

Review

Ca²⁺ Fluxes and CancerSaverio Marchi,¹ Carlotta Giorgi,² Lorenzo Galluzzi,^{3,4,5,6,7,*} and Paolo Pinton^{2,*}¹Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ancona, Italy²Department of Medical Sciences, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology, Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy³Department of Radiation Oncology, Weill Cornell Medical College, New York, NY, USA⁴Sandra and Edward Meyer Cancer Center, New York, NY, USA⁵Caryl and Israel Englander Institute for Precision Medicine, New York, NY, USA⁶Department of Dermatology, Yale School of Medicine, New Haven, CT, USA⁷Université de Paris, Paris, France*Correspondence: deadoc80@gmail.com (L.G.), paolo.pinton@unife.it (P.P.)<https://doi.org/10.1016/j.molcel.2020.04.017>

Ca²⁺ ions are key second messengers in both excitable and non-excitable cells. Owing to the rather pleiotropic nature of Ca²⁺ transporters and other Ca²⁺-binding proteins, however, Ca²⁺ signaling has attracted limited attention as a potential target of anticancer therapy. Here, we discuss cancer-associated alterations of Ca²⁺ fluxes at specific organelles as we identify novel candidates for the development of drugs that selectively target Ca²⁺ signaling in malignant cells.

Historically, the molecular machinery that regulates intracellular calcium (Ca²⁺) fluxes has attracted limited attention as a potential target for cancer therapy, largely reflecting the fact that Ca²⁺ signaling was viewed as uniform across non-excitable cells (Berridge et al., 2000), and hence was considered incompatible with the development of selective agents. However, the role of Ca²⁺ in malignant transformation, tumor progression, and response to treatment has been considerably re-evaluated over the past decade (Monteith et al., 2017). Such a reappraisal has originated not only from structural and functional studies that enabled the development of therapeutic agents targeting Ca²⁺ signaling for non-malignant disorders affecting excitable cells (e.g., arrhythmias, epilepsy) (Frishman, 2007; Weiss and Zamponi, 2019) but also from the ever-increasing deconvolution of intracellular Ca²⁺ fluxes as spatially restricted processes that can be targeted therapeutically. Thus, it is now clear that the molecular machinery that controls intracellular Ca²⁺ signaling in malignant cells is altered as a consequence of changes in expression levels and/or post-translational modifications in its key components or their interactors. Such defects enable malignant transformation, support tumor progression and play a key role in sensitivity to treatment, *de facto* standing out as potential target for the development of targeted therapeutics.

In this review, we critically discuss the molecular mechanisms through which alterations of Ca²⁺ fluxes at specific organelles affect multiple aspects of the malignant phenotype, including aberrant proliferation, resistance to cell death, and metastatic dissemination, with a focus on the possibility to harness such defects for therapeutic purposes. In line with this focus, Ca²⁺-buffering proteins are not discussed herein, although they undoubtedly affect multiple aspects of oncogenesis (Schwaller, 2020).

Global Ca²⁺ Homeostasis in Normal Cells

Cells need to maintain extremely low cytosolic Ca²⁺ levels (~100 nM), hence establishing a 10- to 15,000-fold gradient with the extracellular milieu (in which the Ca²⁺ concentration is

~1–1.5 mM). Although the original evolutionary advantage of such a gradient was probably the avoidance of potentially cytotoxic Ca²⁺-phosphate precipitates, a variety of functions have evolved around such a compartmentalized source of electrochemical energy, including intracellular signal transduction and metabolite transport across membranes (Bootman and Bultynck, 2020; Chen et al., 2020).

Two main ATP-dependent systems extrude Ca²⁺ from the cytosol of mammalian cells: plasma membrane Ca²⁺ ATPases (PMCA), which expel Ca²⁺ to the extracellular space, and sarcoendoplasmic reticular Ca²⁺ ATPases (SERCAs), which accumulate it within the endoplasmic reticulum (ER). Moreover, secretory pathway Ca²⁺ ATPases (SPCAs) promote Ca²⁺ accumulation within the Golgi apparatus (GA), while other organelles such as lysosomes can store Ca²⁺, either as a functional consequence of vesicular trafficking from the extracellular Ca²⁺-rich milieu (e.g., endocytosis) (Galluzzi and Green, 2019) or (at least theoretically) through a hitherto elusive Ca²⁺/H⁺ exchanger (Melchionda et al., 2016). That said, these latter intracellular Ca²⁺ stores are quantitatively limited as compared to the ER (Yang et al., 2019).

Cytosolic Ca²⁺ signaling (during which cytosolic Ca²⁺ concentrations reach 1–2 μM) can be driven by both intracellular and extracellular stores. One of the most common pathways for mammalian cells to evoke Ca²⁺ signaling is initiated by ligand-engaged G protein-coupled receptors (Jain et al., 2018), causing the synthesis of 1,4,5-inositol trisphosphate (IP₃) and IP₃-dependent opening of Ca²⁺ channels of the IP₃ receptor (IP₃R) family at the ER membrane (Prole and Taylor, 2019). Alternatively, IP₃R opening can be initiated by receptor tyrosine kinase signaling and consequent activation of phospholipase C gamma 1 (PLCG1), at least in some cells (Lundgren et al., 2012). Moreover, Ca²⁺ ions can accumulate in cytosol upon the production of reactive oxygen species (ROS) or phosphatidylinositol 3,5-bisphosphate (PI(3,5)P₂) and consequent opening of the ROS- and PI(3,5)P₂-sensitive lysosomal Ca²⁺ channels mucolipin 1



(MCOLN1, also known as TRPML1) (Zhang et al., 2016) and two-pore segment channel 2 (TPCN2) (Li et al., 2019).

Extracellular Ca^{2+} ions (which are the predominant source for cytosolic Ca^{2+} signaling in excitable cells) (Moran et al., 2011) can access the cytosol via a variety of non-voltage-gated non-selective cation channels, including members of the transient receptor potential (TRP) superfamily (which also include MCOLN1) (Venkatchalam and Montell, 2007), and through numerous voltage-dependent Ca^{2+} channels, including L-, R-, N-, P/Q-, and T-type channels (Catterall, 2011). Although voltage-dependent Ca^{2+} channels are widely expressed by excitable cells, they have also been detected in non-excitable (including malignant) cells (Phan et al., 2017). Of note, cytosolic Ca^{2+} fluxes driven by extracellular and intracellular stores are not mutually exclusive but interconnected and highly coordinated. For instance, excitatory Ca^{2+} signaling is initiated by plasma membrane (PM) Ca^{2+} channels but sustained by reticular Ca^{2+} (Roderick et al., 2003). Along similar lines, Ca^{2+} mobilization from intracellular stores in non-excitable cells is generally followed by PMCA-dependent Ca^{2+} extrusion to the extracellular space (Berridge et al., 2003).

Extracellular Ca^{2+} is required to replete intracellular stores in both excitable and non-excitable cells, reflecting the ability of PMCAs to translocate Ca^{2+} across membranes faster than SERCAs and SPCAs (Bootman and Bultynck, 2020). In this setting, Ca^{2+} -depleted cells initiate a slow Ca^{2+} flux from the extracellular space to the ER lumen commonly known as store-operated Ca^{2+} entry (SOCE). At the molecular level, SOCE is mediated by specific members of the ORAI calcium release-activated calcium modulator (ORAI) family, including ORAI1 and ORAI3 (Derler et al., 2016). These PM Ca^{2+} channels relocate to PM-ER junctions upon the oligomerization of members of the stromal interaction molecule (STIM) family. Such an interaction generates so-called Ca^{2+} release-activated Ca^{2+} (CRAC) channels, which enable the accumulation of cytosolic Ca^{2+} ions available for uptake by SERCAs (Derler et al., 2016). Mitochondria are also recruited to neo-formed CRAC channels, where they have been proposed to mediate hitherto unclear regulatory functions (Malli and Graier, 2017). Of note, ORAI1 and ORAI3 can also form channels that support arachidonate-driven Ca^{2+} entry, an activity that does not depend on STIM1 (Thompson et al., 2013).

