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REVIEW

Calcium influx pathways in breast cancer: opportunities for pharmacological intervention

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Ca^{2+} influx through Ca^{2+} permeable ion channels is a key trigger and regulator of a diverse set of cellular events, such as neurotransmitter release and muscle contraction. Ca^{2+} influx is also a regulator of processes relevant to cancer, including cellular proliferation and migration. This review focuses on calcium influx in breast cancer cells as well as the potential for pharmacological modulators of specific Ca^{2+} influx channels to represent future agents for breast cancer therapy. Altered expression of specific calcium permeable ion channels is present in some breast cancers. In some cases, such changes can be related to breast cancer subtype and even prognosis. *In vitro* and *in vivo* models have now helped identify specific Ca^{2+} channels that play important roles in the proliferation and invasiveness of breast cancer cells. However, some aspects of our understanding of Ca^{2+} influx in breast cancer still require further study. These include identifying the mechanisms responsible for altered expression and the most effective therapeutic strategy to target breast cancer cells through specific Ca^{2+} channels. The role of Ca^{2+} influx in processes beyond breast cancer cell proliferation and migration should become the focus of studies in the next decade.

Abbreviations

$[Ca^{2+}]_{CYT}$, cytoplasmic-free calcium; A-804598, N-cyano-N″-[(1S)-1-phenylethyl]-N′-5-quinolinyl-guanidine; EC, endothelial cells; EMT, epithelial to mesenchymal transition; ER^+, oestrogen positive; ERα, oestrogen receptor α; ErbB2 (also known as HER2), human EGF receptor 2; IP_3, inositol 1,4,5-trisphosphate; JNJ41876666, 3-[7-trifluoromethyl-5-(2-trifluoromethyl-phenyl)-1H-benzimid-azol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-eneHydrochloride; NFAT, nuclear factor for activated T-cells; NNC 55-0396, (1S,2S)-2-(2-(N-[(3-benzimidazol-2-yl)propyl]-N-methylamino)ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropanecarboxylate dihydrochloride; PMCA, plasma membrane Ca^{2+}-ATPase; Pyr3, 1-[4-[(2,3,3-trichloro-1-oxo-2-propen-1-yl)amino]phenyl]-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid; SB-209712, 1,6,bis{1-[4-(3-phenylpropyl) piperidinyl]}hexane; SCID, severe combined immune deficiency; SPCA, secretory pathway Ca^{2+}-ATPase; TRP, transient receptor potential

Overview

Cells maintain a large gradient of free Ca^{2+} across the plasma membrane, with intracellular-free Ca^{2+} levels approximately 20 000 fold lower than in the extracellular environment (100 nM vs. 1.8 mM) (Carafoli, 1987; Clapham, 2007). Cells often exploit this Ca^{2+} gradient to initiate and regulate cellular signals through Ca^{2+} influx, usually via the opening of Ca^{2+} permeable ion channels. Many diverse pathways are regulated by increases in intracellular cytoplasmic-free calcium ($[Ca^{2+}]_{CYT}$), including muscle contraction, gene transcription, proliferation and neurotransmitter release (Berridge *et al.*, 2003). Ca^{2+} permeable ion channels are potential pharmacological targets for a variety of conditions. These conditions include hypertension, where L-type voltage-gated Ca^{2+} channel blockers such as nifedipine are used clinically (Aoki *et al.*, 1976), and chronic pain, where the N-type channel inhibitor ziconotide is used (Malmberg and Yaksh, 1995).

This review will focus on research that has assessed calcium influx pathways in breast cancer progression and identified calcium permeable ion channels as pharmacological targets for breast cancer therapy.

Calcium signalling and the important role for calcium influx

There are a variety of reviews detailing the way in which mammalian cells regulate levels of $[Ca^{2+}]_{CYT}$ and the importance of the *nature* of changes in $[Ca^{2+}]_{CYT}$ (such as $[Ca^{2+}]_{CYT}$ oscillations and localized changes in Ca^{2+}) (Berridge *et al.*, 2003; Leybaert and Sanderson, 2012). Figure 1 illustrates some of the main calcium channels, pumps and exchangers involved in calcium signalling pathways. Briefly, $[Ca^{2+}]_{CYT}$ levels are maintained at low levels through the active efflux of Ca^{2+} from the cell via the plasma membrane Ca^{2+}-ATPases (PMCAs), which, along with Na^+/Ca^{2+} exchangers and sarco/endoplasmic reticulum Ca^{2+} ATPases, lower $[Ca^{2+}]_{CYT}$ after activation. Increases in $[Ca^{2+}]_{CYT}$ can occur by several mechanisms. For example, many GPCRs, through activation of PLC and the generation of inositol 1,4,5-trisphosphate (IP_3), release Ca^{2+} from internal calcium stores, such as the sarco/endoplasmic reticulum via IP_3-activated Ca^{2+} channels (Berridge *et al.*, 2003). Two other organelles that are involved in Ca^{2+} signalling are mitochondria, which contain the recently identified mitochondrial Ca^{2+} uniporter (Kirichok *et al.*, 2004) and Na^+/Ca^{2+} exchanger NCLX (Palty *et al.*, 2010),

