channel 1 (VDAC1) before transport through the inner membrane via the mitochondrial Ca^{2+} uniporter (MCU) [51]. The low Ca^{2+} affinity of MCU [52] would render the transporter inefficient at physiological cytosolic Ca^{2+} concentrations. However, regions of close proximity between the ER and a subset of the cells mitochondria leads to creation of a localised micro-domain which, following Ca^{2+} release from the ER, will be of a sufficient Ca^{2+} concentration to stimulate mitochondrial uptake [53]. Transfer of Ca^{2+} across these mitochondria-associated ER membranes appears to play a significant role in tumour development as MCU expression correlates with tumour size and node infiltration and may promote growth and metastasis in triple-negative breast cancers [54, 55]. Mitochondrial Ca^{2+} uptake has also been shown to be crucial to cell movement by regulating cytoskeletal organisation [56, 57].

Store Operated Ca^{2+} Entry

As a certain amount of Ca^{2+} is lost through the activity of efflux pumps, the ER must periodically refill its internal stores from the extracellular environment through activation of store-operated calcium entry (SOCE), a ubiquitous Ca^{2+} influx pathway in eukaryotic cells. SOCE operates through Ca^{2+} release-activated Ca^{2+} (CRAC) channels, the precise nature of which remained unidentified until the mid-2000s, when they were revealed as a complex of the Ca^{2+} detector stromal interaction molecule 1 (STIM1) and the plasma membrane pore-forming protein Orai1 [58-62].

SOCE is activated in response to decreased Ca^{2+} levels within the ER lumen as monitored by intraluminal Ca^{2+}-binding domains of STIM1 [63]. Decreases in Ca^{2} within the ER lumen causes bound Ca^{2+} to dissociate from these domains [64], inducing a conformational change leading to translocation of the active-STIM1 to regions of the ER in close proximity to the plasma membrane. Activated STIM1 proteins can then associate with Orai1. Initially identified in a linkage analysis study of patients with severe combined immunodeficiency (SCID) due to lack of CRAC channel function [58], Orai1 is now known to be one of three mammalian Orai homologs which form hexameric pores in the plasma membrane with high Ca^{2+}-selectivity. Tethering of STIM1 to Orai1 results in activation of the channel and an influx of Ca^{2+} from the extracellular environment.

Although the canonical SOCE pathway is the best described function of the STIM1-Orai1 complex, both proteins can also activate other modes of Ca^2 transport. STIM1 has been shown to activate the transient receptor potential canonical 1 (TRPC1) channel in certain cell types, resulting in the generation of Ca^{2+} influx currents and downstream-signalling effects distinct from those generated by Orai1 activation [65]. Interestingly, Orai1 itself is necessary for TRPC1 activation [66] as Orai1-mediated Ca^{2+} entry first recruits TRPC1-containing vesicles to the plasma membrane [67] where they can subsequently be activated by interaction with STIM1.

Alterations in both expression levels and functional activities of the SOCE pathway have now been demonstrated in several forms of cancer, including breast. Knockdown of either Orai1 or STIM1 significantly decreased migration and invasion of MDA-MB-231 cells while conversely, overexpression increased migration of non-tumourigenic MCF-10A epithelial cells [68]. Overexpression of STIM1 has been reported in breast cancer biopsy samples, with higher expression levels correlating with a decrease in disease-free survival (DFS) [69]. Further work from the same group showed that STIM1 expression is controlled by miR-223 and that decreased miR-223 levels in patients may increase proliferation and metastasis via a loss of STIM1 inhibition [70]. These effects may be linked to EMT as both STIM1 and the related STIM2 (which initiates a weaker but more prolonged SOCE in response to smaller ER Ca^{2+} shifts [71]) promote SOCE in response to TGF-β-induced EMT [72].

Orai1 is upregulated in breast cancer cell lines and is expressed particularly highly in cells of the basal subtype [15, 73]. Different subtypes of breast cancer appear to utilise different combinations of STIM and Orai proteins to mediate SOCE. Patch-clamp analysis of the ERα- MDA-MB-231 cell lines demonstrates an almost total loss of SOCE following knockdown of either STIM1 or Orai1, confirming activity of the canonical SOCE pathway [74]. In contrast, SOCE in the ERα+ MCF-7 cell line was unaffected by loss of Orai1 but was attenuated by knockdown of either STIM1, Orai3 and, to a smaller but significant extent, STIM2. These results indicate a preference for a STIM1/2 and Orai3 combination in ERα+ and the canonical STIM1 and Orai1 pathway in ERα- cells. This difference may be due to a direct impact of ERα on expression levels of SOCE-associated proteins as knockdown of ERα in MCF-7 cells causes decreased expression of Orai3 (but not Orai1), whilst treatment with the ERα ligand 17β-estradiol results in a 3-fold increase in Orai3 mRNA levels [75].

Orai3 may constitute a novel marker and therapeutic target within breast cancer as Orai3 knockdown decreased anchorage-independent growth, Matrigel invasion and *in vivo* tumour development [75] as well as selectively inhibiting cell-cycle progression and proliferation in MCF-7 cells without affecting normal breast epithelial cells [76]. Furthermore, Orai3 expression strongly correlates with expression of the oncogenic transcription factor c-Myc, with knockdown of Orai3 leading to a significant decrease in both expression and activity of c-Myc via the MAPK pathway [77]. Recently, Orai3 was even shown to contribute to development of chemoresistance by increasing p53 degradation [78].

Considering the multitude of tumourigenic properties associated with SOCE, it is of no surprise that development of active agents targeting this pathway are currently under development with many showing significant anti-cancer effect. Carboxyamidotriazole, which is currently in clinical trials for several forms of solid tumour, suppresses several Ca^{2+} transport pathways including SOCE and has shown some promise in the treatment of breast tumours [79, 80]. Phemindole acts a potent apoptotic inducer in triple-negative breast cancer cells by downregulating expression of STIM1 and decreasing SOCE, leading to ER stress mediated cell death and markedly inhibiting growth in an *in vivo* mouse model [81].

SOCE was also recently shown to be decreased by several receptor tyrosine kinase (RTK) inhibitors, used in the treatment of patients with HER2-overexpressing breast tumours [82]. This may represent a novel mechanism of these drugs, with the authors also suggesting that the frequent upregulation of the SOCE pathway in breast cancer could contribute to resistance against such therapies. SOCE may also contribute to resistance against other forms of therapy, as the high sodium content found within the breast tumour microenvironment can activate SOCE and increase expression of P-glycoprotein, a known promoter of drug resistance [83].

