BBA - Molecular Cell Research 1865 (2018) 1786–1794

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

journal homepage: www.elsevier.com/locate/bbamcr

Calcium signaling and the therapeutic targeting of cancer cells

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ARTICLE INFO

Keywords:
Calcium
Calcium signal
Cancer
Cytotoxics
Resistance
Therapeutics

ABSTRACT

The calcium signal is implicated in a variety of processes important in tumor progression (e.g. proliferation and invasiveness). The calcium signal has also been shown to be important in other processes important in cancer progression including the development of resistance to current cancer therapies. In this review, we discuss how Ca^{2+} channels, pumps and exchangers may be drug targets in some cancer types. We consider what factors should be taken into account when considering an optimal Ca^{2+} channel, pump or exchanger as a candidate for further assessment as a novel drug target in cancer. We also present and summarize how some therapies for the treatment of cancer intersect with Ca^{2+} signaling and how pharmacological manipulation of the machinery of Ca^{2+} signaling could promote the effectiveness of some therapies. We also review new therapeutic opportunities for Ca^{2+} signal modulators in the context of the tumor microenvironment.

1. Introduction

The calcium signal is a critical regulator of a variety of cellular processes, many of which intersect with those important in cancer progression, such as proliferation and invasiveness [1,2]. A specific example of the importance of the calcium signal in proliferation is seen in early G1 of the cell cycle where Ca^{2+} is involved in the induction of the early response genes *FOS*, *JUN* and *MYC* [2]. The Ca^{2+} signal is also an essential regulator of other specific events in the cell cycle [2]. Ca^{2+} also plays an important role in invasive pathways and cancer cell migration [3]. Ca^{2+} is involved in the phosphorylation of contractile proteins, the induction of matrix metalloproteinases and is also a major player in the remodeling of peripheral and focal adhesions during cancer cell migration [3]. The calcium signal is also a direct inducer and regulator of cell death as exemplified by the ability of the sarco/endoplasmic reticulum ATPase (SERCA) inhibitor thapsigargin to promote apoptosis in a variety of cell types [4]. The identification that pathways relevant to known oncogenes and/or tumor suppressors can either be calcium sensitive or drive alterations in calcium signaling is further evidence for the relevance of the calcium signal in the context of cancer. The report that PTEN loss (an occurrence in many cancers) can promote the degradation of inositol 1,4,5-trisphosphate (IP_3) receptors and attenuate Ca^{2+}-dependent apoptosis pathways [5] is just one recent example of the intersection between tumor suppressor loss and a remodeling of Ca^{2+} signaling. The contribution of the calcium signal to cancer cell proliferation, metastasis and cell death has been reviewed extensively elsewhere (e.g. [6–10]).

In addition to the critical role of calcium signaling in key elements of disease progression in cancer, a variety of studies have now identified significant remodeling of the expression of specific calcium permeable ion channels or calcium pumps in some types of cancers. In many cases this alteration in expression may be specific for particular cancer subtypes. Examples of such specific remodeling includes enhanced levels of the transient receptor potential vanilloid 6 channel (TRPV6) in breast cancers of the basal molecular subtype, which may be the consequence of increased TRPV6 copy number [11]. Given that the overexpression of HER2 in specific breast cancers through gene amplification is a feature that has been successfully exploited through the use of HER2 targeting agents such as the monoclonal antibody trastuzumab (Herceptin) [12], it stands to reason that where there is pronounced overexpression of a specific calcium permeable ion channel or pump, this too could be exploited to reduce cancer cell proliferation or invasiveness or even to promote cancer cell death. In this review, we will consider the calcium signal and the proteins that directly regulate Ca^{2+} in the context of cancer therapy. It is beyond the scope of this review to consider all of the major contributions made by many laboratories worldwide that are relevant to this topic. We have sought here to reflect on some of the intersections between calcium signaling and the therapeutic targeting of cancer cells and provide some specific examples of the possibilities and constraints in targeting the Ca^{2+} signal.

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https://doi.org/10.1016/j.bbamcr.2018.05.015
Received 8 March 2018; Received in revised form 23 April 2018; Accepted 24 May 2018
Available online 26 May 2018

2. The calcium signal and cancer cells

Readers unfamiliar with the key elements of Ca^{2+} homeostasis and Ca^{2+} signaling in mammalian cells are encouraged to seek out some of the many comprehensive reviews on this topic. Briefly, the major electrochemical gradients for Ca^{2+} between the cytosol (~100 nM), extracellular fluid (> 1 mM) and the intracellular Ca^{2+} stores of the sarco/endoplasmic reticulum (> 100 μM) are maintained by active transport systems such as the plasma membrane Ca^{2+}-ATPases (PMCAs) and sarco/endoplasmic Ca^{2+}-ATPases (SERCAs). Increases in cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$), can be instigated by a variety of mechanisms including direct facilitated diffusion after the opening of plasma membrane Ca^{2+} channels or indirectly via the activation of IP_3 receptors (IP_3Rs) of the sarco/endoplasmic reticulum after stimulation of some G-protein coupled receptors (GPCRs) or some receptor tyrosine kinases (RTKs) coupled to phospholipase C, an enzyme which can convert PIP_2 into the calcium mobilizing agent IP_3. Studies over the last decade have also provided new insights into the molecular components of the Ca^{2+} influx pathway (i.e. store-operated calcium entry) activated after the depletion of internal Ca^{2+} stores (namely the ion channel component Orai1 and the Ca^{2+} store level sensor STIM1) [13]. The last decade has seen the molecular identification of the transporters and exchangers of the mitochondria such as the mitochondrial uniporter (MCU) and the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) [14]. Other well characterized Ca^{2+} influx mechanisms include voltage gated ion channels which include L-type Ca^{2+} channels which can be blocked by anti-hypertensive therapies such as nifedipine and TRP Ca^{2+} permeable ion channels, such as the heat- and capsaicin- (hot chilli component) activated channel TRPV1 [15]. Most of the above examples of Ca^{2+} pumps, channels and exchangers have a variety of isoforms and often splice variants which provide a suite of proteins to precisely control the Ca^{2+} signal. This repertoire of Ca^{2+} channels and pumps allow precise activation of many processes, through controlling the magnitude of the $[Ca^{2+}]_{CYT}$ signal or the temporal and/or spatial aspects of the nature of the calcium signal [16–18].

