HHS Public Access
Author manuscript
Cell Calcium. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:
Cell Calcium. 2018 January ; 69: 46–61. doi:10.1016/j.ceca.2017.07.001.

SERCA Control of Cell Death and Survival

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Abstract

Intracellular calcium (Ca^{2+}) is a critical coordinator of various aspects of cellular physiology. It is increasingly apparent that changes in cellular Ca^{2+} dynamics contribute to the regulation of normal and pathological signal transduction that controls cell growth and survival. Aberrant perturbations in Ca^{2+} homeostasis have been implicated in a range of pathological conditions, such as cardiovascular diseases, diabetes, tumorigenesis and steatosis hepatitis. Intracellular Ca^{2+} concentrations are therefore tightly regulated by a number of Ca^{2+} handling enzymes, proteins, channels and transporters located in the plasma membrane and in Ca^{2+} storage organelles, which work in concert to fine tune a temporally and spatially precise Ca^{2+} signal. Chief amongst them is the sarco/endoplasmic reticulum (SR/ER) Ca^{2+} ATPase pump (SERCA) which actively re-accumulates released Ca^{2+} back into the SR/ER, therefore maintaining Ca^{2+} homeostasis. There are at least 14 different SERCA isoforms encoded by three *ATP2A1-3* genes whose expressions are species- and tissue-specific. Altered SERCA expression and activity results in cellular malignancy and induction of ER stress and ER stress-associated apoptosis. The role of SERCA misregulation in the control of apoptosis in various cell types and disease setting with prospective therapeutic implications is the focus of this review. Ca^{2+} is a double edge sword for both life as well as death, and current experimental evidence supports a model in which Ca^{2+} homeostasis and SERCA activity represent a nodal point that controls cell survival. Pharmacological or genetic targeting of this axis constitutes an incredible therapeutic potential to treat different diseases sharing similar biological disorders.

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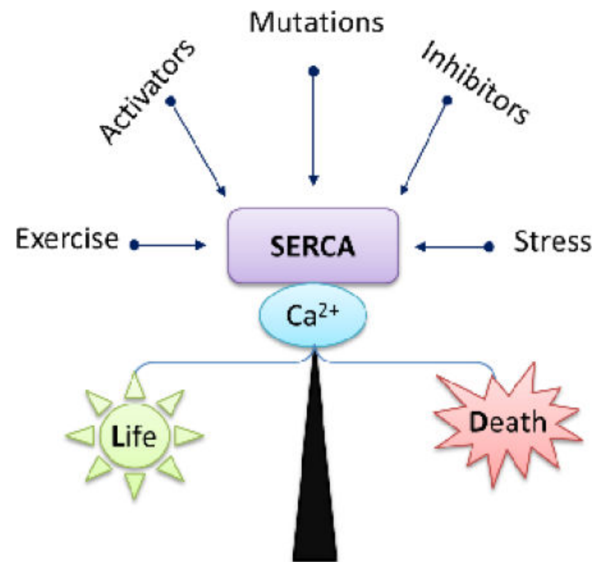
Graphical abstract



Keywords

Calcium; SERCA; SERCA isoforms; Apoptosis; Cell death; ER stress; Cardiovascular diseases; Diabetes; hepatostatosis; Cancer; SERCA Therapies

1. Introduction

1.1. Basic processes of cell death

Three distinct processes of cell death have been recognized to this day: apoptosis, autophagy and necrosis [1] (Figure 1). Autophagy and apoptosis are interrelated since autophagy, and especially mitophagy, can either promote cell survival or enable apoptosis [1]. Furthermore, both autophagy and apoptosis can follow endoplasmic reticulum stress in addition to other death signals. Simultaneous inhibition of both autophagy and apoptosis can lead to cell survival or cell death by necrosis. Necrotic cell death can also occur when autophagy is activated in apoptosis-incompetent cells [1] (Figure 1). The central role of calcium (Ca^{2+}) in cell death and survival is explored in the present issue of *Cell Calcium;* the present review focuses on the specific role of the Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) in cell death and survival (Figures 1 and 2).

1.2. Endoplasmic Reticulum (ER) stress: role of the B-cell lymphoma 2 (Bcl-2) Ca^{2+} rheostat in cell death and survival

The ER plays a critical role in Ca^{2+} handling, protein synthesis and protein processing [2–5]. Impairment of these functions occurs in various pathological conditions resulting in the accumulation of misfolded proteins in the ER, which initiates the ER stress response [6–8]. ER stress triggers the unfolded protein response (UPR) and protein degradation pathways, such as autophagy and apoptosis. The UPR is initiated in response to unfolded proteins and is initially adaptive and pro-survival, but progresses to apoptosis when ER stress becomes

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chronic, irreversible, and when the UPR is ineffective [6–9]. The B-cell lymphoma 2 (Bcl-2) protein family is a central part of protein complexes that modulate the response to ER stress, with apoptosis and autophagy as the possible end-results [2, 10–12]. Bcl-2 is thus described as a rheostat [2] belonging to a large family of proteins comprising pro-apoptotic and anti-apoptotic molecules [2, 12]. The pro-apoptotic members of the Bcl-2 family trigger mitochondrial outer membrane permeabilization (MOMP), leading to the release of cytochrome c and to the assembly of the apoptosome [13–15]. The pro-apoptotic members of Bcl-2, PUMA and NOXA, are BH-3 only proteins participating in ER stress-induced apoptosis in a p53-dependent manner [16]. The CCAAT-enhancer-binding protein homologous protein (CHOP) is induced by ER stress and mediates apoptosis [6]. PUMA was shown to be induced by CHOP, while NOXA is an additional apoptosis mediator induced by the Activation of Transcription 4 (ATF4) [2, 17, 18]. CHOP induces apoptosis by a variety of mechanisms [6]. In particular, CHOP induces the expression of ER oxidoreductin 1α (ERO1α), which activates the ER Ca^{2+} release channel inositol 1,4,5-trisphosphate receptor 1 (IP_3R1) [6]. CHOP also upregulates the pro-apoptotic protein BIM and down-regulates the pro-survival protein Bcl-2 [18].

More complex roles are played by Bcl-2 family members, such as IRE1α (Inositol-Requiring transmembrane kinase/Endonuclease α) which is stabilized by Bax/Bak, two pro-apoptotic members of the Bcl-2 family [2, 19]. In this regard, it has been shown that in apoptosis, Bax and Bak translocate to the ER membrane and promote Ca^{2+} release from the ER lumen [15, 20], either by allowing Ca^{2+} exit through the Bax/Bak oligomerization-formed ionic pores [21] or indirectly by favoring IP_3R opening [13]. In turn, Ca^{2+} leakage from ER recruits more Bax molecules from the cytosol to ER membranes [20], further amplifying the apoptotic signal [22]. Furthermore, earlier studies have addressed the more complex role of Bax and Bak in apoptosis related to ER-mitochondria Ca^{2+} signaling [23]. Bak and Bax double knockout cells had reduced ER Ca^{2+} stores available for ER Ca^{2+} release and mitochondrial Ca^{2+} uptake, making the cells less prone to apoptosis induced by ER Ca^{2+} release; the latter phenotype was reversed by SERCA expression [23]. In the same study, Bax targeting to mitochondria selectively restored apoptosis to "BH3-only" signals [23].

The fine balance between pro- and anti-apoptotic proteins expression at the ER membrane determines the ER Ca^{2+} content along with Ca^{2+} release from ER to cytosol and mitochondria under stimulation [2], thus allowing the Bcl-2 family of proteins to exert a “fine-tuning rheostat” function [2]. In addition to Bax, Bak and Bcl-2, the Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3), when localized at the ER membrane, facilitates ER Ca^{2+} release and mitochondrial Ca^{2+} uptake, and subsequent apoptosis [24, 25]. Multiple specific actions of Bcl-2 family proteins on Ca^{2+} signaling related to apoptosis have been described [23, 26–31]. In line with their role in Ca^{2+} related apoptosis, proteins from the Bcl-2 family also modulate autophagy [1, 2]. Interestingly, Bcl-2 inhibits IP_3R-induced autophagy but not IP_3R-indepdent autophagy induced by ER stress [2].

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1.3. Overview of Calcium homeostasis and the importance of Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA)

Complex spatio-temporal Ca^{2+} signals regulate a multiplicity of cellular processes [3, 32, 33]. Cellular Ca^{2+} homeostasis involves a wide variety of proteins and transporters acting in coordination [3, 34] (Figure 2). Furthermore, numerous diseases are associated with abnormal Ca^{2+} transport [3]. Ca^{2+} ions are present in low concentrations in the cytosol, but high extracellular concentrations and stored in intracellular stores, mainly the Sarco/Endoplasmic Reticulum (SR/ER), the Golgi, the lysosome, the nucleus, in addition to the mitochondria [32–38]. Calcium movements along these concentration gradients are essential to various cellular processes, including muscular contraction and relaxation [34, 39, 40], metabolism, apoptosis, autophagy, proliferation and/or differentiation [32, 34].

The SR/ER is the main intracellular Ca^{2+} storage organelle, with a steady-state Ca^{2+} concentration of approximately 1mM, close to extracellular concentrations, and with significant heterogeneity in Ca^{2+} levels among its different regions [33, 41]. Ca^{2+} in the SR/ER lumen is buffered by Ca^{2+}-binding proteins such as calsequestrin, histidine rich Ca^{2+} binding protein (HRC), calreticulin and the Ca^{2+}-dependent calnexin [3, 39]. Ca^{2+} release from the ER/SR predominantly occurs via the IP_3R and the ryanodine receptor (RyR) [3, 39, 41]. Ca^{2+} release from the ER through IP_3R takes place under the action of two major stimuli: (1) the binding of IP_3 and (2) Ca^{2+} itself, the latter leading to Ca^{2+}-induced Ca^{2+} release [42], although higher cytosolic Ca^{2+} concentrations can inhibit IP_3R [43]. The role of IP_3R expands beyond the release of ER Ca^{2+} stores, and the complex regulation of IP_3R was extensively reviewed elsewhere [42]. A large variety of vital cellular events results from Ca^{2+} release from the ER through IP_3R [42]. The dynamics of the released Ca^{2+} signal leads to the activation of target enzymes such as calcineurin along with its downstream transcription factor NFAT (nuclear factor of activated T lymphocytes), a key regulator of cell growth, survival, and death [32, 34] (Figures 2 and 3).

Ca^{2+} clearance from the cytosol occurs through the plasma-membrane Ca^{2+} ATPase as well as the Na^+/Ca^{2+} exchanger [39] to the extracellular compartment and via SERCA sequestration of Ca^{2+} into the SR/ER. SERCA is the only active Ca^{2+} transporter from the cytosol to the SR/ER. SERCA proteins are coded by three *ATP2A1–3* genes located on 3 different chromosomes, transcribed and processed through alternative splicing into at least 14 SERCA mRNAs encoding a variety of SERCA isoforms. SERCA isoforms are species- and tissue-specific ([35]; reviewed in [34]), with SERCA1 isoform being expressed in adult and neonatal skeletal muscle, SERCA2a in cardiac muscle, SERCA2b ubiquitously expressed in smooth muscle and in all cell types, while SERCA3 has been found co-expressed with SERCA2b in selected cell types such as lung, endothelial cells, β-cells, and Purkinje neurons of cerebellum [44, 45]. Key characteristics of the various SERCA isoforms are presented comparatively in the Table [34, 46].