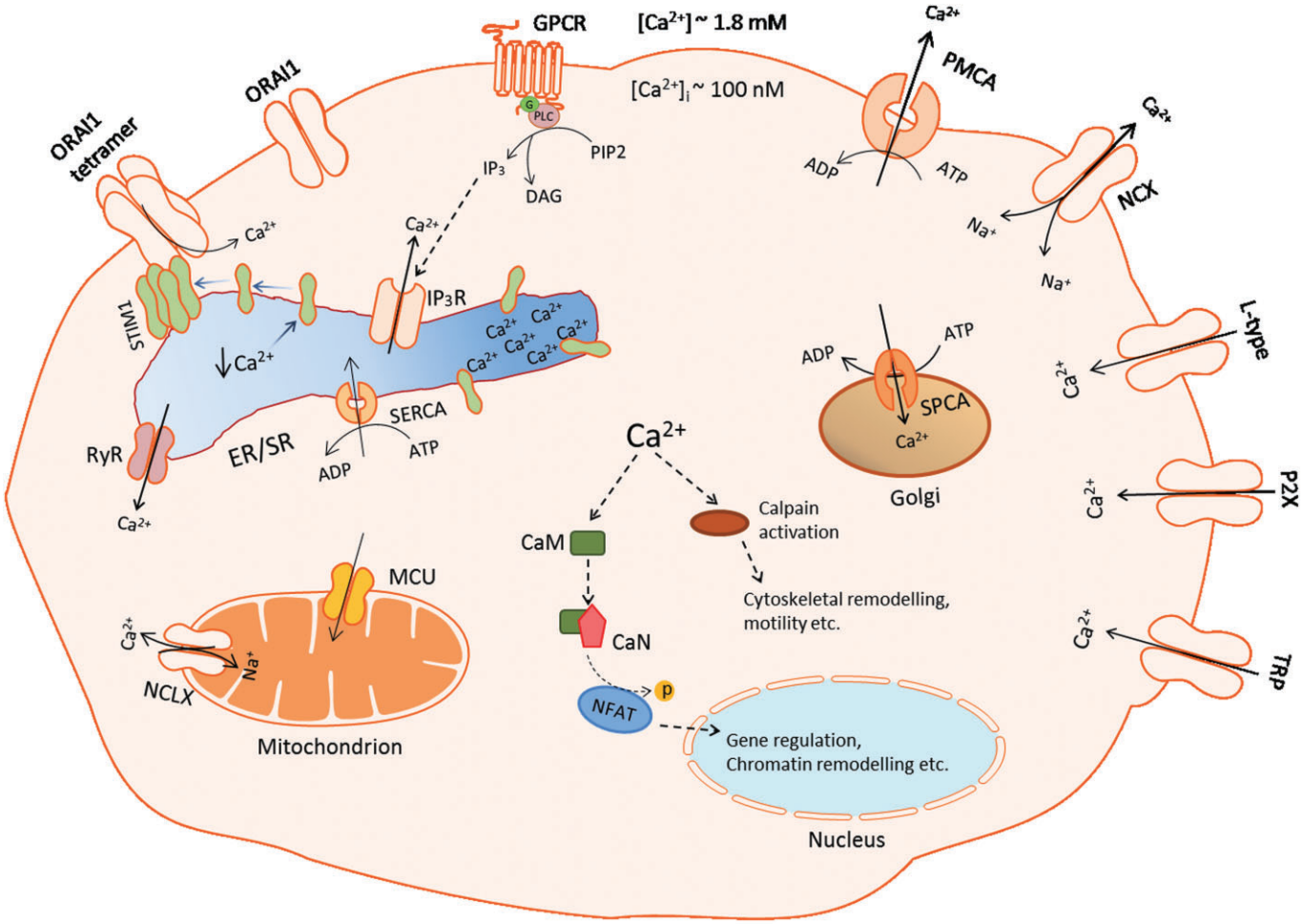

Figure 1

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 (Crabtree, 1999). 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).

and the Golgi, which sequesters intracellular Ca^{2+} via secretory pathway Ca^{2+}-ATPases (SPCAs). Elevations in $[Ca^{2+}]_{CYT}$ are also achieved through the opening of calcium permeable ion channels on the plasma membrane. Calcium influx plays a critical role in many specific physiological events particularly in excitable cells such as excitation–contraction coupling in skeletal muscle (Rios and Brum, 1987; Cheng *et al.*, 1996) and the release of neurotransmitters in neurons (Tsien *et al.*, 1988). Calcium influx also plays an important role in cells on the epithelium, such as in the absorption of Ca^{2+} by the epithelial cells of the intestine (Barley *et al.*, 2001; Hoenderop *et al.*, 2005). In the next section of this review, we will provide a brief overview of the types of calcium permeable ion channels responsible for the influx of calcium in human cells.

Calcium influx pathways in human cells

Although there are many types of calcium permeable ion channels expressed in intracellular organelles, such as the isoforms of IP_3 receptors (IP_3R1, IP_3R2 and IP_3R3) and the mediators of calcium-induced calcium release known as ryanodine receptors (RyR1, RyR2 and RyR3) (Carafoli *et al.*, 2001), there are many more calcium permeable ion channels that are expressed on the plasma membrane of human cells. Figure 1 depicts the mechanisms of intracellular Ca^{2+} signalling, and Figure 2 represents examples of some of the key Ca^{2+} influx pathways and examples of their naturally occurring activators. Below we briefly outline some of the general classes of calcium permeable ion channels with particular attention to some of the ion channels that will be discussed later in this review in the context of studies in breast cancer. Receptor and channel nomenclature conform to BJP's Concise Guide to PHARMACOLOGY (Alexander *et al.*, 2013).

Voltage-gated calcium permeable ion channels

As the name implies, the characterizing feature of voltage-gated calcium permeable ion channels is their sensitivity to changes in membrane potential. However, as reviewed elsewhere, members of this class can differ significantly in physiological, pharmacological and regulatory characteristics (Catterall, 2011).

Voltage-gated calcium channels include L-type, N-type, T-type, R-type and P/Q-type. These channels consist of different subunits, although it is the α_1 subunit that forms the calcium selective pore (Ertel *et al.*, 2000; Cain and Snutch, 2011). The genes that encode the α_1 include *CACNA1S*, *CACNA1C*, *CACNA1D* and *CACNA1F* for L-types; *CACNA1A*, *CACNA1B* and *CACNA1E* for P/Q-, N- and R-type; and *CACNA1G*, *CACNA1H* and *CACNA1I* for T-types (Bidaud *et al.*, 2006).

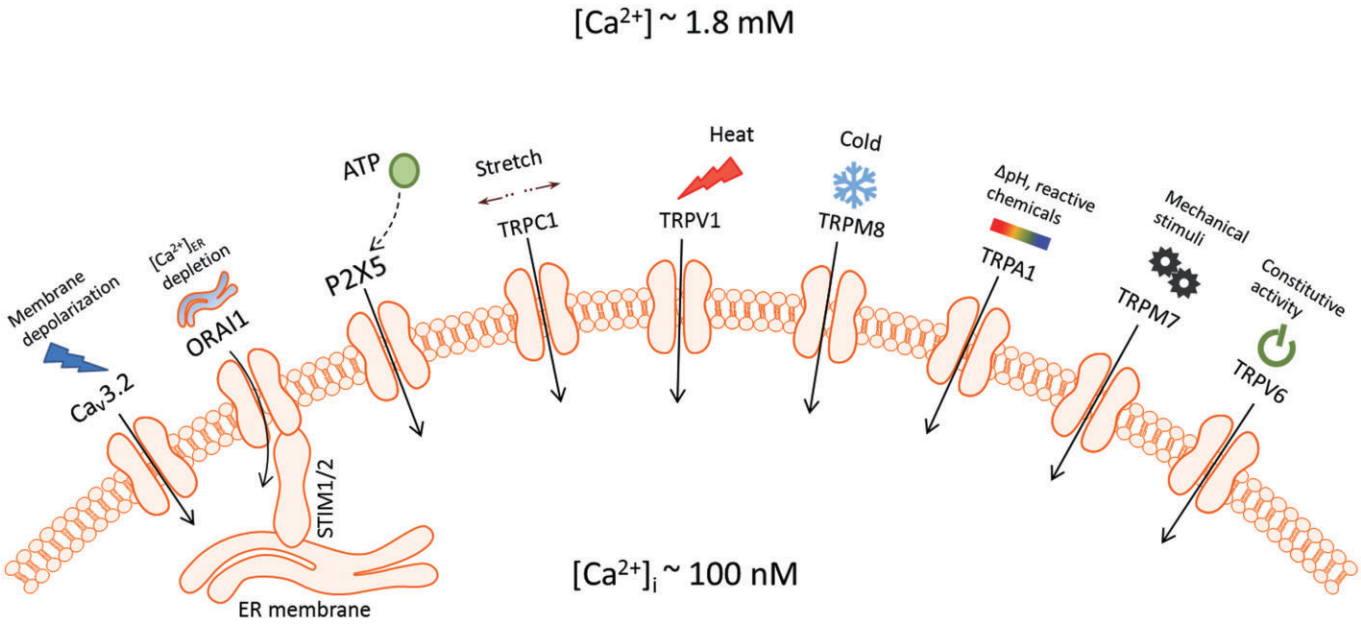

Figure 2

Ca^{2+} influx pathways. Examples of influx pathways and naturally occurring-activation pathways. $Ca_V3.2$ 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 (Benham *et al.*, 2003), 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 (Van de Graaf *et al.*, 2006).

Although voltage-gated calcium channels are predominately thought of in the context of excitable cells, such as those in the CNS and muscle tissue, they also play important roles in some other cell types, as reflected in studies assessing Ca_V1 channels in T-lymphocytes (Fanger *et al.*, 2000; Robert *et al.*, 2011).