The contribution of mitochondria to intracellular Ca^{2+} homeostasis goes way beyond their potential SOCE-regulatory activity. Baseline mitochondrial Ca^{2+} levels resemble their cytosolic counterparts (Giorgi et al., 2018b), but the capacity of the mitochondrial network to accumulate Ca^{2+} upon release from the ER (or entry from the extracellular space) is 10 times higher than that of the cytosol (Giorgi et al., 2018b). Such a capacity, which affects not only mitochondrial metabolism but also Ca^{2+} signaling at extramitochondrial sites and various other cellular processes (e.g., regulated cell death) is commonly referred to as Ca^{2+} buffering (Giorgi et al., 2018b). Ca^{2+} ions readily cross the outer mitochondrial membrane (OMM) via members of the voltage-dependent anion channel (VDAC) family, including VDAC1, VDAC2, and VDAC3 (De Stefani et al., 2012; Shimizu et al., 2015), and then accumulate in the mitochondrial matrix via the mitochondrial calcium uniporter (MCU), a supramolecular

complex under positive and negative regulation by mitochondrial calcium uniporter regulator 1 (MCUR1) and mitochondrial calcium uptake 1 (MICU1), respectively (Kamer and Mootha, 2015; Mallilankaraman et al., 2012a, 2012b). Mitochondria extrude Ca^{2+} via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger solute carrier family 8 member B1 (SLC8B1, best known as NCLX) (Palty et al., 2010) and an $\text{H}^+/\text{Ca}^{2+}$ antiporter, whose molecular nature remains unclear. A potential (but hitherto unconfirmed) candidate for this latter activity is leucine zipper and EF-hand containing transmembrane protein 1 (LETM1) (Jiang et al., 2009).

In summary, normal cells regulate intracellular Ca^{2+} fluxes via a highly interconnected machinery that operates at multiple organelles to allow Ca^{2+} ions to act as second messengers while preventing their potential cytotoxicity (Figure 1). Malignant cells display a variety of defects in such a machinery (Roberts-Thomson et al., 2019), which can be harnessed for the development of novel therapeutic agents, as discussed below.

Plasma Membrane Ca^{2+} Transporters and Cancer

One of the main hallmarks of malignant cells is their ability to boost ROS signaling in support of metabolism, proliferation, and metastatic dissemination while evading the cytotoxicity of ROS overgeneration (Pervaiz, 2018). Besides an extensive metabolic rewiring that supports the generation of endogenous antioxidants such as glutathione (Galluzzi et al., 2013; Gorrini et al., 2013), alterations of Ca^{2+} signaling at mitochondria (see below) and the PM are largely responsible for this feature. While mitochondrial Ca^{2+} promotes ROS generation (see below), the accumulation of ROS at the PM imposes post-translational modifications on some PM Ca^{2+} channels that promote Ca^{2+} entry to boost antioxidant defenses (Takahashi et al., 2018).

Transient receptor potential cation channel subfamily A member 1 (TRPA1) is generally expressed in neurons but is ectopically upregulated in breast and lung tumors, where it mediates Ca^{2+} influx across the PM in response to pro-oxidants, including ROS-generating chemotherapeutics, *de facto* supporting cell survival (Takahashi et al., 2018). At least in part, the ability of TRPA1 to favor chemoresistance originates from the Ca^{2+} -dependent binding of calmodulin 1 (CALM1) to protein tyrosine kinase 2 beta (PTK2B, also known as PYK2), ultimately resulting in the upregulation of the cytoprotective factor MCL1 apoptosis regulator, BCL2 family member (MCL1) (Galluzzi et al., 2018; Porporato et al., 2018; Takahashi et al., 2018). At least in some settings, such a pro-survival effect is compromised by ROS-dependent S-glutathionylation of STIM1, resulting in persistent Ca^{2+} entry via SOCE, mitochondrial permeability transition (MPT), and ultimately cell death (Hawkins et al., 2010).

Ca^{2+} -bound CALM1 directly interacts with (hence regulating the activity of) TRPA1 (and other TRP family members) (Hasan and Zhang, 2018). However, CALM1 potentiates TRPA1 at moderate Ca^{2+} concentrations, whereas it has inhibitory effects in response to robust elevations in Ca^{2+} levels (Zurborg et al., 2007). These data are incompatible with the ability of cancer cells to display chronic TRPA1 hyperactivation, despite increased cytosolic Ca^{2+} concentrations. At least theoretically, such an apparent discrepancy may originate from differences in the nature of Ca^{2+} signals and/or Ca^{2+} -buffering systems operating in malignant versus normal cells. Both pro-oxidants such as H_2O_2

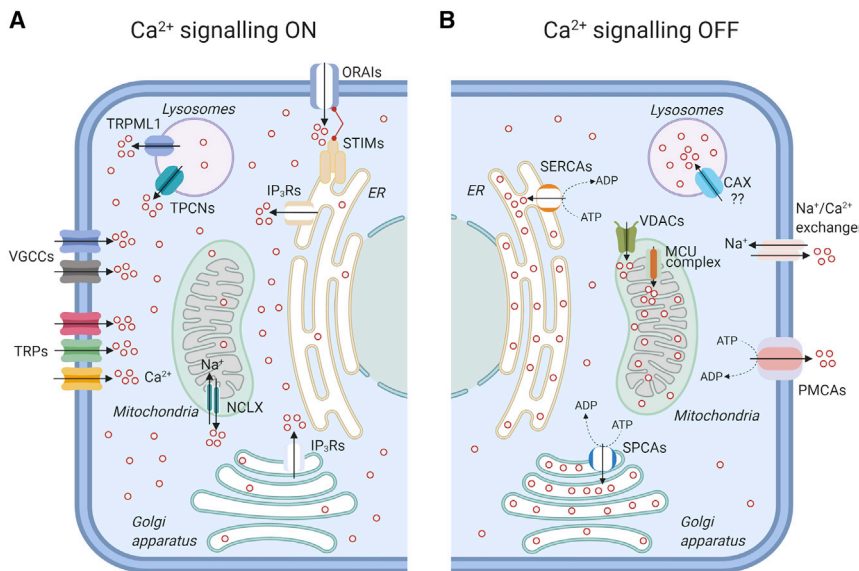


Figure 1. Regulation of Ca²⁺ in Normal Non-excitable Cells

(A) In response to a variety of stimuli, normal non-excitable cells can activate cytosolic Ca²⁺ signaling via a variety of mechanisms, including but not limited to (1) Ca²⁺ release from the endoplasmic reticulum (ER) and the Golgi apparatus (GA), (2) Ca²⁺ release from mitochondria and lysosomes, and (3) Ca²⁺ uptake from the extracellular microenvironment. This causes an increase in cytosolic Ca²⁺ levels coupled to the activation of numerous cellular functions.

(B) Numerous systems contribute to the extinction of Ca²⁺ signaling by reducing cytosolic Ca²⁺ levels upon (1) Ca²⁺ import by the ER, GA, and mitochondria or (2) Ca²⁺ extrusion to the extracellular space. Whether lysosomes accumulate Ca²⁺ via specific transporters, including a hitherto uncharacterized Ca²⁺/H⁺ exchanger (CAX) or from the microenvironment upon endocytosis remains unclear.