Store-operated calcium entry (SOCE) through the plasma membrane is another regulator of intracellular and ER Ca^{2+} that is activated in response to a depletion of ER Ca^{2+} stores (reviewed in [47]). Major components of SOCE are the ER Ca^{2+} sensor Stromal interaction molecule 1 (STIM1), and two channels, the transient receptor potential canonical 1 (TRPC1) and Ca^{2+} pore forming channel Orai [47] (Figure 2). Calcium release from the ER, via IP_3R

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[42], leads to ER Ca^{2+} depletion and subsequent Ca^{2+} entry into the cell through store-operated calcium channels located on the plasma membrane (reviewed in [47, 48]), and Ca^{2+} release-activated Ca^{2+} (CRAC) channels [48]. STIM1 is an ER-based Ca^{2+} sensing protein, which upon ER Ca^{2+} depletion, it couples with Orai channels - the pore-forming unit of CRAC - causing Ca^{2+} influx [48].

2. Calcium signaling in cell survival and cell death

The central role of Ca^{2+} signaling in cell death and survival was recently reviewed [32] and is summarized in figure 2. Ca^{2+} handling in the ER, particularly through SERCA, IP_3R and IP_3-induced Ca^{2+} release [41], along with Ca^{2+} handling in mitochondria [33] and lysosomes all play important roles in the regulation of cell survival, apoptosis and autophagy [32, 37] (Figure 2).

2.1. Role of Ca^{2+} transfer from ER to mitochondria in cell survival and cell death

The major role of the mitochondria in intracellular Ca^{2+} signaling was reviewed extensively elsewhere [33]. The ER and mitochondria physically interact through a domain of the ER called mitochondria-associated membranes (MAMs) [41, 49]. This interaction is important in apoptosis but also in multiple other physiological processes as outlined below and reviewed in more detail elsewhere [50]. Ca^{2+} moves between ER/SR and mitochondria through IP_3R and/or the RyR on the SR/ER side, the voltage-dependent anion channels (VDACs) located on the outer mitochondrial membrane (OMM), as well as the mitochondrial Ca^{2+} uniporter (MCU). MCU moves the Ca^{2+} from the mitochondrial intermembrane space to the mitochondrial matrix [33, 41, 43]. In addition, MCU has a low affinity for Ca^{2+} and can sense an increase in Ca^{2+} concentrations near the ER/SR-mitochondrial junctions, or near the plasma membrane within Ca^{2+} microdomains [33]. Mitochondrial Ca^{2+} uptake is critical to cell survival and to mitochondrial bioenergetics [43], and is critical to cell death, whether necrotic or apoptotic [33] (Figure 2). VDAC1 is linked to IP_3R through the molecular chaperone glucose-regulated protein 75 (GRP75) [41, 51] (Figure 2). It has been shown that palmitoylation was required to target VDAC1, VDAC2 and GRP75, among other proteins, to the MAM [52]. At the MAM, Ca^{2+} cycling between ER and mitochondria through IP_3R and SERCA activates the tricarboxylic acid cycle under resting conditions [50, 53], and lipid metabolism [50, 52]. However, mitochondrial Ca^{2+} overload results in apoptosis through the opening of the mPTP (mitochondrial permeabilization transition pore), release of cytochrome c and other pro-apoptotic factors [41, 50] (Figure 2). Therefore, low-level ER-mitochondria Ca^{2+} transfer maintains bioenergetic processes while excessive Ca^{2+} release from ER to mitochondria results in mitochondrial Ca^{2+} overload and apoptosis [43].

2.2 Fine regulation of ER Ca^{2+} content and IP_3R Ca^{2+} release by proteins of the Bcl-2 family

Ca^{2+} release from the ER through IP_3R, prompted by IP_3 and Ca^{2+} itself, is further modulated by other proteins through post-translational modification (e.g. phosphorylation), and through complex formation with regulatory proteins[3, 27, 42, 53], as reviewed elsewhere [42]. It has been shown that numerous proteins form complexes with IP_3R that

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can enhance IP_3R activity, inhibit IP_3R activity, act as downstream effectors of IP_3R, modify the subcellular distribution of IP_3R or play other roles [42]. ER mitochondrial Ca^{2+} transfer through IP3R and VDAC is the result of a fine balance between oncogenes such as Bcl-2, which promote cell survival by suppressing pro-apoptotic ER-mitochondrial Ca^{2+} transfer, while tumor suppressors like p53 stimulate ER-mitochondrial Ca^{2+} transfer [43]. In the particular case of apoptosis, the anti-apoptotic Bcl-2 inhibits Ca^{2+} release through IP_3R by interacting with IP_3R through the BH4 domain of Bcl-2 and exerts anti-apoptotic effects through this mechanism by preventing mitochondrial Ca^{2+} transfer through VDAC [26, 27]. However, another anti-apoptotic member of the Bcl-2 family, Bcl-XL, was shown to enhance IP_3-mediated ER Ca^{2+} release [27]. These apparently contradictory facts may reflect the finding that the oncogenes Mcl-1, Bcl-2 and Bcl-XL lower the ER store Ca^{2+} content by stimulating IP_3R outside of the MAM, increasing Ca^{2+} escape from the ER [26, 43].

Bcl-2/Bax family proteins are indeed central regulators of cell death in animals [31]. In a previous paragraph, we discussed the “rheostat” function of this family of proteins in the setting of ER stress and apoptosis. More specifically, Bcl-2 inhibits apoptosis through a variety of Ca^{2+}-dependent mechanisms. Bcl-2 reduces ER Ca^{2+} release and ER-mitochondria Ca^{2+} transfer, either directly [27] or by reducing ER Ca^{2+} stores through the stimulation of ER Ca^{2+} leak or the reduction of ER Ca^{2+} uptake by SERCA [35, 43]. Proteins from the Bcl-2 family also regulate VDAC and subsequently mitochondrial Ca^{2+} uptake. Overall, “Bcl-2 was proposed to convert apoptogenic high Ca^{2+} signals to pro-survival Ca^{2+} oscillations” [35]. The antiapoptotic proteins Bcl-XL and Bax-inhibitor 1 (BI-1) also reduce ER Ca^{2+} content, while the pro-apoptotic proteins Bak and Bax exert the opposite effect [31]. In particular, BI-1 induced passive ER Ca^{2+} leak and reduced thapsigargin-induced Ca^{2+} entry in the cytosol and mitochondria [31]. BI-1 action on ER Ca^{2+} leak occurred downstream of Bcl-XL, and did not require Bak or Bax [31].

3. Role of SERCA isoforms in cell death and survival

3.1. Modulation of SERCA activity in ER stress, cell survival and apoptosis

3.1.1 Proteins of the Bcl-2 family and p53 exert anti-apoptotic and pro-apoptotic actions through SERCA—Accumulating evidence supports a critical role for the Bcl-2 family members in modulating ER Ca^{2+} dynamics with different mechanisms proposed. Early studies have established that Bcl-2 lowers ER Ca^{2+} content and mitochondrial Ca^{2+} uptake, with one study showing ER Ca^{2+} leak without inhibition of SERCA [54]. However, this has been controversial, as others have shown that Bcl-2 in fact prevents Ca^{2+} leaking from the ER while maintaining ER Ca^{2+} store [55] potentially by increasing the expression of SERCA [56], and/or preventing IP_3R opening [26, 27, 57]. In subsequent studies, overexpression of Bcl-2 was shown to lower calreticulin levels ([28] and to inhibit several isoforms of SERCA: the skeletal muscle-specific isoform SERCA1a [58], the ubiquitous isoform SERCA2b ([28] and, more recently, SERCA3b [35]. This results in reduced ER Ca^{2+} stores; in the case of Bcl-2, SERCA inhibition is thought to promote cell survival, although excessive Bcl-2 expression led to cell loss, likely due to severe ER Ca^{2+} depletion associated with ER stress and apoptosis [28, 32, 35, 54, 59, 60]. Consistent with the findings on Bcl-2, studies of the pro-apoptotic members of the Bcl-2 family, Bak and

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Bax, have shown that these molecules promote ER Ca^{2+} release during apoptosis by favoring Ca^{2+} mobilization from the ER to the mitochondria thus enhancing cytochrome c release [15]. Interestingly, in double knock-out Bak/Bax cells, ER Ca^{2+} content was reduced, along with apoptosis, due to increased ER Ca^{2+} leak caused by Bcl-2-induced hyperphosphorylation and activation of IP_3R1 [23]. As a consequence, knockdown of Bcl-2 or IP_3R1 expression attenuated ER Ca^{2+} leak and maintained Ca^{2+} levels in knock-out Bak/Bax cells. Likewise, restoration of SERCA2b expression restored ER Ca^{2+} levels and apoptotic sensitivity in these cells [13]. These data imply that the ratio of the anti- and pro-apoptotic Bcl-2 members is critical in controlling ER Ca^{2+} dynamics. There is also growing evidence that the pro-apoptotic protein p53 also regulates ER Ca^{2+} concentration. p53 was shown to localize to MAMs and stimulate apoptosis in a "non-nuclear" manner by directly binding to and activating SERCA, thus promoting ER Ca^{2+} loading under stress conditions, subsequently increasing Ca^{2+} transfer from the ER to the mitochondria [16]. Overall, studies demonstrate that oncogene Bcl-2 decreases ER Ca^{2+} load by inhibiting the SERCA2 pump or by lowering SERCA2 expression levels, and that the pro-apoptotic protein p53 increases the ER Ca^{2+} store by stimulating SERCA pump activity [43]. Taken together, these findings point to a role of SERCA as an effector of apoptosis under specific stimuli.

3.1.2 Expression of truncated SERCA1 in ER stress and apoptosis—Aside from functional SERCA pumps, a truncated SERCA1 (S1T) was described and is characterized by exon 11 splicing and encodes a C-terminally truncated protein unable to pump Ca^{2+}, inducing ER Ca^{2+} leak [46]. It was shown that S1T was induced upon ER stress and increased the docking of mitochondria to ER and Ca^{2+} transfer from the ER to mitochondria [46], providing evidence that S1T was an essential factor in ER stress-related apoptosis, through mitochondrial apoptotic pathways [46].

3.1.3 Potential pro-apoptotic effects of SERCA1 in neonatal rat cardiomyocytes—In a study using adenovirus to over-express SERCA1 in neonatal rat cardiomyocytes (NRCM) and adult rat cardiomyocytes (ARCM), SERCA1 was pro-apoptotic in NRCM but not adult ARCM [61]. The findings were attributed to excessive SERCA activity, SERCA1 being more active than the cardiac-specific SERCA2a. The authors also observed a more marked increase in SERCA1 expression in NRCM, with a replacement of SERCA2a by SERCA1 in NRCM [61]. Overcrowding of the poorly developed SR membranes in NRCM with SERCA1 is also a possible explanation for the findings [61]. This study on SERCA1 was preceded by a study demonstrating the pro-apoptotic effect of SERCA2a expression in Cos cells [62], where SERCA2a over-expression was associated with both cellular and SR Ca^{2+} overload [62] eventually inducing apoptosis. However, these findings were not reproduced in cardiomyocytes, and were largely contradicted in concomitant and subsequent multiple SERCA2a gene therapy studies [63].