Transient receptor potential (TRP) channels

Since the first identification of a TRP channel in Drosophila (Hardie and Minke, 1992), a variety of TRP channels (most of which are permeable to Ca^{2+} ions) have been identified in mammalian cells (Wes *et al.*, 1995; Caterina *et al.*, 1997; Clapham, 2002; Story *et al.*, 2003; Ramsey *et al.*, 2006). TRP channels expressed in human cells belong to the TRPC, TRPA, TRPV, TRPM, TRPML and TRPP families. Many of these channels act as sensors, such as TRPV1 (Caterina *et al.*, 1997; Benham *et al.*, 2003), which is activated by elevated temperatures, and TRPM8, which is activated by lower temperatures (Peier *et al.*, 2002; Prevarskaya *et al.*, 2007). Some in this class are also activated by compounds found in nature, such as capsaicin (the hot component of chilli peppers) and menthol (the cooling component of mint) for the aforementioned TRPV1 and TRPM8 channels respectively (Clapham, 2002). The functional roles and the temperature, mechanical and chemical sensing properties of TRP channels have been extensively reviewed, as have the diseases associated with mutations in these ion channels (Minke, 2006; Nilius, 2007; Prevarskaya *et al.*, 2007). In addition to the role of TRP mutations in human diseases, some cancers such as those of the prostate and breast are associated with the overexpression of specific TRP channels (Prevarskaya *et al.*, 2007; Ouadid-Ahidouch *et al.*, 2013).

Ligand-gated calcium channels

Some calcium permeable ion channels are activated directly by endogenous ligands. Those expressed on the plasma membrane include ion channels such as NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors activated by the neurotransmitter glutamate (Bortolotto *et al.*, 1994; Watkins and Jane, 2006) and P2X receptors, which are a class of purine receptors that respond to extracellular ATP through facilitating the flow of Ca^{2+} across the plasma membrane (Surprenant and North, 2009). There are seven members of the P2X ion channel family, which play important roles in a diverse array of processes, including neuronal signalling and blood coagulation (Pankratov *et al.*, 1998; Hechler *et al.*, 2003).

IP_3 receptors, although clearly ligand-gated, are not generally associated with Ca^{2+} influx due to their predominant expression on the endoplasmic reticulum intracellular Ca^{2+} store. However, there are reports of plasma membrane expression of IP_3R3 in ciliated cells (Barrera *et al.*, 2004) and IP_3R1 in B lymphocytes (Dellis *et al.*, 2008), and in this context, the IP_3 receptor would be regarded as a ligand-gated ion channel responsible for calcium influx.

Store-operated Ca^{2+} entry

Increases in Ca^{2+} influx after the depletion of intracellular calcium stores was a phenomenon first identified in 1986 as capacitative calcium entry (Putney, 1986). However, the complete molecular identity of the components responsible for this important mechanism of Ca^{2+} influx was not achieved until 2006. At this time, the calcium channel ORAI1 was identified using a functional small interfering RNA (siRNA) screen and through the discovery of the mutation responsible for a severe combined immune deficiency syndrome associated with reduced store-operated Ca^{2+} entry (Feske *et al.*, 2006; Zhang *et al.*, 2006). The now well-characterized mechanism for store-operated Ca^{2+} entry has been extensively reviewed (Parekh and Putney, 2005; Varnai *et al.*, 2009; Roberts-Thomson *et al.*, 2010; Lewis, 2011; Putney, 2011). Briefly, the depletion of endoplasmic reticulum Ca^{2+} stores results in the redistribution of the endoplasmic reticulum Ca^{2+} sensor STIM1, which oligomerizes to sections of the endoplasmic reticulum close to the plasma membrane, such that the CRAC activation domain of STIM1 interacts with N-terminal region of ORAI1 proteins (Lewis, 2011). This interaction promotes the influx of Ca^{2+} through a calcium channel formed by ORAI1 oligomers (Mignen *et al.*, 2008). The STIM1-related isoform STIM2, due to its higher affinity for endoplasmic reticulum luminal Ca^{2+} levels, appears to be a critical regulator of basal Ca^{2+} influx in cells via ORAI1 (Brandman *et al.*, 2007).

Calcium signalling and cancer

Calcium signalling is a critical regulator of processes important in cancer, such as apoptosis, proliferation, migration and invasion (Roderick and Cook, 2008; Lee *et al.*, 2011; Prevarskaya *et al.*, 2011). A variety of calcium channels and pumps are associated with different cancers. Generally, these associations have been made by the identification of the overexpression of a calcium channel or pump in a cancer, or the identification of a role for a specific calcium channel or pump in a specific cancer-related process. The association between calcium signalling and cancer and the importance of specific calcium pumps and channels in different cancer types have been reviewed elsewhere (Roderick and Cook, 2008; Prevarskaya *et al.*, 2011; Monteith *et al.*, 2012). Here, we will focus on the studies that have concentrated on calcium signalling in breast cancer.

Breast cancer

Breast cancer is still one of the major causes of mortality in the developed world and breast cancer incidence is rising in developing economies (Shulman *et al.*, 2010). Although often described as one disease, breast cancer is actually a collection of diseases, with very different prognoses and optimal treatment regimes (Sorlie, 2009; Vargo-Gogola and Rosen, 2007). Clinically, breast cancers that express the oestrogen receptor are generally associated with a relatively good long-term prognosis due to their responsiveness to hormonal therapy targeting the oestrogen receptor, such as tamoxifen and the selective oestrogen receptor modulators (Zhang *et al.*, 2000; Park and Jordan, 2002). The development of the monoclonal antibody trastuzumab has revolutionized the treatment of breast cancers that overexpress the human epidermal growth

factor receptor 2 (ErbB2 receptor, also known as HER2 receptor; Baselga *et al.*, 1999). In contrast, breast cancers defined as 'triple negative' are generally associated with a poor prognosis and a lack of long-term effective therapies, in part, due to an absence of ErbB2 receptors, oestrogen and progesterone receptor overexpression (Schneider *et al.*, 2008). The diversity of breast cancer disease is also evident from microarray studies of breast cancer samples. Such studies have used hierarchical clustering to define various breast cancer molecular subtypes. These include luminal A, luminal B, ErbB2 and basal-like and the recently characterized Claudin-low (Sorlie *et al.*, 2001; Herschkowitz *et al.*, 2007). Basal-like and Claudin-low breast cancer subtypes have a significant overlap with triple negative subtype (Prat *et al.*, 2010) and are associated with poor prognosis; these cancers are those for which there is the greatest need for new effective therapies (Perou *et al.*, 2000).

Recent studies have begun to identify specific modifiers of calcium signalling, particularly regulators of calcium influx, as potential new targets for the treatment of breast cancers.

Calcium influx and lactation

There is an obvious association between calcium and the breast. Calcium is a key component of milk, the production of which is the physiological function of the breast to supply neonatal nutrition. The transport of calcium from the maternal blood supply into milk is believed to involve three critical and interrelated processes; the influx of calcium into breast epithelial cells, the sequestration of Ca^{2+} into the secretory pathway and subsequent secretion into milk, and the direct efflux of Ca^{2+} into milk (Neville, 2005; Lee *et al.*, 2006). The additional calcium transport during lactation is achieved by very specific Ca^{2+} transporters. Expression and knockout mice studies have provided direct evidence for the role of the calcium efflux pump typically associated with neurons – PMCA2, in the transport of Ca^{2+} from the cytoplasm of the mouse mammary epithelial cell into milk (Reinhardt *et al.*, 2000; 2004). Expression studies suggest that an isoform of the SPCA, also with restricted tissue distribution – SPCA2, may be responsible for Golgi Ca^{2+} accumulation during lactation and the subsequent secretion of Ca^{2+} into milk (Faddy *et al.*, 2008). In addition to their hypothesized roles in lactation, the expression of PMCA2 and SPCA2 is elevated in some human breast cancers and is associated with cell death and/or proliferation, respectively, in some human breast cancer cell lines (Feng *et al.*, 2010; VanHouten *et al.*, 2010). Hence, there is an association between plasma membrane and secretory pathway calcium pumps that are up-regulated during lactation with some breast cancers. This may also be the case for proteins important in regulating the influx of Ca^{2+} during lactation. Studies assessing store-operated Ca^{2+} entry in mice at different stages of mammary gland development identified ORAI1 isoform up-regulation as a feature of lactation (McAndrew *et al.*, 2011). Store-operated calcium influx may represent an elegant potential mechanism to match the demand for Ca^{2+} sequestration and efflux into milk with supply (Ca^{2+} influx via ORAI1). As will be discussed below, multiple groups have now identified ORAI1 as a potential target for breast cancer therapy.

