

## Opinion

# “Calcium Influx Pathways in Breast Cancer: Opportunities for Pharmacological Intervention”

Hari Sonwani\*

Apollo College of Pharmacy, Anjora, Durg, India

**\*Corresponding author: Hari Sonwani**

Apollo College of Pharmacy, Anjora, Durg, India.

Email: harisonwani10@gmail.com

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Numerous cellular processes, including the release of neurotransmitters and the contraction of muscles, are largely triggered and regulated by Ca<sup>2+</sup> inflow via Ca<sup>2+</sup> permeable ion channels. In addition, Ca<sup>2+</sup> influx regulates cellular migration and proliferation, two mechanisms linked to cancer. This study focuses on calcium influx in breast cancer cells and discusses how future drugs for breast cancer therapy may be pharmacological modulators of particular Ca<sup>2+</sup> influx channels. Certain breast tumors have altered expression of particular calcium permeable ion channels. Such alterations may occasionally be connected to the prognosis and subtype of breast cancer. These days, models both in vivo and in vitro have assisted in identifying particular Ca<sup>2+</sup> channels that are crucial for the growth and invasiveness of cancerous breast cells. Nonetheless, additional research is still needed to fully understand several features of Ca<sup>2+</sup> influx in breast cancer. These include figuring out the processes behind the changed expression and the best treatment plan to target breast cancer cells via particular Ca<sup>2+</sup> channels. In the upcoming ten years, research should concentrate on the function of Ca<sup>2+</sup> influx in mechanisms other than the migration and proliferation of breast cancer cells.

**Keywords:** Breast cancer; Calcium channels; Calcium influx; Calcium signalling; Oncology

**Abbreviations:** N-cyano-N'-[(1S)-1-phenylethyl]; [Ca<sup>2+</sup>] CYT, cytoplasmic-free calcium; IP<sub>3</sub>, inositol 1,4,5-trisphosphate; JNJ41876666, 3-[7-trifluoromethyl-5-(2-trifluoromethyl-phenyl)]; ErbB2 (also known as HER2), human EGF receptor 2; EC, endothelial cells; EMT, epithelial to mesenchymal transition; ER+, oestrogen positive; ER $\alpha$ , oestrogen receptor  $\alpha$ ; Azol-2-yl-1H-benzimid[4.5]dec-1-oxa-2-aza-spiro-2-eneHydrochloride; NNC 55-0396, (1S,2S); NFAT, nuclear factor for activated T-cells[(3-benzimidazol-2-yl)propyl]-2-(2-(N-)]The methylamino)ethyl group tetrahydro-6-fluoro-1,2,3,4-Pyr3, 1-[4-[(2,3,3-trichloro-1-oxo-2-propen-1-yl)amino]phenyl]; PMCA, plasma membrane Ca<sup>2+</sup>-ATPase; -1-isopropyl-2-naphthyl cyclopropanecarboxylate dihydrochloride Trifluoromethyl, or 5-4-carboxylic acid pyrazole -1H; SCID stands for severe combined immune deficiency; SB-209712 is 1,6-bis{1-[4-(3-phenylpropyl) piperidinyl]} hexane; transient receptor potential; TRP, secretory route Ca<sup>2+</sup>-ATPase

**Introduction**

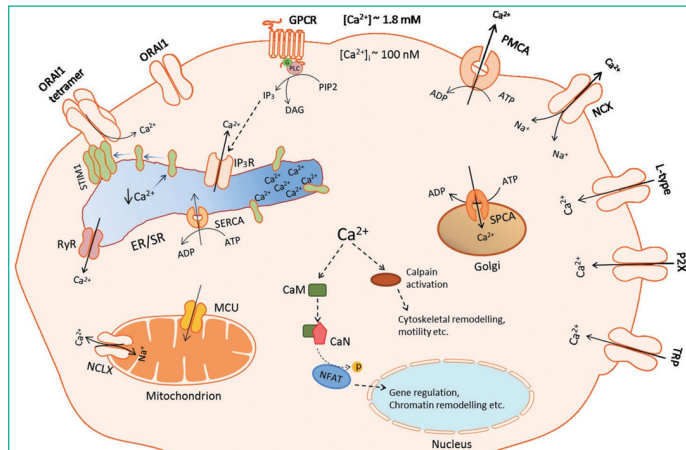
With intracellular free Ca<sup>2+</sup> levels almost 20 000 times lower than in the external environment (100 nM vs. 1.8 mM), cells maintain a significant gradient of free Ca<sup>2+</sup> across the plasma membrane (Carafoli, 1987; Clapham, 2007). Utilizing this Ca<sup>2+</sup> gradient, cells frequently use Ca<sup>2+</sup> influx to start and control cellular signals, typically by opening Ca<sup>2+</sup> permeable ion channels. Numerous varied routes are regularly muscle contraction, gene

transcription, cell division, and neurotransmitter release are all triggered by increases in intracellular cytoplasmic-free calcium ([Ca<sup>2+</sup>]CYT) [14]. For a number of ailments, Ca<sup>2+</sup> permeable ion channels may be useful pharmacological targets. Among these disorders include hypertension, for which nifedipine and other L-type voltage-gated Ca<sup>2+</sup> channel blockers are used clinically [5], and chronic pain. Ziconotide, an N-type channel inhibitor, is