Cancer cells obviously use the same repertoire of calcium channels, pumps and exchangers as non-transformed cells, however as alluded to above and reviewed elsewhere, there is often an aberrant expression of specific Ca^{2+} regulating proteins in some cancer types [1,6]. There is also evidence that cancer cells can be differentially sensitive to specific aspects of calcium signal disruption, such as the induction of cell death instead of pro-survival autophagy after inhibition of IP_3R to mitochondria Ca^{2+} transfer in some cancer cell lines [19]. The remodeling of the expression of calcium channels/pumps or of the Ca^{2+} signal could represent an “Achilles Heel” in the targeting of cancer cells. This review will focus on Ca^{2+} signaling components as targets for cancer therapy and also the intersection between current cancer treatments and calcium signaling. We will discuss this in the context of a) how specific calcium channels or pumps may be targets for cancer therapy, b) how therapies currently used (or in development) for the treatment of some cancers intersect with calcium signaling, c) how modulation of the calcium signal could be exploited to improve the effectiveness of current cancer treatment regimes, d) how calcium signal regulators may play a role in the development of cancer therapy resistant pathways and e) the role of the calcium signal in the targeting of processes in the tumor microenvironment which is important in disease progression.

3. Specific calcium channels or pumps as targets for cancer therapy

The overexpression of specific calcium channels and pumps in some cancer types and/or subtypes has led to the proposal that pharmacological modulators of some calcium channels or pumps may represent future cancer therapies. However, overexpression itself is not a sufficient criterion for a potential pharmacological target in cancer. Pharmacological modulation of the target must alter proliferation, migration and/or induce cancer cell death, analogous to the way anti-HER2 agents exploit the overexpression of the HER2 protein in some breast cancers to control disease progression [12]. The critical role of the calcium signal in many of the hallmarks of cancer [8] certainly gives the potential for an effective therapy. However, a lack of activity, defective membrane trafficking or a limited role of a specific calcium pump or channel in a pathway relevant to tumor progression are just some of the examples where over-expression per se will be insufficient for a calcium pump or channel to be a therapeutic target in a particular cancer type. Another consideration is the likely effects of global pharmacological modulation of the target. Although many cancer therapies work on targets with critical roles in normal cells, as exemplified by some of the major side effects of some anticancer agents (e.g. immunosuppression), this is an important consideration. Tools for target selection and/or prioritization could include consideration of the known toxicity of pharmacological modulators to the target, or where such agents are not currently available, the viability and/or phenotype of knockout animals. It should be noted that despite the diverse expression of some ion channels and ion pumps they still represent targets highly amenable to drug development. Indeed, ion channels have been reported to represent 19% of human protein drug targets and are the targets of existing therapies including L-type Ca^{2+} channel blockers for the control of hypertension [20].

A number of reviews have highlighted the potential of calcium permeable ion channels and calcium pumps as therapeutic targets [1,10]. An example where this approach has recently started to extend towards human clinical trials is for the targeting of the highly Ca^{2+}-selective ion channel TRPV6 [21]. Increased levels of TRPV6 have been reported in a variety of malignant cancers [22] including estrogen receptor negative breast cancers where TRPV6 overexpression may be driven by increases in TRPV6 copy number [11]. Silencing and overexpression studies have identified a critical role for TRPV6 in the proliferation of some cancer cell lines using in vitro and in vivo models [23,24]. TRPV6 inhibitors have now undergone human phase 1 clinical trials in patients with advanced tumors of epithelia origin including those of the ovary, colon, pancreas, breast and prostate [21]. Fig. 1 presents a conceptual overview of how an overexpressed ion channel could be pharmacologically targeted for cancer therapy. The first approach is valid for ion channels that contribute to the proliferation and/or invasive pathways in cancer cells through effects on cytosolic Ca^{2+} signaling. For these targets pharmacological inhibition may attenuate these pathways to reduce proliferation and/or invasion. Example of this approach include the aforementioned TRPV6 as well as inhibitors of store operated calcium entry (SOCE) which target the remodeling of Orai1-mediated Ca^{2+} influx which appears to be a feature of some cancer types [25–27]. TRPV4 is another example, since TRPV4 silencing has recently been shown to reduce the invasiveness of breast cancer cells [28]. In the specific context of TRPV4, it is interesting to note the successful completion of a phase 2 clinical trial of the TRPV4 inhibitor GSK2798745 in patients with congestive heart failure [29], highlighting the potential for the repurposing of this compound and/or similar agents for the treatment of some cancers. Indeed, there is a variety of opportunities for the repurposing of agents targeting calcium permeable ion channels for cancer, such as T-type Ca^{2+} channel blockers such as mibefradil (previously used for cardiovascular disease) which is being assessed in clinical trials for glioblastoma multiforme [30], the most common and aggressive form of brain cancer. The focus of drug development programs for SOCE inhibitors (developed by companies such as CalciMedica and Rhizen Pharmaceuticals) for an array of conditions including autoimmune disorders [31] also represent opportunities for drug repurposing.

Activation of Ca^{2+} permeable ion channels with pronounced overexpression represents another approach to promote cancer cell death, and this potential has now been demonstrated in a variety of models including TRPV4 in breast cancer cells [32] and TRPM8 in prostate cancer cells [33]. However it should be noted as reflected also in Fig. 1,