Lactation-associated Ca^{2+} transport: Relevance to breast cancer

Ca^{2+} transport pathways within breast tissue undergoes a significant remodelling process during mammary gland development preceding lactation [1], and it has been suggested that the oncogenic transformation of breast epithelial cells to a malignant phenotype may recapitulate this process (Fig. 2). The secretory pathway calcium ATPases (SPCA) are responsible for supplying the Golgi apparatus with Ca^{2+} and Mn^{2+} ions, essential to the organelles function of protein sorting and processing [84]. Both SPCA1 and SPCA2 are significantly upregulated during lactation [10, 85], where they are hypothesised to contribute to the extensive transcellular flux of Ca^{2+} into the alveolar lumen [10]. Both isoforms are also upregulated in cases of breast cancer [20, 86], and have been shown to contribute to tumour development [86].

Unexpectedly, Feng *et al* found that knockdown of SPCA2 in MCF-7 cells lead to a significant reduction of intracellular Ca^{2+}[86]. SPCA2 was subsequently shown to traffic to the plasma membrane where it could directly interact with Orai1 leading to channel opening and constitutive Ca^{2+} influx. This unusual method of Orai1 activation was also found to be independent of Ca^{2+} levels within the ER lumen and to contribute to the tumourigenicity of breast cancer, with SPCA2 knockdown leading to decreases in proliferation, anchorage-independent growth and RAS signalling. SPCA2 was also recently shown to play role in the formation of microcalcifications [20], an important mammographic indication leading to early detection of many breast tumours. The precise mechanism by which SPCA2 promotes calcification is not clear but may involve intravesicular concentration of Ca^{2+} and nucleation of hydroxyapatite crystals. Studies from our lab and others have suggested microcalcifications to occur as the result of an active, cellular process involving not only SPCA2 but additional Ca^{2+} transport mechanisms [19, 46], ectopic expression of bone-associated proteins [87] and acquisition of a mesenchymal-phenotype [88]. The presence of microcalcifications has long been recognised for its efficacy in breast tumour detection, with some studies also suggesting links between these calcium deposits and important clinical considerations such as prognosis, HER2 expression and invasion [19]. Experimental evidence suggests that the increased aggressiveness often observed in calcification-associated breast tumours may stem from a direct influence on Ca^{2+} signalling within the tumour. Stimulation of breast cancer cells with hydroxyapatite nanocrystals stimulates proliferation, migration, and expression of inflammatory mediators and metalloproteinases [89-91].

Although the potential contribution of SPCA1 to breast cancer is less understood than SPCA2, SPCA1 has been shown to be upregulated in breast cancer of the basal subtype where it plays a significant role in promoting proteolytic cleavage and activation of IGF1R [92].

In addition to SPCA1/2, transcellular flow of Ca^{2+} ions during lactation is mediated by a second family of ATPase proteins. The plasma membrane Ca^{2+} ATPase (PMCA) family consist of four

isoforms each capable of producing multiple variant proteins through alternative splicing [93], which are expressed at varying levels in different tissues. PMCA1 and PMCA4 are considered to be ubiquitously expressed [94] with studies of breast cancer cell lines demonstrating altered expression of both isoforms [95, 96]. The PMCA2 isoform is crucial for lactation as evidenced by its strong upregulation in murine models of lactation and the 60% reduction in Ca^{2+} levels in milk from PMCA2-null mice [5].

In addition to their physiological role, PMCA transporters have also been shown to be important mediators of response to apoptotic stimuli in breast cancer cells. Silencing of PCMA2 in MDA-MB-231 cells reduced proliferation and enhanced sensitivity to doxorubicin, ionomycin and the Bcl-2 inhibitor ABT-263 [16, 97]. Similar to the PMCA2 isoform, both PMCA1 and PMCA4 appear to act in a protective fashion against different cytotoxic agents. In a study of the relative contributions of both isoforms to Ca^{2+} regulation in MDA-MB-231 cells, knockdown of PMCA1 reduced Ca^{2+} influx in response to a variety of inducers including the SERCA inhibitor cyclopiazonic acid, ATP and ionomycin, indicating this isoform acts as a major modulator of cytoplasmic Ca^{2+} within the MDA-MB-231 cell line [98]. Although cell viability was unaltered by knockdown of either PMCA1 or PMCA4 knockdown, loss of PMCA1 was observed to significantly increase the number of necrotic cells following ionomycin treatment, highlighting the essential role of PMCA1 in preventing excessive Ca^{2+} influx. Interestingly, although knockdown of PMCA4 caused little alteration in cytoplasmic Ca^{2+}, it did significantly increase cellular sensitivity to the pro-apoptotic agent ABT-263, further demonstrating an isoform-specific modulation of cellular death pathways.

PMCA2 is perhaps the best characterised isoform in the context of breast cancer and has been shown to be upregulated in studies of both cell lines and patient samples [16, 96] , with high expression levels correlating with a high tumour grade, lymph node involvement and a decrease in patient survival [12]. Several studies have also identified an association between PMCA2 and HER2 positivity, with PMCA2 activity also appearing to play a vital role in HER2 protein localisation and signalling. This association was amply demonstrated by Jeong et al., who used tissue microarray staining to show correlation between expression of PMCA2 and HER2 and co-localisation of the two proteins within the cell plasma membrane [99]. PMCA2 knockdown reduced levels of total and phosphorylated HER2 in the HER2+ SKBR3 and BT474 cell lines, whilst overexpression in the luminal T47D cell line lead to increased HER2 expression and activity and a significantly enhanced tumour forming capability when implanted into immunocompromised mice.

A subsequent study showed that PMCA2 expression is itself regulated by HER2 signalling, allowing for HER2 protein to promote its own stability and activity via increasing PMCA2 expression and decreasing intracellular Ca^{2+} [100]. Interestingly, the influence of PMCA2 on HER2 was found to be highly dependent on PMCA2's ability to regulate intracellular Ca^{2+}, as expression of a catalytically inactive form of PMCA2 failed to support HER2 activity whilst treatment of SKBR3 cells with a combination of high extracellular Ca^{2+} and ionomycin decreased HER2-PMCA2 interaction. This is in contrast to some studies of PMCA2 function in the MDA-MB-231 cell line, where knockdown decreased proliferation and increased chemosensitivity without altering intracellular Ca^{2+} levels [16, 97]. This may be due to differences in the relative contributions of different PMCA isoforms to the behaviour of individual cell lines. PMCA1 has previously been shown to be the predominant PMCA isoform involved in global Ca^{2+} regulation in MDA-MB-231 cells [98].