3.1.4. Darier's disease—Darier's disease is an autosomal dominant skin disease due to a loss of function mutation of SERCA2b, manifesting with hyperkeratosis [64]. Keratinocytes from Darier's disease patients, from SERCA2+/– mice as well as human keratinocytes treated with SERCA2-small interfering RNA (siRNA), showed an increased expression of

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the TRPC1, an increase in Ca^{2+} entry and a resistance to apoptosis [64]. Thus, in this particular model of SERCA2b deficiency, SOCE is activated and promotes cell survival [64].

3.2. SERCA expression, cell survival and apoptosis in tumor cell lines

Several studies have been published on the effects of SERCA expression and activity on cell survival, as well as apoptosis in a variety of tumoral cell lines. Increased SERCA2 expression was demonstrated in cancer cells and is thought to protect cancer cells from apoptosis [65]. Pharmacologic inhibition of SERCA by curcumin was shown to cause apoptosis in ovarian cancer cells; the pro-apoptotic effect of curcumin was specific to cancer cells and was attenuated on normal epithelial cells [65]. Curcumin exerted its pro-apoptotic effect through the rise of cytosolic Ca^{2+} [65]. Interestingly, curcumin increased the viability of peripheral blood mononuclear cells (PBMC), and did not increase cytosolic Ca^{2+} in PBMC, despite inhibition of SERCA [65]. Similarly, pharmacologic inhibition of SERCA by casearin J (CJ) induced apoptosis in T-cell acute lymphoblastic leukemia (T-ALL) cells [66]. In this study, CJ caused ER Ca^{2+} depletion through IP_3R, resulting in store-operated Ca^{2+} entry through CRAC channels [66]. Ca^{2+} release from the ER leads, subsequently, to Ca^{2+} entry into the mitochondria and oxidative stress [66]. CJ preferentially induced apoptosis in leukemia cells with deregulated Notch signaling, and other cell death mechanisms (i.e. necrosis) in chemo-resistant cells [66]. The study on CJ did not mention any isoform specificity, however [66]. Other SERCA inhibitors that were shown to inhibit the growth of cancer cells include kurahyne, cyclopiazonicacid (CPA), thapsigargin [67] and dihydroartemisinin [68]. In hepatoma cells, palmitic acid (PA) induced ER stress, apoptosis and a reduction in SERCA2b activity, mimicking nonalcoholic steatohepatitis (NASH) [69]. The hepatic stimulator substance (HSS) alleviated ER stress and apoptosis induced by PA, with an accompanying restoration of SERCA2b activity [69]. HSS further prevented the PA-induced release of Ca^{2+} in the cytosol [69]. In another study by Liu et al., ER stress-mediated apoptosis was induced by ceramide in human adenoid cystic carcinoma cells via disruption of ER Ca^{2+} homeostasis, with a down-regulation of the mRNA expression of SERCA2a, SERCA2b and SERCA3 [70].

Triptolide (TTL) was shown to induce apoptosis in the pheochromocytoma cell line PC12 through the up-regulation of SERCA3 and an increase in reticular Ca^{2+} [71]. TTL induced an up-regulation of SERCA3 expression, a down-regulation of RyR expression while the expression of IP_3R and other SERCA isoforms was unchanged [71]. Interestingly, in this study, apoptosis induced by TTL was prevented by the addition of the SERCA inhibitor thapsigargin but not by xestospongin C, an inhibitor of IP_3R [71]. In line with the pro-apoptotic effects of SERCA3 discussed earlier, the SERCA3f isoform, only found in the human SERCA3 family, was shown to promote ER stress when over-expressed in HEK-293 cells [72]. Furthermore, the expression of SERCA3f was increased in failing human hearts, in parallel with the expression of ER stress markers [73]. These observations are consistent with the role of SERCA3 in cellular differentiation, and with a down-regulation of SERCA3 in tumorigenesis [71].

The seemingly conflicting evidence from these studies seems to suggest an isoform-dependent effect of SERCA on apoptosis in tumor cells, SERCA2 being anti-apoptotic and

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favoring tumor growth while SERCA3 favors apoptosis and differentiation. A major limitation of at least some of these studies is the use of pharmacologic agents targeting several components of intracellular Ca^{2+} metabolism, in addition to SERCA.

3.3. SERCA expression, cell survival and apoptosis in anti-tumor immune cells

In line with the pro-apoptotic effects of SERCA3, a previous study by Ghosh et al. concentrated on the effect of SERCA3 on the anti-tumoral immunity effected by CD4(+) T-lymphocytes, in which SERCA2 and SERCA3 are co-expressed [74]. In that study, tumor cells secrete PGE2, leading to the up-regulation of SERCA3 transcription without change in SERCA2b levels. Enhanced SERCA3 is in turn associated with caspase activation and T cell apoptosis [74]. The down-regulation of SERCA3 expression by siRNA or by nifetepimine led to an increase in cytosolic Ca^{2+}, promoted T cell survival and allowed the restoration of anti-tumor immunity [74]. Nifetepimine downregulated the expression of SERCA3 but did not alter the expression of SERCA2b [74]. Nonetheless, doses of nifetepimine twice higher than the dose used to promote T cell survival were associated with decreased viability of CD4(+) T-lymphocytes and PBMC [74]. While CD4(+) T-lymphocytes were protected from tumor-induced apoptosis by nifetepimine, CD8(+) T-lymphocytes were not [74].

4. SERCA, Cell death and Survival in specific cell types

4.1. Cardiomyocytes and heart failure

A decrease in SERCA2a activity and Ca^{2+} uptake have been shown to be responsible for abnormal Ca^{2+} homeostasis in both experimental and human failing hearts [75, 76]. At the cellular level, depressed relaxation reflects impaired clearance of cytosolic/diastolic Ca^{2+} and reduced cardiac SR loading. This could be due to decreased SERCA2a protein levels and/or increased inhibition of it function by many regulatory peptides including phospholamban (PLN), sarcolipin (SLN), myoregulin (MLN) and dwarf open reading frame (DWORF) [77] [78–81]. Importantly, progression to heart failure, with the hallmark decline in cardiac function, has been associated with cardiomyocytes loss through activation of apoptotic pathways [77, 82]. This occurs through multiple signaling cascades, which include cytochrome c release from the toxically Ca^{2+}-overloaded mitochondria, activation of caspases, and protein and DNA degradation [83] (Figure 3).

Elevated intracellular Ca^{2+} also provides a stimulus for induction of ER stress, as well as cell death since a variety of kinases and signaling cascades are directly activated by Ca^{2+} or use Ca^{2+} as a cofactor. Indeed, an association between altered Ca^{2+} cycling and ER stress response has been reported in ischemic myocardium besides hypertrophic and human and animal models of failing hearts [84–86]. Overexpression of the ER stress regulator Activating Transcription Factor 6 (ATF6) in transgenic mouse hearts attenuated ischemic damage and improved contractility most likely through upregulation of GRP78, while a dominant-negative mutant of ATF6 leads to increased apoptosis, ventricular dilatation and reduced functional recovery followed by heart failure and death [87]. This is consistent with the finding that a decline in ER Ca^{2+} or myocardial infarction trigger the activation of ATF6 in cardiomyocyte, and upregulates its expression, respectively [88–90], further suggesting that ATF6 is essential in promoting cardiac protection in this context. This observation is

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interesting as ATF6 may serve as a potential regulator SERCA2a. SR/ER Ca^{2+} depletion in cultured cardiac myocytes promoted the translocation of ATF6 from the ER to the nucleus and the activation of the SERCA2a promoter, leading to augmented SERCA2a protein levels [91]. Likewise, Silent information regulator 1(Sirt1) was found to activates SERCA2a promoter in a dose-dependent manner in cardiomyocytes [92], indicating that the reported anti-apoptotic benefits of Sirt1 are SERCA2a-mediated. Conversely, SERCA2a activity can epigenetically be regulated by the tumor necrosis factor-α via enhancing the methylation of the SERCA2a promoter region in cardiomyocytes and subsequently compromising its expression together with its activity [93], further curtailing SERCA2a's intrinsic role in cell survival and Ca^{2+} cycling.

In addition, insulin and insulin-like growth factor 1 increased SERCA2a protein levels in cardiac myocytes and this increase was directly mediated by Akt [94]. As a consequence, myocytes from mice with overexpressed Akt demonstrated enhanced contractility and relaxation with increased protein expression of SERCA2a, suggesting that Akt signaling is involved in the modulation of SERCA2a and the pro-viability pathway. This is important since Akt is a powerful survival signal in many systems including the heart that blocks apoptosis insults [95–97].

Ischemia-reperfusion (I/R) injury is associated with ER stress-dependent apoptosis, including activation of C/EBP homolog protein (CHOP) and Caspase-12. This occurs in part as a consequence of increased intracellular (i.e. diastolic) Ca^{2+} overload during I/R. SERCA2a is reduced under ischemia injury. Using a model of I/R injury in the rat we demonstrated that SERCA2a overexpression significantly improved cardiomyocytes survival [98], indicating that a decrease in diastolic Ca^{2+} and better handling of intracellular ions during the rush of reperfusion are critical for the protection of the cardiomyocytes. These effects are specific to SERCA2a as forced expression of paravalbumin, a muscle-specific Ca^{2+} sink protein, failed to mitigate the I/R effects, and therefore had much less pronounced cardioprotection against I/R-induced cell death [98]. These findings corroborate our earlier data demonstrating improved survival and enhanced energetic state following SERCA2a overexpression in heart failure model [99], and are in sharp contrast to the findings noted earlier in COS cells [62] reporting pro-apoptotic effects of SERCA2a (section 3.1.3).

There are other protein partners reported to interact with SERCA with various degrees and modulate its activity, further disrupting its pro-survival characteristics. For instance, the HS1 associated protein X-1 (HAX-1), a mitochondria protein with antiapoptotic function, was also shown to localize to the SR and directly interact with PLN and regulates its activity via decreasing PLN phosphorylation state [100]. Although HAX-1 overexpression protected cardiomyocytes against hypoxia/reoxygenation-induced cell death [101], it also decreased SERCA2a Ca^{2+} affinity as well as depressed myocyte Ca^{2+} kinetics and mechanics [102]. HAX-1 binds directly to SERCA2a and regulates its expression levels but this interaction is less significant as HAX-1 appears to exert its action primarily on PLN; HAX-1 fails to translocate to the SR in the absence of PLN and the antiapoptotic effects of HAX-1, after hypoxia/reoxygenation-induced apoptosis, were only enhanced in the presence of PLN [100, 102]. These studies suggest that, as a mitochondrial protein, HAX-1 anti-apoptotic properties are favorably associated with reduced mitochondria Ca^{2+} load. As mentioned

