Ca2+ Fluxes and Cancer

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Abstract

Ca^{2+} ions are key second messengers in both excitable and non-excitable cells. Owing to the rather pleiotropic nature of Ca^{2+} transporters and other Ca^{2+}-binding proteins, however, Ca^{2+} signaling has attracted limited attention as potential target for anticancer therapy. Here, we discuss cancer-associated alterations of Ca^{2+} fluxes at specific organelles as we identify novel candidates for the development of drugs that selectively target Ca^{2+} signaling in malignant cells.

Introduction

Historically, the molecular machinery that regulates intracellular calcium (Ca^{2+}) fluxes has attracted limited attention as a potential target for cancer therapy, largely reflecting the fact that Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} fluxes as spatially restricted processes that can be targeted therapeutically. Thus, it is now clear that the molecular machinery that controls intracellular Ca^{2+} 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 Ca^{2+} fluxes at specific organelles impact on 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, Ca^{2+}-buffering proteins are not discussed herein, although they undoubtedly impact multiple aspects of oncogenesis (Schwaller, 2020).

Global Ca^{2+} homeostasis in normal cells

Cells need to maintain extremely low cytosolic Ca^{2+} levels (~100 nM), hence establishing a 10- to 15,000-fold gradient with the extracellular milieu (where Ca^{2+} concentration is approx. 1-1.5 mM). Although the original evolutionary advantage of such a gradient was the avoidance of potentially cytotoxic Ca^{2+}-phosphate precipitates, a variety of functions have evolved around such a compartmentalized source of electrochemical energy, including intracellular signal transduction as well metabolite transport across membranes (Bootman and Bultynck, 2020; Chen et al., 2020).

Two main ATP-dependent systems extrude Ca^{2+} from the cytosol of mammalian cells: plasma membrane Ca^{2+} ATPases (PMCAs), which expel Ca^{2+} to the extracellular space, and sarcoendoplasmic reticular Ca^{2+} ATPases (SERCAs), which accumulate it within the endoplasmic reticulum (ER). Moreover, secretory pathway Ca^{2+} ATPases (SPCAs) promote Ca^{2+} accumulation within the Golgi apparatus (GA), while other organelles such as lysosomes can store Ca^{2+}, either as a functional consequence of vesicular trafficking from the extracellular Ca^{2+}-rich milieu (*e.g.*, endocytosis) (Galluzzi and Green, 2019) or (at least theoretically) through an hitherto elusive Ca^{2+}/H^{+} exchanger (Melchionda et al., 2016). That said, these latter intracellular Ca^{2+} stores are quantitatively limited as compared to the ER (Yang et al., 2019).

Cytosolic Ca^{2+} signaling (during which cytosolic Ca^{2+} 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 Ca^{2+} signaling is initiated by ligand-engaged G-protein coupled receptors (Jain et al., 2018), causing the synthesis of 1,4,5-inositol trisphosphate (IP_3) and IP_3-dependent opening of Ca^{2+} channels of the IP_3 receptor (IP_3R) family at the ER membrane (Prole and Taylor, 2019). Alternatively, IP_3R 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, Ca^{2+} ions can accumulate in cytosol upon the production of reactive oxygen species (ROS) or phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) and consequent opening of the ROS- and $PI(3,5)P_2$-sensitive lysosomal Ca^{2+} 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) (Venkatachalam 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).

Importantly, 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 ORAI calcium release-activated calcium modulator 1 (ORAI1) and ORAI calcium release-

activated calcium modulator 1 (ORAI3) (Derler et al., 2016). These PM Ca^{2+} channels relocalize to PM-ER junctions upon 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 sited 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; Mallilankaraman et al., 2012b). Mitochondria extrude Ca^{2+} via the Na^{+}/Ca^{2+} exchanger solute carrier family 8 member B1 (SLC8B1, best known as NCLX) (Palty et al., 2010) and a H^{+}/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 support the generation of endogenous antioxidants like 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. Indeed, 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.; 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 stromal interaction molecule 1 (STIM1), resulting in persistent Ca^{2+} entry via SOCE, mitochondrial permeability transition (MPT) and ultimately cell death (Hawkins et al., 2010).