IP₃R, 1,4,5-inositol trisphosphate receptor; MCU, mitochondrial calcium uniporter; NCLX (official name: SLC8B1), solute carrier family 8 member B1; PMCA, plasma membrane Ca²⁺ ATPase; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase; SPCA, secretory pathway Ca²⁺ ATPase; STIM, stromal interaction molecule; TPCN, two-pore segment channel; TRP, transient receptor potential cation channel member; TRPML1 (official name: MCOLN1), mucolipin 1; VDACC, voltage-dependent anion channel; VGCC, voltage-gated calcium channel.

stromal interaction molecule; TPCN, two-pore segment channel; TRP, transient receptor potential cation channel member; TRPML1 (official name: MCOLN1), mucolipin 1; VDACC, voltage-dependent anion channel; VGCC, voltage-gated calcium channel.

and chemotherapeutics (i.e., carboplatin) trigger slow and moderate oscillations in cytosolic Ca²⁺ levels, which differ from those generated by TRPA1-activating stimuli (e.g., mustard oil), but may resemble those originating from Ca²⁺ pulses by the uncaging of 1-(4,5-dimethoxy-2-nitrophenyl)-EDTA, which induces TRPA1 potentiation without inactivation (Wang et al., 2008). These observations support the potential utility of agents that would selectively inhibit TRPA1 or target the CALM1 → TRPA1 axis in support of common chemotherapeutics that compromise antioxidant defenses in cancer cells.

TRPA1 is upregulated in breast and lung tumors in which the master antioxidant regulator nuclear factor, erythroid 2-like 2 (NFE2L2, best known as NRF2) is hyperactive (Takahashi et al., 2018), which orchestrates ROS resistance via canonical and non-canonical (i.e., via TRPA1) mechanisms. The oncogenic activity of NRF2 is also associated with other TRP channels. In particular, Ca²⁺ entry through the redox-sensitive channel TRP cation channel subfamily M member 2 (TRPM2) drives NRF2 activation and consequent upregulation of various antioxidant enzymes and IQ motif containing GTPase activating protein 1 (IQGAP1), a Ca²⁺-dependent modulator of NRF2 stability (Bao et al., 2019). TRPA1 and TRPM2 are often co-expressed in malignant lesions (Takahashi et al., 2018), suggesting a synergistic role of different TRP members in defining a specific malignant phenotype.

Other TRP channels are frequently overexpressed in human tumors, including TRPM3 (Hall et al., 2014), TRP cation channel subfamily C member 1 (TRPC1) (Azimi et al., 2017), TRPC6 (Guilbert et al., 2008), TRP cation channel subfamily V member 4 (TRPV4) (Peters et al., 2017), and TRPV6 (Fixemer et al., 2003). Moreover, TRPM7 and TRPV2 appear to be upregulated at sites of metastatic dissemination (Canales et al., 2019). Mechanistically, TRPM3 has been shown to promote the progression of clear cell renal cell carcinomas by stimulating autophagy (an

evolutionary conserved cytoprotective mechanism) (Galluzzi et al., 2018c) via calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2) (Hall et al., 2014). Conversely, TRPM7 is the main TRP channel involved in the generation of short-lived Ca²⁺ flickers that drive cancer cell migration (Wei et al., 2009). Finally, elevated cytosolic Ca²⁺ levels correlate with the increased secretion of matrix metalloproteinases by cancer cells (Monet et al., 2010; Rybarczyk et al., 2017), *de facto* favoring a remodeling of the local microenvironment in support of metastatic dissemination. Non-transformed cells experiencing TRP activation rapidly undergo cytosolic Ca²⁺ and cell death (Shapovalov et al., 2011). Thus, malignant cells must acquire additional features that allow them to control the amplitude and kinetics of Ca²⁺ fluxes and hence harness the beneficial effects of Ca²⁺ signaling while avoiding its potential cytotoxicity. Besides a superior resistance to cell death induction (Hanahan and Weinberg, 2011), these changes include but are not limited to an increased mitochondrial capacity for Ca²⁺ buffering (see below).

SOCE is also frequently altered in malignant cells, although defining the contribution of SOCE defects to malignant transformation, tumor progression, or sensitivity to treatment is complex, given the multifactorial nature of the CRAC channel. Nonetheless, the upregulation of STIM1 alone or together with ORAI1 correlates with increased migratory capacity, metastatic dissemination, and poor overall survival in different human tumors (Yang et al., 2009). Mechanistically, this ensures the establishment of oscillatory Ca²⁺ signals at specialized PM areas that enable the invadopodium formation, extracellular matrix degradation (Sun et al., 2014), and PYK2 activation (Chen et al., 2011).

Polarized SOCE in malignant cells is also driven by the interaction of ORAI1 with potassium calcium-activated channel subfamily N member 3 (KCNN3) at specific glycolipoprotein- and cholesterol-rich microdomains of the PM called lipid rafts (Chantôme et al., 2013). Such a polarization appears to be controlled

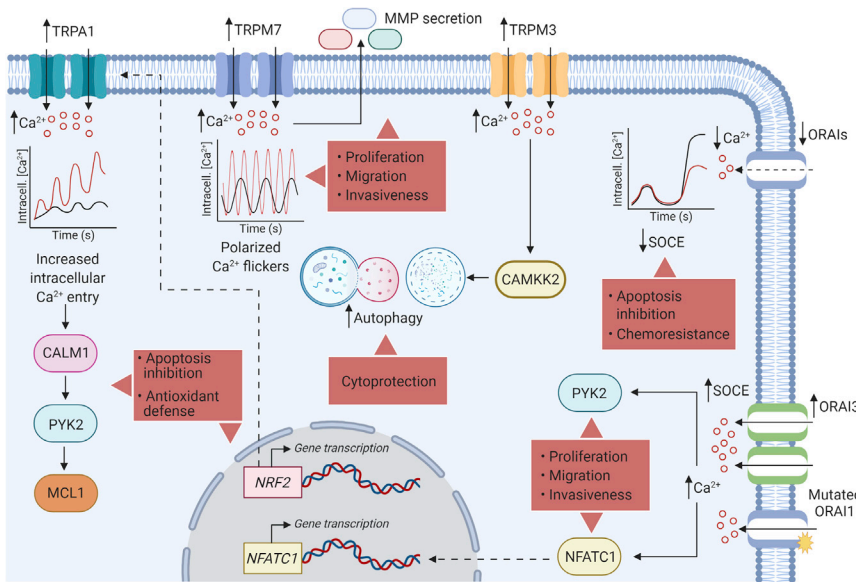


Figure 2. Cancer-Associated Alterations of Ca^{2+} Fluxes at the Plasma Membrane
Malignant cells can harness multiple alterations of Ca^{2+} fluxes at the plasma membrane in support of tumor progression or resistance to treatment. In particular, increased Ca^{2+} entry upon the overexpression of various transient receptor potential cation channel (TRP) family members, ORAI calcium release-activated calcium modulator 3 (ORAI3), or *ORAI1* mutations, as well as store-operated calcium entry (SOCE) inhibition downstream of ORAI downregulation, have been linked to superior resistance to cell death and improved metastatic potential as a consequence of (1) activation of antiapoptotic and mitogenic pathways; (2) establishment of antioxidant defenses; (3) autophagy initiation; (4) acquisition of increased motility; and (5) secretion of matrix metalloproteinases (MMPs). CALM1, calmodulin 1; CAMKK2, calcium/calmodulin-dependent protein kinase 2; MCL1, MCL1 apoptosis regulator, BCL2 family member; NFATC1, nuclear factor of activated T cells 1; NRF2 (official name: NFE2L2), nuclear factor, erythroid 2 like 2; PYK2 (official name: PTK2B), protein tyrosine kinase 2 beta.

(at least in part) by the microtubule system under regulation by the tubulin-modifying enzyme histone deacetylase 6 (HDAC6) (Chen et al., 2013). In support of this notion, SOCE favors cellular migration when adhesion to the matrix is weak (i.e., in metastatic cells displaying considerable microtubular rewiring), whereas it inhibits migration when adhesion is strong (i.e., in normal cells) (Tsai et al., 2014). These observations identify a key role for SOCE remodeling in tumor progression. Further supporting this notion, gain-of-function *ORAI1* mutations causing constitutive Ca^{2+} influx and Ca^{2+} -dependent activation of nuclear factor of activated T cells 1 (NFATC1) have been associated with cancer (Frischauf et al., 2017). Moreover, SOCE inhibition by pharmacological agents limits the migration and proliferation of cultured human breast cancer cells (Azimi et al., 2018).