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earlier, the anti-apoptotic protein Bcl-2 has been reported to directly interact with and inactivate SERCA1 [58], SERCA2 [28] and SERCA3 [35] although others have shown the opposite, an increase in SERCA2 expression following Bcl-2 overexpression in vivo [103]. Interestingly, Bcl-2 inactivation of SERCA was alleviated by the heat shock proteins HSP70, HSP90, HSP27 and crystalin [104]. S100A, a small EF-hand type Ca^{2+}-binding protein preferentially expressed in myocardial tissue, co-immunoprecipitates with SERCA2a in a Ca^{2+}- dependent manner and enhances its activity [105]. Acylphosphatase, a cytosolic highly basic enzyme of 11 kDa, was found to compete with PLN and attenuates its inhibitory action on SERCA2a [106]. ERp57, a ubiquitous ER thiol-dependent oxido-reductase, was demonstrated to associate with SERCA2b (but not SERCA2a) at higher Ca^{2+} concentrations resulting in inhibition of the pump with reduced Ca^{2+} oscillations, while depletion of ER Ca^{2+} displaces ERp57 from SERCA2b leading to stimulation of its Ca^{2+}-ATPase activity [107]. Silencing of the ER chaperone calnexin in cardiomyocytes resulted in induction of ER stress and initiation of apoptosis through disruption of Ca^{2+} cycling and SERCA2a reduced expression, highlighting a specific role for calnexin in cell survival and Ca^{2+} homeostasis [108]. Indeed, Roderick et al. have reported that under resting state calnexin binds to SERCA2b and inhibits its activity; however, under condition of ER stress such as ER Ca^{2+} depletion, calnexin is phosphorylated in a Ca^{2+}-dependent manner causing it to dissociate from SERCA2b thus freeing it for Ca^{2+} uptake [109]. Interestingly, palmitoylation of calnexin has been demonstrated to also regulate its localization on the mitochondria-associated membrane (MAM) and control its interaction with SERCA2a and other ER chaperone proteins [52, 53]. The role of calnexin in the regulation of ER Ca^{2+} signaling is largely dictated by ER homeostasis. Un-palmitoylated calnexin function preferentially as an ER quality control and protein chaperoning molecule in partnership with ERp57, but palmitoylation recruits it to MAM shifting its function towards the control and fine-tuning of Ca^{2+} delivery from the ER to the mitochondria through its interaction with and activation of SERCA2a [53]. This finding is somehow surprising and contradicts the findings of Roderick et al. [109] who reported rather an inhibitory effect of calnexin on SERCA2b. Although the explanation of this discrepancy is not clear, it is tempting though to speculate that phosphorylation [109] and palmitoylation [53] may influence calnexin differently. It is also possible that calnexin's effects are SERCA isoforms specific (2a vs. 2b, due to the fact that SERCA2b possesses a c-terminal extension tail which is absent in SERCA2a). Regardless, this remains to be determined.

4.2. Vascular Smooth Muscle Cells and Atherosclerosis

Vascular proliferative disorders such as atherosclerosis and hypertension are the most common causes of severe cardiovascular diseases. The proliferative response of vascular smooth muscle cells (VSMCs) is essential in injury recovery after coronary angioplasty and stent implantation. Although VSMCs are normally located in the arterial media and maintained in a contractile/quiescent state, injury or mechanical stress of arteries causes migration of VSMCs into the intima layer of the arterial wall, where they switch their phenotype and start to proliferate and synthesize extracellular matrix proteins, resulting in expansion of the arterial intima [110]. Chronic alteration in intracellular Ca^{2+} signaling plays an important role in neointima hyperplasia and vessel remodeling. In the VSMC the two SERCA isoforms 2a and 2b are present but their respective roles are still not elucidated.

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Ca^{2+} cycling in contractile/quiescent VSMCs requires the expression of the SERCA2a isoform, whereas Ca^{2+} cycling in proliferating/synthetic VSMCs is associated only with the ubiquitous isoform SERCA2b [111], suggesting that proliferation of VSMCs is associated with the loss of SERCA2a. Loss of SERCA2a in turn triggers a more sustained store-operated Ca^{2+} influx leading to the activation of the calcineurin-NFAT axis and restenosis. Therefore, SERCA2a gene transfer [112] or inhibition of the NFAT transcription pathway [113] normalized SR Ca^{2+} cycling and attenuated VSMCs proliferation and neointima formation, thus reducing the risk of atherogenesis.

Furthermore, experimental studies have shown that macrophage infiltration is involved in neointimal hyperplasia and atherogenesis with accelerated macrophage apoptosis as a major event driving lesion progression and plaque instability and necrotic core formation [114, 115]. As such, macrophage ER stress-associated cell death, caused by accumulation of toxic lipids in macrophage foam cells in the arterial wall, has been identified as an emerging underlying factor in the pathogenesis of atherosclerosis [115, 116]. For instance, insulin-resistant macrophages express high levels of CHOP, making them more susceptible to apoptosis striking while atherosclerosis-prone mice deficient in CHOP showed suppressed macrophage cell death and plaque necrosis [117]. Similarly, downregulation of insulin receptor signaling in macrophages and macrophages from diabetic mice displayed reduced SERCA2b mRNA and protein expression, in addition to activated ER stress pathways, apoptosis and ER Ca^{2+} stores exhaustion, partly due to attenuation of the MEK/ERK signaling cascade [118], suggesting a direct effect of SERCA2b dysfunction and ER stress in macrophage apoptosis. Furthermore, defective SERCA2b activity and alterations in Ca^{2+} signaling in macrophages in obesity have been shown to involve Ca^{2+}/calmodulin dependent-protein kinase II (CaMKII). Ca^{2+} overload triggered by lipid-mediated SERCA2b inhibition activates CaMKII in macrophages resulting in the activation of downstream apoptosis pathways, including mitochondrial permeabilization and release of cytochrome c, induction of NOX-mediated ROS production and CHOP induction, and activation of proapoptotic signal transducer and activator of transcription-1 (STAT1) [119, 120]. In this regard, mice with macrophage-specific deletion of CaMKII displayed less plaque necrosis [115].

Taken together, these data support a model in which Ca^{2+} homeostasis and SERCA2 activity represent a nodal point linking vascular remodeling and cell survival.

4.3. Hepatocytes and Hepatosteatosis

In the liver, cell death is induced by apoptosis and by caspase-independent necrosis. Signs of ER stress have been observed in many liver diseases where sustained ER stress leads to apoptosis and cell injury. In particular, perturbations in ER Ca^{2+} homeostasis are linked to apoptosis effectors since ER Ca^{2+} depletion inundates the cytosol with Ca^{2+} leading to mitochondrial Ca^{2+} accumulation and the triggering of mitochondrial permeabilization, reactive oxygen species (ROS) production and release of apoptosis executioners from mitochondria into the cytoplasm. Emerging evidence shows that a major cause of ER stress is attributed to a reduction in the SERCA2b function, as SERCA2b dysfunction leads to elevated cytoplasmic Ca^{2+}, causing ER stress-induced toxicity and cell death. In fact,

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restoration of SERCA2b expression in the liver of diabetic mice for instance reduced ER stress [121] [122], while SERCA2b silencing had the opposite effects [123]. Furthermore, inhibition of SERCA2b by agents such as thapsigargin results in activation of ER-stress response along with the simultaneous activation of apoptotic pathways within the ER and the mitochondria (Figure 4). Increasing SERCA activity maintains ER Ca^{2+} and thus ER function in spite of stressors. SERCA2 activation can sequester more cytosolic Ca^{2+} and prevent apoptosis induced by mitochondrial signaling [124]. As a consequence of elevation in intracellular Ca^{2+} concentration, many Ca^{2+}-dependent kinases and signaling cascades are activated. For instance, CaMKII, a Ca^{2+}-responsive kinase, is activated in obesity and induces ER stress [125, 126], resulting in heightened expression of the proapoptotic CHOP which promotes apoptosis through its effects on Bcl-2 family members and modulation of calcium handling [14, 124, 127]. CHOP-induced apoptosis was demonstrated to be primarily caused by depleted ER Ca^{2+} and is activated by CaMKII. However, it remains to be demonstrated what causally triggers apoptosis, a decrease in ER Ca^{2+} content or an increase in cytosolic Ca^{2+} concentration, although some reports favor depletion of ER Ca^{2+} as the cause of cell death [128, 129].

What causes SERCA2b reduction/dysfunction in hepatocytes is not well defined, however recent research has reported that, in the context of obesity and insulin resistance for instance, the total ER membrane content is reduced and disorganized likely leading to decreased SERCA2b expression and depletion of ER Ca^{2+} and activation of apoptotic pathways. Furthermore, changes in ER membrane fluidity caused by increased obesity-induced phospholipids incorporation into the ER membrane results in dysfunctional SERCA2b and amplification of ER Ca^{2+} imbalance [123, 130]. Exposure of hepatocytes to toxic levels of palmitate induced increased intracellular lipid accumulation and activation of ER stress associated with decreased ER Ca^{2+} content, augmented mitochondrial Ca^{2+} levels and accumulation of ROS, are events that all precede the onset of apoptosis [130]. The extensive accumulation of lipids in the hepatocytes not only destabilizes the fluidity of the endomembrane and subsequent inactivation of SERCA2b but also leads to increased peroxidation of fatty acids and generation of ROS, further resulting in the disruption of the ER membrane, amplification of ER stress and ER stress-mediated cell death. ROS in turn, with peroxidation, can directly target SERCA2b, further inactivating its function (Figure 4). The lipotoxic effects of palmitate on SERCA2b and ER stress were effectively prevented by treatment with free radical scavengers or the Ca^{2+} chelator BAPTA (1,2-bis(o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid), hence highlighting the sensitivity of SERCA2 to ROS [69, 130]. Using a small molecule SERCA2 agonist, we recently demonstrated that activation of SERCA2b in obese diabetic mice normalized ER Ca^{2+} dyshomeostasis, attenuated ER stress response and ER stress-induced apoptosis, as well as reduced hepatic steatosis [121], which is consistent with previous studies of short term SERCA2b overexpression by adenoviral gene transfer in the liver [69, 123]. The consequence of this lipid-mediated SERCA2b inactivation is the development of fatty liver, insulin resistance and diabetes [115, 116]. Nevertheless, there are other non-lipid-mediated mechanisms of SERCA2 dysfunction demonstrated in the context of other organs such as oxidation, nitrosylation and inflammatory cytokines associated with obesity (Figure 4).

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Whether these cytokines and the post-translational modifications are also active actors in the liver remains to be investigated.

4.4. Pancreatic β-cells and Diabetes

Like the liver, signs of ER stress have also been found in islets from mice [131] and human diabetic patients [132]. As described above, provoked increase in cytosolic Ca^{2+} and ER stress appears to have an implication on cell death and survival in the context of β-cell and diabetes as well. In fact, it is now recognized that obesity/lipotoxicity, oxidative stress and chronic high glucose all cause β-cell ER stress as well as ER luminal Ca^{2+} depletion [124, 133], leading to disruption of insulin signaling and induction of insulin resistance [134], resulting in the consequential β-cell mass decline and death, and progression to overt diabetes [135, 136]. Alterations in SERCA function and ER Ca^{2+} cycling also seem to underlie the mechanism of apoptosis in the β-cell. Several studies have reported significant reduction in pancreatic islet SERCA2b and SERCA3 expression and function under diabetic and inflammatory conditions [121, 122, 137–139]. Inhibition of SERCA2b by thapsigargin or cyclopiazonic acid in pancreatic β-cells initiates ER stress response, followed by cell death [139]. Moreover, we recently demonstrated that SERCA2b deficiency, combined with high fat died stress, led to increased basal cytosolic Ca^{2+} levels, decreased insulin secretion, reduced β-cell proliferation and β-cell mass, along with increased β-cell ER stress and death [131]. In addition, free fatty acids treatment activated ER stress in β-cell and triggered cell death likely through ER Ca^{2+} depletion and attenuation of SERCA2b activity and expression [140–143]. Importantly, ER stress-induced cell death in cultured β-cells was shown to be primarily caused by impaired SERCA2b function rather than increased IP_3R or RyR [144]. Indeed, SERCA2b gene transfer or treatment with the peroxisome proliferator-activated receptor-γ (PPARγ) agonist pioglitazone restored SERCA2b and SERCA3 expression and attenuated ER stress [138, 143] while treatment with small molecule SERCA2 activators significantly alleviated ER stress-induced cell death of β-cells [131]. These results suggest a critical and direct role for SERCA2b activity and the maintenance of ER Ca^{2+} homeostasis in β-cells apoptosis.