In addition, PMCA isoforms are now thought to operate through a "fine-tuning" process, whereby they may affect processing of Ca^{2+} signals without significant alteration of global Ca^{2+} concentrations. One method by which this may be accomplished is through binding with Ca^{2+}-

regulated proteins and isolating them in localised regions of low Ca^{2+}, generated by the Ca^{2+} efflux activity of PCMA itself [101]. An example of this is the calcineurin/ nuclear factor of activated T-cells (NFAT) pathway. Calcineurin is a serine/threonine phosphatase that becomes activated in response to increased intracellular Ca^{2+}, triggering further activation of multiple signalling factors including NFAT. Activation of the calcineurin/NFAT pathway is often considered tumourigenic as it has been shown to promote migration and invasion in breast cancer cells [102]. However, high levels of calcineurin activation may also promote apoptosis [103].

PMCA2 regulates activation of the calcineurin/NFAT pathway through direct interaction with calcineurin, sequestering it within "cellular micro-domains" [104, 105]. Baggott *et al* demonstrated that this interaction could be blocked by overexpression of a PMCA2 peptide fragment responsible for calcineurin binding, which competes with endogenous PMCA2 and reduces the levels of calcineurin sequestered within low-Ca^{2+} plasma membrane micro domains, allowing for its activation. Disruption of PMCA2-calcineurin interactions was also shown to increase levels of pro-apoptotic FasL with a consequent activation of apoptosis and enhancement of paclitaxel cytotoxicity.

Transient receptor potential channels

Transient receptor potential (TRP) channels represent a large family of ion channel proteins which can be further divided into multiple subfamilies including TRPC (canonical), TRPV (vanilloid) and TRPM (melastatin). These diverse channels can be gated by a variety of stimuli, including temperature, mechanical stimulation, chemical ligands and ion concentrations. Many TRP channels have been implicated in breast tumourigenesis [106].

The canonical TRP channels (TRPC1-7) are the most conserved members of the TRP family to the originally identified drosophilia protein. As previously discussed, the TRPC1 channel can activate an alternative mechanism of SOCE in certain cell types [66, 67] and is also the best characterised TRPC channel in relation to breast cancer. TRPC1 is upregulated in breast cancer [18], especially within the basal subtype where it appears to act as an important mediator of hypoxic response. Knockdown of TRPC1 decreased hypoxia-induced phosphorylation of STAT3 and EGFR and subsequent induction of the EMT marker Snail [107]. Induction of EMT in breast cancer cells is known to be a Ca^{2+} dependent process [108] and other studies have also highlighted a role for TRPC1 (and other TRP channels) in promoting EMT. TGF-β stimulation promotes SOCE in murine mammary epithelial cells, which acts to increase migration and expression of mesenchymal markers [109]. These increases were found to be abrogated following silencing of the TRPC1 channel and conversely increased by TRPC1 overexpression. Similarly, knockdown of TRPC1 in MDA-MB-468 cells blocks EGF-mediated upregulation of ATP-binding cassette, sub-family C, member 3 (ABCC3), a known promoter of multidrug resistance [110]. In addition to TRPC1, studies have also shown upregulation of TRPC3 and TRPC6 in breast cancer samples [13]. TRPC3 expression is elevated in the triple-negative MDA-MB-231 cell line compared to luminal MCF-7 cells, where its inhibition markedly reduced proliferation and increased apoptosis [111]. Similarly, TRPC6 is upregulated in breast cancer cell lines [112], where it promotes proliferation, migration and invasion, likely through regulating localisation of Orai1 and Orai3 channels [13, 113].

The TRP-melastatin (TRPM) subfamily contains 8 members, named after the first identified member melastatin 1 (TRPM1). Several members of the TRPM family contains enzymatic domains in addition to their ion channel function. For example, TRPM7 which is overexpressed

in breast cancer patients [11, 18, 112] and has been shown to correlate with decreased patient survival [114], contains a kinase domain which promotes a mesenchymal phenotype in breast cancer cells with associated increases in migration and invasion [115]. Knockdown of TRPM7 reduced vimentin levels and activation of STAT3 pathways in response to EGF stimulation in MDA-MB-468 cells [108], as well as significantly reducing focal adhesion, cell migration and invasion and tumour formation in a xenograft model [11, 114]. TRPM7 may also play a role in formation of microcalcifications as pharmacological inhibition and siRNA knockdown reduced calcification in an *in vitro* model [19].

The TRPM2 channel appears to protect breast cancer cells from genomic damage, as knockdown has been shown to increase both sensitivity to chemotherapeutic agents and levels of DNA damage under resting conditions, as well as reducing proliferation [116, 117]. The fact that these effects were observed only in transformed breast cancer cells and not in noncancerous cells raises the possibility of targeting TRPM2 as a viable therapeutic strategy. Additionally, bioinformatic analysis has suggested it may be of prognostic value in ERα- and HER2+ patients [118]. Interestingly, although reduced expression of TRPM2 renders breast cancer cells more susceptible to chemotherapeutic damage, it conversely increases resistance to neutrophil-cytotoxicity and metastatic potential, suggesting that expression of this channel may be a positive or negative factor, depending on the stage of tumour growth and spread [119]. TRPM8 is important in the perception of cold, and is responsive to decreases in temperature and menthol. It is also highly expressed in many breast tumours [112, 120], with expression levels being regulated in part by estrogen signalling [121]. Knockdown of TRPM8 decreased migration, invasion and expression of mesenchymal markers in MDA-MB-231 cells, with overexpression in MCF-7 conversely promoting an EMT effect via activation of the AKT/GSK-3β pathway [120].

TRP- vanilloid (TRPV) channels are a family of 6 TRP channels, several of which are thermosensitive and play a role in temperature detection. A recent examination of the subcellular distribution of TRPV1 channels found that patients with aggregation of TRPV1 protein within the ER or Golgi apparatus had a significantly reduced survival rate compared to patients with a more diffuse pattern of staining [122]. Activation of TRPV1 may promote cellular death in breast cancer cells suggesting that channel agonists could have some therapeutic efficacy as was demonstrated in a study by Wu et al., which demonstrated a TRPV1-dependent induction of necrosis in MCF-7 cells following treatment with the TRPV1 agonist capsaicin [123]. Other studies have also found that proliferation could be disrupted by both agonists and antagonists of the channel, suggesting a range of potential clinical strategies [124].