Apparently at odds with this, *ORAI1* downregulation and consequent SOCE abolition protects prostate cancer cells from cell death induced by thapsigargin, which evokes a sustained Ca^{2+} influx to the cytoplasm (Flourakis et al., 2010). Similar observations have been obtained in colorectal cancer cells harboring oncogenic *KRAS* mutations, although in this case SOCE inhibition originated from decreased STIM1 levels (Pierro et al., 2018). Thus, the downregulation of various components of the CRAC channel may promote the resistance of cancer cells to stressors that favor Ca^{2+} influx, including hypoxia.

Cancer cells can take advantage of both SOCE activation and inhibition, depending on contextual factors, including the configuration of the molecular machinery for cell death. In some cases, such as advanced, androgen-independent prostate cancer, an alternative ORAI variant (i.e., *ORAI3*) has been shown to form heteromultimeric complexes with *ORAI1* to ensure store-independent arachidonic acid-regulated Ca^{2+} entry in the context of conventional, *ORAI1*-dependent SOCE inhibition (Dubois et al., 2014). These changes ensure oscillatory Ca^{2+} waves that promote tumor progression and support antioxidant defenses (see above), along with cell death resistance. Of note, *ORAI3* me-

diates oncogenic functions in the mammary tissue (Hasna et al., 2018; Motiani et al., 2010, 2013), and increased *ORAI3:ORAI1* expression ratio correlates with poor prognosis in colorectal cancer patients (Ibrahim et al., 2019). Thus, while *ORAI3* stands out as a prominent candidate for the development of therapeutic agents specific for cancer cells, the pharmacological inhibition of conventional SOCE may be detrimental as (1) it would limit the activity of some chemotherapeutics that trigger ER stress (e.g., cisplatin) (Gualdani et al., 2019) and (2) it would compromise anti-cancer immune responses by CD8^+ T cells, which strictly rely on SOCE (Weidinger et al., 2013).

Thus, multiple steps of the oncogenic cascade are influenced by the deregulation of Ca^{2+} fluxes at the PM (Figure 2), but targeting such alterations remains challenging given the pleiotropism of the system, perhaps with the sole exception of *ORAI3*. An alternative approach that remains to be pursued is the development of agents specific for mutant *ORAI1*, although they would be useful only for cancers bearing *ORAI1* mutations.

Oncogenic Ca^{2+} Dynamics at the ER

Alterations in reticular Ca^{2+} fluxes affect Ca^{2+} homeostasis at large, not only because the ER is the major cellular store for Ca^{2+} but also because Ca^{2+} levels at extrareticular sites strictly depend on reticular Ca^{2+} dynamics (Giorgi et al., 2018a). Of note, specialized regions of the ER that are preferentially juxtaposed to mitochondria, the so-called mitochondria-associated ER membranes (MAMs) (Box 1), are sites for preferential Ca^{2+} transfer to mitochondria (Wu et al., 2018). Reflecting the key role of mitochondrial Ca^{2+} in the control of proliferation, metabolism, and cell death (see below), several oncogenic and oncosuppressive proteins strategically localize to MAMs to regulate cell fate by interfering with ER Ca^{2+} fluxes (Marchi et al., 2014).

The precise role of ER Ca^{2+} uptake in oncogenesis and tumor progression is difficult to ascertain as SERCAs are encoded by 3 different genes (*ATP2A1*, *ATP2A2*, *ATP2A3*) and 14 splicing

Box 1. Mitochondria-Associated ER Membranes

The smooth endoplasmic reticulum (ER) forms structural and functional connections with virtually all organelles, notably mitochondria. ER-mitochondria contact sites are closely opposed and tethered to each other, but membranes do not fuse as they maintain a typical distance of 20–50 nm (Wu et al., 2018). These so-called mitochondria-associated ER membranes (MAMs) have distinct biochemical properties and can be isolated by subcellular fractionation, which has been harnessed to identify the preferential localization to MAMs of multiple enzymes and regulatory proteins (Wieckowski et al., 2009). The proteomic profile of MAMs suggests that MAMs participate in numerous cellular functions, including lipid transfer, inflammatory responses, autophagy, the control of redox homeostasis, and Ca^{2+} signaling. Thus, MAMs act as molecular platforms that decode a plethora of inputs for orchestrating various cellular responses (Galluzzi et al., 2012). It is therefore not surprising that defects in MAM integrity or composition have been linked to various pathological conditions, including cancer. In many cases, MAM alterations result in a drastic remodeling of ER-mitochondria Ca^{2+} transfer that supports malignant transformation or tumor progression. This occurs not only because several oncogenic and oncosuppressive factors reside at MAMs, where they control the expression or function of different Ca^{2+} transporters (see main text), but also as a consequence of MAM breakdown. Notably, the correct architecture of MAMs is ensured by structural proteins, including PDZ domain containing 8 (PDZD8), VAMP associated protein B and C (VAPB, VAPC), and regulator of microtubule dynamics 3 (RMDN3, also known as PTPIP51). The preservation of a proper spacing between ER and mitochondrial membranes at MAMs is essential to regulate Ca^{2+} fluxes, metabolism, and sensitivity to cell death, thus constituting a key factor for multiple cancer-related processes (Morciano et al., 2018).

variants, and the downregulation of specific transcripts is generally associated with compensatory mechanisms (Arbajian et al., 2011). In line with this notion, ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 3 (ATP2A3, best known as SERCA3) expression decreases during colorectal carcinogenesis (Brouland et al., 2005), but reticular Ca^{2+} levels appear to remain unaffected, potentially upon compensatory ATP2A2 (best known as SERCA2) upregulation (Fan et al., 2014).

The initial interest in SERCAs as a target for anticancer therapy stemmed from the highly cytotoxic but virtually unselective activity of the pan-SERCA inhibitor thapsigargin (Lyttton et al., 1991). To circumvent limited selectivity, thapsigargin has been engineered for activation by folate hydrolase 1 (FOLH1, also known as PSMA), which is abundant in the microenvironment of some malignant (but not normal) tissues (Denmeade et al., 2012). Moreover, it seems that malignant cells driven by NOTCH or WNT signaling are particularly sensitive to low-dose thapsigargin (Roti et al., 2013; Suisse and Treisman, 2019), which may open a therapeutic window. However, while the abolition of reticular Ca^{2+} uptake is highly cytotoxic, lowered Ca^{2+} concentrations may support tumor progression. In line with this notion, heterozygous loss-of-function mutations in *Atp2a2* predispose mice to gastric carcinogenesis (Prasad et al., 2005), and mutations in each of the SERCA-coding genes have been documented in a variety of tumors, including head and neck cancer (Stransky et al., 2011). Moreover, chemoresistance supported by tumor protein p53 (*TP53*) mutations or thioredoxin-related transmembrane protein 1 (TMX1) downregulation is accompanied by inhibition of SERCA activity (Giorgi et al., 2015; Raturi et al., 2016). Finally, truncated ATP2A1 (best known as SERCA1) splice variants not only reduce reticular Ca^{2+} levels at baseline but also favor Ca^{2+} leakage, which supports at least some degree of mitochondrial signaling (Chami et al., 2001). Thus, genetic defects in various SERCAs appear to endow (pre-)malignant cells with a dual advantage: protection from Ca^{2+} overload-driven cell death and generation of spontaneous Ca^{2+} oscillations that promote mitochondrial activity (see below).