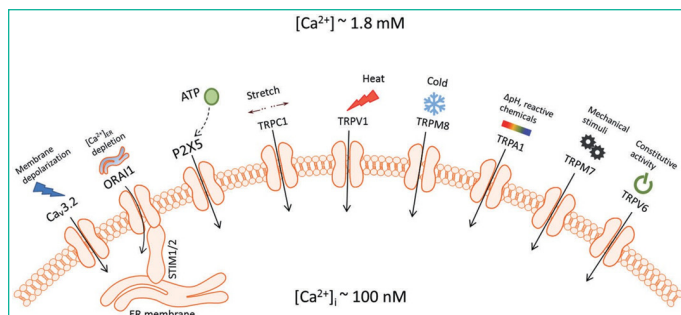
employed (Malmberg and Yaksh, 1995). The research that has evaluated calcium influx routes in the development of breast cancer and identified calcium permeable ion channels as pharmacological targets for breast cancer therapy will be the main emphasis of this review.

### Calcium Signaling: The Critical Function of Calcium Influx

Numerous reviews [14 Leybaert and Sanderson, 2012] describe how mammalian cells control  $[Ca^{2+}]_{CYT}$  levels and the significance of the nature of variations in  $[Ca^{2+}]_{CYT}$  (such as  $[Ca^{2+}]_{CYT}$  oscillations and localized changes in  $Ca^{2+}$ ). As Figure 1 shows a few of the primary calcium exchangers, pumps, and channels in the pathways that signal calcium. In summary, the active efflux of  $Ca^{2+}$  from the cell through the plasma membrane  $Ca^{2+}$ -ATPases (PMCA) maintains  $[Ca^{2+}]_{CYT}$  levels at low levels. Activation of these enzymes, together with  $Na^{+}/Ca^{2+}$  exchangers and sarco/endoplasmic reticulum  $Ca^{2+}$  ATPases, lowers  $[Ca^{2+}]_{CYT}$ . There are various mechanisms that can lead to increases in  $[Ca^{2+}]_{CYT}$ . For instance, several GPCRs, via means of activation of Through  $IP_3$ -activated  $Ca^{2+}$  channels, PLC and the production of inositol 1,4,5-trisphosphate ( $IP_3$ ) release  $Ca^{2+}$  from internal calcium reserves, such as the sarco/endoplasmic reticulum [14]. The recently discovered mitochondrial  $Ca^{2+}$  uniporter (Kirichok et al., 2004) and the  $Na^{+}/Ca^{2+}$  exchanger NCLX (Palty et al., 2010) are two more organelles that are involved in  $Ca^{2+}$  signaling. and the Golgi, which uses the secretory route  $Ca^{2+}$ -ATPases (SPCAs) to sequester intracellular  $Ca^{2+}$ . The opening of calcium permeable ion channels on the plasma membrane also results in increases in  $[Ca^{2+}]_{CYT}$ . Many distinct physiological activities, especially those involving excitable cells, such the release of neurotransmitters in neurons and the excitation–contraction coupling in skeletal muscle (Rios and



**Figure 1:** Schematic depiction of some of the  $Ca^{2+}$  channels, pumps and exchangers involved in  $Ca^{2+}$  signalling in mammalian cells.  $Ca^{2+}$  influx channels include the ORAI1 channel (an example of a store-operated  $Ca^{2+}$  entry channel), L-type  $Ca^{2+}$  channels (an example of a voltage-gated  $Ca^{2+}$  channel), P2X receptor channel (an example of a ligand-gated  $Ca^{2+}$  channel) and TRP channels (channels that vary in their  $Ca^{2+}$  selectivity). GPCRs increase  $[Ca^{2+}]_{CYT}$  via PLC-mediated generation of  $IP_3$  and activation of  $IP_3R$ .  $[Ca^{2+}]_{CYT}$  levels are sustained at low levels through the active efflux of  $Ca^{2+}$  by PMCAs and  $Na^{+}/Ca^{2+}$  exchangers on the plasma membrane. Sequestration of  $Ca^{2+}$  into the ER  $Ca^{2+}$  store is mediated by SERCA, into the mitochondria by Mitochondrial  $Ca^{2+}$  Uniporter (MCU) and into the Golgi by secretory pathway  $Ca^{2+}$ -ATPase (SPCA). Increases in  $[Ca^{2+}]_{CYT}$  can result in the activation of calcineurin (CaN) that phosphorylates the transcription factor NFAT, which after translocation into the nucleus regulates gene transcription [30]. Calcium can also activate many cytosolic proteins with  $Ca^{2+}$ -sensitivity confirmation and activities such as calpain, which can regulate a number of important cellular processes including cytoskeletal remodelling and motility (Storr et al., 2011).