Importantly, 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 elevation 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. Indeed, both pro-oxidants like H_2O_2 and chemotherapeutics (*i.e.*, carboplatin) trigger slow and moderate oscillations in cytosolic Ca^{2+} levels, which differ from those generated by TRPA1 activating stimuli (like mustard oil), but may resemble those originated from Ca^{2+} pulses by 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 to 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, Ca^{2+} entry through the redox-sensitive channel transient receptor potential cation channel subfamily M member 2 (TRPM2) drives NRF2activation and consequent upregulation of various antioxidant enzymes and IQ motif containing GTPase activating protein 1 (IQGAP1), a Ca^{2+}-dependent modulator of NRF2 stability (Bao et al., 2019). Importantly, 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 transient receptor potential cation channel subfamily M member 3 (TRPM3) (Hall et al., 2014), transient receptor potential cation channel subfamily C member 1 (TRPC1) (Azimi et al., 2017), transient receptor potential cation channel subfamily C member 6 (TRPC6) (Guilbert et al., 2008), transient receptor potential cation channel subfamily V member 4 (TRPV4) (Peters et al., 2017), and transient receptor potential cation channel subfamily V member 6 (TRPV6) (Fixemer et al., 2003). Moreover, transient receptor potential cation channel subfamily M member 7 (TRPM7) and transient receptor potential cation channel subfamily V member 2 (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 (CAMMK2) (Hall et al., 2014). Conversely, TRPM7 is the main TRP channel involved in the generation of short-lived Ca^{2+} flickers that drive cancer cell migration (Wei et al., 2009). Finally, elevated cytosolic Ca^{2+} levels correlate with 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. Importantly, non-transformed cells experience TRP activation rapidly undergo cytosolic Ca^{2+} and cell death (Shapovalov et al., 2011). Thus, malignant cells must acquire additional features that allow them to control the amplitude and kinetics of Ca^{2+} fluxes and hence harness the beneficial effects of Ca^{2+} signaling while avoid 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 Ca^{2+} 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, 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 ensues the establishment of oscillatory Ca^{2+} 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 termed lipid rafts (Chantome et al., 2013). Such as polarization appears to be controlled (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). Altogether, 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 CRAC channels may promote the resistance of cancer cells to stressors that favor Ca^{2+} influx, including hypoxia.

Taken together, these observations that 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). Altogether, 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 mediates oncogenic functions also in the mammary tissue (Hasna et al., 2018; Motiani et al., 2010; Motiani et al., 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, 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 anticancer 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 deregulation of Ca^{2+} fluxes at the PM (**Figure 2**), but targeting such alterations remain 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 only be useful for cancers bearing *ORAI1* mutations.

Oncogenic Ca^{2+} dynamics at the endoplasmic reticulum

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 (*i.e.*, *ATP2A1*, *ATP2A2*, *ATP2A3*) in 14 splicing variants, and the downregulation of specific transcript is generally associated with compensatory mechanisms (Arbabian et al., 2011). In line with this notion ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 3 (ATP2A3, best known as SERCA3) expression decrease 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).

Initial interest in SERCAs as target for anticancer therapy stemmed from the highly cytotoxic, but virtually unselective, activity of the pan-SERCA inhibitor thapsigargin (Lytton 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 ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 1 (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 IP_3Rs (Pinton et al., 2000; White et al., 2005). In this context, inositol 1,4,5-trisphosphate receptor type 3 (ITPR3, best known as IP_3R3) and ITPR2 (best known as IP_3R2) may play a predominant role as compared to ITPR1 (best known as IP_3R1), 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 IP_3Rs by inhibiting them (Ivanova et al., 2019; Rong et al., 2009), promoting some degree of activation at baseline by increasing the sensitivity to IP_3 (Eckenrode 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; Pinton et al., 2001) remains to be clarified. At least in part, these apparently contrasting observations may relate to the ability of both pro- and anti-apoptotic Bcl-2 family members to regulate 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 IP_3R3 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, IP_3R3 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., 2019). In these settings, additional mechanisms must be at play to inhibit cell death, as transient IP_3R3 overexpression is generally sufficient to kill both normal and cancer cells (Guerra et al., 2019; Ueasilamongkol et al., 2019). Although the precise antiapoptotic pathways supporting oncogenesis in IP_3R3-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_3R3-overexpressing cancer cells to hydrogen peroxide (another trigger of MPT) will provide additional insights into this possibility.

Altogether, 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, inhibition of Ca^{2+} efflux stands out as 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 derivation 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 that promotes glycolysis and favors local immunosuppression (Choudhry and Harris, 2018; Vitale et al., 2019), as well as the ROS-dependent secretion of the pro-metastatic enzyme matrix metallopeptidase 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 MCU

inhibitor (Csordas et al., 2013; Mallilankaraman et al., 2012b). Accordingly, reduced MICU1 levels and high MCU/MICU1 ratios have been associated with poor disease outcome 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 to 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 of 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

antioxidants defenses (Bansal and Simon, 2018), as well as 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). Altogether, 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 in 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 impact of other mitochondrial Ca^{2+} transporters in malignant transformation, tumor

progression and response to therapy remains obscure. NCLX controls SOCE via a sophisticated redox circuitry (Ben-Kasus Nissim et al., 2017), but its role 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. Indeed, 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 Ca^{2+}/H^+ antiporter remains matter of debate (Austin et al., 2017). Irrespective of these unresolved issues, it would be interesting to investigate whether LETM1 levels affect basal mitochondrial Ca^{2+} in cancer cells, 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+}.