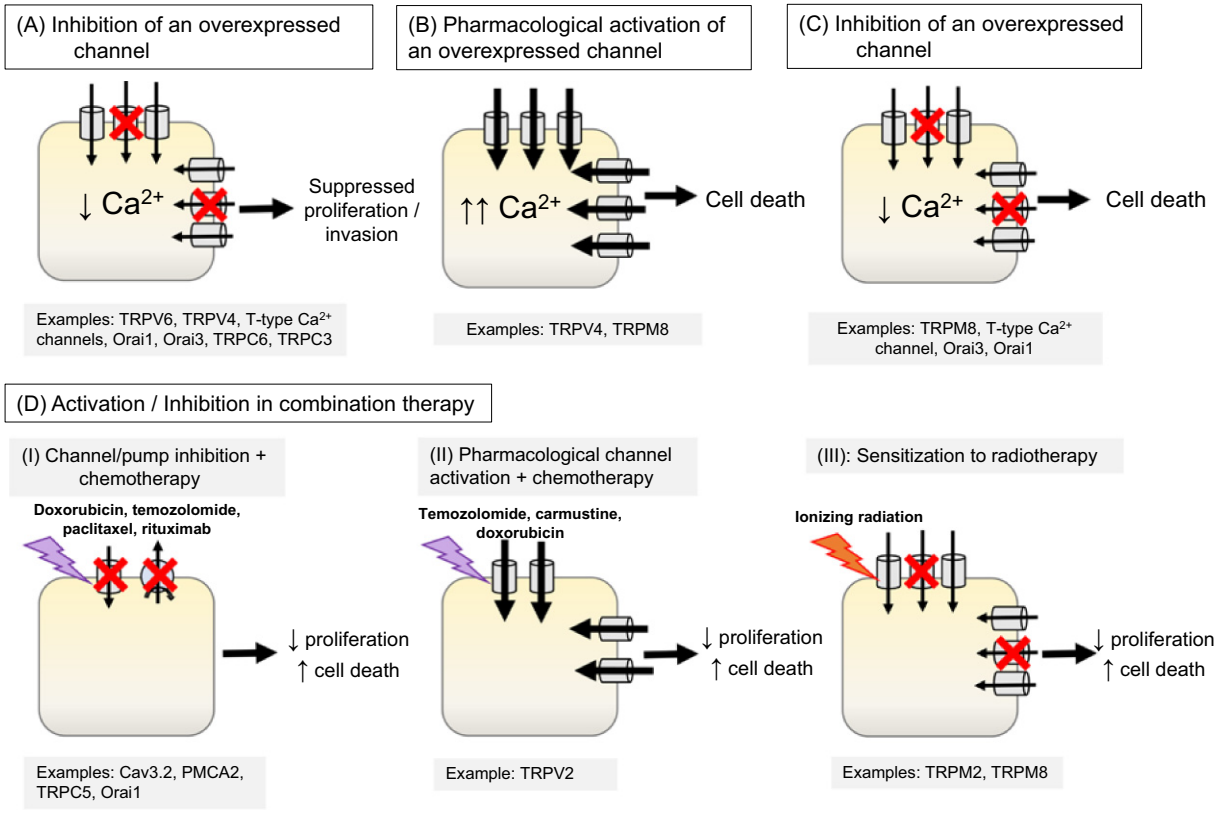

Fig. 1. Strategies to target Ca^{2+} signaling for cancer therapy. The overexpression of Ca^{2+} channels in cancer cells may be targeted by: (A) inhibition to suppress pro-proliferative or pro-migratory Ca^{2+} signals, examples of such channels include TRPV6 [24], T-type voltage-gated Ca^{2+} channels, i.e. Cav3.2 [57], Orai1 [27], Orai3 [95], TRPC6 [96] and TRPC3 [97]; (B) activation to promote Ca^{2+} overload and activating cell death (eg: apoptosis and oncosis), examples are TRPV4 [32] and TRPM8 [33]; or (C) inhibition to suppress pro-survival Ca^{2+} signals to induce cell death, examples include TRPM8 [33], T-type Ca^{2+} channels [34], Orai1 [98] and Orai3 [99]. Alternatively, Ca^{2+} signaling can also be strategically targeted in combination with anti-cancer drugs to promote killing of cancer cells (D). Proposed mechanisms include (I) inhibition of a Ca^{2+} channel such as Cav3.2 [57], TRPC5 [67] and Orai1 [51] or a plasmalemmal Ca^{2+} efflux pump such as PMCA2 [55]; (II) activation of a Ca^{2+} channel, eg: TRPV2 [100] to promote the cytotoxic effect of chemotherapy drugs such as temozolomide, doxorubicin and carmustine; or (III) inhibition of specific Ca^{2+} influx channels such as TRPM2 [60] and TRPM8 [59] in combination with radiotherapy to promote tumor-suppressing and/or cytotoxic effects.

that there may also be examples where inhibition of a plasmalemmal Ca^{2+} permeable ion channel may also induce cancer cell death. In these examples rather than direct "Ca^{2+} overload" inducing necrotic or apoptotic cell death, the attenuation of a Ca^{2+} influx pathway may induce cancer death through other pathways. Examples includes the ability of T-type Ca^{2+} channel inhibition to produce apoptosis in p53-competent HCT116 colon cancer cells through activation of p38-MAPK [34]. Another area of study has been the identification of calcium transporters, pumps and channels of intracellular organelles that when silenced can also attenuate pathways important in tumor progression. These examples extend to the recently identified components of mitochondrial Ca^{2+} transport and the less studied Ca^{2+} pumps of the Golgi apparatus – secretory pathway Ca^{2+} ATPases (SPCAs). Reduced expression of MCU in MDA-MB-231 breast cancer cells suppresses their migration in vitro and metastasis in vivo [35], and reduced expression of SPCA2 attenuates the proliferation of MCF-7 cells in vitro and tumor growth in vivo [26].

The discussion above has mostly focused on targeting specific calcium channels, pumps or exchangers through exploitation of their overexpression and/or critical role in a proliferation or invasive pathways. However, there may be circumstances where mutations in a Ca^{2+} regulating protein may be a feature of cancer cells. In some cases these events may be rare and confined to only a very few individual cancers, such as appears to be the case for gain of function mutations in Orai1 identified from the cBioPortal database [36]. Although further work on other calcium channels, pumps and exchangers is required, there is an example which does point to the potential significance of a mutation of a calcium pump in a cancer which leads to a significant clinical impact. Somatic mutations in the PMCA3 Ca^{2+} efflux pump in some aldosterone-producing adenomas reduces the activity of the PMCA3 pump and remodels Ca^{2+} signaling which appears to promote aldosterone production and the associated severe arterial hypertension in patients with these adenomas [37,38]. Whether the future will see highly selective agents targeting specific mutations of a calcium channel, pump or channel in a specific cancer type is still unclear, however such agents are theoretically possible given the successful development of CFTR ion channel mutation specific drugs for cystic fibrosis therapy [39].