**Figure 2:**  $Ca^{2+}$  influx pathways. Examples of influx pathways and naturally occurring-activation pathways.  $CaV3.2$  is an example of a voltage-gated  $Ca^{2+}$  channel that is activated by membrane depolarization (Panner and Wurster, 2006); ORAI1 is an example of a store-operated  $Ca^{2+}$  channel that is activated upon depletion of endoplasmic reticulum  $Ca^{2+}$  stores (Lewis, 2011); P2X5 is an example of a purine receptor that facilitates the flow of  $Ca^{2+}$  across the plasma membrane in response to extracellular ATP (Surprenant and North, 2009); examples of TRP channels include the canonical mechanosensitive cation channel TRPC1, which can be activated by membrane stretch (Maroto et al., 2005), the vanilloid TRPV1 channels activated by high temperatures [13], the melastatin TRPM8 channel activated by lower temperatures (Prevarskaya et al., 2007), the sole member of ankyrin TRPA family TRPA1, which is a key chemoreceptor responsive to reactive chemicals (Moran et al., 2011), TRPM7, which can be directly activated by mechanical stress (Numata et al., 2007), and TRPV6, which has constitutive activity at low  $[Ca^{2+}]_i$  and physiological membrane potential [52].

Brum, 1987) [26], depend critically on calcium influx. (Tsien et al., 1988). In cells in the epithelium, calcium influx is also crucial for processes like the intestinal epithelial cells' absorption of  $Ca^{2+}$  [7,61]. We will give a quick summary of the many kinds of calcium permeable materials in the next section of this review.

### Human Cell Mechanisms for Calcium Influx

Many other types of calcium permeable ion channels are also expressed in intracellular organelles, including the isoforms of  $IP_3$  receptors ( $IP_3R1$ ,  $IP_3R2$ , and  $IP_3R3$ ) and the ryanodine receptors ( $RyR1$ ,  $RyR2$ , and  $RyR3$ ), which are mediators of calcium-induced calcium release [22]. That is expressed on human cells' plasma membranes. The processes of intracellular  $Ca^{2+}$  signaling are shown in Figure 1, and some of the major  $Ca^{2+}$  influx pathways and examples of their naturally occurring activators are shown in Figure 2. The general kinds of calcium permeable ion channels are briefly described below, with special emphasis on some of the ion channels that will be covered.

### Calcium Permeable Ion Channels That are Voltage-Gated

Voltage-gated calcium permeable ion channels are characterized by their sensitivity to changes in membrane potential, as their name suggests. Members of this class can, however, differ greatly in their physiological, pharmacological, and regulatory traits, as has been discussed elsewhere [24]. Calcium channels that are voltage-gated include the L-type, N-type, T-type, R-type, and P/Q-type. Various subunits make up these channels, but the calcium-selective pore is formed by the  $\alpha_1$  subunit [40]. For L-types, the  $\alpha_1$  is encoded by *CACNA1S*, *CACNA1C*, *CACNA1D*, and *CACNA1F* genes; for C-types, *CACNA1A*, For P/Q, N, and R kinds, use *CACNA1B* and *CACNA1E*; for T types, use *CACNA1G*, *CACNA1H*, and *CACNA1I* [16]. Studies evaluating  $CaV1$  channels in T-lymphocytes have shown that, despite being primarily associated with excitable cells like those in the central nervous system and muscle tissue, voltage-gated calcium channels also have significant functions in other cell types [42,53].