Altered Ca^{2+} homeostasis in breast cancer

The role of calcium signalling in the regulation of a variety of key processes in tumourigenesis such as proliferation, migration, invasion, cell death and angiogenesis is well established and has been extensively reviewed (Berridge *et al.*, 2003; Monteith *et al.*, 2007). Additionally, it is now well understood that some cancers are characterized by alterations in specific aspects of calcium signalling. Examples of such alterations are seen in cancers, including those of the prostate where increased TRPV6-mediated Ca^{2+} entry is associated with enhanced transcription factor nuclear factor for activated T-cell (NFAT) activation and proliferation (Lehen'kyi *et al.*, 2007), and in ovarian cancer where increases in Ca^{2+} influx mediated via TRPC3 lead to increased proliferation (Yang *et al.*, 2009b).

Although it appears that alterations in calcium signalling are not the driving force for the initiation of breast tumourigenesis, such changes could be pharmacologically exploited to reduce breast cancer proliferation and metastasis or even promote breast cancer cell death. The remodelling of calcium signalling appears to differ between subtypes of breast cancer and can be mediated via very different mechanisms and lead to different consequences. For example, the secretory pathway Ca^{2+} ATPase I isoform (SPCA1) is significantly elevated in basal-like breast cancers, and silencing of SPCA1 in the basal-like breast cancer cell line MDA-MB-231 reduces proliferation. This functional consequence is associated with inhibition of the production of active insulin-like growth factor 1 receptor (Grice *et al.*, 2010). This is via a mechanism likely to involve the modulation of Ca^{2+}-dependent proprotein convertases resident in the Golgi lumen (Grice *et al.*, 2010). Overexpression of the calcium efflux pump PMCA2 appears to be associated more with ErbB2 receptor positive breast cancers (VanHouten *et al.*, 2010). In T-47D breast cancer cells, exogenous overexpression of PMCA2 and the subsequent increased capacity for effluxing Ca^{2+} imparts resistance to cell death, suggests that inhibitors of PMCA2 could promote cell death pathways in breast cancers that overexpress PMCA2. The remainder of this review will focus on Ca^{2+} influx pathways that are remodelled in some breast cancers and the issues associated with targeting specific Ca^{2+} channels in the treatment of breast cancer.

Remodelling of Ca^{2+} influx pathways in breast cancer

There is evidence of remodelling of Ca^{2+} influx in breast cancer cells via specific calcium influx pathways that represent different classes of calcium channels. Table 1 lists some of the Ca^{2+} channels and/or Ca^{2+} channel regulators that have altered expression in breast cancer cell lines and/or breast tumours. In other cases, Ca^{2+} channels are linked to important pathways in breast cancer cells through pharmacological modulators.

Voltage-gated Ca^{2+} channels in breast cancer

One of the classes of calcium permeable ion channels that has received the least attention in breast cancer is voltage-gated

Table 1

Changes in the mRNA or protein expression of some of the Ca^{2+} channels and Ca^{2+} channel regulators in breast cancer (cell lines and patient samples)

Channel/ Regulator	Sample	Change in expression		Functional studies completed
		mRNA	Protein	
$Ca_V3.1$ $Ca_V3.2$	Breast cancer cell lines – MCF-7 and MDA-MB-231 (Taylor *et al.*, 2008)	↑ in sub-confluent	–	$Ca_V3.1$/$Ca_V3.2$ silencing reduces proliferation of MCF-7 cells (Taylor *et al.*, 2008)
ORAI1	Breast cancer cell lines (Motiani *et al.*, 2010)		↓ in ER^+, ↔ in ER^-	ORAI1 silencing decreases MDA-MB-231 migration and invasion *in vitro* and metastasis *in vivo* (Yang *et al.*, 2009a)
	Breast cancer cell lines (McAndrew *et al.*, 2011)	↑	–	
	Clinical breast cancer samples (McAndrew *et al.*, 2011)	↑ (basal vs. non-basal)	–	
ORAI3	Breast cancer cell lines (Faouzi *et al.*, 2011)	↑	–	ORAI3 silencing reduces cell proliferation and induces cell cycle arrest in MCF-7 and T-47D cells (Faouzi *et al.*, 2011; 2013) and inhibits invasion of MCF-7 cells and breast tumour growth in mice models injected with MCF7 cells (Motiani *et al.*, 2013)
	Breast cancer samples (Faouzi *et al.*, 2011)	↑	–	
	Breast cancer cell lines (Motiani *et al.*, 2010)	–	↑ in ER^+	
	Breast cancer cell lines (McAndrew *et al.*, 2011)	↔		
STIM1	Breast cancer cell lines (Motiani *et al.*, 2010)	–	↓ in ER^+	STIM1 siRNA decreases the migration and invasion of MDA- MB-231 and metastasis of the MDA-MB-231 tumour cells in immunodeficient NOD/SCID mice (Yang *et al.*, 2009a)
	Breast cancer samples (McAndrew *et al.*, 2011)	↑ in basal and poor prognosis breast cancers	–	
P2X5	Breast cancer cell lines and breast cancer samples (Overes *et al.*, 2009)	↑	–	P2X5 silencing reduces EGF-mediated induction of the EMT marker vimentin (Davis *et al.*, 2011)
P2X7	Breast cancer cell line (MDA-MB-435S) (Jelassi *et al.*, 2011)	↑	↑	P2X7 receptor silencing reduces ATP-induced cell migration and invasion in MDA-MB-435S (Jelassi *et al.*, 2011)
TRPC1	Breast cancer samples (Dhennin-Duthille *et al.*, 2011)	↑	–	TRPC1 silencing reduces CaR-induced proliferation in MCF-7 cells (El Hiani *et al.*, 2009)
	Breast cancer cell line MCF7 (El Hiani *et al.*, 2006)	–	–	
TRPC3	Breast cancer samples and breast cancer cell lines (Aydar *et al.*, 2009)	↑	–	ND
TRPC6	Breast cancer samples and breast cancer cell lines (Aydar *et al.*, 2009)	↑	–	
	Breast cancer cell lines (Guilbert *et al.*, 2008)	↑	↑	ND
	Breast cancer samples (Dhennin-Duthille *et al.*, 2011)	–	–	
TRPV4	Endothelial cells (ECs) derived from human breast carcinomas (Pla *et al.*, 2012)	–	↑ compared with normal human ECs	TRPV4 silencing reduces migration of endothelial cells derived from human breast carcinomas (Pla *et al.*, 2012)
TRPV6	Breast cancer samples (Bolanz *et al.*, 2008)	↑	–	TRPV6 silencing decreases proliferation of T-47D cells (Bolanz *et al.*, 2008) and migration and invasion of MDA-MB-231 cells (Dhennin-Duthille *et al.*, 2011)
	Breast cancer samples (Dhennin-Duthille *et al.*, 2011)	↑	–	
	Breast cancer samples (Peters *et al.*, 2012)	–	–	
	Breast cancer cell lines (Peters *et al.*, 2012)	↑ in ER^- and poor prognosis	–	
TRPM7	Breast cancer samples (Dhennin-Duthille *et al.*, 2011)	↑	–	TRPM7 silencing reduces cell proliferation in MCF-7 cells (Guilbert *et al.*, 2009); and reduces metastatic potential of MDA-MB-231 *in vivo* and reduces their migration *in vitro* (Middelbeek *et al.*, 2012)
	Breast cancer samples (Guilbert *et al.*, 2009)	↑	–	
TRPM8	Breast cancer samples (Tsavaler *et al.*, 2001)	↑	–	ND
	Breast cancer samples (Dhennin-Duthille *et al.*, 2011)	↑	–	

. In many cases, alterations in expression were reported to occur only in a selection of breast cancer cell lines or samples. ↑: increased, ↓: decreased; ↔: no significant difference; ND, not determined; SCID, severe combined immune deficiency.