Taken together, 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, transcription factors from the microphthalmia family, including melanocyte inducing transcription factor (MITF), transcription factor EB (TFEB), transcription factor EC (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). Importantly, the activity of these transcription factors is largely regulated by lysosomal Ca^{2+} efflux via TRPML1. This results 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 transcription factors (Medina et al., 2015).

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 PIKFYVE complex (*VAC14*) as they repress myotubularin 1 (*MTM1*), hence establishing a circuitry whereby both TRPML1 and its major activator ($PI(3,5)P_2$, which is synthesized by VAC14 and degraded by MTM1) are abundant (Jung et al., 2019). Thus, in $HRAS^{G12V}$-driven tumors exhibit constitutive release of lysosomal Ca^{2+} that feed 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 as 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 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 (Kasitinon 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) (Rybstein et al., 2018).

Since also endolysosomal Ca^{2+} exit through TPCN2 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 also reflect the ability lysosomes to evoke localized Ca^{2+} puffs that promote migration (Wei et al., 2009). Moreover, under specific conditions lysosomes relocalize 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 as or excessive Ca^{2+} influx from the extracellular space, *de facto* mediating robust cytoprotective effects. These 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 an 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 Ca2+ transporting 1 (ATP2C1, best known as SPCA1) inhibition in triple-negative breast cancer cells does not impose profound changes to cytosolic Ca^{2+} signaling (Grice et al., 2010). Upregulation of ATPase secretory pathway Ca2+ 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 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 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 impact 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 that block components of the molecular machinery for Ca^{2+} homeostasis 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 antineoplastic effects in mice, in the absence of significant toxicity. Notably, SOR-C13 is currently under 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 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 characterized by mental and motor retardation (Frei et al., 1998).

As an alternative, pharmacological activation Ca^{2+} channels overexpressed by malignant cell 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-positive 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 results 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 (source www.clinicaltrials.gov).

IP_3Rs have also attracted attention as potential target for the development of anticancer agents. On the one hand, IP_3R 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 (Cardenas et al., 2016). On the other hand, molecules that modulate IP_3R activity, including small peptides that release IP_3R from BCL2-mediated inhibition (Akl et al., 2013; Bittremieux et al., 2019; Zhong et al., 2011) as well as chemical inhibitors of IP_3R3 degradation (Kuchay et al., 2017), have been investigated for the capacity to support Ca^{2+} overload culminating with cell death or sensitization to conventional chemotherapeutics. However, the toxic effects of these molecules remain largely unexplored.

Thus, to translate Ca^{2+}-based anticancer agents from the bench to the bedside, it will be important not only to identify molecules that selectively target Ca^{2+} homeostasis in malignant cells, but also to consider the metabolic heterogeneity of the latter and the mechanisms though which such a heterogeneity is connected to cell death regulation. Moreover, it will be critical to link altered Ca^{2+} 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 Ca^{2+}-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 MHC Class I molecules (Raghavan et al., 2013), but also since 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 on the effect of deranged Ca^{2+} homeostasis on the immunostimulatory effects of CALR in cancer cells. In conclusion, alterations in Ca^{2+} 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 network.

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Legends to Figures

Figure 1. **Regulation of Ca^{2+} in normal non-excitable cells.** In response to a variety to stimuli, normal non-excitable cells can activate cytosolic Ca^{2+} signaling via a variety of mechanisms, including (but not limited to): (1) Ca^{2+} release from the endoplasmic reticulum (ER) and the Golgi apparatus; (2) Ca^{2+} release from mitochondria and lysosomes; as well as (3) Ca^{2+} uptake from the extracellular microenvironment. This causes an increase in cytosolic Ca^{2+} levels coupled to the activation of numerous cellular functions (**A**). Numerous systems contribute to the extinction of Ca^{2+} signaling by reducing cytosolic Ca^{2+} levels upon: (1) Ca^{2+} import by the ER, Golgi apparatus and mitochondria; or (2) Ca^{2+} extrusion to the extracellular space. Whether lysosomes accumulate Ca^{2+} via specific transporters including a hitherto uncharacterized Ca^{2+}/H^{+} exchanger (CAX) or from the microenvironment upon endocytosis remains unclear (**B**). IP_3R, 1,4,5-inositol trisphosphate receptor; MCU, mitochondrial calcium uniporter; NCLX (official name: SLC8B1), solute carrier family 8 member B1; PMCA, plasma membrane Ca^{2+} ATPase; SERCA, sarcoendoplasmic reticular Ca^{2+} ATPase; SPCA, secretory pathway Ca^{2+} ATPase; STIM, stromal interaction molecule; TPCN, two pore segment channel; TRPML1 (official name: MCOLN1), mucolipin 1; TRP, transient receptor potential cation channel member; VDAC, voltage-dependent anion channel; VGCC, voltage-gated calcium channel.