4. The intersection between current and potential cancer therapies with Ca^{2+} signaling: modulation of the calcium signal to improve the effectiveness of current cancer treatment regimes

Given that anti-cancer agents target proliferative or invasive pathways, and that these pathways involve either global or localized changes in $[Ca^{2+}]_{CYT,}$ the use of anti-cancer agents may thus indirectly alter Ca^{2+} signaling in effected cancer cells. Agents which induce cell death and may compromise cellular integrity or reduce ATP production will also indirectly alter Ca^{2+} signaling through promoting the influx of Ca^{2+} ions or through the eventual lack of ATP leading to an inability to maintain ion gradients across the cell. Agents which block specific growth factors that act in part through receptors coupled to IP_3 production will also have an impact on $[Ca^{2+}]_{CYT}$ signaling. In the section

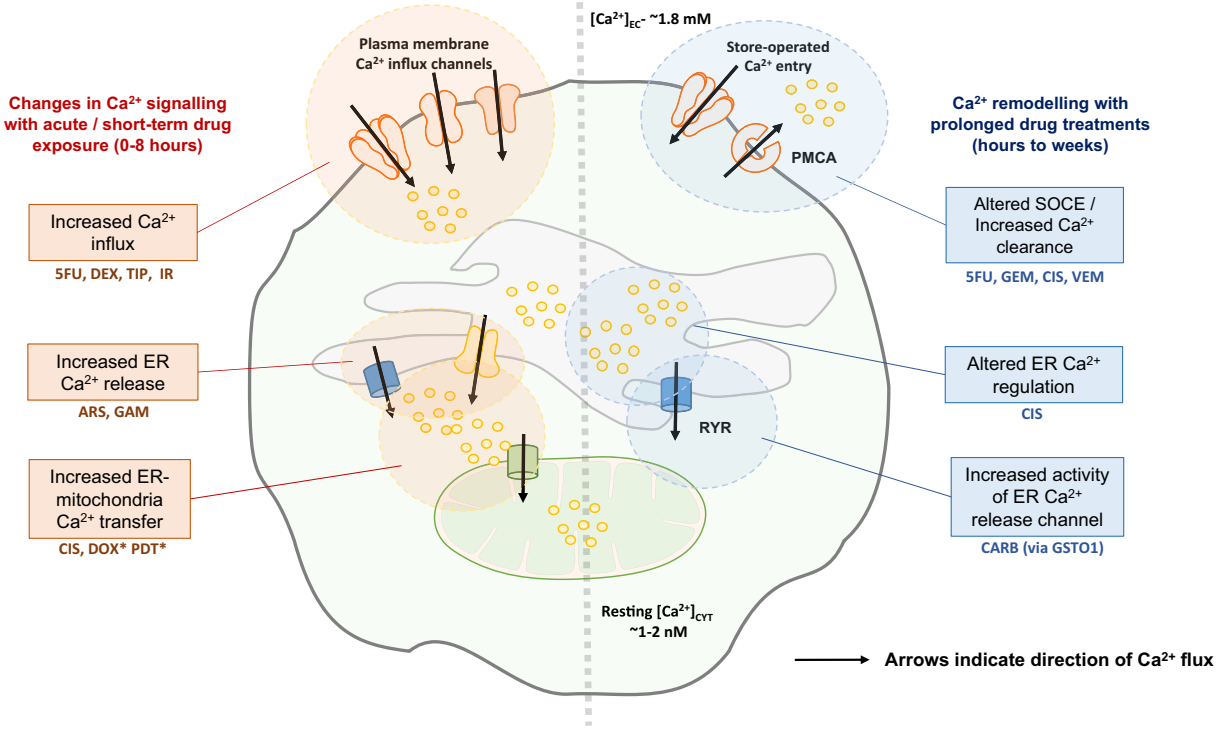

Fig. 2. Ca^{2+} signal modulation in cancer cells by anti-cancer treatments. Examples of short-term (i.e. 0 to 8 h; shown in red boxes) and prolonged exposure (> 8 h treatment and including resistant cell lines; shown in blue boxes) to specific anti-cancer drugs in different cancer models. Drugs or treatments that appear to increase Ca^{2+} influx as a result of short-term exposure include 5-Fluorouracil (5FU) [40], dexamethasone (DEX) [44], Tipifarnib (TIP) [42] or ionizing radiation (IR) [60]. Drugs such as arsenic trioxide (ARS) [49] and gamitrinib (GAM) [101] have been suggested to promote endoplasmic reticulum (ER) Ca^{2+} release during short term exposure. Short-term exposure of cancer cells to cisplatin [43,74], doxorubicin (DOX) [46], or photodynamic therapy (PDT) [45], may enhance ER-mitochondria Ca^{2+} transfer in some models. Examples of the remodeling of Ca^{2+} signaling with longer-term drug treatments include altered store-operated Ca^{2+} entry (SOCE) as a result of treatment with 5-Fluorouracil [47,54], gemcitabine (GEM) [47] or cisplatin [48]. Long term treatment with vemurafenib (VEM) [102] can enhance cytosolic Ca^{2+} removal after SOCE. Altered ER Ca^{2+} release mediated by SERCA inhibition is observed in cisplatin-resistant cancer cells (generated from prolonged exposure of cancer cells to cisplatin), i.e. reduced in resistant lung cancer cell lines [103] and increased in resistant ovarian cancer cell lines [48]. Carboplatin (CARB) treatment can increase the activity of ryanodine receptor Ca^{2+} release channels on the ER via GSTO1 [75].
Abbreviations: 5FU: 5-Fluorouracil; DEX: Dexamethasone; TIP: Tipifarnib (in clinical trials); IR: Ionizing radiation; CIS: Cisplatin; ARS: Arsenic trioxide; GAM: Gamitrinib; DOX: Doxorubicin; PDT: Photodynamic therapy; GEM: Gemcitabine; VEM: Vemurafenib; CARB: Carboplatin. *Denotes effect only seen in p53-competent cells

below, we will discuss less obvious intersections between agents used to treat cancer with calcium signaling.