The TRPV6 channel may also be a promising target as its expression is elevated in breast cancer and associated with poor survival [112, 125]. Knockdown of TRPV6 decreased proliferation and increased apoptosis in T47D cells [126]. The ERα-modulator tamoxifen has also been shown to inhibit both expression and activity of TRPV1, suggesting that reduced TRPV-1-mediated Ca^{2+} could play a role in the anti-proliferative effect of tamoxifen [126, 127]. Interestingly, similar levels of TRPV6 inhibition were observed in the ERα- MDA-MB-231 cell line as ERα+ MCF-7, indicating that this effect was independent of estrogen signalling and could be effective even in patients with ERα- tumours [127]

The TRPV4 channel was also found to be upregulated in breast cancer, with further increased levels observed in metastatic lesions and expression correlated with tumour grade, size, and decreased survival **[128]**. Although this upregulation confers breast cancer cells with increased tumourigenic properties including loss of E-cadherin expression and supporting migration and invasion **[128, 129]**, excessive activation can also promote cell

death **[130]** and may improve response to chemotherapy via normalisation of the tumour vasculature **[131]**.

Conclusion

Considering the diverse range of physiological activities regulated by Ca^{2+} signalling, it is of little surprise that alterations within this complex signalling network have the potential to influence tumour behaviour. Ca^{2+} signalling within tumours is controlled by an ever-expanding family of transporters, many of which have now been demonstrated to be dysregulated. Many studies have correlated expression levels of these channels with important clinical attributes such as proliferative rate and HER2 status, suggesting they may be clinically useful as prognostic tests. Looking towards therapeutic options, *in vitro* studies have demonstrated clear proof of concept in both inhibiting and activating certain channels to alter key tumour properties such as proliferation and apoptosis. Although promising, some challenges remain before translation of these findings can truly begin. Perhaps the most significant hurdle to be overcome is the generation of novel agents with improved specificity, as many inhibitors commonly used in *in vitro* studies are capable of inhibiting related channels, and in some cases, even activating them [132]. However, this work is currently under way [133], with several promising agents in development, some of which have undergone early clinical trial [134]. In addition, many Ca^{2+} transporters have differential expression patterns between tumour types and even subtypes, requiring a careful selection of targets to maximise clinical benefit and reduce the risk of side-effects. It is hoped that continuing study and improved understanding of tumourigenic Ca^{2+} signalling pathways will soon allow for translation of these findings to the clinic in order to improve patient outcome.

Acknowledgements

The authors acknowledge the support of Breast Cancer Now grant 2013NovPhD147

Conflict of Interest statement

The authors declare that there are no conflicts of interest.

References

1. Cross, B.M., et al., *Cellular calcium dynamics in lactation and breast cancer: from physiology to pathology.* Am. J. Physiol. Cell Physiol., 2014. **306**(6): p. C515-26.
2. Chen, Y.F., et al., *Remodeling of calcium signaling in tumor progression.* J. Biomed. Sci., 2013. **20**: p. 23.
3. So, C.L., et al., *Calcium signalling and breast cancer.* Semin. Cell Dev. Biol., 2019. **94**: p. 74-83.
4. Reinhardt, T.A., et al., *Ca^{2+}-ATPase protein expression in mammary tissue.* Am. J. Physiol. Cell Physiol., 2000. **279**(5): p. C1595-C602.

5. Reinhardt, T.A., et al., *Null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 2 impairs calcium transport into milk.* J. Biol. Chem., 2004. **279**(41): p. 42369-73.
6. Reinhardt, T.A. and R.L. Horst, *Ca2+-ATPases and their expression in the mammary gland of pregnant and lactating rats.* Am. J. Physiol., 1999. **276**(4): p. C796-802.
7. VanHouten, J.N., M.C. Neville, and J.J. Wysolmerski, *The Calcium-Sensing Receptor Regulates Plasma Membrane Calcium Adenosine Triphosphatase Isoform 2 Activity in Mammary Epithelial Cells: A Mechanism for Calcium-Regulated Calcium Transport into Milk.* Endocrinology, 2007. **148**(12): p. 5943-54.
8. McAndrew, D., et al., *ORAI1-mediated calcium influx in lactation and in breast cancer.* Mol. Cancer Ther., 2011. **10**(3): p. 448-60.
9. Davis, F.M., et al., *Essential role of Orai1 store-operated calcium channels in lactation.* Proc. Natl. Acad. Sci. U. S. A., 2015. **112**(18): p. 5827-32.
10. Cross, B.M., et al., *SPCA2 Regulates Orai1 Trafficking and Store Independent Ca2+ Entry in a Model of Lactation.* PloS one, 2013. **8**(6): p. e67348.
11. Meng, X., et al., *TRPM7 mediates breast cancer cell migration and invasion through the MAPK pathway.* Cancer Lett., 2013. **333**(1): p. 96-102.
12. VanHouten, J., et al., *PMCA2 regulates apoptosis during mammary gland involution and predicts outcome in breast cancer.* Proc. Natl. Acad. Sci. U.S.A., 2010. **107**(25): p. 11405-10.
13. Aydar, E., et al., *Abnormal expression, localization and interaction of canonical transient receptor potential ion channels in human breast cancer cell lines and tissues: a potential target for breast cancer diagnosis and therapy.* Cancer Cell Int., 2009. **9**: p. 23-.
14. Guilbert, A., et al., *Evidence that TRPM7 is required for breast cancer cell proliferation.* Am. J. Physiol. Cell Physiol., 2009. **297**(3): p. C493-C502.
15. McAndrew, D., et al., *ORAI1-Mediated Calcium Influx in Lactation and in Breast Cancer.* Molecular Cancer Therapeutics, 2011. **10**(3): p. 448.
16. Peters, A.A., et al., *The calcium pump plasma membrane Ca^{2+}-ATPase 2 (PMCA2) regulates breast cancer cell proliferation and sensitivity to doxorubicin.* Sci. Rep., 2016. **6**: p. 25505.
17. Azimi, I., S.J. Roberts-Thomson, and G.R. Monteith, *Calcium influx pathways in breast cancer: opportunities for pharmacological intervention.* Br. J. Pharmacol., 2014. **171**(4): p. 945-60.
18. Mandavilli, S., B.B. Singh, and A.E. Sahmoun, *Serum calcium levels, TRPM7, TRPC1, microcalcifications, and breast cancer using breast imaging reporting and data system scores.* Breast Cancer (Dove Med Press) 2012. **2013**(5): p. 1-7.
19. O'Grady, S. and M.P. Morgan, *Deposition of calcium in an in vitro model of human breast tumour calcification reveals functional role for ALP activity, altered expression of osteogenic genes and dysregulation of the TRPM7 ion channel.* Sci. Rep., 2019. **9**(1): p. 542-.
20. Dang, D., H. Prasad, and R. Rao, *Secretory pathway Ca(2+) -ATPases promote in vitro microcalcifications in breast cancer cells.* 2017. **56**(11): p. 2474-85.
21. Qi, H., et al., *Frequency and relative prevalence of calcium blips and puffs in a model of small IP_3R clusters.* Biophys. J., 2014. **106**(11): p. 2353-63.