Ca^{2+} channels. The T-type Ca^{2+} channel blocker, NNC 55-0396, is far more potent at inhibiting the proliferation of MCF-7 breast cancer cells than a non-cancer-derived breast cell line, MCF-10A (Taylor *et al.*, 2008). The association between T-type Ca^{2+} channels and breast cancer cell proliferation is also seen through the high levels of mRNA for the α_{1G} ($Ca_V3.1$) and α_{1H} ($Ca_V3.2$) T-type Ca^{2+} channel subunits in proliferating sub-confluent MCF-7 and MDA-MB-231 breast cancer cells but not confluent cultures of these cell lines (Taylor *et al.*, 2008). Further support for a potential role for T-type-mediated Ca^{2+} influx in breast cancer is the ability of the T-type Ca^{2+} channel blocker, mibefradil, to inhibit the growth of MCF-7 breast cancer cells (Bertolesi *et al.*, 2002). As discussed later in this review, mRNA levels of the voltage-gated Ca^{2+} channel subunit encoded by the gene *CACNA2D3* ($\alpha 2\delta 3$ subunit) are reduced in some metastatic breast cancers (Palmieri *et al.*, 2012). How down-regulation of *CACNA2D3* could contribute to the development of metastasis of breast cancer is unclear and changes in *CACNA2D3* levels may not be a causative factor in metastasis. However, mechanisms could involve the promotion of a remodelling of Ca^{2+} homeostasis through compensatory up-regulation of other calcium transporters, the results of which could be enhanced migration or invasion capacity and/or an altered sensitivity to apoptotic stimuli.

TRPC channels in breast cancer

Calcium permeable ion channels of the TRP family have been widely studied in some cancers. Examples include TRPM8 and TRPV6, which have been extensively studied in *in vitro* and *in vivo* models of prostate cancer and also human prostate cancer samples (Tsavaler *et al.*, 2001; Fixemer *et al.*, 2003). TRP channels are also increasingly studied in breast cancers (Ouadid-Ahidouch *et al.*, 2013). A recent extensive assessment of the expression of TRP channels in breast cancer identified significantly elevated TRPC1 mRNA in 5 of 10 human breast cancers with a greater than 30-fold increase evident in one breast cancer sample compared with its paired non-tumour sample (Dhennin-Duthille *et al.*, 2011). However, TRPC1 expression is mostly overexpressed in small and low proliferation capacity tumours, suggesting that TRPC1 may not be the optimal target for therapies against advanced or aggressive breast cancers (Dhennin-Duthille *et al.*, 2011). These same studies also reported significantly elevated (up to 200-fold) TRPC6 mRNA in 7 of 10 breast tumour samples compared with paired control samples (Dhennin-Duthille *et al.*, 2011), a finding consistent with the work of Aydar *et al.* who found elevated TRPC6 mRNA in breast cancer biopsy samples compared with normal biopsy tissue (Aydar *et al.*, 2009). This group also reported that TRPC6 silencing reduces the growth of MDA-MB-231 breast cancer cells (Aydar *et al.*, 2009). Elevation in TRPC3 mRNA also appears to be a feature of some breast cancer cells (Aydar *et al.*, 2009). Given the identification of TRPC3 as a potential therapeutic target in ovarian cancer (Yang *et al.*, 2009b), further assessment of TRPC3 in breast cancer is warranted. The relationship between TRPC isoform overexpression and breast cancer subtype and the use of *in vivo* models to determine the utility of this class of ion channel for breast cancer therapy are obvious studies for the future.

TRPM channels in breast cancer

Although the first work reporting the overexpression of the low temperature activated ion channel TRPM8 in some cancers resulted in TRPM8 receiving the most attention in the context of prostate cancer (Tsavaler *et al.*, 2001), these studies also reported the overexpression of TRPM8 in breast cancer. The role of TRPM8 in breast cancer cells and its potential as a therapeutic target in breast cancer has not been fully explored, although recent studies suggest that TRPM8 overexpression may be more common in oestrogen receptor positive breast cancers and well-differentiated lower grade breast cancers (Chodon *et al.*, 2010). Another isoform of the TRPM family that is receiving increasing attention in the context of breast cancer is TRPM7. TRPM7 is a unique TRP channel, as it is Ca^{2+} permeable as well as permeable to Mg^{2+} and it has its own kinase domain, for which some substrates have been identified (Runnels *et al.*, 2001; Bates-Withers *et al.*, 2011; Paravicini *et al.*, 2012). Immunohistochemistry studies assessing TRPM7 levels in human breast cancers indicate that TRPM7 overexpression may be a feature of higher grade and highly proliferative breast cancers and may play a role in the proliferation of MCF-7 breast cancer cells (Guilbert *et al.*, 2009). More recent studies suggest that TRPM7 may be particularly important in breast cancer metastasis. TRPM7 levels are high in high-grade tumours, and high levels of TRPM7 mRNA are predictive of poor survival and the occurrence of distant metastasis (Middelbeek *et al.*, 2012). Silencing of TRPM7 reduces key *in vitro* parameters for invasiveness in MDA-MB-231 breast cancer cells, including cellular elongation and migration rate. MDA-MB-231 cells with TRPM7 silencing also have less metastatic potential *in vivo* as assessed by reduced formation of metastasis after tail vein injection (Middelbeek *et al.*, 2012). Collectively, recent studies of TRPM7 suggest that this channel may be a pharmacological target for breast cancer therapy. In this context, it is interesting to note that the Hawaiian soft coral derived compound Waixenicin A is an inhibitor of MCF-7 breast cancer growth (Zierler *et al.*, 2011).

TRPV channels in breast cancer

The most studied TRPV channel in breast cancer is TRPV6. TRPV6 is elevated in cancer of the breast along with those of the prostate, thyroid, colon and ovary (Zhuang *et al.*, 2002; Lehen'kyi *et al.*, 2012). However, further detail was provided by Bolanz *et al.* who obtained the first evidence that TRPV6 was likely to be up-regulated in only a specific subset of breast cancers (Bolanz *et al.*, 2008), with subsequent studies by Dhennin-Duthille *et al.* also reporting elevated levels of TRPV6 in a subset of breast cancer biopsies (Dhennin-Duthille *et al.*, 2011). Peters *et al.* recently demonstrated that breast tumours with elevated TRPV6 mRNA were more likely to belong to breast cancers of the basal molecular subtype and were also more likely to be oestrogen receptor negative (Peters *et al.*, 2012). Moreover, breast cancers with high levels of TRPV6 mRNA were associated with poorer survival (Peters *et al.*, 2012). TRPV6 may also be a potential therapeutic target in breast cancer given that TRPV6 silencing is associated with reduced proliferation in T-47D breast cancer cells (Peters *et al.*, 2012) and attenuation of migratory and invasive properties in MDA-MB-231 breast cancer cells (Dhennin-Duthille *et al.*, 2011). The TRPV4 ion channel was recently associated

with breast cancer, but in a very different way. Endothelial cells derived from breast cancers have higher levels of TRPV4 than those isolated from normal breast tissue and exhibit a greater $[Ca^{2+}]_{CYT}$ response to TRPV4 activation via arachidonic acid and 4α-phorbol 12,13-didecanoate (Pla *et al.*, 2012). These results implicate TRPV4 as a potential target in angiogenesis. Indeed, activation of TRPV4 promotes the migration of breast cancer-derived endothelial cells but not endothelial cells derived from normal breast and TRPV4 silencing reduces arachidonic acid-stimulated migration of breast cancer-derived endothelial cells (Pla *et al.*, 2012). *In vivo* experiments are required to determine how effective TRPV4 inhibition is at inhibiting angiogenesis in breast cancer compared with clinically used vascular endothelial growth factor A inhibitors.