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 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; (5) acquisition of increased motility; and (6) secretion of matrix metallopeptidases (MMPs). CALM1, calmodulin 1; CAMKK2, calcium/calmodulin dependent protein kinase 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.

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 1,4,5-inositol trisphosphate 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 expand 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 as well as 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, as well as to the ability of anti-apoptotic Bcl-2 family member 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, etc..) 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.

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 higher Ca^{2+} levels in mitochondria at baseline, resulting in increased proliferation as a consequence of accrued ATP and reactive oxygen species (ROS) production. ROS also favors metastatic dissemination by promoting hypoxia inducible factor 1 subunit alpha (HIF1A)-dependent transcription programs and matrix metallopeptidase 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, upregulation of mitochondrial calcium uptake regulator 1 (MCUR1) results in increased Ca^{2+} uptake by mitochondria along with 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.

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 channel 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 proto-oncogene, GTPase (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 cause by alterations in the levels of ATPase secretory pathway Ca2+ 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 ORAI calcium release-activated calcium modulator 1 (ORAI1). CALM1, calmodulin 1; CALN (official name: PPP3CA), protein phosphatase 3 catalytic subunit alpha.

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 support 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) 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).

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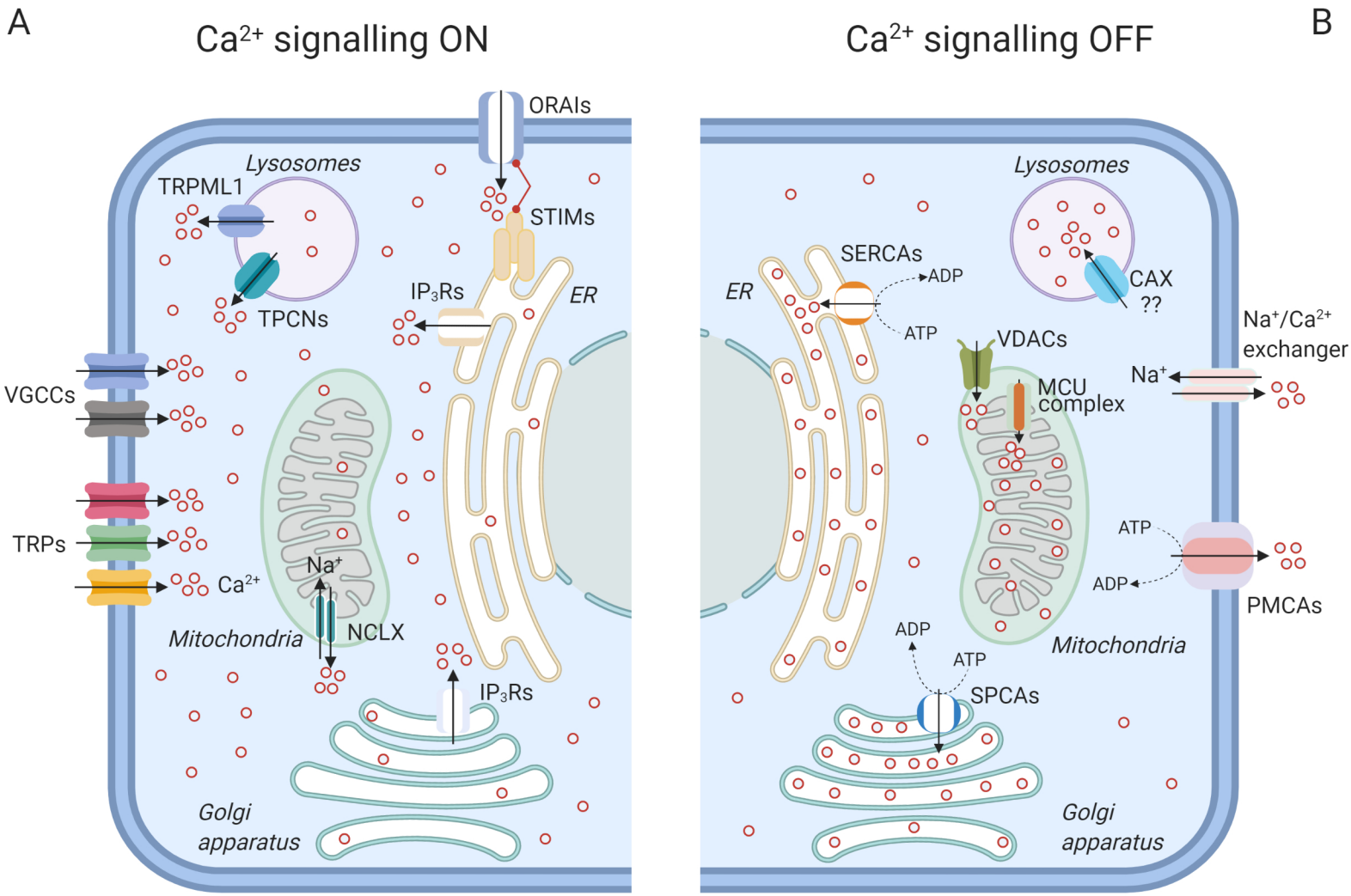
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TRPML1
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ER
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VGCCs
TRPs
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Na+
NCLX
Mitochondria
IP3Rs
Golgi apparatus
B
Ca2+ signalling OFF
Lysosomes
SERCAs
ADP
ATP
ER
CAX
??
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ATP
ADP
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ADP
ATP
SPCAs
Mitochondria
Golgi apparatus