22. Samanta, K. and A.B. Parekh, *Spatial Ca^{2+} profiling: decrypting the universal cytosolic Ca^{2+} oscillation.* J. Physiol., 2017. **595**(10): p. 3053-62.
23. Rizaner, N., et al., *Intracellular calcium oscillations in strongly metastatic human breast and prostate cancer cells: control by voltage-gated sodium channel activity.* Eur. Biophys. J., 2016. **45**(7): p. 735-48.
24. Monteith, G.R., F.M. Davis, and S.J. Roberts-Thomson, *Calcium channels and pumps in cancer: changes and consequences.* J. Biol. Chem., 2012. **287**(38): p. 31666-73.
25. Stewart, T.A., K.T. Yapa, and G.R. Monteith, *Altered calcium signaling in cancer cells.* Biochim. Biophys. Acta, 2015. **1848**(10 Pt B): p. 2502-11.
26. Bronner, F., *Extracellular and intracellular regulation of calcium homeostasis.* Sci. World J., 2001. **1**: p. 919-25.
27. Schwarz, D.S. and M.D. Blower, *The endoplasmic reticulum: structure, function and response to cellular signaling.* Cell. Mol. Life Sci., 2016. **73**(1): p. 79-94.
28. Prins, D. and M. Michalak, *Organellar Calcium Buffers.* Cold Spring Harb. Perspect. Biol., 2011. **3**(3).
29. Coe, H. and M. Michalak, *Calcium binding chaperones of the endoplasmic reticulum.* Gen. Physiol. Biophys., 2009. **Focus Issue 28**: p. F96-f103.
30. Oslowski, C.M. and F. Urano, *Measuring ER stress and the unfolded protein response using mammalian tissue culture system.* Methods Enzymol., 2011. **490**: p. 71-92.
31. Hu, R., et al., *NF-κB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer.* Mol. Cell. Biol., 2015. **35**(2): p. 379-90.
32. Wang, J., et al., *Blockade of GRP78 sensitizes breast cancer cells to microtubules-interfering agents that induce the unfolded protein response.* J. Cell. Mol. Med., 2009. **13**(9B): p. 3888-97.
33. Giorgi, C., et al., *Mitochondrial Ca^{2+} and apoptosis.* Cell Calcium, 2012. **52**(1): p. 36-43.
34. Brini, M. and E. Carafoli, *Calcium Pumps in Health and Disease.* Physiol. Rev., 2009. **89**(4): p. 1341-78.
35. Vandecaetsbeek, I., et al., *Structural basis for the high Ca^{2+} affinity of the ubiquitous SERCA2b Ca^{2+} pump.* Proc. Natl. Acad. Sci. U.S.A., 2009. **106**(44): p. 18533-8;.
36. Wang, Y., et al., *SGK3 sustains ERα signaling and drives acquired aromatase inhibitor resistance through maintaining endoplasmic reticulum homeostasis.* Proc. Natl. Acad. Sci. U.S.A., 2017. **114**(8): p. E1500-E8.
37. Papp, B. and J.-P. Brouland, *Altered Endoplasmic Reticulum Calcium Pump Expression during Breast Tumorigenesis.* Breast Cancer (Auckl.), 2011. **5**: p. 163–74.
38. Varga, K., et al., *Expression of calcium pumps is differentially regulated by histone deacetylase inhibitors and estrogen receptor alpha in breast cancer cells.* BMC Cancer, 2018. **18**(1): p. 1029.
39. Mahdi, S.H.A., et al., *The effect of TGF-beta-induced epithelial–mesenchymal transition on the expression of intracellular calcium-handling proteins in T47D and MCF-7 human breast cancer cells.* Arch. Biochem. Biophys., 2015. **583**: p. 18-26.