Various studies have indeed described changes in intracellular Ca^{2+} signals induced by some anti-cancer agents, and Fig. 2 illustrates some examples of different agents in different cancer cell types and models. One such example of the intersection between an anti-cancer drug and calcium signaling was described by Can et al., when they defined the effects of 5-Fluorouracil (5-FU) in HCT116 colon cancer cells [40]. 5-FU is an anti-metabolite agent used in the treatment of a variety of cancers including those of the colon. 5-FU was reported to produce an increase in $[Ca^{2+}]_{CYT}$ at 90 min and 5-FU mediated activation of p53 was calcium-dependent and involved calmodulin [40]. This requirement for increased $[Ca^{2+}]_{CYT}$ driven by Ca^{2+} influx was also observed with other agents, including the proteasome inhibitor bortezomib [41] and a clinically trialled drug, tipifarnib which works by inhibiting the farnesyltransferase enzyme [42]. In HeLa cervical cancer cells, cisplatin appears to induce a delayed increase in Ca^{2+} levels in the cytosol and mitochondria (~6 h) and the cytotoxic effects of cisplatin is reduced by cytosolic free Ca^{2+} buffering, indicating a role for these Ca^{2+} changes in the effects of cisplatin [43]. In acute lymphoblastomic leukemia cells, treatment with dexamethasone (an anti-cancer treatment strategy for these cancers) promotes Ca^{2+} influx (within 5 min), however in this case the Ca^{2+} influx appears be a survival mechanism, since chelation of intracellular Ca^{2+} increases the sensitivity of acute lymphoblastomic leukemia cells to dexamethasone [44] Photodynamic therapy of tumors with functional p53 is also associated with increases in $[Ca^{2+}]_{CYT}$ as assessed in vivo using a skinfold chamber and the of tumor cells with a Ca^{2+} sensitive indicator [45]. Doxorubicin treatment (~6 h) is also able to remodel Ca^{2+} reuptake rates in the endoplasmic reticulum in p53-expressing HCT-116 colon cancer cells [46]. Prolonged treatments with anti-cancer agents such as cisplatin and 5FU also result in a remodeling of Ca^{2+} signaling as shown in Fig. 2, with some studies showing aberrant SOCE in treatment-resistant cell lines derived from prolonged drug treatments [47,48]. Collectively, these and other studies [49] indicate that some cancer therapies result in rapid or longer-term changes in calcium homeostasis in cancer cells. The greater use of fluorescence-based genetically encoded Ca^{2+} sensors [50] will be a particularly powerful tool in defining how cancer therapies change Ca^{2+} on the time scale of hours or days as small molecule dye leakage and sequestration has arguably limited some of the previous studies. Studies assessing Ca^{2+} in specific domains may also be insightful, given reports that the anti-CD20 monoclonal antibody Rituximab in SUDHL4 B Lymphoma cells produces localized Orai1-mediated Ca^{2+} influx [51].

One of the most clearly defined associations between pharmacological modulators of a cancer target and the calcium signaling machinery is seen for Bcl-2 inhibitors. Bcl-2 family members are complex regulators of endoplasmic reticulum Ca^{2+} store release (via IP3Rs) and mitochondrial Ca^{2+} uptake (via the voltage dependent anion channel (VDAC)) [52]. This intersection between the Bcl-2 family and Ca^{2+} transport may be important in the regulation of cancer cell death pathways, cell metabolism and proliferation. Indeed, studies have defined the ability of various Bcl-2 family members to regulate aspects of