β-cells express three different SERCA isoforms, SERCA2a, -2b and -3 (a–c), with SERCA2b highly abundant and SERCA2a minimally expressed, while SERCA3 is half the level of SERCA2b in mouse islets [137, 138]. This is important as it implies that SERCA2b isoform is the most crucial regulator of basal ER calcium transport in β-cells [145]. Additionally, we demonstrated that overexpression of just SERCA2b in SERCA2 knockout INS-1 cells is sufficient to rescue Ca^{2+} homeostasis and ER stress-induced cell death [131]. The role of SERCA2a in β-cell biology is unclear, given its low expression levels. The role of SERCA3 on the other hand seems to be conflicting at best. Although single nucleotide polymorphisms in the SERCA3 locus have been linked independently to diabetes susceptibility [137], SERCA3-null mice exhibited normal glucose tolerance, normal basal cytosolic Ca^{2+} levels and no sign of ER stress [145, 146]. Importantly, we did not observe any compensatory upregulation of SERCA3 in our SERCA2-deficient mouse model or in the clonal SERCA2-null β-cell line, indicating that SERCA3 may not play a role in patterning β-cell Ca^{2+} handling and ER stress-mediated apoptosis [131].

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ER stress and mitochondrial dysfunction are closely associated and constitute two major defects of type 2 diabetes. Since the ER and mitochondria both store and functionally depend on Ca^{2+}, recent evidence indicates that disruption of Ca^{2+} homeostasis – as it occurs in diabetic conditions – induces ER stress and mitochondrial dysfunction [147, 148] (Figure 5). This ultimately leads to disruption of Ca^{2+} cycling and decreased energy status with lower ATP levels, resulting in reduced SERCA2b activity which further potentiates ER stress–mitochondria miscommunication, and activates ER stress-dependent and intrinsic mitochondrial cell death pathways in β-cells as well as diabetes [149, 150].

In the context of both type 1 and type 2 diabetes, there is an activation of inflammatory pathways and increased production of pro-inflammatory cytokines, including interleukins and interferons, with subsequent cytokine-induced Ca^{2+} disruptions and β-cell mass loss and death [124, 151]. SERCA2b is a direct target of cytokines in β-cells destruction. Immune cells-generated interleukin (IL)-1β in combination with interferon (IFN)-γ and/or tumor necrosis factor (TNF)-α stimulates nitric oxide ($NO^•$) production in pancreatic islets via NF-kB activation leading to β-cell elimination. $NO^•$ disturbs ER Ca^{2+} homeostasis and directly targets SERCA2b, first by suppressing its expression and activity [139, 151] through the inhibition of the transcription factor Sp1 [152], and second by interacting with superoxide anion to generate peroxynitrate which further inhibits SERCA2b via tyrosine nitration and reduces β-cell viability [139, 153–155] (Figure 4). However, $NO^•$ appears to play only a minor role in cytokine-induced apoptosis in human islets [156], suggesting that other undetermined pathways independent of $NO^•$ production are responsible for cytokine-induced apoptosis in this case. Of note, there is evidence that cytokines induce ER stress in mouse β-cells independent of $NO^•$ production [157] or in fact ER stress itself was not necessary for cytokine-induced β-cell death [158]. Furthermore, cytokines may also promote β-cell apoptosis through pathways other than SERCA2b, albeit still in response to a rise in cytosolic Ca^{2+}. Exposure to the proinflammatory cytokines IL-1β, IFN-γ, and TNF-α provokes a Ca^{2+}-activated, calcineurin-dependent dephosphorylation of the pro-apoptotic BH3-only protein Bad, causing β-cells to undergo apoptosis [159]

4.5. Podocytes and nephropathy/Hypertension

In diabetic nephropathy, advanced glycation end-products in podocytes lead to ER stress and Ca^{2+} release from the ER, and podocyte apoptosis [3]. The unfolded protein response is also activated in the tubules. Decreased IP_3R1 in the afferent arteriole and mesangial cell leads to smaller increase in cytosolic Ca^{2+} release in response to vasoconstrictors, promoting glomerular hyperfiltration and damage [3]. In a more recent study, using db/db diabetic mice treated with the natural compound astragaloside-IV, features of diabetes and diabetic nephropathy were found to be improved *in vivo* with restoration of SERCA2b activity and expression in the renal cortex [160]. In cultured podocytes, palmitate induced ER stress and a rise in intracellular Ca^{2+}, leading to podocyte apoptosis [160]; however, SERCA2b overexpression protected podocytes from palmitate-induced ER stress and apoptosis [160]. Moreover, astragaloside-IV was protective by increasing SERCA2b expression and activity [160]. Interestingly, similar effects were obtained with astragaloside-IV and with the PPARγ agonist rosiglitazone, suggesting a role for PPARγ in the effect of astragaloside-IV [160].

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5. SERCA and Cancer

As noted earlier, normal Ca^{2+} equilibrium is an important regulator of cell proliferation and differentiation. Disruption of Ca^{2+} homeostasis and signaling contributes to the development of cancer [161, 162]. As such, there is increasing evidence for the importance of SERCA pumps dysfunction in malignant transformation. SERCA2$^{+/-}$ mice develop squamous cell tumors. Loss or reduced SERCA expression and different mutations in SERCA isoforms, namely SERCA3 and SERCA2, have been linked to many cancers, such as lung, prostate and colon cancers, head and neck squamous cell carcinoma [163, 164]. In colon and gastric carcinomas, choroid plexus tumors and myeloid leukemia, SERCA3 was significantly reduced [165, 166]. Interestingly, exposure of colon and gastric cells to differentiation-promoting factors markedly upregulated SERCA3 expression and normalized cytosolic Ca^{2+} concentrations [167]. Similarly, other studies demonstrated that Epstein-Barr virus-induced immortalization of B cells leads to down-regulation of SERCA3 and remodeling of ER Ca^{2+} homeostasis [168], while progressive decrease in SERCA3 expression levels inversely correlation with tumor differentiation in colonic epithelium [165]. The human hepatitis B virus DNA has been shown to integrate into the SERCA1 gene in a liver tumor, resulting in multiple slice variants of mutated proteins that triggered increased ER Ca^{2+} depletion and apoptosis [169]. On the other hand, upregulation of SERCA2 was found in colorectal cancer cells, which may drive proliferation and migration; thus, inhibiting SERCA2 in this case would actually enhance cell differentiation and induction of apoptosis. This paradoxical behavior of the SERCA isoforms may be attributed to their Ca^{2+} affinity and transport characteristics and to the stages of tumor progression and type of cell or tissue affected [170]. Nevertheless, unlike the minor role it plays in β-cell biology discussed above, SERCA3 appears to be an integral player in the differentiation process of cancer cells. These observations suggest that SERCA3 in particular is a useful tool to study colon cell differentiation.

6. SERCA-related Prospective Therapies

Given the fundamental roles SERCA plays in biological systems and pathological states, a growing campaign to capitalize on the potential therapeutic effects of SERCA has been expanding. Efforts to either activate/restore or inhibit SERCA function have yielded promising initial results, and in some cases have led to pre-advanced clinical trials such as SERCA2a upregulation in heart failure and SERCA inhibition for advanced treatment of cancer. Given the importance and the recent advances in prostate cancer-specific SERCA inhibition using traditional drugs such as thapsigargin and its new prodrug G202, we will discuss in this section how manipulation of Ca^{2+} signals has led to success stories in overcoming cancer burden. The reader is also encouraged to refer to other reviews addressing this subject [170–173]. We will also try to discuss new emerging both biological and pharmacological approaches to modulate SERCA function in cancer as well as other disease conditions.

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6.1. Viral-mediated SERCA gene delivery

To date, pharmaceutical compounds have failed to ameliorate Ca^{2+} cycling defects in the myocardium; thus, approaches that use molecular targeting to correct such abnormalities have been pursued as new therapeutic modalities for patients with advanced heart failure. Improvements in delivery techniques and in vector technology, including the development of recombinant adeno-associated vectors, have allowed for safe, long-term, and efficient gene transfer to the myocardium. Following positive results of contractile improvement in pre-clinical large animal studies [174, 175], small-sized early-phase clinical trials of SERCA2a restoration via intracoronary delivery of adeno-associated virus type 1 (AAV1) in patients with advanced heart failure have shown great promise with a significant reduction of clinical events [176, 177]. Unfortunately, a subsequent larger multinational clinical trial has failed to duplicate the efficacy of AAV1-SERCA2a gene transfer reported in the early-phase studies and the number of adverse events in these trials were not different between the treated patients and the placebos [178, 179]. While several factors may explain the failed outcome in these trials, the staggering low level of viral infectivity observed in the human hearts may account for the large part of the negative results, less than 1% of cardiomyocytes being infected (only 20 to 561 copies of vector per milligram of DNA) compared to the viral uptake observed in pre-clinical animal models (20 000–350 000 copies of vector per milligram of DNA).

Studies in rodent and large animal models of pulmonary arterial hypertension (PAH) have shown that gene transfer of SERCA2a via intratracheal delivery of aerosolized AAV1 carrying the human SERCA2a gene (AAV1.SERCA2a) has decreased pulmonary artery smooth muscle cells proliferation and cyclin D1 expression leading to improvement in PAH symptoms [180].

6.2. SERCA Activators

Developing pharmacological therapies that directly target defective endogenous SERCA provides a novel approach to treat diabetes for instance and other conditions where SERCA function is compromised. Pharmacological agonists of SERCA are currently rare. We have recently demonstrated that the small molecule SERCA2 allosteric activator CDN1163 activated SERCA2b Ca^{2+}-ATPase activity, normalized liver ER Ca^{2+} dyshomeostasis *in vivo* and *in vitro*, improved hepatic steatosis and corrected multiple metabolic abnormalities associated with ER stress as well as mitochondrial inefficiency [121]. In addition, CDN1163 also attenuated ER stress in liver and pancreatic β-cells [121, 131] and inhibited ER stress-induced apoptosis in liver tissue leading to improvement in glucose tolerance along with dyslipidemia [121].

SERCA2a activity has been shown to be modulated by post-translational modification mechanisms (figure 4). Kho et al. found that sumoylation of SERCA2a was significantly reduced in cardiac tissue from heart failure patients and in mouse and pig models of heart failure. Restoring sumoylation levels appeared to increase the intrinsic activity of SERCA2a in failing cardiomyocytes and prolong its lifetime [181]. Likewise, sumoylation of the SERCA2a transcription factor Sp1 also led to an increase in SERCA2a transcription in the failing heart [182]. Interestingly, the flavonoid Luteolin has recently been shown to enhance

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SERCA2a expression and stability and to stimulate the upregulation of small ubiquitin-related modifier (SUMO) 1 [183]. In addition, Luteolin upregulated the ratio of Bcl-2/Bax, and caspase-3/cleaved-Caspase3, leading to reduction in apoptosis of failing cardiomyocytes [183]. These studies indicate that sumoylation might be a viable pathway to regulate cell death in cardiomyocytes; whether a similar process occurs in other tissues prone to apoptosis-driven failure is to be determined. Similar to sumoylation, the post-translational effect of glutathionaylation on SERCA has been recognized. Glutathionaylation of SERCA at cysteine 674 residue activates SERCA and increases Ca^{2+} uptake [184, 185]. However, under pathological conditions such as atherosclerosis, cysteine 674 residue is irreversibly oxidized, preventing SERCA glutathionaylation and activation [184, 185].