40. Azeez, J.M. and R. Vini, *VDAC1 and SERCA3 Mediate Progesterone-Triggered Ca^{2+} Signaling in Breast Cancer Cells.* J Proteome Res., 2018. **17**(1): p. 698-709.
41. Sehgal, P., et al., *Inhibition of the sarco/endoplasmic reticulum (ER) Ca^{2+}-ATPase by thapsigargin analogs induces cell death via ER Ca^{2+} depletion and the unfolded protein response.* J. Biol. Chem., 2017. **292**(48): p. 19656-73.
42. Pimentel, A.A., et al., *The marine sponge toxin agelasine B increases the intracellular Ca2+ concentration and induces apoptosis in human breast cancer cells (MCF-7).* Cancer Chemother. Pharmacol., 2012. **69**(1): p. 71-83.
43. Thillaiappan, N.B., et al., *IP3 receptors and Ca^{2+} entry.* Biochim. Biophys. Acta, 2019. **1866**(7): p. 1092-100.
44. Van Petegem, F., *Ryanodine Receptors: Structure and Function.* J. Biol. Chem., 2012. **287**(38): p. 31624-32.
45. Abdul, M., S. Ramlal, and N. Hoosein, *Ryanodine Receptor Expression Correlates with Tumor Grade in Breast Cancer.* Pathol. Oncol. Res., 2008. **14**(2): p. 157-60.
46. Zhang, L., et al., *Functional SNP in the microRNA-367 binding site in the 3'UTR of the calcium channel ryanodine receptor gene 3 (RYR3) affects breast cancer risk and calcification.* Proc. Natl. Acad. Sci. U.S.A., 2011. **108**(33): p. 13653-8.
47. Lu, H., et al., *Chemotherapy-Induced Ca^{2+} Release Stimulates Breast Cancer Stem Cell Enrichment.* Cell Rep., 2017. **18**(8): p. 1946-57.
48. Szatkowski, C., et al., *Inositol 1,4,5-trisphosphate-induced Ca^{2+} signalling is involved in estradiol-induced breast cancer epithelial cell growth.* Mol. Cancer, 2010. **9**: p. 156-.
49. Mound, A., et al., *Downregulation of type 3 inositol (1,4,5)-trisphosphate receptor decreases breast cancer cell migration through an oscillatory Ca^{2+} signal.* Oncotarget, 2017. **8**(42): p. 72324-41.
50. Davis, F.M., et al., *Assessment of gene expression of intracellular calcium channels, pumps and exchangers with epidermal growth factor-induced epithelial-mesenchymal transition in a breast cancer cell line.* Cancer Cell Int., 2013. **13**(1): p. 76.
51. Kerkhofs, M., et al., *Emerging molecular mechanisms in chemotherapy: Ca^{2+} signaling at the mitochondria-associated endoplasmic reticulum membranes.* Cell Death Dis., 2018. **9**(3): p. 334.
52. De Stefani, D., et al., *A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter.* Nature, 2011. **476**(7360): p. 336-40.
53. Rizzuto, R., et al., *Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses.* Science, 1998. **280**(5370): p. 1763-6.
54. Tosatto, A., et al., *The mitochondrial calcium uniporter regulates breast cancer progression via HIF-1α.* EMBO Mol. Med., 2016. **8**(5): p. 569-85.
55. Tang, S., et al., *Mitochondrial Ca^{2+} uniporter is critical for store-operated Ca^{2+} entry-dependent breast cancer cell migration.* Biochem. Biophys. Res. Commun., 2015. **458**(1): p. 186-93.
56. Prudent, J., et al., *Mitochondrial Ca^{2+} uptake controls actin cytoskeleton dynamics during cell migration.* Sci. Rep., 2016. **6**: p. 36570-.
57. Watson, R. and A.B. Parekh, *Mitochondrial regulation of CRAC channel-driven cellular responses.* Cell Calcium, 2012. **52**(1): p. 52-6.

58. Feske, S., et al., *A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function.* Nature, 2006. **441**(7090): p. 179-85.
59. Liou, J., et al., *STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx.* Curr. Biol., 2005. **15**(13): p. 1235-41.
60. Vig, M., et al., *CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry.* Science, 2006. **312**(5777): p. 1220-3.
61. Roos, J., et al., *STIM1, an essential and conserved component of store-operated Ca²⁺ channel function.* The Journal of Cell Biology, 2005. **169**(3): p. 435-45.
62. Zhang, S.L., et al., *Genome-wide RNAi screen of Ca²⁺ influx identifies genes that regulate Ca²⁺ release-activated Ca²⁺ channel activity.* Proceedings of the National Academy of Sciences, 2006. **103**(24): p. 9357-62.
63. Hewavitharana, T., et al., *Location and function of STIM1 in the activation of Ca^{2+} entry signals.* J. Biol. Chem., 2008. **283**(38): p. 26252-62.
64. Soboloff, J., et al., *STIM proteins: dynamic calcium signal transducers.* Nat. Rev. Mol. Cell Biol., 2012. **13**(9): p. 549-65.
65. Ong, H.L., S.I. Jang, and I.S. Ambudkar, *Distinct contributions of Orai1 and TRPC1 to agonist-induced Ca^{2+} signals determine specificity of Ca^{2+}-dependent gene expression.* PLoS One, 2012. **7**(10): p. e47146.
66. Cheng, K.T., et al., *Functional requirement for Orai1 in store-operated TRPC1-STIM1 channels.* J. Biol. Chem., 2008. **283**(19): p. 12935-40.
67. Cheng, K.T., et al., *Local Ca^{2+} entry via Orai1 regulates plasma membrane recruitment of TRPC1 and controls cytosolic Ca^{2+} signals required for specific cell functions.* PLoS Biol., 2011. **9**(3): p. e1001025.
68. Yang, S., J.J. Zhang, and X.Y. Huang, *Orai1 and STIM1 are critical for breast tumor cell migration and metastasis.* Cancer Cell, 2009. **15**(2): p. 124-34.
69. Yang, Y., et al., *Expression of STIM1 is associated with tumor aggressiveness and poor prognosis in breast cancer.* Pathol. Res. Pract., 2017. **213**(9): p. 1043-7.
70. Yang, Y., et al., *MicroRNA-223 Targeting STIM1 Inhibits the Biological Behavior of Breast Cancer.* Cell. Physiol. Biochem., 2018. **45**(2): p. 856-66.
71. Chen, Y.-F., L.-H. Chen, and M.-R. Shen, *The distinct role of STIM1 and STIM2 in the regulation of store-operated Ca^{2+} entry and cellular function.* J. Cell. Physiol., 2019. **234**(6): p. 8727-39.
72. Zhang, S., et al., *STIM1 and STIM2 differently regulate endogenous Ca^{2+} entry and promote TGF-β-induced EMT in breast cancer cells.* Biochem. Biophys. Res. Commun., 2017. **488**(1): p. 74-80.
73. Azimi, I., et al., *ORAI1 and ORAI3 in Breast Cancer Molecular Subtypes and the Identification of ORAI3 as a Hypoxia Sensitive Gene and a Regulator of Hypoxia Responses.* Cancers (Basel), 2019. **11**(2): p. 208.
74. Motiani, R.K., 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., 2010. **285**(25): p. 19173-83.
75. Motiani, R.K., et al., *Orai3 is an estrogen receptor α-regulated Ca^{2+} channel that promotes tumorigenesis.* FASEB J., 2013. **27**(1): p. 63-75.
76. Faouzi, M., et al., *Down-regulation of Orai3 arrests cell-cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells.* J. Cell. Physiol., 2011. **226**(2): p. 542-51.