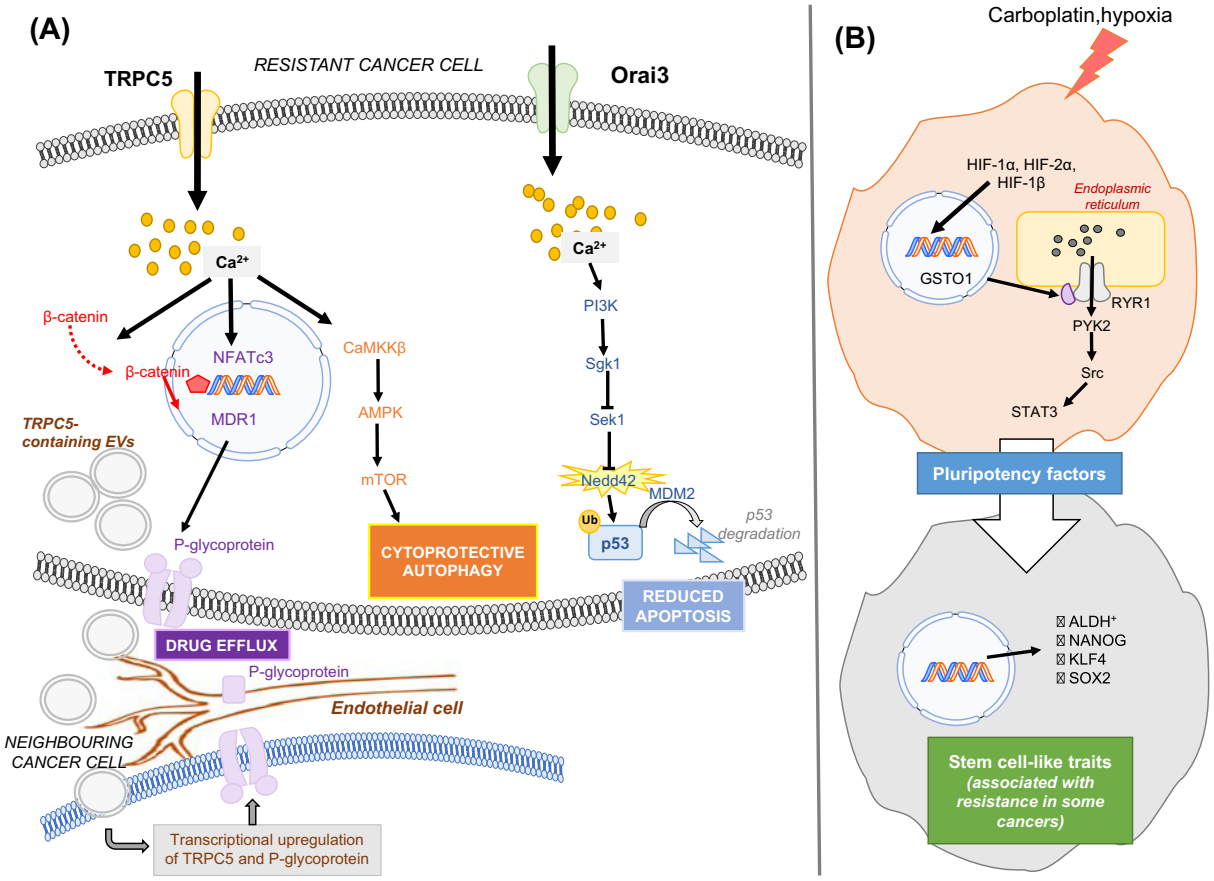

Fig. 3. Specific examples by which the Ca^{2+} signaling machinery may be modulated to promote resistance to cancer therapies. (A) shows how overexpression or increased activity of the plasmalemmal Ca^{2+} channels, TRPC5 and Orai3 can contribute to therapeutic resistance. *Left*: Increased Ca^{2+} influx through upregulated TRPC5 expression activates a variety of signaling pathways which promotes and/or sustains chemo-resistance. TRPC5-regulated Ca^{2+} influx may promote the nuclear localization of β-catenin (in red), where it binds to the promoter region of the MDR1 gene and increases the transcription of P-glycoprotein drug efflux pumps [70]. Increased P-glycoprotein expression can also be mediated through transcriptional activity of NFATc3 (in purple) [67]. In breast cancer cells, TRPC5 may be contained in vesicles (in brown) which can be externalized and transferred to drug-sensitive cells [66] and vascular endothelial cells [104] which promotes P-glycoprotein upregulation. Increased Ca^{2+} influx through TRPC5 can also activate pro-survival autophagy in response to chemotherapy (i.e. doxorubicin) via activation of CaMKKβ/AMPK/mTOR pathway (in orange) [68]. *Right*: Elevated Orai3 expression in breast cancer cells results in increased Ca^{2+} influx which activates the phosphoinositide-3-kinase (PI3K)/Sgk1 pathway (in blue), which in turn phosphorylates (inhibiting) the action of Sek1, a negative regulator of Nedd42, the ubiquitin ligase. This frees Nedd42 to ubiquitinate p53, marking it for degradation, thereby inhibiting the pro-apoptotic p53 pathway leading to greater apoptotic resistance [72]. (B) shows how the kinetics of Ca^{2+} release from the ER through Ryanodine receptor subtype 1 (RYR1) channels can be an important player in the acquisition of stem cell-like traits which promote cancer cell survival in response to chemotherapy (carboplatin) [75]. Cancer cell stemness is implicated in resistance to anti-cancer therapies in various other cancer models.

Ca^{2+} homeostasis with consequences on cell survival [52]. The complexity of the link between the calcium signal and the actions of some Bcl-2 inhibitors is exemplified by the selective ability of PMCA4 silencing but not silencing of the PMCA1 isoform to promote the death-inducing effects of the Bcl-2 inhibitor Navitoclax in MDA-MB-231 breast cancer cells [53]. There are other examples where the modulation of calcium channels, pumps or exchangers can sensitize cancer cells to the effects of some therapies. These include the ability of Orai1 silencing to promote the death-inducing effects of 5-FU in HepG2 hepatocarcinoma cells [54], the promotion of tumor-suppressing effects of doxorubicin by PMCA2 silencing in MDA-MB-231 breast cancer cells [55] and the ability of the T-type Ca^{2+} channel blocker mibefradil to promote the effects of carboplatin in an in vivo model of platinum-resistant ovarian tumors [56]. More recently, mibefredil has also been shown to suppress glioblastoma growth and promote the effectiveness of temozolomide in vivo [57]. The potential overlap between the effectiveness of cancer therapies and calcium transport machinery is also seen in the regulation of HER2 signaling by PMCA2 in breast cancer cells [58] and the role of Ca^{2+} permeable ion channels in cell death via ionizing radiation [59,60]. It could be argued that combination therapy consisting of calcium signal modulators and existing anti-cancer therapies with defined mechanisms of actions will be the most likely regimen to have lasting clinical impact.

There are also examples where agents used in cancer therapy or even to treat other diseases have been reported to have effects on calcium signaling which have then been attributed to their effects on cancer cells. For example, the antipsychotic drug Trifluoperazine was identified as a potent inhibitor of the invasiveness of glioblastoma cells in vitro and in vivo via disinhibition of IP3Rs through its interactions with calmodulin subtype 2 (CaM2) [61]. Similarly, the anti-estrogenic drug Tamoxifen has been reported to inhibit TRPV6 activity in breast cancer cells independently of the estrogen receptor [62]. Some calcium channels, pumps or exchangers may also afford an ability to control pathways which are difficult to pharmacologically target, such as the proposal to use SERCA inhibitors for cancers associated with *NOTCH1* mutations, after genomic screens identified SERCA inhibition as a way to induce cell cycle arrest in *NOTCH1*-mutated human leukemia cells [63].

The intersection between current therapies and calcium signaling are not confined to pharmacological agents used to treat cancer.