Recent work has provided evidence that SERCA activity is positively regulated by SR/ER transmembrane micropeptides and redox regulators. DWORF, a micropeptide predominantly expressed in mouse and human heart muscle, was demonstrated to bind to SERCA2a, increases SR Ca^{2+} load and enhances cardiac contractility. Mutant mice with a frameshift in DWORF sequence induced a reduction in SERCA2a function and Ca^{2+} clearance rates [81]. Mechanistically, DWORF binds to SERCA2a and shields it from the inhibitory effects of PLN, SLN and MLN peptides by displacing their binding to it (discussed below in 6.3). In this way, DWORF may functionally compete with PLN, SLN and MLN for the binding to the SERCA2a. Likewise, selenoprotein N (SEPN1), an oxidoreductase ER membrane protein, physically interacts with SERCA2b and stimulates its Ca^{2+} uptake activity [186]. SEPN1 enhances SERCA2 activity by reducing SERCA2's luminal loop (L4) cysteines that are hyperoxidized by ER oxidoreductin-1 (ERO1)-generated peroxides [186], while deletion of SEPN1 leads to depletion of ER Ca^{2+} and elevation of basal cytosolic Ca^{2+} content [187], a phenotype resembling that observed with calnexin deficiency discussed earlier.

Multiple studies have also reported other SERCA activation mechanisms. For example, adiponectin is able to mitigate I/R cardiac cell injury by upregulating SERCA2a and inhibiting ER stress [188]. Exercise and adiponectin gene therapy provided protection against skeletal muscle dysfunction in type 2 diabetes by upregulating SERCA1 [189]. Although the precise mechanism underlying adiponectin's activation of SERCA2a is not known, it is believed that it occurs through enhancement of PLN phosphorylation, thus relieving its inhibition of SERCA2a [188]. Resveratrol and histone deacytelase inhibitors increased SERCA3, but not SERCA2, expression in breast cancer cells leading to changes in Ca^{2+} transport activity and induction of apoptosis, although the mechanisms by which they upregulate SERCA is not well defined [190]. The reported beneficial effect of short chain fatty acids, such as n-butyrate, in preventing colon carcinogenesis has been attributed to their ability to inhibit histone deacytelases [191]. Given the pro-SERCA influence of the histone deacytelase inhibitors, it is conceivable to speculate that short chain fatty acids also work by restoring SERCA3 expression and inducing differentiation as well as apoptosis of colon and other cancer cells. Indeed, butyrate, valerate and a score of other butyrate-releasing pro-drugs are shown to effectively induce SERCA3 expression [170], further reinforcing the notion that SERCA is an integral player in the control of apoptosis.

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6.3. SERCA Inhibitors

Experimental evidence suggests that there is a macromolecular complex involved in the negative regulation of SERCA. The histidine-rich calcium binding protein (HRC) directly binds to SERCA2a and regulates its activity in cardiomyocytes. Studies in vitro and in transgenic mice overexpressing HRC in the heart showed depressed SERCA2a activity and SR Ca^{2+} uptake, leading to heart failure [77, 192]. Surprisingly, overexpression of HRC in ischemia injury model provided cardioprotection partially conferred by reduction in mitochondrial Ca^{2+} content and attenuation of apoptosis and necrosis [77].

PLN and SLN are two well established endogenous negative inhibitors of SERCA2a and function as critical regulators of contractility and disease [78, 79]. Both peptides directly bind to the cytosolic and/or transmembrane domains of SERCA2a lowering its affinity for Ca^{2+}, eventually leading to development of cardiac disease, including heart failure [77, 193]. More recently, myoregulin (MLN), another peptide that shares structural and sequence similarity with PLN and SLN, was identified to be specifically expressed in skeletal muscle in mice and human [80], and was shown to bind to SERCA and decreases Ca^{2+} uptake [80]. Interestingly, the fruit fly Drosophila melanogaster also expresses an inhibitory micropeptide called sarcolamban (SCL) that is found in cardiac and somatic muscle which directly binds to drosophila and human SERCAs [194]. SCL deficient flies displayed cardiac arrhythmias due to severe muscle contraction defects [194]. Although the distribution of PLN, SLN, MLN and SCL appears to be muscle-specific, recent work has identified and functionally characterized two SERCA-inhibitory transmembrane peptides in non-muscle cell types that share structural similarity with the muscle counterpart peptides [195]. Endoregulin (ELN) co-localizes primarily with SERCA3 isoform in the epithelial cells from liver, pancreas, lung, intestine and trachea, while another-regulin (ALN) predominantly distributes with SERCA2b in the salivary glands, brown tissue, heart, and epidermal epithelium [195]. ELN and ALN appear to bind to the same groove of the SERCA as PLN and form a stable interaction with it. Given the large degree of structural and functional similarities amongst the various SERCA inhibitory peptides, it is quite possible that some sort of functional redundancy may exist among these peptides. Regardless, considering the importance of the broad role of SERCA in maintaining Ca^{2+} homeostasis, these peptides will likely have profound consequences in muscle and non-muscle tissues in health and disease.

Other mechanisms known to modify the expression and activity of SERCA2 involve microRNAs, a class of small, non-coding RNAs that act as post-transcriptional repressors of target genes by antisense binding to 3′-untranslated regions (3′-UTRs) of target mRNAs, resulting in mRNA degradation and/or translational repression [196]. MicroRNA-25 for instance has been identified as a direct negative regulator of SERCA2a expression in heart failure. Adenoviral overexpression of microRNA-25 resulted in 35% decrease in SERCA2a expression, while inhibition of miroRNA-25 *in vivo* produced a significant increase in SERCA2a abundance [197]. The finding that microRNA-25 expression is enhanced in human left ventricular samples from patients with severe heart failure at the time of cardiac transplantation [197] raises the prospect that measures to inhibit microRNA-25 in heart failure conditions may be of clinical benefit in this case.

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Acetylation, another form of post-translational modification, may regulate SERCA2a activity and influence Ca^{2+} cycling in a manner opposite to that conferred by sumoylation. Increased levels of SERCA2a acetylation in failing human hearts have been reported which are reversed by the NAD-dependent deacetylase sirtuin-1 [181]. Also, glycosylation has been demonstrated to influence SERCA2a function directly or through the regulation of PLN. Under diabetic or hyperglycemic conditions, SERCA2a glycosylation is increased resulting in reduced SERCA2a activity and diastolic Ca^{2+} clearance [198, 199]. Increased levels of glycosylation are associated with notable decreases in SERCA2a mRNA and proteins levels and enhanced PLN expression [198, 199]. The closely related glycosylation process of *O-GlcNA*cylation has also been shown to regulate SERCA2a expression in cardiac tissue and to negatively affect its function. Diabetes-induced *O-GlcNA*cylation caused significant decreases in SERCA2a protein expression and prolonged Ca^{2+} decay [198], possibly through the *O-GlcNA*cylation of the of the transcription factor SP1, known to transcriptionally controls SERCA2a expression [200]. Furthermore, under conditions of increased *O-GlcNA*cylation, PLN *O-GlcNA*cylation correlated with reduced PLN phosphorylation and augmented SERCA2a-PLN interaction in cardiomyocytes, further inhibiting SERCA2a function [201]. Conversely, reducing cellular *O-GlcNA*cylation decreased PLN protein expression and increased its phosphorylation in diabetic hearts [202], suggesting that lowering excess cellular *O-GlcNA*cylation has beneficial influence on SERCA2 activity.

Chemotherapy targeting SERCA has led to many initial promising results. Known selective SERCA pump inhibitors such as thapsigargin, cyclopiazonic acid as well as curcumin have been proposed and used as anticancer drugs [203, 204]. Thapsigargin in particular has been successfully tested as a potential targeted treatment for prostate cancer [173]. Sustained-thapsigargin inhibition of SERCA pump triggers cellular events leading to activation of apoptotic pathways within the ER and the mitochondria of cancer cells. However, thapsigargin would be toxic *in vivo* as SERCA is ubiquitously expressed in all cells. To circumvent this hurdle and obtain cell-specific effects, scientists have devised specific protease activated prodrug targeting strategies to deliver thapsigargin to prostate cancer cells. Thapsigargin derivatives have been covalently coupled to a protease-specific peptide carriers that can be restrictively hydrolyzed by the prostate-specific antigen (PSA) [205], a serine protease that is only secreted by prostate luminal epithelial cells [206] or the carboxypeptidase prostate-specific membrane antigen (PMSA) [207], a peptide highly expressed by a large fraction of prostate cancer cells [208, 209]. The PSA prodrug is inactive and would only become active in the prostate tumor microenvironment where the cytotoxic thapsigargin is liberated free from the inactive complex inhibiting SERCA locally and causing apoptosis of the prostate malignant cells [205]. G202, a PSMA-activated drug for instance, has been demonstrated to selectively eradicate PSMA-expressing cells *in vitro* and caused significant tumor regression in two mouse xenograft models of human prostate cancer and a model of human breast cancer [207]. Because of these successful findings and the low toxicity of G202, a human phase I clinical has been initiated in advanced cancer patients. The study has demonstrated the safety and tolerability of G202 in these patients [210]. Interestingly, a subset of patients suffering from hepatocellular cancer showed a prolonged period of disease stabilization [210], an observation that invigorated phase II trial

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in these patients, which is currently underway. In addition, a cell-based thapsigargin delivery platform has recently been developed. Human mesenchymal stem cells, generally known to preferentially home to cancer sites [211, 212] including prostate tissue [213], were loaded with the thapsigargin PSA-activated prodrug G114 [214], selectively induced apoptosis of the prostate cancer cell line LNCaP and inhibited tumor growth *in vivo* [215]. Regrettably, G114 has a limited half-life and unfavorable pharmacological properties.

Other novel chemotherapeutic agents, like the Bcl-2 inhibitor HA14-1, artemisinin, Saikosaponin-d, AlisolB, and 2,5-dimethyl-celecoxil all have been reported to induce autophagy, ER stress and apoptosis as a result of SERCA pump inhibition (reviewed in [171]).

7. Concluding Remarks

Given the central importance of Ca^{2+} and proper Ca^{2+} cycling in disease cases like diabetes, fatty liver, heart failure, atherosclerosis and cancer, SERCA-targeted therapies have emerged as viable approaches for alleviating these conditions.

The Paradox imposed by the differential expression of SERCA2/3 isoforms in different cancer cells for instance indicates that the modulation of SERCA expression is not as simple as it may look, and therefore the challenge on hand is to develop SERCA isoform-specific and less off-target small molecule modulators of SERCA function in disease specific setting. Besides specific pharmacological SERCA inhibition, SERCA isoforms switching may as well constitute a feasible strategy to induce or inhibit cancer cell differentiation and subsequent apoptosis. Cancer cells have managed to evade and bypass apoptosis and hijack the delicate Ca^{2+} architecture to their advantage; designing “smart SERCA signals” appears beneficial in alleviating the detrimental effect of cancer and other states of ER stress and apoptosis.