## TRP Channels, or Transient Receptor Potentials

Numerous TRP channels, the majority of which are permeable to Ca<sup>2+</sup> ions, have been discovered in mammalian cells since the discovery of the first TRP channel in *Drosophila* [58]. These findings have been reported by Wes et al. (1995), Caterina et al. (1997), Clapham (2002), Story et al. (2003), and Ramsey et al. (2006). The families of TRP channels that are expressed in human cells are TRPC, TRPA, TRPV, TRPM, TRPML, and TRPP. A lot of these channels function as sensors. For example, TRPM8 is activated by lower temperatures (Peier et al., 2002; Prevarskaya et al., 2007) while TRPV1 is triggered by higher temperatures [13,23]. Certain members of this class are also triggered by substances that can be found in nature, such as menthol, which cools the body, and capsaicin, which is the spicy part of chilli peppers. These compounds activate the aforementioned TRPM8 and TRPV1 channels, respectively [29]. The disorders linked to mutations in these ion channels, as well as the functional roles and mechanical, chemical, and temperature sensing characteristics of TRP channels, have all been well examined (Minke, 2006; Nilius, 2007; Prevarskaya et al., 2007). Apart from the function of TRP mutations certain TRP channel overexpression is linked to certain malignancies in humans, including those of the breast and prostate (Prevarskaya et al., 2007; Ouadid-Ahidouch et al., 2013).

### Ca<sup>2+</sup> Ligand-Gated Channels

Certain endogenous ligands directly activate specific calcium permeable ion channels. Ion channels like NMDA and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which are triggered by the neurotransmitter glutamate [20] (Watkins and Jane, 2006), as well as P2X receptors, a class of purine receptors that react to extracellular ATP by promoting the flow of Ca<sup>2+</sup> across the plasma membrane (Surprenant and North, 2009), are among those that are expressed on their membrane. The P2X ion channel family consists of seven members that are crucial to a wide range of procedures, such as blood coagulation and neural signaling (Pankratov et al., 1998) [59].

Despite being ligand-gated, IP<sub>3</sub> receptors are not generally linked to Ca<sup>2+</sup> influx since they are primarily expressed on the internal Ca<sup>2+</sup> store of the endoplasmic reticulum. Nonetheless, findings of IP<sub>3</sub>R3 plasma membrane expression in ciliated cells [8] and IP<sub>3</sub>R1 plasma membrane expression in B lymphocytes [34] support the idea that the IP<sub>3</sub> receptor is a ligand-gated ion channel that facilitates calcium influx.

### Store-Based Ca<sup>2+</sup> Entrance System

The phenomenon known as capacitive calcium entry was initially described in 1986 and refers to increases in Ca<sup>2+</sup> influx following the depletion of intracellular calcium reserves (Putney, 1986). Still, the Not until 2006 was the full chemical identity of the elements causing this significant Ca<sup>2+</sup> influx mechanism discovered. At this point, the mutation causing a severe combined immune deficiency syndrome linked to decreased store-operated Ca<sup>2+</sup> entry was found, and a functional small interfering RNA (siRNA) screen was used to identify the calcium channel ORAI1 [47] (Zhang et al., 2006). Numerous reviews have been written about the now-well-characterized mechanism for store-operated Ca<sup>2+</sup> entry (Parekh and Putney, 2005; Varnai et al., 2009; [53] (Putney, 2011). In summary, the endoplasmic reticulum Ca<sup>2+</sup> sensor STIM1 is redistributed in response to the depletion of Ca<sup>2+</sup> stores in the endoplasmic re-

ticulum. It oligomerizes to regions of the endoplasmic reticulum that are near the plasma membrane, allowing the N-terminal portion of ORAI1 proteins to engage with the CRAC activation domain of STIM1 (Lewis, 2011). Through a calcium channel created by ORAI1 oligomers, this interaction facilitates the influx of Ca<sup>2+</sup> (Mignen et al., 2008). Owing to its increased affinity for endoplasmic reticulum luminal Ca<sup>2+</sup> levels, the STIM1-related isoform STIM2 seems to be a crucial modulator of basal Ca<sup>2+</sup> influx in cells through ORAI1 [21].

## Cancer and Calcium Signaling

Apoptosis, proliferation, migration, invasion, and other processes relevant to cancer are all regulated by calcium signaling (Roderick and Cook, 2008; Lee et al., 2011; Prevarskaya et al., 2011). Numerous calcium channels and pumps have been linked to various types of cancer. These connections have often been established via the finding that a calcium channel or pump is overexpressed in cancer, or the finding that a particular calcium channel or pump plays a part in a particular cancer-related process. Previous reviews (Roderick and Cook, 2008; Prevarskaya et al., 2011; Monteith et al., 2012) have examined the connection between calcium signaling and cancer as well as the significance of particular calcium pumps and channels in various cancer types. Here, our attention will be on the research that has looked specifically at calcium signaling in breast cancer.

### Cancer of the Breast

According to Schulman et al. (2010), breast cancer incidence is rising in developing economies, while it remains one of the leading causes of death in the developed world. Breast cancer is actually a group of diseases, despite being referred to as one.