Similar oncogenic functions have been attributed to the antiapoptotic proteins BCL2 apoptosis regulator (BCL2) and BCL2 like 1 (BCL2L21, best known as BCL-X_L), although these may operate on reticular Ca^{2+} efflux via IP₃Rs (Pinton et al., 2000; White et al., 2005). In this context, inositol 1,4,5-trisphosphate receptor type 3 (ITPR3, best known as IP₃R3) and ITPR2 (best known as IP₃R2) may play a predominant role as compared to ITPR1 (best known as IP₃R1), at least potentially reflecting their elevated capacity to transmit Ca^{2+} signals to mitochondria (Bartok et al., 2019; Mendes et al., 2005; Sun et al., 2019). However, whether BCL2 and other antiapoptotic Bcl-2 proteins limit agonist-induced Ca^{2+} release through IP₃Rs by inhibiting them (Ivanova et al., 2019; Rong et al., 2009), promoting some degree of activation at baseline by increasing the sensitivity to IP₃ (Eck-enrode et al., 2010; White et al., 2005) or supporting a cytoprotective Ca^{2+} leak from the ER via other mechanisms (Bassik et al., 2004; Palmer et al., 2004; Pinton et al., 2000, 2001), remains to be clarified. These apparently contrasting observations may at least in part relate to the ability of both pro- and antiapoptotic Bcl-2 family members to regulate mitochondrial VDAC opening (Chong et al., 2020; Shimizu et al., 1999; Tajeddine et al., 2008), and the highly divergent expression of these regulators of apoptosis in cells from different tissues or tumor types, ultimately resulting in different priming of the apoptotic system at mitochondria (Potter and Letai, 2016).

Consistent with this, multiple MAM-resident oncogenic proteins other than BCL2 and BCL-X_L, such as promyelocytic leukemia (PML), AKT serine/threonine kinase 1 (AKT1), and KRAS^{G13D}, inhibit IP₃R3 at MAMs to promote tumor progression (Betz et al., 2013; Bononi et al., 2017; Giorgi et al., 2010; Kuchay et al., 2017; Marchi et al., 2012; Pierro et al., 2014). However, IP₃R3 upregulation has also been attributed oncogenic roles in some tissues, especially the gastric epithelium, bile ducts, and liver (Guerra et al., 2019; Mangla et al., 2020; Ueasilamongkol et al., 2020). In these settings, additional mechanisms must be at play to inhibit cell death, as transient IP₃R3 overexpression is generally sufficient to kill both normal and cancer cells (Guerra et al., 2019; Ueasilamongkol et al., 2020). Although the precise antiapoptotic

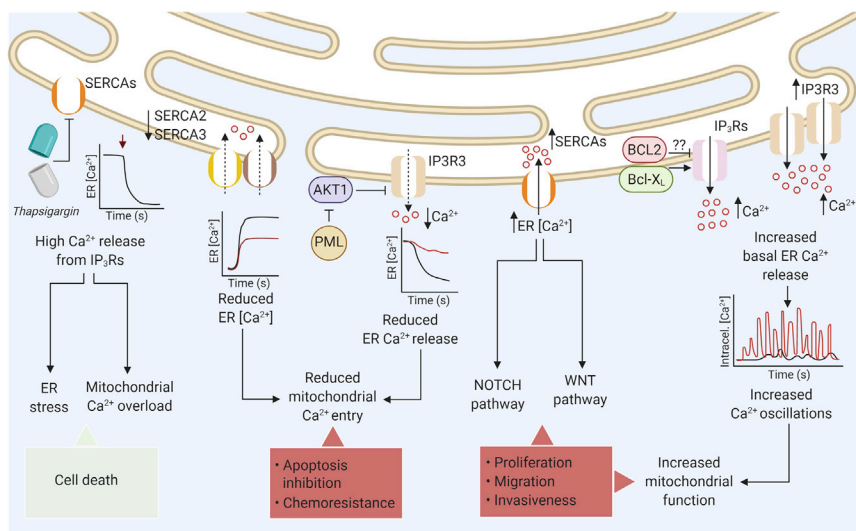


Figure 3. Cancer-Associated Defects of Reticular Ca^{2+} Homeostasis

Acute sarcoendoplasmic reticular Ca^{2+} ATPase (SERCA) inhibition with thapsigargin is highly toxic for malignant (and non-malignant) cells as a consequence of IP_3 receptor (IP_3R)-dependent cytosolic Ca^{2+} overload. Conversely, SERCA downregulation in cancer cells results in limited Ca^{2+} uptake by the endoplasmic reticulum (ER), hence, limiting the pool available for release by IP_3Rs in response to ER-targeting chemotherapeutics, which *de facto* supports chemoresistance. A similar effect is mediated by AKT serine/threonine kinase 1 (AKT1) and antiapoptotic Bcl-2 family members, although the precise mechanisms whereby the latter deplete reticular Ca^{2+} remain to be elucidated. Conversely, mild upregulation of SERCAs expands the pool of Ca^{2+} that is available for release by IP_3Rs , resulting in increased Ca^{2+} release in baseline conditions. This results in improved mitochondrial functions and the activation of NOTCH and WNT signaling in the absence of over-cytotoxicity, ultimately promoting cancer cell proliferation and invasiveness. Similar oncogenic effects have been attributed to IP_3R

upregulation and to the ability of antiapoptotic Bcl-2 family members to modulate IP_3R opening or promote ER Ca^{2+} leak. Representative Ca^{2+} fluctuations in the ER or cytoplasm of cells experiencing the depicted processes (e.g., thapsigargin administration, SERCA-dependent Ca^{2+} uptake by the ER) in wild-type settings (black curves) or in the presence of cancer-associated alterations (red curves) are represented. BCL2, BCL2 apoptosis regulator; BCL-X_L (official name: BCL2L1), BCL2-like 1; PML, promyelocytic leukemia.

pathways supporting oncogenesis in IP_3R -overexpressing cells remain to be elucidated, it is tempting to invoke defects in MPT, the major cell death routine triggered by Ca^{2+} overload (Galluzzi et al., 2018b). Testing the responsiveness of IP_3R -overexpressing cancer cells to hydrogen peroxide (another trigger of MPT) will provide additional insights into this possibility.

These observations exemplify how SERCA and IP_3R defects can contribute to oncogenesis and tumor progression (Figure 3). In this setting, while activation of Ca^{2+} release may cause the death (or at least increase the chemosensitivity) of cancer cells with intact ER stores, the inhibition of Ca^{2+} efflux stands out as a potential strategy to inhibit malignant cells that rely on constitutive Ca^{2+} to mitochondria for metabolism and proliferation, as discussed below.

Mitochondrial Ca^{2+} Homeostasis and Cancer Progression

Mitochondrial Ca^{2+} accumulation upon cytosolic Ca^{2+} signaling regulates intra- and extramitochondrial metabolism and has a major influence on the propensity of cells to undergo cell death via MPT (Bonora et al., 2019). Cancer cells of different histological derivations overexpress channels of the VDAC family (which enable Ca^{2+} to cross the OMM) (Mazure, 2017), as well as MCU, the pore-forming unit of the complex responsible for Ca^{2+} accumulation in the mitochondrial matrix (Marchi et al., 2019b; Vultur et al., 2018). Although such alterations would theoretically increase the propensity of cancer cells to undergo MPT and die in response to a variety of stressors, mitochondria from malignant cells are highly protected from permeabilization as they contain increased levels of MCL1 and other anti-apoptotic proteins of the Bcl-2 family (Singh et al., 2019). Such an increased capacity for Ca^{2+} uptake boosts mitochondrial respiration by favoring the activity of multiple dehydrogenases involved in the

tricarboxylic acid cycle (TCA), ultimately resulting in enhanced ATP and ROS production (Denton, 2009). At least in part, accrued ROS synthesis as driven by Ca^{2+} involves specialized MAM regions coupling cytosolic Ca^{2+} oscillations to H_2O_2 generation in nanodomains localized to mitochondrial cristae (Booth et al., 2016).