Ligand-gated Ca^{2+} channels in breast cancer

Ligand-activated Ca^{2+} channels are the subject of some studies focused on understanding pathways and processes important in breast cancer. However, further studies are required. This is exemplified by studies assessing P2X7 receptors, which have linked this receptor to cancer cell invasiveness (Jelassi *et al.*, 2011) and the anti-invasive properties of the anthraquinone emodin (Jelassi *et al.*, 2013). These studies have mostly used MDA-MB-435S cells, a basal breast cancer cell with strong melanoma-like characteristics (Chambers, 2009; Afrasiabi *et al.*, 2010). Studies on other basal-like and non-basal-like breast cancer cell lines as well as other P2X receptor isoforms may identify which breast cancer subtype(s) and which P2X receptors may offer the best therapeutic potential for the control of breast cancer metastasis.

ORAI calcium channels in breast cancer

As described earlier, the ability of cells to promote the influx of Ca^{2+} upon depletion of their internal Ca^{2+} stores was an elegant mechanism first described over 26 years ago and referred to as capacitative calcium entry (Putney, 1986). Numerous studies have now linked either the calcium channel component (ORAI channels) or the endoplasmic reticulum Ca^{2+} level sensors (STIM proteins) to breast cancer. However, the *nature* of the remodelling of the expression of these proteins appears to be highly dependent on the breast cancer subtype. The ORAI isoform most up-regulated during lactation (McAndrew *et al.*, 2011), ORAI1, has increased mRNA levels in the widely studied T-47D, MCF-7 and MDA-MB-468 cell lines compared with the non-breast cancer-derived 184A1 and 184B5 cell lines. ORAI1 up-regulation appears to be a feature of the poor prognosis basal breast cancer subtype (McAndrew *et al.*, 2011). Basal molecular subtype breast cancers are also more likely to have higher mRNA levels of the canonical ORAI1 activator STIM1 and lower levels of its related isoform STIM2. Breast cancers with a high level of STIM1 and a low level of STIM2 are associated with a significantly poorer prognosis, suggesting that a remodelling of store-operated Ca^{2+} entry may be a feature of breast cancers with greater aggressiveness and metastasis (McAndrew *et al.*, 2011). However, increased ORAI1-mediated Ca^{2+} influx may also be an important feature in some non-basal breast cancers as some of these cancers have high levels of the secretory pathway Ca^{2+} ATPase 2 isoform (SPCA2), which as discussed below has a domain that can activate ORAI1-mediated Ca^{2+} influx (Feng *et al.*, 2010). Studies assessing the effect of ORAI1 silencing in breast cancer cells support a role for ORAI1 in breast cancer progression. Silencing of ORAI1 reduces the proliferation of breast cancer cell lines *in vitro* (Feng *et al.*, 2010; McAndrew *et al.*, 2011) and *in vivo* (Feng *et al.*, 2010), and ORAI1 silencing reduces the invasiveness of MDA-MB-231 breast cancer cells *in vitro* and metastasis *in vivo* (Yang *et al.*, 2009a). The mechanism by which ORAI1 silencing reduces breast cancer cell migration appears to involve alterations in the turnover of focal adhesions (Yang *et al.*, 2009a) and its effects on proliferation are linked to reduced extracellular-signal regulated kinase (ERK1/2) phosphorylation (Feng *et al.*, 2010). Reduced ORAI1-mediated Ca^{2+} influx was also recently shown to be an important aspect by which reduced human ether a-gogo K^+ channel 1 inhibits the migration of MDA-MB-231 breast cancer cells (Hammadi *et al.*, 2012). Collectively, these studies suggest that pharmacological agents that inhibit ORAI1-mediated Ca^{2+} influx may represent a new approach to attenuating breast cancer growth and/or metastasis.

ORAI1 is not the only ORAI isoform to be linked to breast cancer. ORAI3 has recently been associated with oestrogen positive breast cancer (Motiani *et al.*, 2013). There are elevated ORAI3 protein levels in oestrogen receptor positive breast cancer cell lines and a greater dependence of store-operated Ca^{2+} entry on ORAI3 in MCF-7 oestrogen receptor positive breast cancer cells compared with oestrogen receptor negative MDA-MB-231 breast cancer cells (Motiani *et al.*, 2010). ORAI3 may be a highly effective target for oestrogen receptor positive breast cancer because ORAI3 silencing reduces the anchorage-independent cell growth and invasiveness of MCF-7 cells *in vitro* and the growth of MCF-7 tumours *in vivo*, via mechanisms involving ERK1/2 and focal adhesion kinase and calcium activated transcription factor NFAT (Motiani *et al.*, 2013). The recent report that the proliferation of MCF-7 cells but not non-cancer breast-derived MCF-10A cells is sensitive to ORAI3 silencing, and the significant elevation of ORAI3 mRNA in 10 of 13 breast cancers compared with normal breast tissue, provides further evidence that ORAI3 represents a novel target for oestrogen receptor positive breast cancers (Faouzi *et al.*, 2011). ORAI3 silencing appears to reduce proliferation in breast cancer cells via the c-Myc pathway and MAPK pathways (Faouzi *et al.*, 2013). Further studies are required to determine the potential of ORAI3 inhibition to either increase the effectiveness of current therapies for oestrogen positive breast cancers or to target oestrogen receptor positive breast cancers that have developed resistance to anti-oestrogen-based therapies.

Mechanisms responsible for altered plasma membrane Ca^{2+} channel expression in breast cancer cells

Although neglected for some time, recent studies have begun to explore the mechanisms by which specific Ca^{2+} 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

oestrogen receptor α (ERα). Silencing of ERα in human MCF-7 breast cancer cells reduces ORAI3 mRNA and protein levels but does not affect levels of ORAI1 (Motiani *et al.*, 2013). This provides a potential mechanistic link between ORAI3 overexpression and breast cancer cells expressing ERα. TRPM8 levels are also reduced by ERα silencing in MCF-7 cells and are increased by 17-β-oestradiol (Chodon *et al.*, 2010). The observation that the expression of the Ca^{2+} permeable ion channel TRPV4 is decreased by progesterone in T-47D breast cancer cells (Jung *et al.*, 2009) also suggests that altered expression of some calcium channels in breast cancers are driven by hormonal mechanisms. Further assessment of this mechanism for other calcium channels and the consequences of anti-oestrogen therapy on the expression of calcium channels in clinical breast cancer now appear warranted.