#### Breast Cancer

Breast cancer incidence is rising in developing economies, and it remains one of the leading causes of death in the developed world (Shulman et al., 2010). Breast cancer is essentially a group of disorders with widely distinct prognoses and ideal treatment regimens, while being referred to as a single illness frequently (Sorlie, 2009; Vargo-Gogola and Rosen, 2007). Because they respond well to hormonal therapy that targets the oestrogen receptor, such as tamoxifen and selective oestrogen receptor modulators, breast cancers that express the oestrogen receptor are generally associated with a relatively good long-term prognosis (Zhang et al., 2000; Park and Jordan, 2002). The treatment of breast tumors that overexpress human epidermal growth factor has been completely transformed by the discovery of the monoclonal antibody trastuzumab.

Factor receptor 2 (ErbB2 receptor) [9], commonly referred to as the HER2 receptor. On the other hand, "triple negative" breast cancers are typically linked to a poor prognosis and a dearth of long-term effective medicines. This is partly because these tumors overexpress the oestrogen and progesterone receptors and lack ErbB2 receptors (Schneider et al., 2008). Microarray analysis of breast cancer samples also demonstrates the heterogeneity of the disease. Hierarchical clustering has been utilized in these research to identify different molecular subtypes of breast cancer. These consist of the recently described Claudin-low, basal-like, luminal A, luminal B, ErbB2, and luminal (Sorlie et al., 2001) [60]. Triple negative and basal-like and Claudin-low breast cancer subtypes significantly overlap (Prat et al., 2010) and have a dismal prognosis; novel, efficient treatments are most urgently needed for these tumors (Perou et al., 2000).



Certain modifiers of calcium signaling, in particular regulators of calcium influx, have been identified in recent research as possible new targets for the treatment of breast cancer.

### Inflow of Calcium and Lactation

The relationship between calcium and the breast is evident. One essential component of milk is calcium, which the breast produces as part of its physiological role to nourish newborns. Three crucial and connected mechanisms are thought to be involved in the transport of calcium from the maternal blood supply into milk: the entry of calcium into breast epithelial cells, the sequestration of Ca<sup>2+</sup> into the secretory pathway and later release into milk, as well as the Ca<sup>2+</sup>'s direct outflow into milk (Neville, 2005; Lee et al., 2006). During lactation, extra calcium transport is made possible by highly specialized Ca<sup>2+</sup> transporters. Studies using expression and mutant animals have directly demonstrated the function of PMCA2, a calcium efflux pump commonly linked to neurons, in the movement of Ca<sup>2+</sup> from the cytoplasm of mouse mammary epithelial cells into milk (Reinhardt et al., 2000; 2004). Based on expression studies, Golgi Ca<sup>2+</sup> accumulation during lactation and the subsequent release of Ca<sup>2+</sup> into milk may be caused by an isoform of the SPCA, called SPCA2, which likewise has restricted tissue distribution [41]. Apart from its potential functions during breastfeeding, PMCA2 and SPCA2 expression is linked to cell death and/or proliferation, respectively, in certain human breast cancer cell lines and is raised in some human breast malignancies [46] (VanHouten et al., 2010). Therefore, some breast cancers are associated with secretory pathway calcium pumps that are up-regulated during lactation and the plasma membrane. This might also apply to proteins that play a key role in controlling the Ca<sup>2+</sup> influx during lactation. ORAI1 isoform up-regulation was found to be a characteristic of breastfeeding in studies evaluating store-operated Ca<sup>2+</sup> entry in mice at various stages of mammary gland development (McAndrew et al., 2011). An elegant potential mechanism to balance the supply and demand for Ca<sup>2+</sup> outflow and sequestration in milk could be store-operated calcium influx (Ca<sup>2+</sup> inflow via ORAI1). Since As will be covered later, ORAI1 has recently been recognized by a number of groups as a possible target for breast cancer treatment.