In line with these notions, MCU levels in malignant cells positively correlate with mitochondrial Ca^{2+} uptake, ROS production, migratory capacity, and propensity for metastatic dissemination. Besides improved ATP availability, the ROS-driven activation of hypoxia inducible factor 1 subunit alpha (HIF1A), a transcription factor (TF) that promotes glycolysis and favors local immunosuppression (Choudhry and Harris, 2018; Vitale et al., 2019), and the ROS-dependent secretion of the pro-metastatic enzyme matrix metalloproteinase 2 (MMP2) (Conlon and Murray, 2019) play a major role in the ability of mitochondrial Ca^{2+} to drive tumor progression (Ren et al., 2017; Tosatto et al., 2016). Ca^{2+} -dependent ROS generation also occurs when MICU1 is downregulated, reflecting the physiological role of MICU1 as an MCU inhibitor (Csordás et al., 2013; Mallilankaraman et al., 2012b). Accordingly, reduced MICU1 levels and high MCU:MICU1 ratios have been associated with poor disease outcomes in patients with hepatocellular carcinoma (Ren et al., 2017) and breast cancer (Curry et al., 2013), respectively. Of note, MCU, whose conductivity for Ca^{2+} is positively regulated by ROS-driven S-glutathionylation (Dong et al., 2017), also controls cell-cycle progression by generating spontaneous mitochondrial Ca^{2+} transients that coordinate mitotic entry in support of proliferation (Koval et al., 2019; Zhao et al., 2019), which identifies yet another mechanism for ROS-driven alterations in mitochondrial Ca^{2+} fluxes to support tumor progression.

That said, some tumors display reduced MCU or high MICU1 levels, which underlies (at least some degree of) cell death resistance (Chakraborty et al., 2017; Hong et al., 2017; Marchi et al.,

2013). In this setting, restoring normal mitochondrial Ca^{2+} uptake results in overt cytotoxicity or sensitization to conventional therapeutic agents (Chakraborty et al., 2017; Marchi et al., 2013). Although the reasons why some cancer cells acquire diametrically opposed alterations in mitochondrial Ca^{2+} dynamics remain to be clarified, it is tempting to invoke the extraordinary metabolic and functional flexibility that generally accompanies malignant transformation as a main factor. Thus, while cancer cells that synthesize ATP by glycolysis may achieve increased resistance to cell death by MCU inhibition (via MCU downregulation or MICU1 upregulation), malignant cells that prevalently rely on mitochondrial respiration for ATP synthesis are expected to require a hyperactive MCU complex (upon MCU upregulation or MICU1 downregulation), calling for the establishment of alternative cytoprotective pathways.

Consistent with this view, highly glycolytic ovarian cancer cells exhibit high MICU1 expression, reduced mitochondrial Ca^{2+} levels, and resistance to cisplatin (Chakraborty et al., 2017). Cytoprotective alterations potentially at work in malignant cells that rely on mitochondrial Ca^{2+} signaling for bioenergetic metabolism and proliferation include reinforced antioxidant defenses (Bansal and Simon, 2018) and endogenous MPT inhibition (Antony et al., 2016; Marchi et al., 2019c). Notably, the oncogenic protein AKT1 and the oncosuppressor TP53, whose ability to inhibit or drive cell death, respectively, has been linked to Ca^{2+} regulation, control MPT by phosphorylating (AKT1) or physically interacting with (TP53) the key MPT regulator peptidylprolyl isomerase F (PPIF, best known as CYPD) (Ghosh et al., 2015; Vaseva et al., 2012). Moreover, AKT1-expressing tumors require high mitochondrial Ca^{2+} and ROS production at baseline to proliferate, largely as a result of phosphorylation-dependent MICU1 inhibition (Marchi et al., 2019a). These findings delineate a complex mechanism whereby mitochondrial Ca^{2+} signaling and cell death resistance co-evolve with metabolic alterations in the context of tumor progression.

The oncogenic activity of MCUR1 provides additional insights into the role of mitochondrial Ca^{2+} in cancer. In line with the ability of MCUR1 to positively regulate MCU activity, MCUR1 expression levels positively correlate with mitochondrial Ca^{2+} accumulation (Mallilankaraman et al., 2012a). In hepatocellular carcinoma cells, MCUR1 is strongly upregulated in the context of ROS overproduction, resulting in ROS-dependent TP53 degradation and consequent resistance to cell death (Ren et al., 2018). In this context, antioxidant defenses are also elevated downstream of NRF2 activation, which further lowers cellular susceptibility to cell death in the presence of increased ROS levels that sustain proliferation (Jin et al., 2019). That said, MCUR1 has also been suggested to operate as an assembly factor for respiratory complex IV (Paupe et al., 2015). In this scenario, the correlation of MCUR1 levels with mitochondrial Ca^{2+} uptake and ROS production may reflect the impact of MCUR1 on oxidative phosphorylation, suggesting an alternative, Ca^{2+} -independent mechanism through which MCUR1 promotes tumor progression. This possibility, however, remains to be experimentally verified.

While the impact of MCU complex activity on oncogenesis has been investigated by multiple groups, the role of other mitochondrial Ca^{2+} transporters in malignant transformation, tumor pro-

gression, and response to therapy remains obscure. NCLX controls SOCE via a sophisticated redox circuitry (Ben-Kasus Nissim et al., 2017), but its function in cancer cell proliferation, cell death, and migration is unclear. The impact of VDACs on the biology of (pre-)malignant cells appears to (1) be independent of their permeability to Ca^{2+} ions and (2) display considerable variability. VDACs support tumor progression as integral parts of the molecular machinery that exchanges key metabolites (e.g., ADP, ATP) across the OMM (Mazure, 2017), but also operate as a key mediator of MPT, *de facto* favoring the death of (pre-)malignant cells exposed to chemotherapy (Tajeddine et al., 2008). LETM1 is markedly overexpressed in various neoplasms, and it reportedly supports cancer cell survival and metabolic fitness (Piao et al., 2009), but it remains unclear whether these effects depend on Ca^{2+} signaling. It has been proposed that LETM1 exchanges mitochondrial Ca^{2+} for H^+ when cytosolic Ca^{2+} levels are high and MCU mediates its import, whereas it acts as an alternative Ca^{2+} influx when cytosolic Ca^{2+} is low (Jiang et al., 2009). However, the role of LETM1 as a $\text{Ca}^{2+}/\text{H}^+$ antiporter remains a matter of debate (Austin et al., 2017). Irrespective of these unresolved issues, it would be interesting to investigate whether LETM1 levels affect mitochondrial Ca^{2+} in cancer cells at baseline, as the remodeling of Ca^{2+} spikes in the mitochondrial matrix of malignant cells may constitute a pivotal factor in the regulation of tumor progression by mitochondrial Ca^{2+} .

These observations delineate multiple mechanisms whereby alterations in mitochondrial Ca^{2+} signaling influence malignant transformation, tumor progression, and response to therapy in the context of metabolic rewiring, ROS generation, and resistance to cell death (Figure 4). In this context, agents targeting deregulated MCU activity (either directly or via MICU1 and MCUR1) may constitute promising candidates for the development of new anticancer drugs, especially for tumors that exhibit elevated oxidative phosphorylation.

Other Intraorganellar Ca^{2+} Defects in Cancer Cells

Ca^{2+} concentrations in the lysosomal lumen are similar to reticular Ca^{2+} levels (Christensen et al., 2002; Lloyd-Evans et al., 2008). Thus, despite their limited volume, lysosomes can release considerable amounts of Ca^{2+} , mostly via the ROS- and PI(3,5) P_2 -sensitive channel TRPML1 (Fine et al., 2018). Lysosomal functions are critical for cancer cells undergoing autophagy as a consequence of nutrient deprivation or exposure to therapeutic agents (Galluzzi et al., 2017). Moreover, lysosomes located to the cell periphery support metastasis by releasing metalloproteases that digest the extracellular matrix (Naegeli et al., 2017). In line with this notion, TFs from the microphthalmia family, including melanocyte-melanocyte inducing transcription factor (MITF), TFEB, TFEC, and transcription factor binding to IGHM enhancer 3 (TFE3), are upregulated in a variety of tumors, where they support lysosomal biogenesis, autophagy, and metabolism (Slade and Pulinilkunnil, 2017). The activity of these TFs is largely regulated by lysosomal Ca^{2+} efflux via TRPML1, resulting in the Ca^{2+} -dependent activation of calcineurin, an oligomeric enzyme with phosphatase activity (Park et al., 2019), and consequent dephosphorylation-dependent nuclear relocalization of the TFs (Medina et al., 2015).