The phenomenon of gene amplification is well appreciated in breast cancer. The gene amplification of ErbB2 receptors in many aggressive breast cancers is exploited by the humanized monoclonal antibody trastuzumab (Baselga *et al.*, 1999). Very few studies have assessed the possibility of gene amplification of calcium channels in breast cancer. However, *TRPV6* gene amplification appears to be one potential mechanism for the overexpression of TRPV6 in SK-BR-3, ZR-75-1 and T-47D breast cancer cell lines (where copy numbers are between 6 and 9) and in some breast cancers, where TRPV6 elevated copy number is associated with oestrogen receptor negative, triple negative and basal-like breast cancers (Peters *et al.*, 2012). Other mechanisms for altered expression in breast cancer that have not yet been fully explored are epigenetic-mediated changes, such as gene methylation. The gene for the voltage-gated calcium channel regulatory subunit, *CACNA2D3*, is associated with higher methylation in breast cancers with metastasis to the CNS, and DNA demethylation results in a pronounced increase in *CACNA2D3* levels in MDA-MB-453 breast cancer cells (Palmieri *et al.*, 2012). The significance of this for calcium signalling and breast cancer pathways is still unclear, but the methylation of the *CACNA2D3* gene is proposed as a potential biomarker for the development of metastases (Palmieri *et al.*, 2012). The future should see an increased focus on these and other potential mechanisms for altered expression of Ca^{2+} channels in breast cancer cells.

Regulation of Ca^{2+} channel activity

Studies have begun to identify complexity in the regulation of calcium channels in breast cancer cells. The ability of the N-terminal domain of the SPCA2 calcium pump to activate Ca^{2+} influx via ORAI1 and promote activation of NFAT suggests that, in some cases, it may be enhanced activation of a calcium channel (in this case through overexpression of another protein) rather than the overexpression of the calcium channel itself that is the driving force for tumour progression (Feng *et al.*, 2010). Further support for the significance of such indirect mechanisms is provided by Kim *et al.*, who showed that the tumour suppressor Numb1 is a negative regulator of TRPV6 activity (Kim *et al.*, 2013b). Numb1 silencing increases proliferation and basal Ca^{2+} influx in MCF-7 breast cancer cells where it directly interacts with TRPV6 (Kim *et al.*, 2013b). Another level of complexity in the role of calcium influx channels in cancer is through alterations in calcium channel localization. Bidaux *et al.* demonstrated that in prostate cancer cells, some of the overexpressed TRPM8 protein is present on the endoplasmic reticulum where this localization is implicated in prostate cancer progression through alteration of the calcium content of internal stores (Bidaux *et al.*, 2007). Studies are required to determine if similar localization changes occur in breast cancer cells. We know that silencing of TRPC1 in MDA-MB-468 breast cancer cells attenuates the high levels of ORAI1-mediated basal Ca^{2+} influx in this cell line (Davis *et al.*, 2012). Suggestions are that this is mediated, in part, through the expression of TRPC1 on the endoplasmic reticulum of MDA-MB-468 breast cancer cells and promotion of Ca^{2+} leakage from that calcium store. However, further studies are required to establish if non-plasmalemmal localization of TRPC1 and other calcium channels is a feature of some breast cancers.

Pharmacological targeting of calcium influx pathways in breast cancer

As described throughout this review, one of the major potential advantages of regulators of calcium influx as novel drug targets for cancer is the clear ability to design pharmacological modulators of Ca^{2+} permeable ion channels. As outlined in Table 2, there are a variety of reported activators and inhibitors to many of the calcium permeable ion channels that are associated with cancers. The majority of studies described throughout this review have identified specific calcium permeable ion channels as potential therapeutic targets using pharmacological inhibitors and/or siRNA or short hairpin (sh)RNA-mediated silencing. Such approaches are clearly suitable given the role of calcium signalling in the promotion of cellular proliferation and motility. Indeed, *in vivo* studies show the ability of pharmacological inhibitors of calcium influx pathways to inhibit breast cancer proliferation and/or invasion (Taylor and Simpson, 1992; Belpomme *et al.*, 2000). Another mechanism for oncology therapy is the promotion of cancer cell death. There is a lack of studies of this aspect of calcium influx in breast cancer, even though high and sustained levels of $[Ca^{2+}]_{CYT}$ can be a trigger for apoptosis and large increases in $[Ca^{2+}]_{CYT}$ can even trigger necrosis (Frandsen *et al.*, 2012). Hence, one suggested approach to target an overexpressed calcium permeable ion channel is to administer a channel activator to produce sustained calcium influx sufficient to induce cell death. As discussed earlier, TRPM8 is overexpressed in many prostate cancers, and early studies in the LNCaP prostate cancer cell line show that the TRPM8 activator menthol could induce apoptosis (Zhang and Barritt, 2004). Despite the reported overexpression of TRPM8 in some breast cancers (Tsavaler *et al.*, 2001; Chodon *et al.*, 2010; Dhennin-Duthille *et al.*, 2011), the consequence of activation of this and other calcium permeable ion channels has not been fully explored in breast cancer cells.

It is likely that cell death via calcium channel activation will only occur in breast cancer cells where there is sufficient overexpression of the channel such that an activator will produce enough calcium influx to promote cell death pathways. One of the potential risks of using an activator to

Table 2

Pharmacological modulators of some of the Ca^{2+} channels with altered expression in breast cancer

Channel	Regulator	Activator/Inhibitor	Studies in cancer cells (*in vitro* and *in vivo*)
TRPV4	4α-PDD	Activator (Watanabe *et al.*, 2002)	4α-PDD-induced Ca^{2+} responses are greater in endothelial cells derived from breast cancers (Pla *et al.*, 2012)
	GSK1016790A	Activator (Thorneloe *et al.*, 2008)	ND
	BAA	Inhibitor (Smith *et al.*, 2006)	ND
	GSK2193874	Inhibitor (Thorneloe *et al.*, 2012)	ND
TRPM7	Waixenicin	Inhibitor (Kim *et al.*, 2013a)	Waixenicin inhibits cell proliferation of human Jurkat T-cells and rat basophilic leukaemia cells (Zierler *et al.*, 2011) and the growth and survival of human gastric (AGS) and breast (MCF-7) adenocarcinoma cell (Kim *et al.*, 2013a)
TRPM8	AMTB	Inhibitor (Lashinger *et al.*, 2008)	AMTB reduces the proliferation of PNT1A, LNCap, PC3 and DU145 prostate cancer cells (Valero *et al.*, 2012)
	JNJ41876666	Inhibitor (Parks *et al.*, 2011)	JNJ41876666 reduces proliferation rates of PNT1A, LNCap, PC3 and DU145 prostate tumour cells (Valero *et al.*, 2012)
	PBMC	Inhibitor (Knowlton *et al.*, 2011)	ND
	RQ-00203078	Inhibitor (Okamoto *et al.*, 2012)	ND
	WS-12	Activator (Ma *et al.*, 2008)	WS-12 suppresses the migration and invasion of HSC3 and HSC4 oral squamous cell carcinoma cell lines (Okamoto *et al.*, 2012)
TRPV1	Capsaicin	Activator (Caterina *et al.*, 1997)	Capsaicin inhibits proliferation and induces apoptosis of PC-3 prostate cancer cells (Sanchez *et al.*, 2006), induces apoptosis of glioma (Amantini *et al.*, 2007) and human nasopharyngeal carcinoma NPC-TW 039 cells (Ip *et al.*, 2012), and promotes Fas/CD95-mediated apoptosis of urothelial cancer cells (Amantini *et al.*, 2009)
	DkTx	Activator (Bohlen *et al.*, 2010)	ND
	ABT-102	Inhibitor (Gomtsyan *et al.*, 2008)	ND
	SB-705498	Inhibitor (Rami *et al.*, 2006)	ND
TRPC3	Pyr3	Inhibitor (Kiyonaka *et al.*, 2009)	Pyr3 has no effect on cell viability in gastric cancer cells (Kim *et al.*, 2012)
TRPC6	Hyperforin	Activator (Leuner *et al.*, 2007)	Hyperforin promotes apoptosis in HT-1080 human fibrosarcoma and SK-N-BE human neuroblastoma, decreases proliferation of HT-1080 cells, reduces metastasis of HT-1080 in a mouse model (Dona *et al.*, 2004) and reduces the growth and viability of MCF-7 and MDA-MB-231 cells (Aydar *et al.*, 2009)
	20-HETE	Activator (Basora *et al.*, 2003)	ND
P2X7	A-804598	Inhibitor (Donnelly-Roberts *et al.*, 2009)	ND
	A-740003	Inhibitor (Honore *et al.*, 2006)	A-70003 decreases irradiation- induced cell death in the M059J human glioma cell line (Gehring *et al.*, 2012)
	A- 438079	Inhibitor (Nelson *et al.*, 2006)	Reduces ATP-induced cell death in human RPMI 8226 multiple myeloma cells (Farrell *et al.*, 2010)
T-type	Mibefradil	Inhibitor (Mishra and Hermsmeyer, 1994)	Inhibits proliferation of MCF-7 breast cancer cells (Bertolesi *et al.*, 2002)
	NNC 55-0396	Inhibitor (Huang *et al.*, 2004)	Suppresses proliferation of MCF-7 cells (Taylor *et al.*, 2008)
	SB-209712	Inhibitor (McNaughton *et al.*, 2000)	ND
ORAI1	SKF-96365	Inhibitor (Chung *et al.*, 1994)	SKF-96365 inhibits invasion of MDA-MB-231 cells, and inhibits breast tumour metastasis in a mouse model (Yang *et al.*, 2009a)
	2-APB	Inhibitor (Maruyama *et al.*, 1997)	2-APB decreases the cell growth of MCF-7 and MDA-MB-231 cells (McAndrew *et al.*, 2011)