It is worthy to note that although our discussion was entirely focused on SERCA pumps, other Ca^{2+} regulatory proteins are also involved in one way or the other in the life and death cycle of the cell, although the magnitude and the mechanisms of involvement depend on the cell type. Therefore, we do not ignore the fact that there is a great degree of cross-talk between SERCA pumps and other Ca^{2+} channels, pumps and transporters in fine-tuning the calcium signal in matters of life (health) and death (disease).

Acknowledgments

This work was supported in part by grants from the National Institutes of Health, HL097357, DK100624 and DK020541 (to DL).

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Highlights

- Changes in cellular Ca2+ dynamics contribute to the regulation of cell growth and survival.
- Temporal and spatial Ca2+ signals are therefore tightly fine-tuned by a variety of Ca2+ handling enzymes, channels and transporters, including the sarco/endoplasmic reticulum Ca2+ ATPase pump (SERCA) which is responsible for Ca2+ uptake.
- Mutations and intrinsic/extrinsic factors modulate SERCA function. Altered SERCA expression and activity leads to elevated cytoplasmic calcium, resulting in cellular malignancy and induction of ER stress and ER stress-associated apoptosis and organ damage.
- Ca2+ homeostasis and SERCA activity represent a nodal point that controls cell survival. Pharmacological or genetic targeting of this axis constitutes a great therapeutic potential for many diseases.

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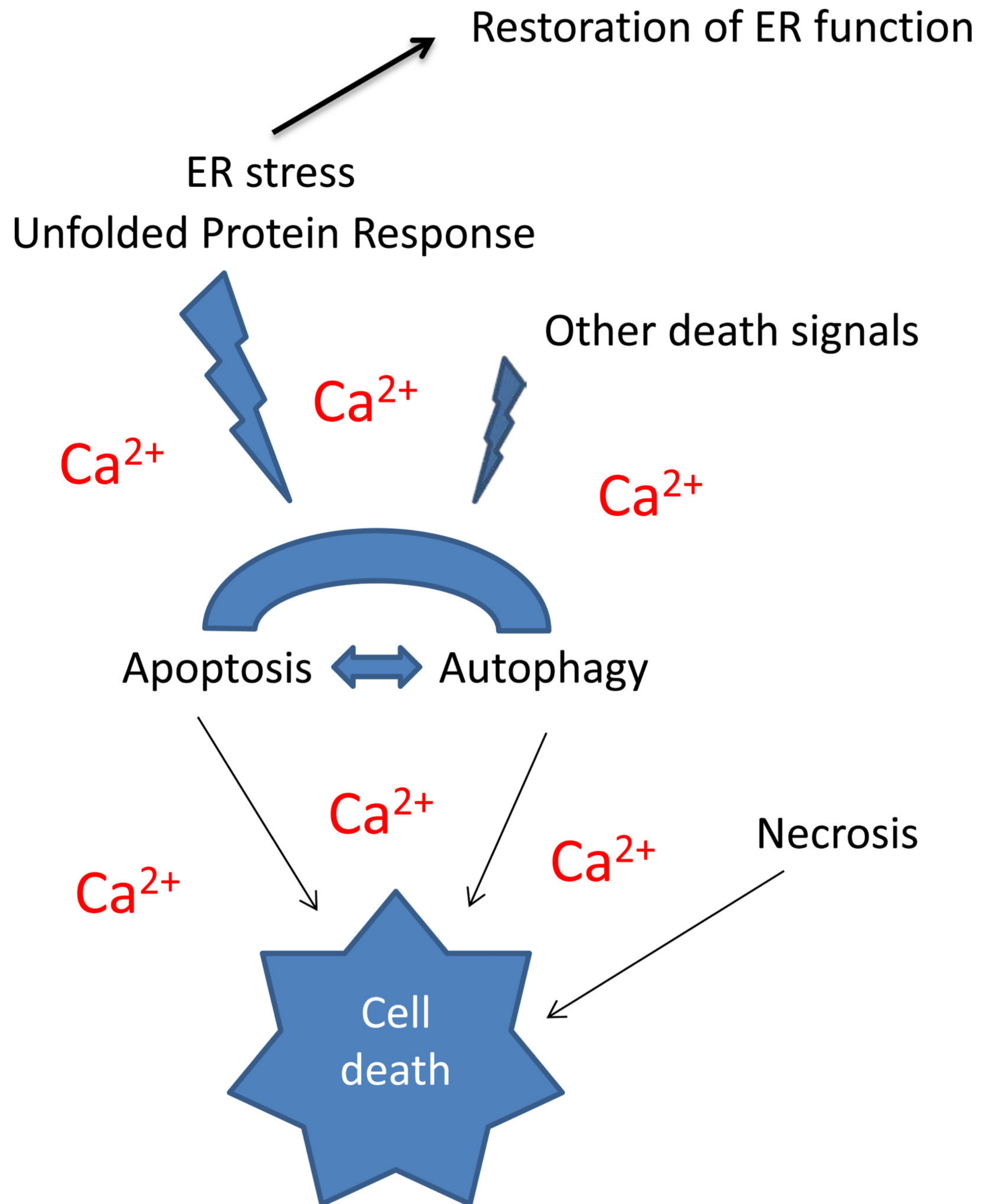

Figure 1. Basic processes of cell death

Cell death occurs through three different means: apoptosis, autophagy and necrosis

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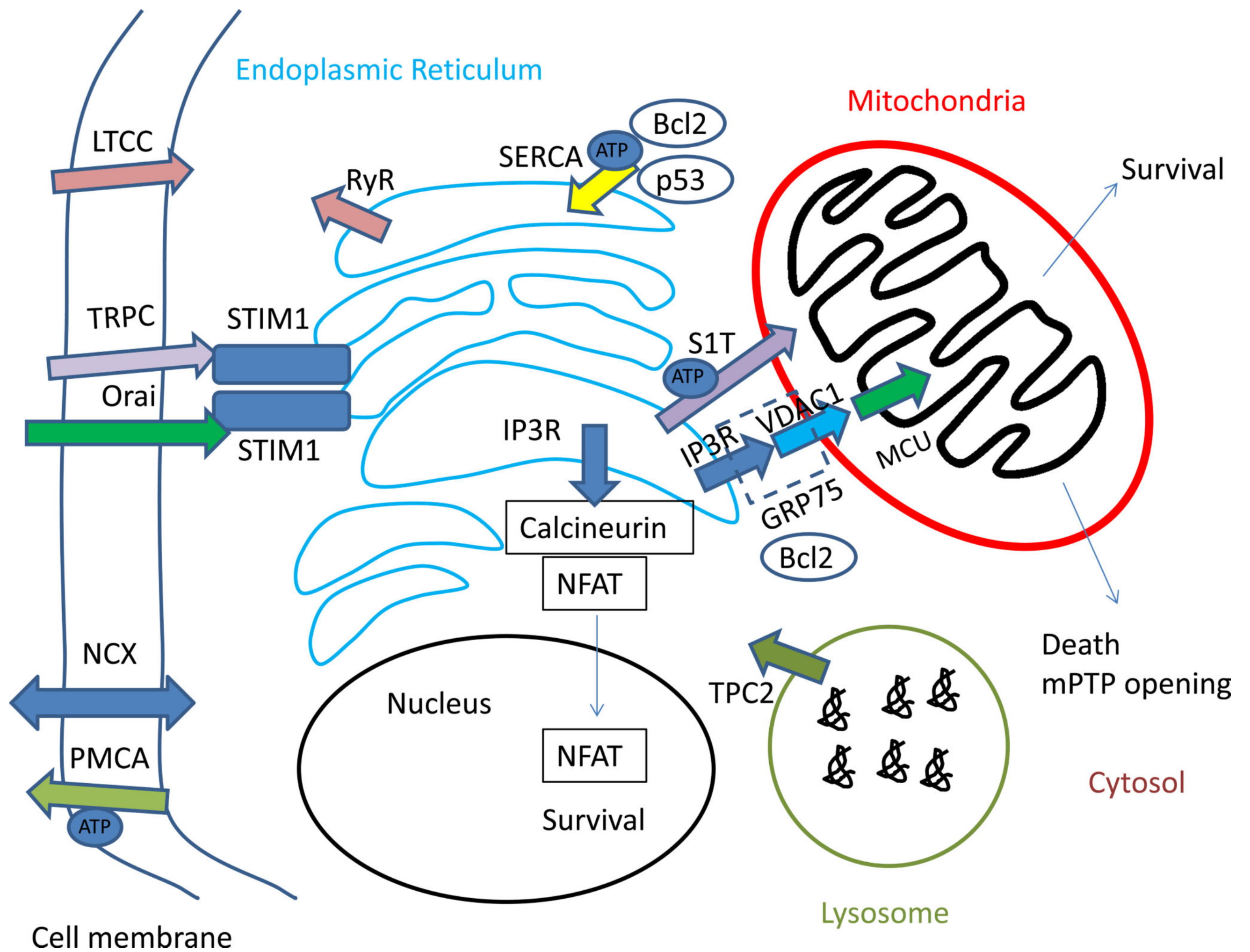

Figure 2. Overview of intracellular Ca^{2+} signaling and its implications in cell death and survival

Major organelles and players regulating Ca^{2+} influx and efflux during the process of cell death. and Bcl-2, B-cell lymphoma 2; GRP75, glucose-regulated protein 75; IP_3R, inositol1,4,5-trisphosphate (IP_3) receptor; LTCC, L-type Ca^{2+} channel; MCU, mitochondrial Ca^{2+} uniporter; mPTP, mitochondrial permeabilization transition pore; NCX, Na^+/Ca^{2+} exchanger; NFAT, nuclear factor of activated T lymphocytes; PMCA, plasma-membrane Ca^{2+} ATPase; RyR, Ryanodine Receptor; SERCA, Sarco/Endoplasmic Reticulum Ca^{2+} ATPase; STIM1, Stromal interaction molecule 1; TPC2, two-pore channel 2; TRPC, transient receptor potential canonical; VDAC, voltage-dependent anion channel. See text for further explanations