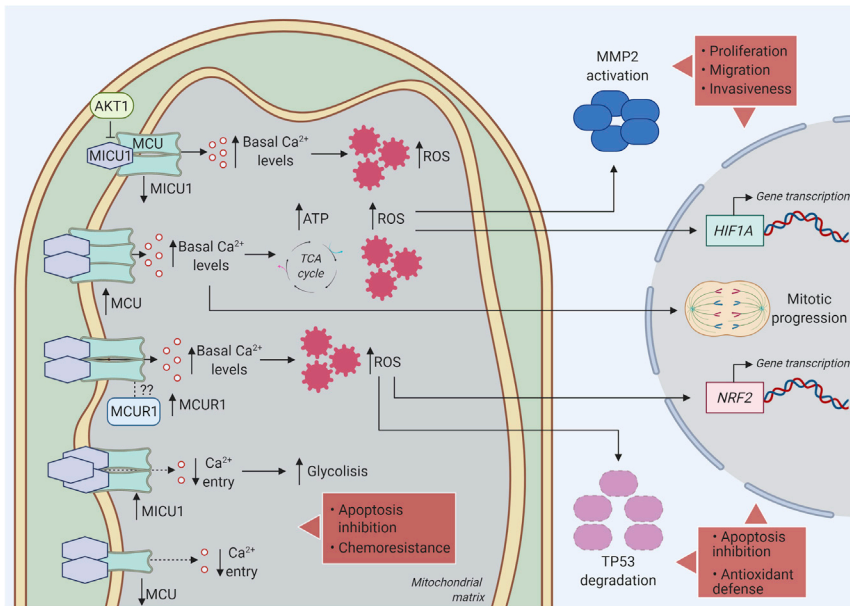


Figure 4. Alterations of Mitochondrial Ca^{2+} Signaling in Cancer Cells

At high mitochondrial calcium uniporter (MCU) to mitochondrial calcium uptake 1 (MICU1) ratios, cancer cells accumulate increased Ca^{2+} levels in mitochondria at baseline, resulting in accelerated proliferation as a consequence of accrued ATP and reactive oxygen species (ROS) production. ROS also favor metastatic dissemination by promoting hypoxia-inducible factor 1 subunit alpha (HIF1A)-dependent transcription programs and matrix metalloproteinase 2 (MMP2) activation. Similar effects can result from MICU1 inhibition by AKT serine/threonine kinase 1 (AKT1). Conversely, low MCU:MICU1 ratios limit mitochondrial Ca^{2+} entry and hence protect cancer cells from death induced by chemotherapeutics. Finally, the upregulation of mitochondrial calcium uptake regulator 1 (MCUR1) results in increased Ca^{2+} uptake by mitochondria along with the activation of a ROS-dependent antioxidant response orchestrated by nuclear factor, erythroid 2 like 2 (NFE2L2, best known as NRF2) and involving tumor protein p53 (TP53) degradation. Whether MCUR1 is a bona fide component of the MCU complex remains unclear.

This is particularly relevant for HRAS^{G12V}-driven tumors, which are characterized by high levels of MITF and TFEB (Urbanelli et al., 2014). Both MITF and TFEB transactivate *TRPML1* and *VAC14* component of the PIKFYVE complex (*VAC14*) as they repress myotubularin 1 (*MTM1*), hence establishing a circuitry whereby both *TRPML1* and its major activator (PI(3,5)P₂, which is synthesized by *VAC14* and degraded by *MTM1*) are abundant (Jung et al., 2019). Thus, HRAS^{G12V}-driven tumors exhibit the constitutive release of lysosomal Ca^{2+} that feeds into a positive loop to further sustain MITF and TFEB activity (Jung et al., 2019). Moreover, high Ca^{2+} efflux from lysosomes favors cancer growth by the mitogen-associated protein kinase (MAPK) pathway and by supporting HRAS^{G12V} activation at the PM via the formation of nanoclusters (Jung et al., 2019). Of note, lysosomal Ca^{2+} release is also sensed by *CALM1*, resulting in the activation of the mechanistic target of rapamycin complex 1 (MTORC1) at the lysosomal surface and the MTORC1-dependent transduction of a mitogenic signal (Li et al., 2016; Sun et al., 2018), at least in triple-negative breast cancer (Xu et al., 2019). Curiously, *TRPML1* inhibits rather than activates MTORC1 and MAPK signaling in melanoma, but still supports tumor progression (Kasitinton et al., 2019). The precise mechanisms underlying the ability of *TRPML1* to drive melanoma progression in the context of MTORC1 and MAPK inhibition remain obscure, but may be linked to autophagy activation (given the major inhibitory role of MTORC1 on autophagy) (Rybinski et al., 2018).

Since endolysosomal Ca^{2+} exit through *TPCN2* also promotes proliferation and metastasis in breast cancer cells (Favia et al., 2014; Nguyen et al., 2017), lysosomal Ca^{2+} release may constitute a general oncogenic factor, pointing to *VAC14*, *TRPML1*, and *TPCN2* as potential candidates for the development of novel therapeutic agents. Besides the ability of lysosomal Ca^{2+} to initiate transcriptional programs that support proliferation and

adaptation to adverse microenvironmental conditions, such a key role may reflect the ability of lysosomes to evoke localized Ca^{2+} puffs that promote migration (Wei et al., 2009). Moreover, under specific conditions lysosomes relocate in the proximity of the ER and PM, hence influencing ER Ca^{2+} release (Atakpa et al., 2018) and SOCE (Sbano et al., 2017). Finally, lysosomes positioned at strategic intracellular sites could act as alternative Ca^{2+} -buffering systems, limiting the continuous Ca^{2+} transfer from the ER to mitochondria or excessive Ca^{2+} influx from the extracellular space, *de facto* mediating robust cytoprotective effects. The latter possibility remains to be formally investigated.

Ca^{2+} homeostasis at the GA has also been linked to malignant transformation and tumor progression. Aging *Atp2c1*^{+/-} mice (which are heterozygous for a ubiquitous SPCA) display an increased incidence of squamous cell carcinomas (Okunade et al., 2007). However, it is not clear whether this phenotype can be ascribed to defects in Ca^{2+} fluxes, since ATPase secretory pathway Ca^{2+} transporting 1 (ATP2C1, best known as SPCA1) inhibition in triple-negative breast cancer cells does not impose profound changes on cytosolic Ca^{2+} signaling (Grice et al., 2010). The upregulation of ATPase secretory pathway Ca^{2+} transporting 2 (ATP2C2, best known as SPCA2) occurs physiologically during lactation (Faddy et al., 2008) and has been documented in numerous cases of breast cancer (Feng et al., 2010). In this context, SPCA2 mediates constitutive Ca^{2+} influx by enhancing ORAI1 activity in a store-independent manner, ultimately favoring the nuclear translocation of NFATC1 in support of proliferation and disease progression (Feng et al., 2010). Thus, the activity of SPCA2 in breast cancer resembles that of ORAI3 in prostate carcinoma, also constituting a potential target for the development of novel anticancer agents. Although the actual contribution of Ca^{2+} fluxes within the GA to malignant transformation and tumor progression has never been investigated in detail, circumstantial evidence points to a potential

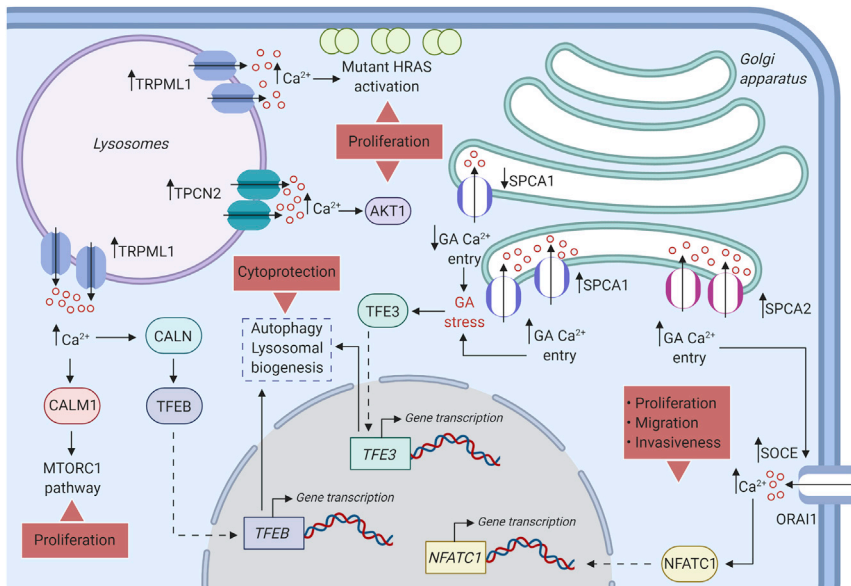


Figure 5. Defects of Ca^{2+} Fluxes in the Lysosomes and Golgi Apparatus of Malignant Cells