Figure 1

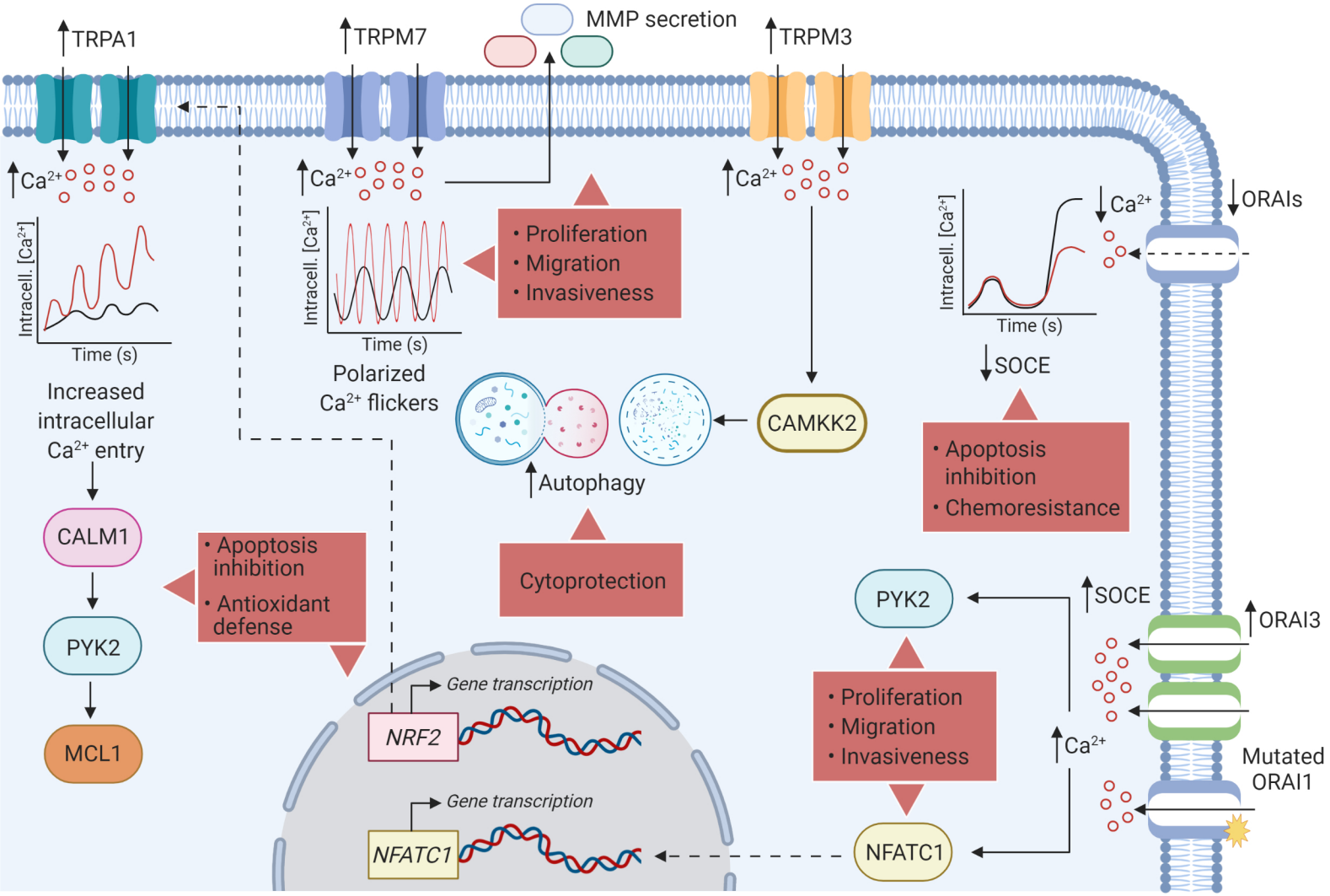