77. Faouzi, M., et al., *ORAI3 silencing alters cell proliferation and cell cycle progression via c-myc pathway in breast cancer cells.* Biochim. Biophys. Acta, 2013. **1833**(3): p. 752-60.
78. Hasna, J., et al., *Orai3 calcium channel and resistance to chemotherapy in breast cancer cells: the p53 connection.* Cell Death Differ., 2018. **25**(4): p. 691-705.
79. Xie, J., et al., *SOCE and cancer: Recent progress and new perspectives.* Int. J. Cancer, 2016. **138**(9): p. 2067-77.
80. Lodola, F., et al., *VEGF-induced intracellular Ca^{2+} oscillations are down-regulated and do not stimulate angiogenesis in breast cancer-derived endothelial colony forming cells.* Oncotarget, 2017. **8**(56): p. 95223-46.
81. Chakraborty, S., et al., *Phemindole, a Synthetic Di-indole Derivative Maneuvers the Store Operated Calcium Entry (SOCE) to Induce Potent Anti-Carcinogenic Activity in Human Triple Negative Breast Cancer Cells.* Front. Pharmacol., 2016. **7**: p. 114-.
82. Emeriau, N., et al., *Store operated calcium entry is altered by the inhibition of receptors tyrosine kinase.* Oncotarget, 2018. **9**(22): p. 16059-73.
83. Babaer, D., et al., *High salt induces P-glycoprotein mediated treatment resistance in breast cancer cells through store operated calcium influx.* Oncotarget, 2018. **9**(38): p. 25193-205.
84. Van Baelen, K., et al., *The Ca^{2+}/Mn^{2+} pumps in the Golgi apparatus.* Biochim. Biophys. Acta, 2004. **1742**(1): p. 103-12.
85. Faddy, H.M., et al., *Localization of plasma membrane and secretory calcium pumps in the mammary gland.* Biochem. Biophys. Res. Commun., 2008. **369**(3): p. 977-81.
86. Feng, M., et al., *Store-independent activation of Orai1 by SPCA2 in mammary tumors.* Cell, 2010. **143**(1): p. 84-98.
87. Cox, R.F., et al., *Osteomimicry of mammary adenocarcinoma cells in vitro; increased expression of bone matrix proteins and proliferation within a 3D collagen environment.* PLoS One, 2012. **7**(7): p. e41679-e.
88. Scimeca, M., et al., *Microcalcifications in breast cancer: an active phenomenon mediated by epithelial cells with mesenchymal characteristics.* BMC Cancer, 2014. **14**: p. 286-.
89. Cooke, M.M., et al., *Phosphocitrate inhibits calcium hydroxyapatite induced mitogenesis and upregulation of matrix metalloproteinase-1, interleukin-1beta and cyclooxygenase-2 mRNA in human breast cancer cell lines.* Breast Cancer Res. Treat., 2003. **79**(2): p. 253-63.
90. Cox, R.F., et al., *Microcalcifications in breast cancer: novel insights into the molecular mechanism and functional consequence of mammary mineralisation.* Br. J. Cancer, 2012. **106**(3): p. 525-37.
91. Morgan, M.P., et al., *Calcium hydroxyapatite promotes mitogenesis and matrix metalloproteinase expression in human breast cancer cell lines.* Mol. Carcinog., 2001. **32**(3): p. 111-7.
92. Grice, D.M., et al., *Golgi calcium pump secretory pathway calcium ATPase 1 (SPCA1) is a key regulator of insulin-like growth factor receptor (IGF1R) processing in the basal-like breast cancer cell line MDA-MB-231.* J. Biol. Chem., 2010. **285**(48): p. 37458-66.
93. Brini, M., *Plasma membrane Ca(2+)-ATPase: from a housekeeping function to a versatile signaling role.* Pflugers Archiv : European journal of physiology, 2009. **457**(3): p. 657-64.

94. Brini, M., *Plasma membrane Ca^{2+}-ATPase: from a housekeeping function to a versatile signaling role.* Pflug Arch Eur J Phy, 2008. **457**(3): p. 657.
95. Lee, W.J., et al., *Expression of plasma membrane calcium pump isoform mRNAs in breast cancer cell lines.* Cell. Signal., 2002. **14**(12): p. 1015-22.
96. Lee, W.J., S.J. Roberts-Thomson, and G.R. Monteith, *Plasma membrane calcium-ATPase 2 and 4 in human breast cancer cell lines.* Biochem. Biophys. Res. Commun., 2005. **337**(3): p. 779-83.
97. Curry, M., S.J. Roberts-Thomson, and G.R. Monteith, *PMCA2 silencing potentiates MDA-MB-231 breast cancer cell death initiated with the Bcl-2 inhibitor ABT-263.* Biochem. Biophys. Res. Commun., 2016. **478**(4): p. 1792-7.
98. Curry, M.C., et al., *Distinct Regulation of Cytoplasmic Calcium Signals and Cell Death Pathways by Different Plasma Membrane Calcium ATPase Isoforms in MDA-MB-231 Breast Cancer Cells.* Journal of Biological Chemistry, 2012. **287**(34): p. 28598-608.
99. Jeong, J., et al., *PMCA2 regulates HER2 protein kinase localization and signaling and promotes HER2-mediated breast cancer.* Proceedings of the National Academy of Sciences of the United States of America, 2016. **113**(3): p. E282-E90.
100. Jeong, J., et al., *HER2 signaling regulates HER2 localization and membrane retention.* PloS one, 2017. **12**(4): p. e0174849-e.
101. Holton, M.L., et al., *Plasma membrane calcium ATPase proteins as novel regulators of signal transduction pathways.* World J. Biol. Chem., 2010. **1**(6): p. 201-8.
102. Quang, C.T., et al., *The calcineurin/NFAT pathway is activated in diagnostic breast cancer cases and is essential to survival and metastasis of mammary cancer cells.* Cell Death Dis., 2015. **6**(2): p. e1658-e.
103. Ryeom, S., et al., *Targeted Deletion of the Calcineurin Inhibitor DSCR1 Suppresses Tumor Growth.* Cancer Cell, 2008. **13**(5): p. 420-31.
104. Blanc, M.C., et al., *Disruption of the interaction between PMCA2 and calcineurin triggers apoptosis and enhances paclitaxel-induced cytotoxicity in breast cancer cells.* Carcinogenesis, 2012. **33**(12): p. 2362-8.
105. Holton, M., et al., *The interaction between endogenous calcineurin and the plasma membrane calcium-dependent ATPase is isoform specific in breast cancer cells.* FEBS Letters, 2007. **581**(21): p. 4115-9.
106. Fels, B., et al., *The Role of TRP Channels in the Metastatic Cascade.* Pharmaceuticals (Basel), 2018. **11**(2).
107. Azimi, I., et al., *TRPC1 is a differential regulator of hypoxia-mediated events and Akt signalling in PTEN-deficient breast cancer cells.* J. Cell Sci., 2017. **130**(14): p. 2292.
108. Davis, F.M., et al., *Induction of epithelial-mesenchymal transition (EMT) in breast cancer cells is calcium signal dependent.* Oncogene, 2014. **33**(18): p. 2307-16.
109. Schaar, A., et al., *TRPC1-STIM1 activation modulates transforming growth factor beta-induced epithelial-to-mesenchymal transition.* Oncotarget, 2016. **7**(49): p. 80554-67.
110. Stewart, T.A., et al., *A role for calcium in the regulation of ATP-binding cassette, sub-family C, member 3 (ABCC3) gene expression in a model of epidermal growth factor-mediated breast cancer epithelial-mesenchymal transition.* Biochem. Biophys. Res. Commun., 2015. **458**(3): p. 509-14.