Radiotherapy is still widely used to control the growth of specific cancers including glioblastoma. Klumpp et al. have reported that TRPM8 is elevated in some glioblastomas and that in a cell line model, ionizing radiation promotes Ca^{2+} signaling via TRPM8 [59]. Furthermore, TRPM8 silencing promoted the anti-proliferative effects of ionizing radiation, leading the authors to suggest that TRPM8 inhibitors may be a target to reduce radioresistance in glioblastomas [59]. On this note, radiation was also found to promote TRPM2-mediated Ca^{2+} influx and induction of G_2M cell cycle arrest in Jurkat leukemia cells [60]. Another example of the importance of intracellular Ca^{2+} and calcium transport proteins is seen in the effectiveness of calcium electroporation, a process similar to electroporation used with chemotherapy agents which is used in treatment of some cancers [64]. In calcium electroporation therapy, a high voltage pulse is applied after injecting a highly concentrated solution of Ca^{2+} ions into the tumor site. This produces an influx of Ca^{2+} into the cancer cell leading to necrosis [65]. It now appears that cancer cells are much more sensitive to cell death from calcium electroporation than cells of the normal surrounding tissue. This increased sensitivity of some cancer cells to calcium electroporation may be due to the fact that these cancer cells have a lower level of calcium efflux pumps (PMCAs), compared to surrounding normal tissue [65]. The consequence of this is that cancer cells are more likely to reach the sustained high levels of $[Ca^{2+}]_{CYT}$ required to trigger necrosis due to a reduced ability to extrude the massive influx of calcium ions after electroporation [65]. Thus, there may be future opportunities to combine pharmacological modulators of specific Ca^{2+} pumps or Ca^{2+} channels to enhance the effectiveness of a range of cancer therapies, from cytotoxic agents to molecular targeted therapies and ionizing radiation to electroporation.

5. The calcium signal and its machinery as regulators of cancer therapy resistance pathways

Our understanding of how the calcium signal may contribute or even drive pathways important in therapeutic resistance in cancer has seen major advancements in the last five years. The contribution of the Ca^{2+} permeable ion channel TRPC5 in multidrug resistance development in breast cancer is arguably the most well-characterized (Fig. 3). TRPC5 protein contained within extracellular vesicles can be transferred from a resistant breast cancer cell to a non-resistant breast cancer cell, where this transfer promotes TRPC5 mediated Ca^{2+} influx in the therapy sensitive recipient cell [66]. This Ca^{2+} influx then triggers the expression of multi-drug resistant ATPase 1 (MDR-ATPase 1) also referred to as P-glycoprotein via a Ca^{2+}-dependent transcription factor (NFATc3) [67]. This increased expression of MDR-ATPase 1 then promotes the efflux of a variety of anti-cancer agents and then bestows resistance to many cancer therapies. Hence, a resistance cascade is initiated whereby calcium channels can effectively promote the development of MDR-ATPase 1 resistance pathways even more efficiently than the transfer of MDR-ATPase 1 would itself. The contribution of TRPC5 in breast cancer resistance appears to be multifaceted since TRPC5 is also a contributor to an autophagy cell survival mechanism that promotes therapy resistance in breast cancer cells through a CaMKKβ/AMPKα/mTOR pathway [68]. The importance of TRPC5 in this multidrug resistance cascade is reflected not only in the ability of TRPC5 silencing to reverse multi-drug resistance in MCF-7 cells made resistant to doxorubicin [67] but also the ability of levels of TRPC5-containing circulating exosomes to predict chemotherapy resistance in breast cancer patients [69]. The role of TRPC5 in therapy resistance is not restricted to breast cancer; increased expression of TRPC5 in resistant colon cancer cell lines has also been shown to enhance the expression of ABCB1 drug efflux pump through the Wnt/β-catenin signaling pathway [70]. Breast cancer cell line models have also identified specific ion channels with altered expression with drug resistance, such as the increased levels of Cav3.2 mRNA in SKBR3 breast cancer cells with acquired and intrinsic trastuzumab-resistance [71]. In clinical datasets, elevated levels of Orai3 is associated with chemo-resistance and this may involve a p53 mechanism through Mdm2 and Nedd4-2 [72].

The role of ion channels in drug resistance in cancer also applies in other cancers. For example in hepatocarcinoma, TRPC6 has been proposed as a target to reverse multidrug resistance [73]. In ovarian cancer, mitochondrial calcium uptake 1 (MICU1) silencing has been reported to drive chemo-resistance in some ovarian cancers, where it may do so through promoting glycolysis. Indeed, MICU1 silencing promoted the effectiveness of cisplatin in an OV90 ovarian cancer cell line in vivo model [74]. Another dimension of therapeutic resistance in cancer is cancer stem cells. In this context the Ca^{2+} signal is again implicated, but the mechanism is indirect and involves a calcium channel which may have been understudied due to perceptions that it may play only a minor role in cells with epithelial features – the Ryanodine Receptor. Lu and colleagues identified that carboplatin treatment of breast cancer cells increases levels of the glutathione-S-transferase (GST) protein GSTO1 via a hypoxia-inducible factor (HIF) mechanism [75]. GSTO1 appears to interact with Ryanodine receptor 1 and increase its activity. Then through a Ca^{2+} dependent mechanism involving PYK2, SRC and finally STAT3 signaling, GSTO1 is able to increase the proportion of cells with an apparent breast cancer stem cell phenotype [75]. The link between Ryanodine Receptor 1 and resistance is not only reflected by its regulation of pluripotency factors in breast cancer cell lines, but also in the ability of Ryanodine Receptor 1 silencing to delay tumor recurrence in a MDA-MB-231 in vivo carboplatin tumor relapse model. In this context it is interesting to note that a number of studies have now identified Ca^{2+} permeable ion channels as regulators of aspects of epithelial to mesenchymal transition (EMT) [76,77]. EMT is associated with the acquisition of “stemness” and therapeutic resistance [78,79] and as discussed later in this review is also Ca^{2+} signal-dependent [80].

6. Targeting of processes in the tumor microenvironment through Ca^{2+} signaling

The approaches and studies described so far have focused on the cancer cell itself as the target for modifying the Ca^{2+} signal. However, as reviewed elsewhere the tumor microenvironment is a key element of tumor progression [81,82]. The contribution of the microenvironment of the primary tumor can either suppress tumor growth or promote survival or even metastasis [82,83]. The microenvironment of metastatic sites and at pre-metastatic niches will also be a powerful force in how cancer progresses in a particular patient [84]. A variety of currently available agents work in the context of the tumor microenvironment such as aromatase inhibitors to suppress estrogen production and angiogenesis inhibitors to reduce blood flow to the tumor [85]. An immediate example of the overlap between the tumor microenvironment and the calcium signal is seen in the agent mipsagargin, which underwent phase 2 clinical trials. Mipsagargin is a prodrug form of the SERCA inhibitor and almost ubiquitous apoptosis inducer thapsigargin [4]. The prodrug linker is an enzyme substrate for prostate specific membrane antigen (PSMA), hence the higher levels of PMSA expressed on the surface of tumor cells results in localized accumulation of thapsigargin and in turn the death of cancer cells and other cells which make up and/or support the tumor [4]. The tumor mass and tumor microenvironment are an amalgam of different cell types, a cocktail of growth factors and cytokines and is often defined by a significant gradient in oxygen and nutrient levels. The varied cell types that make up a tumor mass and its surroundings include the cancer cells which can be heterogeneous in their mutation or gene expression profile [86], immune cells, cells that make up the tumor vasculature and non-transformed cells that surround tumor cells such as fibroblasts and adipocytes, which can also contribute to tumor progression. Although the tumor microenvironment is the least studied area of how calcium signaling may contribute to cancer progression,