### Ca<sup>2+</sup> Homeostasis Changes in Breast Cancer

Numerous critical processes in carcinogenesis, including invasion, migration, angiogenesis, cell death, and proliferation, are regulated by calcium signaling. This function has been thoroughly examined and is well-established [14] (Monteith et al., 2007). Furthermore, it is now widely known that certain malignancies are defined by changes in particular calcium signaling components. Such alterations are observed in various cancers, such as prostate cancer (Lehen'kyi et al., 2007) where increased Ca<sup>2+</sup> entry mediated by TRPV6 is linked to enhanced transcription factor nuclear factor for activated T-cell (NFAT) activation and proliferation, and ovarian cancer (Yang et al., 2009b) where increases in Ca<sup>2+</sup> influx mediated by TRPC3 lead to increased proliferation. However, It seems that changes in calcium signaling do not initiate breast tumorigenesis; instead, these changes may be pharmacologically manipulated to decrease the growth and spread of breast cancer or even stimulate the death of breast cancer cells. distinct subtypes of breast cancer seem to alter calcium signaling in distinct ways, which can be mediated by very different mechanisms and have different outcomes. For instance, basal-like breast cancers have much higher levels of the secretory route Ca<sup>2+</sup> ATPase I isoform (SPCA1), and in the basal-like breast cancer cell line MDA-MB-231, silencing SPCA1

lowers proliferation. According to Graci et al. (2010), this functional outcome is linked to suppression of the synthesis of active insulin-like growth factor 1 receptor. This is by a method that probably includes the modification of Golgi lumen-resident pro-protein convertases that are Ca<sup>2+</sup>-dependent [46]. It seems that breast tumors that are positive for the ErbB2 receptor are more closely linked to the overexpression of the calcium efflux pump PMCA2 (VanHouten et al., 2010). The resistance to cell death that T-47D breast cancer cells exhibit when exogenous PMCA2 is overexpressed implies that PMCA2 inhibitors may facilitate the pathways leading to cell death in breast malignancies that overexpress PMCA2. The remaining sections of this study will concentrate on the challenges surrounding the targeting of specific Ca<sup>2+</sup> channels in the therapy of breast cancer, as well as the Ca<sup>2+</sup> influx pathways that are remodeled in some cases of breast cancer.

### Ca<sup>2+</sup> Influx Channels are altered in Breast Cancer

Evidence of the Ca<sup>2+</sup> influx remodeling is present. Breast cancer and ligand-gated Ca<sup>2+</sup> channels Certain research that aim to comprehend key pathways and processes in breast cancer focus on ligand-activated Ca<sup>2+</sup> channels. Still, further research is needed. Studies evaluating P2X7 receptors provide as examples of this, as they have connected this receptor to the invasiveness of cancer cells (Jelassi et al., 2011) and the anti-invasive characteristics of the anthraquinone emodin (Jelassi et al., 2013). MDA-MB-435S cells, a basal breast cancer cell with strong melanoma-like properties, have been used in the majority of these researches [1,25]. Research on alternative P2X receptor isoforms and basal-like and non-basal-like breast cancer cell lines may help determine which subtype(s) of breast cancer and which P2X receptors may have the greatest therapeutic promise for the control of the disease of metastases from breast cancer.

### Mechanisms Responsible for Altered Plasma Membrane Ca<sup>2+</sup> Channel Expression in Breast Cancer Cells

Although neglected for some time, recent studies have begun to explore the mechanisms by which specific Ca<sup>2+</sup> channels are overexpressed in some breast cancers. One possible mechanism for the overexpression of some calcium permeable ion channels is through hormone receptors, such as receptor  $\alpha$  for estrogen (ER $\alpha$ ). In human MCF-7 breast cancer cells, silencing ER $\alpha$  lowers the levels of ORAI3 mRNA and protein but has no effect on ORAI1 levels (Motiani et al., 2013). This suggests a possible molecular connection between breast cancer cells expressing ER $\alpha$  and ORAI3 overexpression. In MCF-7 cells, ER $\alpha$  silencing also lowers TRPM8 levels, while 17- $\beta$ -oestradiol raises them [27]. The discovery that progesterone inhibits the expression of the Ca<sup>2+</sup> permeable ion channel TRPV4 in T-47D breast cancer cells (Jung et al., 2009) further implies that hormonal mechanisms may be responsible for the altered expression of certain calcium channels in breast malignancies. Additional evaluation of this process for additional calcium channels and the effects of antioestrogen treatment it now seems appropriate to focus on the expression of calcium channels in clinical breast cancer.

It is widely acknowledged that gene amplification has a role in breast cancer. The humanized monoclonal antibody trastuzumab takes advantage of the gene amplification of ErbB2 receptors in many aggressive breast tumors [9]. The likelihood that calcium channel gene amplification contributes to breast cancer has not been examined in many studies. The overexpression of

TRPV6 in SK-BR-3, ZR-75-1, and T-47D breast cancer cell lines, where copy numbers range from 6 to 9, and in certain breast cancers, where an elevated copy number of TRPV6 is linked to oestrogen receptor negative, triple negative, and basal-like breast cancers, suggests that TRPV6 gene amplification may be one possible mechanism for this overexpression (Peters et al., 2012). Others Gene methylation is one example of an epigenetic modification that is one of the causes for altered expression in breast cancer that has not yet been well investigated. According to Palmieri et al. (2012), DNA demethylation causes a substantial increase in CACNA2D3 levels in MDA-MB-453 breast cancer cells. The gene for the voltage-gated calcium channel regulatory subunit, CACNA2D3, is linked to greater methylation in breast cancers with metastases to the central nervous system. The methylation of the CACNA2D3 gene is suggested as a potential biomarker for the development of metastases, however its importance for calcium signaling and breast cancer pathways is yet unknown (Palmieri et al., 2012). Future research on this and other putative mechanisms for altered Ca<sup>2+</sup> channel expression in breast cancer cells ought to receive more attention.