111. Wang, Y., et al., *TRPC3 Regulates the Proliferation and Apoptosis Resistance of Triple Negative Breast Cancer Cells through the TRPC3/RASA4/MAPK Pathway.* Cancers (Basel), 2019. **11**(4).
112. Dhennin-Duthille, I., et al., *High expression of transient receptor potential channels in human breast cancer epithelial cells and tissues: correlation with pathological parameters.* Cell. Physiol. Biochem., 2011. **28**(5): p. 813-22.
113. Jardin, I., et al., *TRPC6 Channels Are Required for Proliferation, Migration and Invasion of Breast Cancer Cell Lines by Modulation of Orai1 and Orai3 Surface Exposure.* Cancers (Basel), 2018. **10**(9): p. 331.
114. Middelbeek, J., et al., *TRPM7 Is Required for Breast Tumor Cell Metastasis.* Cancer Res., 2012. **72**(16): p. 4250.
115. Guilbert, A., et al., *Transient receptor potential melastatin 7 is involved in oestrogen receptor-negative metastatic breast cancer cells migration through its kinase domain.* European Journal of Cancer, 2013. **49**(17): p. 3694-707.
116. Hopkins, M.M., et al., *Inhibition of the transient receptor potential melastatin-2 channel causes increased DNA damage and decreased proliferation in breast adenocarcinoma cells.* Int. J. Oncol., 2015. **46**(5): p. 2267-76.
117. Koh, D.W., et al., *Enhanced cytotoxicity in triple-negative and estrogen receptor-positive breast adenocarcinoma cells due to inhibition of the transient receptor potential melastatin-2 channel.* Oncol. Rep., 2015. **34**(3): p. 1589-98.
118. Sumoza-Toledo, A., M.I. Espinoza-Gabriel, and D. Montiel-Condado, *Evaluation of the TRPM2 channel as a biomarker in breast cancer using public databases analysis.* Bol. Med. Hosp. Infant. Mex., 2016. **73**(6): p. 397-404.
119. Gershkovitz, M., et al., *TRPM2 Mediates Neutrophil Killing of Disseminated Tumor Cells.* Cancer Res., 2018. **78**(10): p. 2680.
120. Liu, J., et al., *TRPM8 promotes aggressiveness of breast cancer cells by regulating EMT via activating AKT/GSK-3β pathway.* Tumor Biol., 2014. **35**(9): p. 8969-77.
121. Chodon, D., et al., *Estrogen regulation of TRPM8 expression in breast cancer cells.* BMC Cancer, 2010. **10**: p. 212-.
122. Lozano, C., et al., *Intracellular aggregated TRPV1 is associated with lower survival in breast cancer patients.* Breast cancer (Dove Medical Press), 2018. **10**: p. 161-8.
123. Wu, T.T.L., et al., *Consequences of activating the calcium-permeable ion channel TRPV1 in breast cancer cells with regulated TRPV1 expression.* Cell Calcium, 2014. **56**(2): p. 59-67.
124. Vercelli, C., et al., *Transient receptor potential vanilloid 1 expression and functionality in mcf-7 cells: a preliminary investigation.* J. Breast Cancer, 2014. **17**(4): p. 332-8.
125. Peters, A.A., et al., *Calcium Channel TRPV6 as a Potential Therapeutic Target in Estrogen Receptor–Negative Breast Cancer.* Mol. Cancer Ther., 2012. **11**(10): p. 2158.
126. Bolanz, K.A., M.A. Hediger, and C.P. Landowski, *The role of TRPV6 in breast carcinogenesis.* Mol. Cancer Ther., 2008. **7**(2): p. 271.
127. Bolanz, K.A., et al., *Tamoxifen Inhibits TRPV6 Activity via Estrogen Receptor–Independent Pathways in TRPV6-Expressing MCF-7 Breast Cancer Cells.* Mol. Cancer Res., 2009. **7**(12): p. 2000.

128. Lee, W.H., et al., *TRPV4 plays a role in breast cancer cell migration via Ca2+-dependent activation of AKT and downregulation of E-cadherin cell cortex protein.* Oncogenesis, 2017. **6**: p. e338.
129. Lee, W.H., et al., *TRPV4 Regulates Breast Cancer Cell Extravasation, Stiffness and Actin Cortex.* Sci. Rep., 2016. **6**: p. 27903-.
130. Peters, A.A., et al., *Oncosis and apoptosis induction by activation of an overexpressed ion channel in breast cancer cells.* Oncogene, 2017. **36**(46): p. 6490-500.
131. Adapala, R.K., et al., *Activation of mechanosensitive ion channel TRPV4 normalizes tumor vasculature and improves cancer therapy.* Oncogene, 2015. **35**: p. 314.
132. Colton, C.K. and M.X. Zhu, *2-Aminoethoxydiphenyl borate as a common activator of TRPV1, TRPV2, and TRPV3 channels.* Handb. Exp. Pharmacol., 2007(179): p. 173-87.
133. Gautier, M., et al., *New insights into pharmacological tools to TR(i)P cancer up.* Br. J. Pharmacol., 2014. **171**(10): p. 2582-92.
134. Fu, S., et al., *First-in-human phase I study of SOR-C13, a TRPV6 calcium channel inhibitor, in patients with advanced solid tumors.* Invest. New Drugs, 2017. **35**(3): p. 324-33.