Increased Ca^{2+} release from lysosomes as a consequence of mucolipin 1 (MCOLN1, also known as TRPML1) or two-pore segment 2 (TPCN2) upregulation can favor cancer cell proliferation via mechanistic target of rapamycin complex 1 (MTORC1), AKT serine/threonine kinase 1 (AKT1), or mutant HRAS, as well as the activation of lysosomal biogenesis and autophagy via transcription factor EB (TFEB). Similar effects can occur downstream of Golgi apparatus (GA) stress caused by alterations in the levels of ATPase secretory pathway Ca^{2+} transporting 1 (ATP2C1, best known as SPCA1) or SPCA2. In this latter setting, cytoprotective and mitogenic effects have been attributed to the activation of transcription factor binding to IGHM enhancer 3 (TFE3) and nuclear factor of activated T cells 1 (NFATC1)-dependent transcriptional programs, respectively. Notably, NFATC1 driven by GA stress involves a compensatory increase in Ca^{2+} uptake via calcium release-activated calcium modulator 1 (ORAI1). CALM1, calmodulin 1; CALN (official name: PPP3CA), protein phosphatase 3 catalytic subunit alpha.

link. Notably, TFE3 activation, which characterizes multiple tumors including pancreatic ductal adenocarcinoma (Perera et al., 2015), also ensues GA stress caused by alterations in GA Ca^{2+} levels upon SPCA1 overexpression (Smaardijk et al., 2018) or downregulation (Lissandron et al., 2010). It would be interesting to investigate whether the propensity of aging *Atp2c1*^{+/-} mice to develop squamous cell carcinomas can be abrogated by blocking or deleting TFE3.

Irrespective of these and other open issues, lysosomes and the GA stand out as important but underestimated regulators of Ca^{2+} homeostasis that affect multiple steps of the carcinogenic process (Figure 5). We surmise that additional investigation may reveal potential targets other than SPCA2 for the development of novel anticancer agents that modulate Ca^{2+} fluxes at lysosomes and the GA.

Therapeutic Perspectives and Concluding Remarks

Although Ca^{2+} deregulation has long been viewed as a bystander of malignant transformation, tumor progression, and resistance to therapy, accumulating preclinical and clinical evidence support a central role for alterations in Ca^{2+} homeostasis in cancer. Thus, Ca^{2+} signaling has begun to attract attention as a potential target for the development of novel anticancer therapies.

In this context, promising Ca^{2+} -based anticancer therapeutics include agents blocking components of the molecular machinery for Ca^{2+} homeostasis that are highly overexpressed in malignant versus non-malignant tissues, as well as molecules that inhibit Ca^{2+} transporters conferring low susceptibility to cell death and concomitantly sustaining cancer cell proliferation. In line with this notion, the TRPA1 inhibitor AM-0902, alone or in combination with chemotherapeutics (Takahashi et al., 2018), as well as the TRPV6-antagonistic peptides SOR-C13 and SOR-C27 (Bowen et al., 2013; Xue et al., 2018), mediate robust anti-neoplastic effects in mice in the absence of significant toxicity. Notably, SOR-C13 is in clinical testing in patients with advanced

refractory solid tumors (NCT03784677). An orally available blocker of TRPC6 (i.e., BI 749327) also mediates beneficial effects *in vivo*, in models of cardiac and renal disease (Lin et al., 2019), but it has not yet been investigated for its anticancer properties. That said, it will be crucial to determine whether these agents impair anticancer immunosurveillance (Galluzzi et al., 2018a; Rao et al., 2019), in thus far resembling SOCE and TRPM2 inhibitors (Gershkovitz et al., 2018; Weidinger et al., 2013). Finally, systemic TRPML1 inhibition may not be achievable as loss-of-function mutations in *MCOLN1* cause a lysosomal storage disease that is characterized by mental and motor retardation (Frei et al., 1998).

As an alternative, pharmacological activation Ca^{2+} channels overexpressed by malignant cells may be harnessed to evoke cytosolic Ca^{2+} overload and consequent MPT-driven cell death. Consistent with this notion, the TRPV4 activator GSK1016790A inhibits the growth of TRPV4⁺ human breast carcinomas established in immunodeficient mice (Peters et al., 2017). However, this approach may also favor the proliferation of cancer cell clones with limited sensitivity to cell death induction, *de facto* selecting and favoring the progression of chemoresistant disease. At least theoretically, concentrating Ca^{2+} overload in a restricted area of the cell may result in superior cytotoxicity, as demonstrated by the potent effect of PSMA-activatable thapsigargin (G-202 or mipsagargin) (Denmeade et al., 2012). However, despite encouraging results from early-phase clinical trials enrolling patients with advanced solid tumors (Mahalingam et al., 2019; Mahalingam et al., 2016), the clinical development of G-202 for oncological indications appears to be at an impasse (<https://www.clinicaltrials.gov>).

IP₃Rs have also attracted attention as potential targets for the development of anticancer agents. On the one hand, IP₃R inhibitors have been evaluated for their ability to limit ER-to-mitochondria Ca^{2+} transfer, hence blocking the proliferation of cancer cells relying on oxidative phosphorylation for ATP

synthesis (Cárdenas et al., 2016). On the other hand, molecules that modulate IP₃R activity, including small peptides that release IP₃R from BCL2-mediated inhibition (Akl et al., 2013; Bittremieux et al., 2019; Zhong et al., 2011) and chemical inhibitors of IP₃R3 degradation (Kuchay et al., 2017), have been investigated for the capacity to support Ca²⁺ overload culminating with cell death or sensitization to conventional chemotherapeutics. However, the toxic effects of these molecules remain largely unexplored.

Thus, to translate Ca²⁺-based anticancer agents from the bench to the bedside, it will be important not only to identify molecules that selectively target Ca²⁺ homeostasis in malignant cells but also to consider the metabolic heterogeneity of the latter and the mechanisms through which such heterogeneity is connected to cell death regulation. Moreover, it will be critical to link altered Ca²⁺ fluxes in cancer cells to the ability of the latter to evade immunosurveillance, which is now recognized as a key hallmark of oncogenesis (Galluzzi et al., 2018a; Hanahan and Weinberg, 2011). The ER-resident Ca²⁺-buffering chaperone calreticulin (CALR) is expected to play a major role in this context, not only because CALR is critical for the proper loading of antigenic epitopes on the surface of major histocompatibility complex (MHC) class I molecules (Raghavan et al., 2013) but also because CALR is intimately involved in the ability of stressed and dying cancer cells to deliver stimulatory, adjuvant-like signals to immune cells (Galluzzi et al., 2020; Rodriguez-Ruiz et al., 2020; Salvagno and Cubillos-Ruiz, 2019). However, little is known of the effect of deranged Ca²⁺ homeostasis on the immunostimulatory effects of CALR in cancer cells.

In conclusion, alterations in Ca²⁺ fluxes influence malignant transformation, tumor progression, and response to therapy by affecting an intricate network of cancer cell-intrinsic (e.g., metabolism, redox homeostasis) and extrinsic (e.g., antigen presentation, danger signaling) functions. Additional work is urgently awaited to disentangle the molecular and functional complexity of such a network.

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