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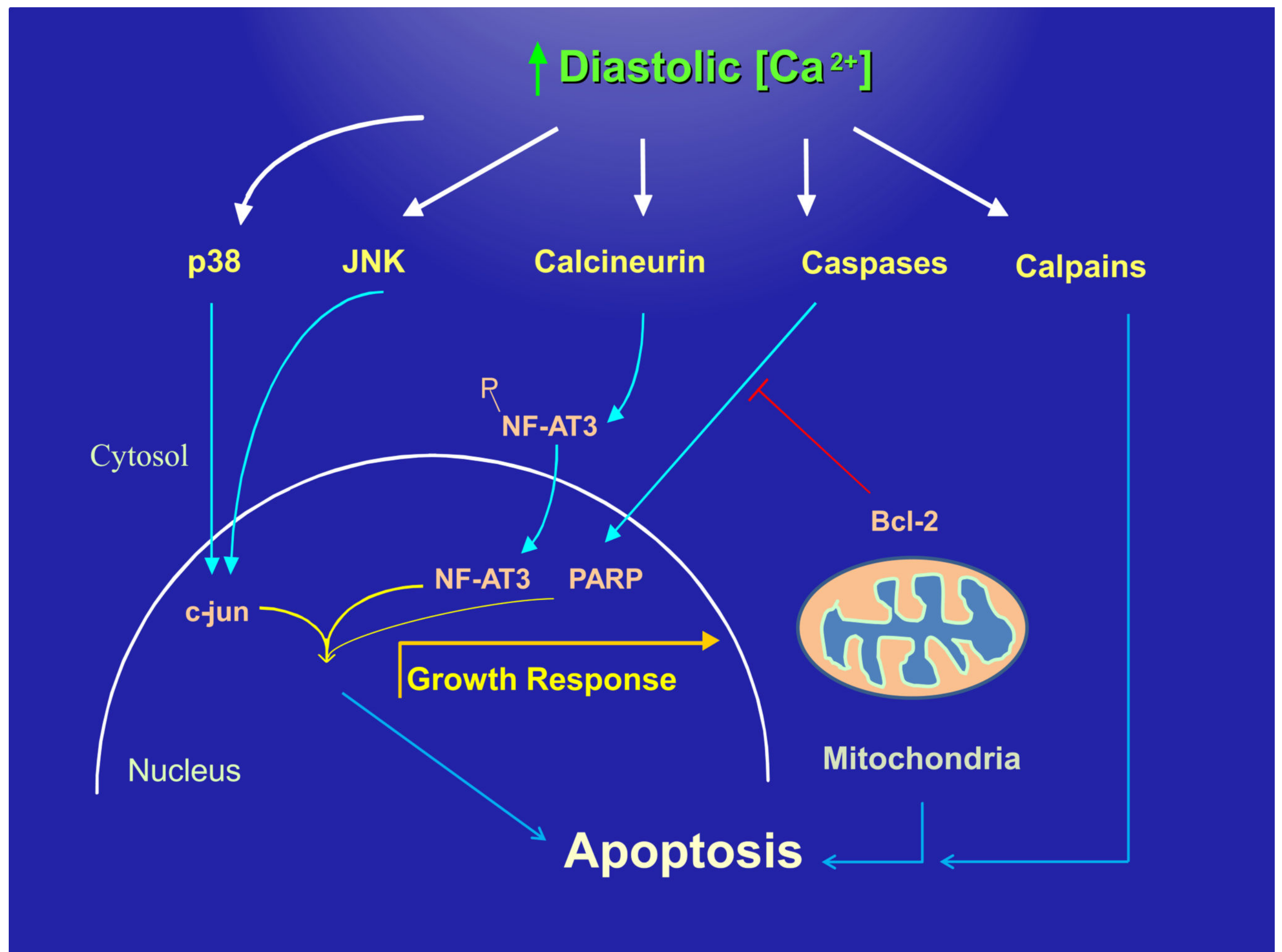

Figure 3. Consequences of elevated diastolic Ca^{2+}

Increased cytosolic/diastolic Ca^{2+} activates multiple Ca^{2+}-dependent kinases and proteases and triggers a cascade of signaling pathways that regulate cell growth, survival and death. Mitochondrial Ca^{2+} overload results in apoptosis through the opening of the mPTP. Mitochondria-associated Bcl-2 plays a pro-survival role.

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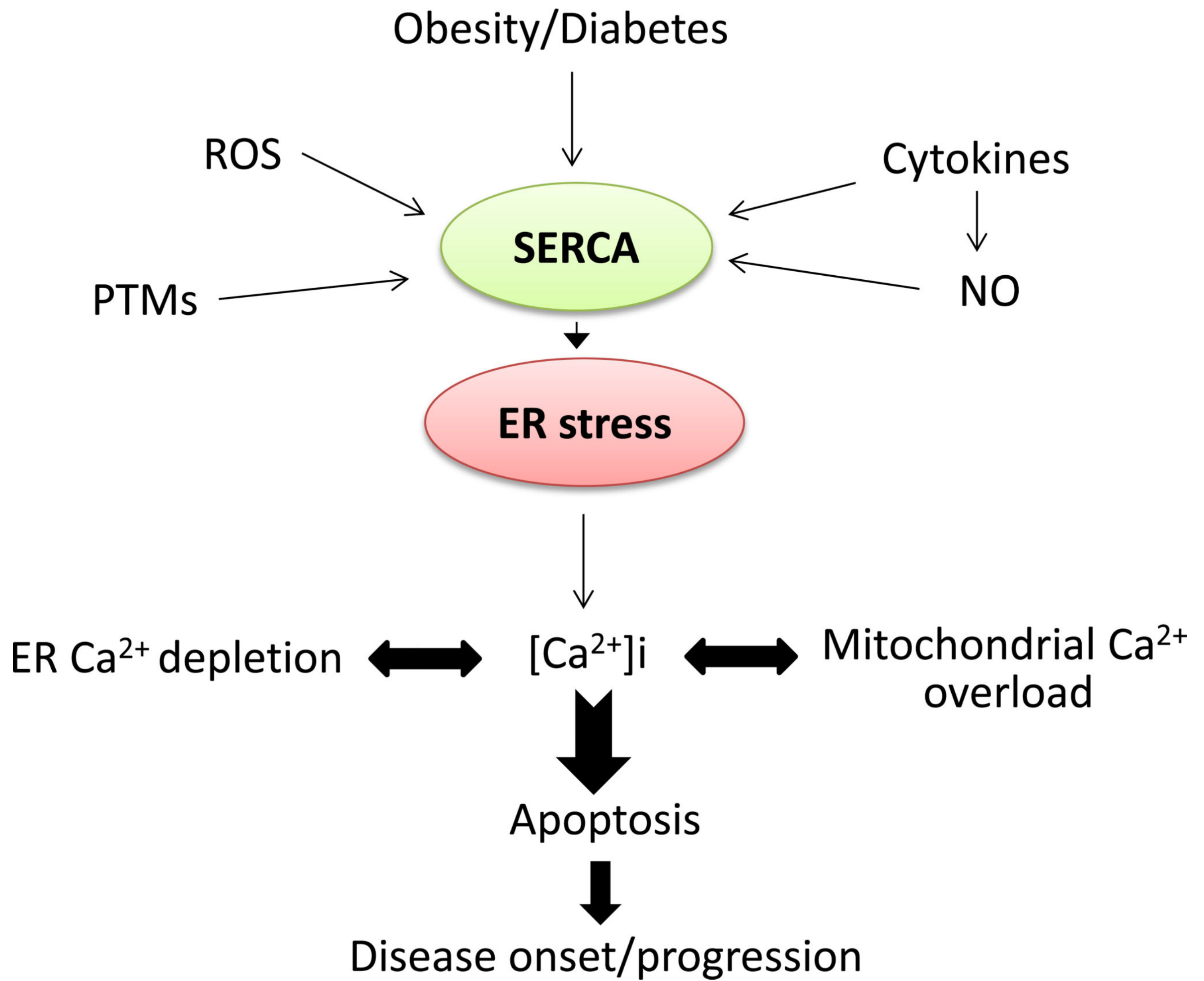

Figure 4. ER stress-induced toxicity and cell death

External factors such as obesity, reactive oxygen species (ROS), post-translational modifications (PTMs), cytokines and nitric oxide (NO) negatively regulate the activity and/or expression of SERCA leading to ER stress activation and cytosolic Ca^{2+} rise and initiation of apoptosis and organ damage.

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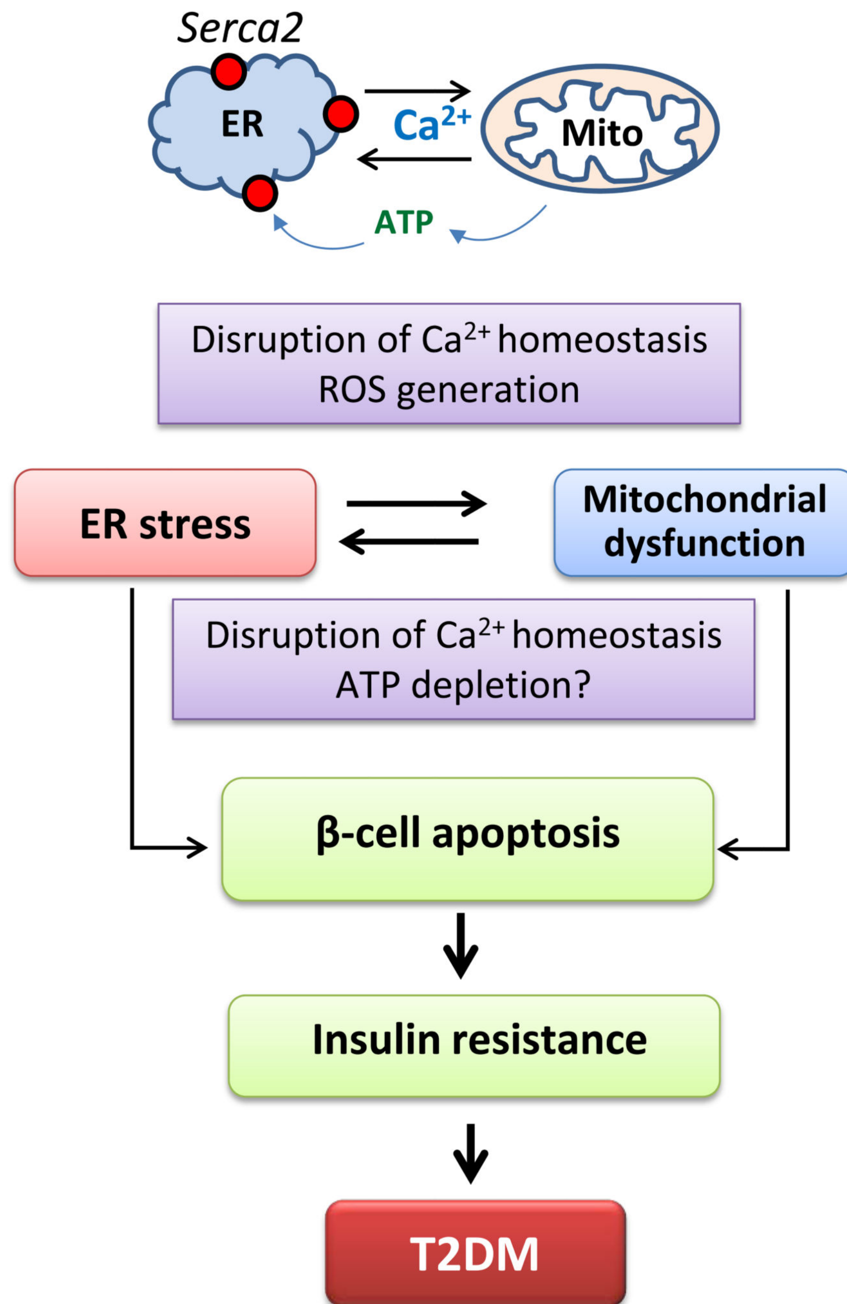

Figure 5. Interaction between ER and Mitochondria

Ca^{2+} is dynamically cycled between the ER and mitochondria; Serca2 utilizes ATP generated by the mitochondria to clear up cytosolic Ca^{2+} accumulation. This cycle is disrupted under pathological conditions. The ER and mitochondria are structurally and functionally connected. Functional defects in either organelle contribute to insulin resistance and diabetes (type 2 diabetes, T2DM) through induction of β-cell death and mass decline.