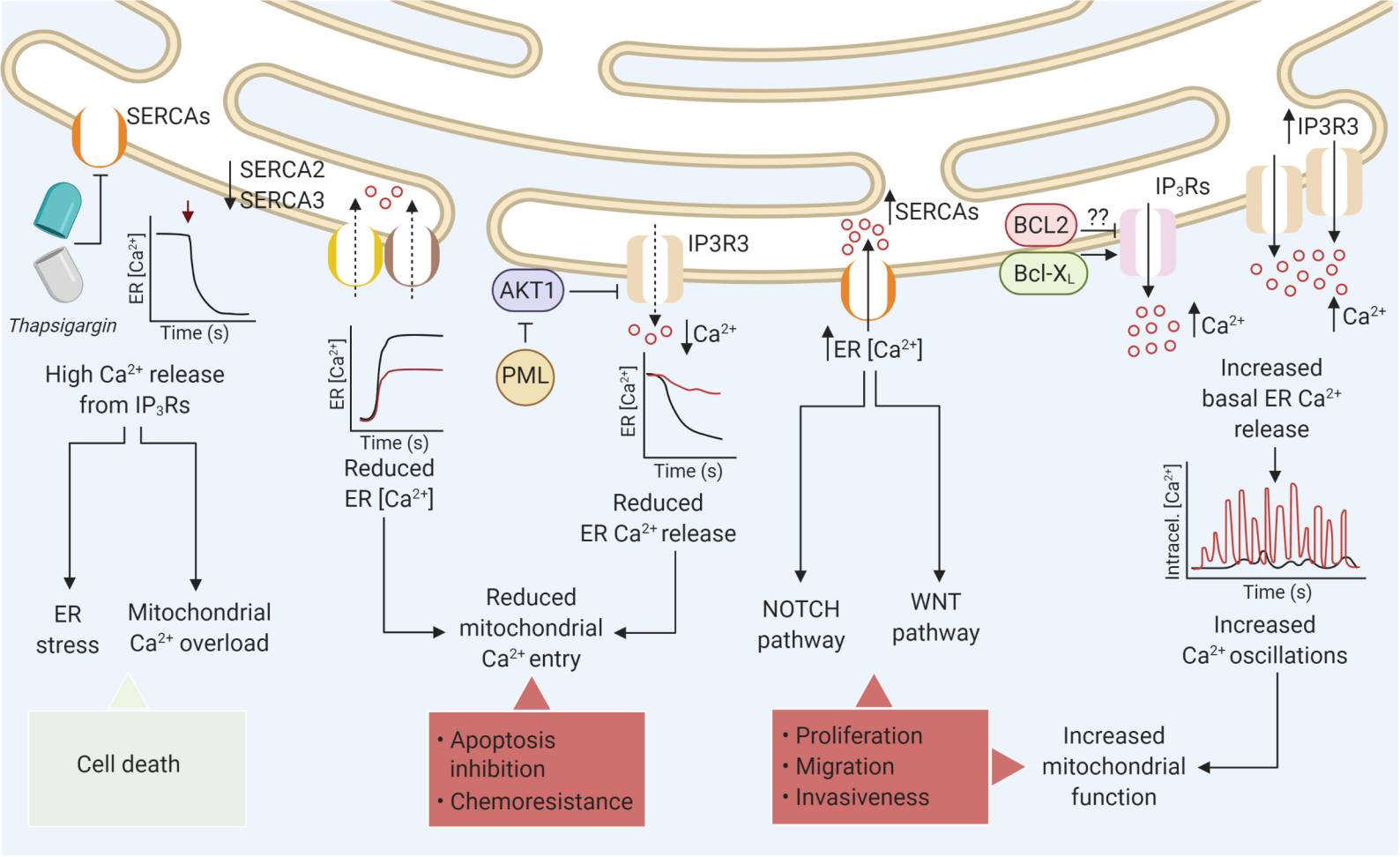