there are some studies which do define or suggest a role for Ca^{2+} signaling and/or specific calcium channels, pumps or exchangers. The calcium signal is used by a variety of different cell types, so it is expected that in some cases it will also be used for tumors to interact with, modify and respond to its microenvironment.

The Ca^{2+} signal is a critical regulator of immune cell function, and the immune system may either be key to the destruction of cancer cells or may promote pro-metastatic pathways. Hence, the contribution of calcium channels and pumps to immune cell function may be an important factor when considering potential side effects of a calcium channel modulator or as a way to promote immune cell function or attenuate pro-metastatic pathways [7,87]. One example of the intersect between cells of the immune system and cancer cells is the ability of the cancer associated macrophage released cytokine - CCL18, to promote invasiveness through eliciting Ca^{2+} signals in breast cancer cells [88]. Moreover, the Ca^{2+} signal itself can be key in the way immune cells destroy cancer cells as previously reviewed [9]. There may even be an optimal level of Ca^{2+} influx in this process such that even inhibitors of Ca^{2+} channels that may have been thought to suppress immune cell function may actually promote tumor cell destruction when sub-maximally inhibited [89]. There are now clear opportunities to explore the roles of specific calcium channels and pumps of immune cells to better understand how immune cells can promote metastasis or target and destroy cancer cells. A better understanding of this interaction may define new strategies for combination therapies.

The growth factors, cytokines and low O_2 levels present in the tumor microenvironment can be promoters of a more invasive and chemoresistant phenotype. One of the processes which may contribute to cancer cells acquiring these tumorigenic capabilities which can be induced by growth factors (e.g. epidermal growth factor) and hypoxia is EMT. Both epidermal growth factor and hypoxia-induced EMT are inhibited by chelation of intracellular Ca^{2+} [80]. Specific calcium channels may therefore represent therapeutic opportunities to disrupt EMT induction pathways in the tumor microenvironment, as exemplified by the ability of TRPM7 silencing and pharmacological inhibition to reduce the induction of the EMT marker vimentin in MDA-MB-468 breast cancer cells [80]. Similarly, specific Ca^{2+} permeable ion channels appear to regulate responses to hypoxia in the tumor microenvironment such as the ability of TRPC1 silencing to suppress hypoxia-induced increases in STAT3 and epidermal growth factor receptor phosphorylation in MDA-MB-468 breast cancer cells [90]. Another example of the role of the Ca^{2+} signal in the tumor microenvironment is in angiogenesis pathways promoting vascularisation and providing nutrients to support the growing tumor mass. Indeed, many studies have collectively shown the bidirectional dependence between the pro-angiogenesis factor, vascular endothelial growth factor and calcium signaling. One ion channel which appears to be important in angiogenesis in cancer is TRPV4 [91,92]. The critical role of TRPV4 in tumor angiogenesis means that inhibitors of TRPV4 could represent anti-angiogenesis therapies and deprive cancer cells of their needed blood supply. Nevertheless, while solely targeting angiogenic pathways may have limited effectiveness, improving tumor vasculature in combination with cytotoxic chemotherapy agents may promote the effectiveness of cytotoxic chemotherapy. Hence, TRPV4 activators could represent vascular normalization therapies able to improve the entry of cytotoxic agents to cancer cells in the tumor mass [91]. Indeed, it was recently demonstrated that the TRPV4 activator GSK1016790A, improves the effectiveness of cisplatin in an in vivo model by promoting the maturation of blood vessels supplying tumors [91].

An area which is still in its infancy in relation to our understanding of the tumor microenvironment is how calcium signaling may be important in the interaction between the non-transformed stromal cells that surround the primary tumor mass/metastasis and the cancer cells at those sites. There are however, examples that demonstrate that this should be an area for further study. In serous ovarian cancer, a Ca^{2+} dependent pathway is important in the way in which adipocytes in adipocyte-rich metastatic deposits promote metastatic growth [93]. In a study looking at gene expression in brain metastasis from breast cancer, lower levels of the expression of the gene for IP3R1 was found [94]. This study suggests that a remodeling of the expression of some calcium channels may be driven by the metastatic environment and/or specific clones with altered expression of calcium channels may have an improved ability to colonize specific metastatic sites. Understanding how cells of the tumor stroma use calcium signaling to modify processes in cancer cells and vice-versa should be a priority for future studies seeking to identify novel mechanisms to target processes in the tumor microenvironment.

7. Conclusion

The studies cited throughout this review are a reflection of the increased number of research groups around the world helping to define changes in calcium signaling in cancer cells. Many of these studies are also providing insights into what calcium channels, pumps and exchangers may be best to target in specific cancer types. Although work is also increasingly focused on the potential of combination therapies, this is an area which still requires increased focus for a number of cancer types and therapies, particularly in the area of pharmacological inhibitors to specific calcium channels and pumps, rather than silencing approaches. The future should also see an acceleration in the number of studies which will define the importance of calcium signaling in the context of the tumor microenvironment.

Transparency document

The http://dx.doi.org/10.1016/j.bbamcr.2018.05.015 associated with this article can be found, in online version.

Acknowledgments

The research was supported by the National Health and Medical Research Council (NHMRC; project grants 1079672 and 1079671). G.R.M. is supported by the Mater Foundation. The Translational Research Institute is supported by a grant from the Australian Government.

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