ND, not determined, # maximum of five regulators listed and some regulators are not specific for their listed target.
4α-PDD, 4α-phorbol 12,13-didecanoate; 2-APB, 2-aminoethoxydiphenyl borate; AMTB, N-(3-aminopropyl)-2-{[(3-methylphenyl)methyl]oxy}-*N*-(2-thienylmethyl)benzamide hydrochloride salt; BAA, bisandrographolide A; DkTx, double-knot toxin; GSK1016790A, N-((1S)-1-{[4-((2S)-2-{[(2,4-dichlorophenyl)sulfonyl]amino}-3-hydroxypropanoyl)-1-piperazinyl]carbonyl}-3-methylbutyl)-1-benzothiophene-2-carboxamide; JNJ41876666, 3-[7-trifluoromethyl-5-(2-trifluoromethyl-phenyl)-1H-benzimid-azol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-eneHydrochloride; PBMC, (1-phenylethyl-4-(benzyloxy)-3-methoxybenzyl(2-aminoethyl)carbamate); SB-705498, (R)-1-(2-bromophenyl)-3-(1-(5-(trifluoromethyl)pyridin-2-yl)pyrrolidin-3-yl)urea; SB-209712, (1,6,bis{1-[4-(3-phenylpropyl) piperidinyl]}hexane); SKF-96365, 1-[2-(4-methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl] imidazole, 1-[β-(3-(4-methoxyphenyl)propoxy)-4-methoxyphenethyl]-1H-imidazole hydrochloride; WS-12, *N*- (4-methoxyphenyl)-p-menthone-3-carboxamide.

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 tumour 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 $[Ca^{2+}]_{CYT}$ changes. Sustained Ca^{2+} 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 (see Table 1) do not have both widely available selective inhibitors and activators (see Table 2). However, such studies, particularly *in vivo*, would greatly advance our understanding of the best therapeutic strategies for targeting calcium channels in breast cancer.

Calcium influx pathways in breast cancer: emerging areas

Major contributions have been made to our understanding of calcium influx in breast cancer cells by many different studies across different research groups. Most studies have naturally focused on either assessing the role of the calcium signalling in key cancer progression events and/or identifying specific calcium permeable channels with altered expression in breast cancer cells and the mechanisms involved. However, some recent studies are beginning to identify specific Ca^{2+} permeable channels in other contexts and may reflect emerging areas that may progress rapidly in the next decade. One such area is chemotherapeutic resistance. Ma *et al.* recently showed that the silencing of TRPC5 restores sensitivity to adriamycin in adriamycin-resistant MCF-7 breast cancer cells (Ma *et al.*, 2012). This work provides evidence that targeting a specific Ca^{2+} channel may represent a novel approach to reverse resistance of breast cancer cells to some types of chemotherapies.

In addition to the direct association between specific calcium permeable ion channels with cellular migration and invasiveness, studies have also begun to associate specific channels with other processes important in breast cancer metastasis such as epithelial to mesenchymal transition (EMT) (Hu *et al.*, 2011; Davis *et al.*, 2012). Triggers of EMT in breast cancer cells include growth factors (e.g. EGF) and hypoxia (Lester *et al.*, 2007; Lo *et al.*, 2007). Changes in the expression of a repertoire of proteins occur during EMT, with the changes bestowing increased migratory and invasive properties and resistance to cell death. Recent studies have provided evidence that Ca^{2+} influx pathways may be remodelled as a consequence of EMT. EGF-induced EMT in MDA-MB-468 breast cancer cells is associated with altered purine receptor Ca^{2+} signalling and increased levels of P2X5 mRNA (Davis *et al.*, 2011). Studies using the same model indicate that EMT reduces basal, agonist and store-operated Ca^{2+} influx (Davis *et al.*, 2012). Further links between EMT and calcium signalling are seen through the association of EMT induced by the down-regulation of the transcription factor Oct4 and associated changes in store-operated Ca^{2+} entry in MCF-7 cells (Hu *et al.*, 2011). Calcium signalling is also a key event in the induction of EGF and hypoxia-mediated EMT in MDA-MB-468 breast cancer cells, with TRPM7 playing a role in the induction of some EMT markers by EGF. This probably occurs through effects on the phosphorylation of signal transducer and activator of transcription 3 (Davis *et al.*, 2013).

In their most recent review, Hanahan and Weinberg (2011) defined the hallmarks and emerging inidcators and enabling characteristics of cancer and highlighted the importance of the cellular heterogeneity of tumours and the microenvironment. Although, as outlined previously, calcium influx has been studied and associated with many of the hallmarks of cancer, there are still areas of tumour biology where the study of calcium signalling is still in its infancy. To take one example, despite the clear importance of calcium signalling in responses to growth factors in the tumour microenvironment, there is a clear lack of studies assessing calcium signalling between breast cancer cells in tumours and with those cells that surround them (e.g. immune inflammatory cells) (Hanahan and Weinberg, 2011). There is also a particular paucity in the study of the role of calcium signalling in cancer stem cells. This is probably due, in part, to the technical difficulties in measuring Ca^{2+} in three-dimensional culture models and *in vivo*. However, advancements in imaging and genetically targeted Ca^{2+} sensors may now lead to studies that expand our understanding of how Ca^{2+} influx pathways may contribute to tumour progression.

Conclusion

Alterations in the expression and/or activity of Ca^{2+} permeable ion channels are a characteristic of some breast cancer cells. Our understanding of why these changes in expression occur is gradually improving with some mechanistic insights. The clearly established sensitivity of some Ca^{2+} channels to selective pharmacological modulators makes them attractive targets for breast cancer therapy. Although both *in vitro* and *in vivo* studies in many cases support such an approach, further studies are required to define the optimal therapeutic strategy and to determine what resistance mechanisms may develop to such agents.

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Conflict of interest

The authors declare no conflict of interest.

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