TRPA1
TRPM7
MMP secretion
TRPM3
Ca2+
Ca2+
Ca2+
Intracell. [Ca2+]
Time (s)
Increased intracellular Ca2+ entry
Intracell. [Ca2+]
Time (s)
Polarized Ca2+ flickers
• Proliferation
• Migration
• Invasiveness
CAMKK2
Autophagy
Cytoprotection
CALM1
PYK2
MCL1
• Apoptosis inhibition
• Antioxidant defense
Gene transcription
NRF2
Gene transcription
NFATC1
PYK2
• Proliferation
• Migration
• Invasiveness
NFATC1
Ca2+
SOCE
ORAI3
Mutated ORAI1
Ca2+
ORAIs
Intracell. [Ca2+]
Time (s)
SOCE
• Apoptosis inhibition
• Chemoresistance

Figure 2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SERCA2
SERCA3
Thapsigargin
ER [Ca2+]
Time (s)
High Ca2+ release from IP3Rs
ER stress
Mitochondrial Ca2+ overload
Cell death
ER [Ca2+]
Time (s)
Reduced ER [Ca2+]
AKT1
PML
IP3R3
Ca2+
ER [Ca2+]
Time (s)
Reduced ER Ca2+ release
Reduced mitochondrial Ca2+ entry
• Apoptosis inhibition
• Chemoresistance
SERCAs
ER [Ca2+]
NOTCH pathway
WNT pathway
• Proliferation
• Migration
• Invasiveness
BCL2
??
Bcl-XL
IP3Rs
Ca2+
IP3R3
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Intracel. [Ca2+]
Time (s)
Increased Ca2+ oscillations
Increased mitochondrial function

Figure 3

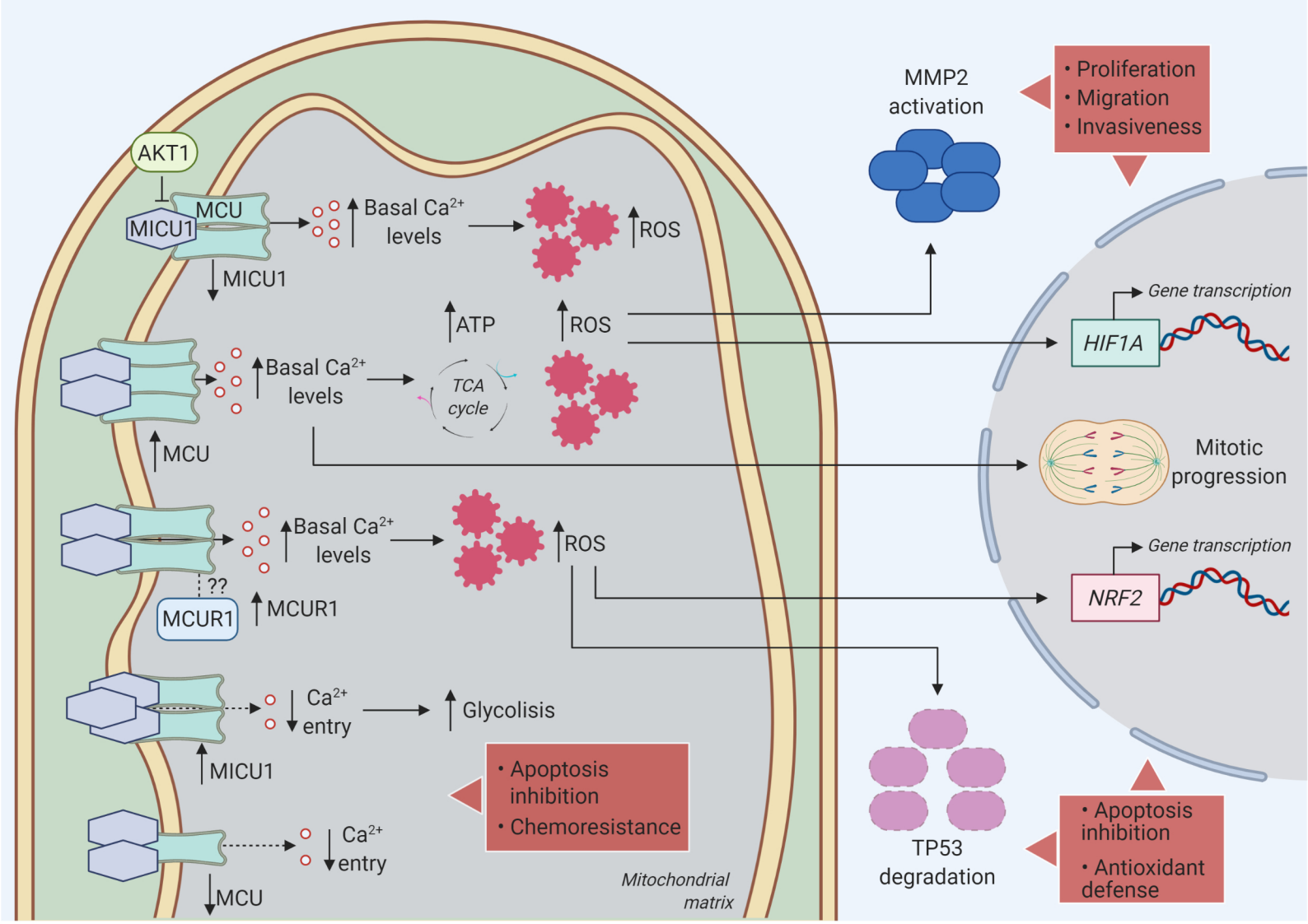
AKT1
MICU1
MCU
Basal Ca2+ levels
ROS
MICU1
ATP
ROS
Basal Ca2+ levels
TCA cycle
MCU
Basal Ca2+ levels
ROS
??
MCUR1
MCUR1
Ca2+ entry
Glycolisis
MICU1
Ca2+ entry
MCU
Apoptosis inhibition
Chemoresistance
Mitochondrial matrix
MMP2 activation
Proliferation
Migration
Invasiveness
Gene transcription
HIF1A
Mitotic progression
Gene transcription
NRF2
TP53 degradation
Apoptosis inhibition
Antioxidant defense