### Control in Ca<sup>2+</sup> Channel Function

Research has started to show that the control of calcium channels in breast cancer cells is complex. It is possible that enhanced activation of a calcium channel (in this case, through overexpression of another protein) rather than overexpression of the calcium channel itself is the driving force for tumor progression in some cases, as suggested by the ability of the SPCA2 calcium pump's N-terminal domain to activate Ca<sup>2+</sup> influx via ORAI1 and promote activation of NFAT [46]. Kim et al.'s discovery that the tumor suppressor Numb1 is a negative regulator of TRPV6 activity lends more credence to the significance of these indirect processes (Kim et al., 2013b). Proliferation is increased by number one silencing, where it directly interacts with TRPV6 through basal Ca<sup>2+</sup> influx in MCF-7 breast cancer cells (Kim et al., 2013b). Changes in calcium channel location add another layer of complexity to the involvement of calcium influx channels in cancer. Bidaux et al. showed that a portion of the overexpressed TRPM8 protein is found on the endoplasmic reticulum in prostate cancer cells. This location is linked to the advancement of prostate cancer via changing the calcium level of internal stores [17]. To find out if comparable localization changes happen in breast cancer cells, more research is needed. It is known that in MDA-MB-468 breast cancer cells, suppressing TRPC1 reduces the high levels of basal Ca<sup>2+</sup> influx mediated by ORAI1 in this cell line [32]. Observations suggest that TRPC1 expression on the endoplasmic reticulum of MDA-MB-468 breast cancer cells and the stimulation of Ca<sup>2+</sup> leakage from that calcium storage are partially responsible for this. To determine whether non-plasmalemmal localization of TRPC1 and other calcium channels is a characteristic of some breast tumors, more research is necessary.

### Targeting Calcium Influx Pathways with Pharmaceuticals in Breast Cancer

One of the main potential benefits of using calcium influx regulators as new cancer treatment targets is their obvious capacity to create pharmacological modulators of Ca<sup>2+</sup> permeable ion channels, as this review has explained. Many of the calcium permeable ion channels that are known to have activators and inhibitors are listed in Table 2. connected to malignancies. Most of the investigations included in this review have used pharmacological inhibitors, siRNA or short hairpin (sh)RNA-mediated silencing, or both, to identify particular calcium perme-

able ion channels as possible therapeutic targets. Considering the part that calcium signaling plays in promoting cellular motility and proliferation, such strategies are obviously appropriate. Indeed, pharmacological inhibitors of calcium influx pathways have been shown in vivo studies to prevent invasion and/or proliferation of breast cancer (Taylor and Simpson, 1992) [12]. The induction of cancer cell death is an additional mechanism of oncology therapy. Studies on this feature of calcium influx in breast cancer are scarce, despite the fact that sustained high levels of [Ca<sup>2+</sup>]CYT might promote apoptosis and Necrosis can even be induced by significant elevations in [Ca<sup>2+</sup>]CYT [49]. Hence, administering a channel activator to create a prolonged calcium influx strong enough to cause cell death is one recommended strategy to target an overexpressed calcium permeable ion channel. As was previously mentioned, many prostate tumors overexpress TRPM8, and preliminary research using the prostate cancer cell line LNCaP indicates that menthol, a TRPM8 activator, may cause apoptosis (Zhang and Barritt, 2004). Although TRPM8 has been found to be overexpressed in certain types of breast cancer (Tsavaler et al., 2001) [27,35], the effects of activating this and other calcium permeable ion channels on breast cancer cells have not been thoroughly investigated.

It is most likely that calcium causes cell death channel opening only take place in breast cancer cells when the channel is sufficiently overexpressed to allow an activator to generate enough calcium influx to encourage cell death pathways. Among the possible dangers of utilizing an activator to induce the death of breast cancer cells is the consequence of activation in cells that have only a moderate overexpression of the ion channel. In this latter scenario, channel activation could actually promote proliferation and/or invasion. Clinically, this could result in an initial reduction in tumor volume (via cell death), followed by a period of accelerated proliferation and metastasis. In vitro and in vivo experiments are required to address this possibility. However, another outcome of channel activation in breast cancer cells could be a reduction in proliferation and invasion due to a change in the nature of [Ca<sup>2+</sup>]CYT changes. Sustained Ca<sup>2+</sup> influx induced by a channel activator in breast cancer cells could interfere with processes such as proliferation and motility. Studies of this possible phenomenon may be hampered in breast cancer cells, as many of the calcium influx channels overexpressed in breast cancer cells (Table 1) do not have both widely available selective inhibitors and activators. However, such studies, particularly in vivo, would greatly advance our understanding of the best therapeutic strategies for targeting calcium channels in breast cancer.

### New Developments in the Mechanisms of Calcium Influx in Breast Cancer

Numerous investigations by many research groups have significantly advanced our understanding of calcium influx in breast cancer cells. Naturally, the majority of research has concentrated on determining the mechanisms underlying the increased expression of particular calcium permeable channels in breast cancer cells, as well as the involvement of calcium signaling in significant events in the evolution of the disease. On the other hand, some recent research is starting to pinpoint particular Ca<sup>2+</sup> permeable channels in different settings, which might indicate new fields that could advance quickly over the course of the next ten years. Chemotherapeutic resistance is one of these topics. Recent research by Ma et al. demonstrated that adriamycin-resistant MCF-7 breast cancer cells can become adriamycin-sensitive again when TRPC5 is silenced (Ma et al.,

(2012)). This study offers evidence that focusing on a particular Ca<sup>2+</sup> channel could be a potential strategy for reversing breast cancer cells' resistance to certain chemotherapies.

Apart from the direct correlation between particular calcium permeable ion channels and invasiveness and cellular migration, research has also started to link these channels to other critical processes in breast cancer metastasis, including the Epithelial to Mesenchymal Transition (EMT) [32,63]. Growth factors, such as EGF, and hypoxia are known to trigger EMT in breast cancer cells (Lester et al., 2007; Lo et al., 2007). During EMT, a variety of proteins express themselves differently, giving rise to enhanced migratory and invasive characteristics as well as resistance to cell death. Current Research has shown that Ca<sup>2+</sup> influx routes may undergo modifications due to epithelial-mesenchymal transition. P2X5 mRNA levels are elevated and purine receptor Ca<sup>2+</sup> signaling is changed in response to EGF-induced EMT in MDA-MB-468 breast cancer cells [31]. Research employing the identical model demonstrate that EMT diminishes basal, agonist, and store-operated Ca<sup>2+</sup> calcium signaling. This is demonstrated by the correlation between EMT, which is brought about by the transcription factor Oct4 being down-regulated, and alterations in store-operated Ca<sup>2+</sup> entry in MCF-7 cells [63]. In MDA-MB-468 breast cancer cells, calcium signaling is also a critical step in the development of EGF and hypoxia-mediated EMT, with TRPM7 contributing to the induction of some EMT markers by EGF. This most likely happens as a result of interactions with signal transducer and transcription activator 3's phosphorylation [32].

Hanahan and Weinberg (2011) outlined the hallmarks, developing predictors, and enabling aspects of cancer in their most current review. They also emphasized the significance of the microenvironment and the cellular heterogeneity of tumors. While calcium influx has been extensively researched and linked to some cancer characteristics, as previously said, the field of calcium signaling research in certain tumor biology domains is still in its early stages. For instance, there are surprisingly few research examining calcium signaling between breast cancer cells in tumors, despite the obvious significance of this process in reactions to growth factors in the tumor microenvironment. interactions with the surrounding cells (immune inflammatory cells, for example) [57]. Additionally, research on the function of calcium signaling in cancer stem cells is particularly lacking. This is most likely partially caused by the technological challenges associated with measuring Ca<sup>2+</sup> in vivo and in three-dimensional culture models. But recent developments in genetically targeted Ca<sup>2+</sup> sensors and imaging could result in investigations that broaden our knowledge of the potential roles that Ca<sup>2+</sup> influx pathways may play in the development of tumors.

### In Summary

Certain breast cancer cells have changes in the expression and/or activity of Ca<sup>2+</sup> permeability ion channels. With certain molecular insights, our knowledge of why these variations in expression occur is progressively becoming clearer. The indubitably demonstrated sensitivity Certain Ca<sup>2+</sup> channels are appealing targets for breast cancer treatment due to their selectivity towards pharmacological modulators. While studies conducted in vitro and in vivo frequently lend support to this method, further research is needed to identify the best course of treatment and identify potential resistance pathways to these drugs.

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