Figure 4

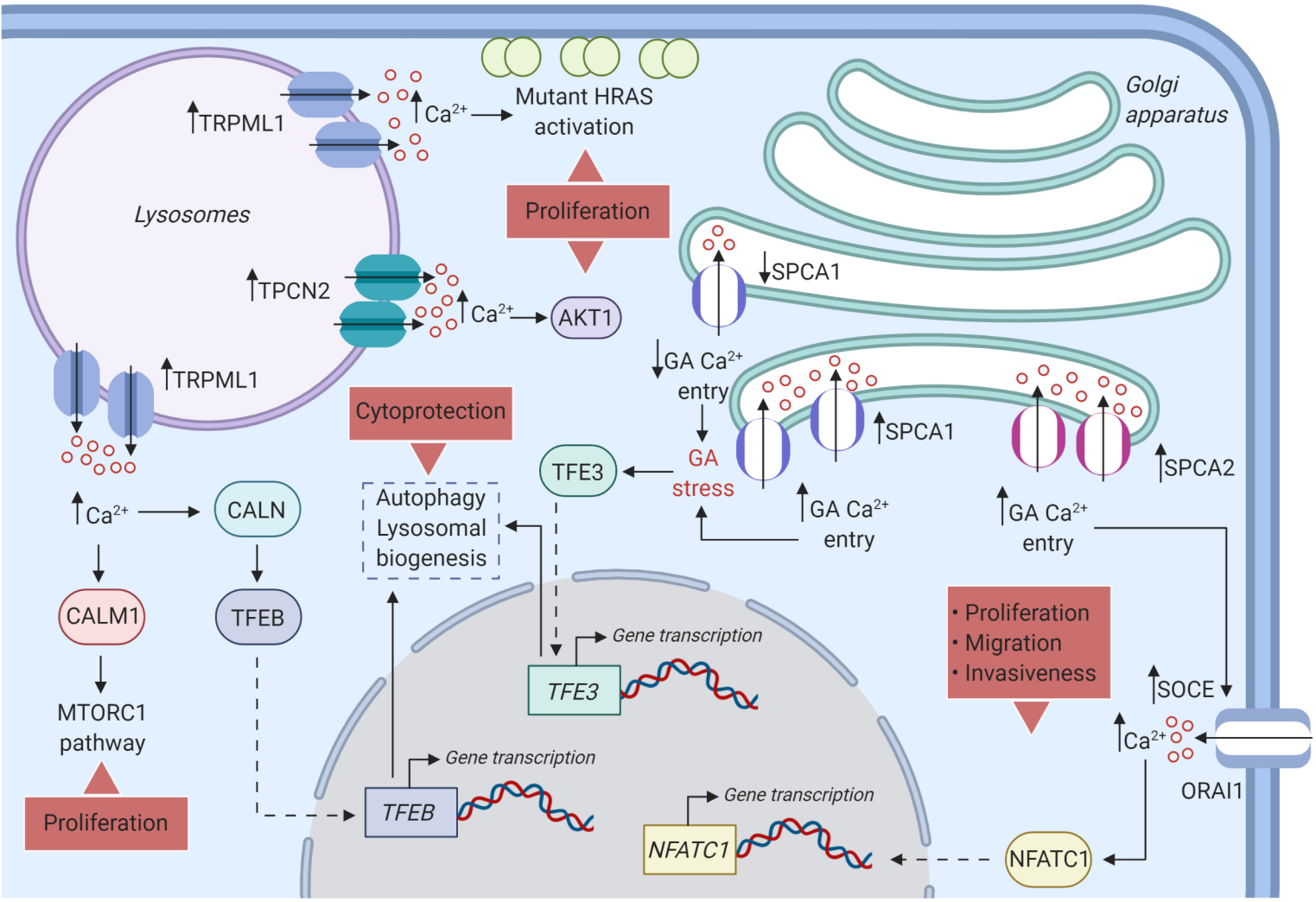
TRPML1
Ca2+
Mutant HRAS activation
Golgi apparatus
Lysosomes
Proliferation
SPCA1
TPCN2
Ca2+
AKT1
GA Ca2+ entry
TRPML1
Cytoprotection
SPCA1
SPCA2
TFE3
GA stress
Ca2+
CALN
Autophagy Lysosomal biogenesis
GA Ca2+ entry
GA Ca2+ entry
CALM1
TFEB
Gene transcription
TFE3
• Proliferation
• Migration
• Invasiveness
SOCE
MTORC1 pathway
Gene transcription
TFEB
Ca2+
ORAI1
Proliferation
Gene transcription
NFATC1
NFATC1

Figure 5