Biochimica et Biophysica Acta 1858 (2017) 615–627

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Calcium regulates cell death in cancer: Roles of the mitochondria and mitochondria-associated membranes (MAMs)☆

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ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form 30 December 2016
Accepted 8 January 2017
Available online 10 January 2017

Keywords:
Calcium (Ca^{2+})
Apoptosis
Autophagy
Mitochondria associated membranes (MAMs)
Endoplasmic reticulum
Tumor

ABSTRACT

Until 1972, the term 'apoptosis' was used to differentiate the programmed cell death that naturally occurs in organismal development from the acute tissue death referred to as necrosis.

Many studies on cell death and programmed cell death have been published and most are, at least to some degree, related to cancer. Some key proteins and molecular pathways implicated in cell death have been analyzed, whereas others are still being actively researched; therefore, an increasing number of cellular compartments and organelles are being implicated in cell death and cancer. Here, we discuss the mitochondria and subdomains of the endoplasmic reticulum (ER) that interact with mitochondria, the mitochondria-associated membranes (MAMs), which have been identified as critical hubs in the regulation of cell death and tumor growth. MAMs-dependent calcium (Ca^{2+}) release from the ER allows selective Ca^{2+} uptake by the mitochondria. The perturbation of Ca^{2+} homeostasis in cancer cells is correlated with sustained cell proliferation and the inhibition of cell death through the modulation of Ca^{2+} signaling. This article is part of a Special Issue entitled Mitochondria in Cancer, edited by Giuseppe Gasparre, Rodrigue Rossignol and Pierre Sonveaux.

1. Introduction

Cell death is a crucial and essential aspect of life. Although this statement may be contradictory, cell death itself is directly connected to cell proliferation and cell survival [1]. (See Fig. 1)

Programmed cell death has been established as an anti-cancer defense mechanism; therefore, any modification to the related pathways leads to uncontrolled cell proliferation and oncogenesis.

Early classifications of cell death were based on morphological assays, and apoptosis was one of the first processes to be described. The initial morphological observations were described as the rounding-up of the cell, a reduction in cellular volume, chromatin condensation, cytoplasmic shrinkage, the retraction of pseudopods, nuclear fragmentation, and a particular boiling-like process termed blebbing [2].

Apoptosis is the most important and well-studied mechanism of cell death; approximately 10 million cells undergo the apoptotic process in an adult human under physiological conditions each day [3]. The primary proteins involved in apoptotic cell death and their respective activities will be discussed in the subsequent sections.

The apoptotic process is classified as type I cell death. Type II cell death is classified as autophagy, a pro-survival process that also acts as a pro-death pathway, as it is involved in several biological events, such as aging, development, protein turnover, neurodegeneration and cancer [4]. In addition to the canonical proteins mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK), the most well-studied proteins that act as initiation sensors, BECLIN1 and autophagy and BECLIN1 regulator 1 (AMBRA1) are also involved in the initial steps of autophagosome formation [5].

Necrosis is defined as type III cell death and has long been considered as an accidental and unscheduled form of cell death. Nonetheless, according to several recent studies, the execution of the necrotic process may be regulated by a set of catabolic mechanisms and signal transduction pathways [6]. The Bcl-interacting protein 3 (BNIP3), Bcl-2-modifying factor (BMF) (pro-death proteins and members of the Bcl-2 family), NIP3-like protein X (Nix), the kinase receptor-interacting protein 1

☆ This article is part of a Special Issue entitled Mitochondria in Cancer, edited by Giuseppe Gasparre, Rodrigue Rossignol and Pierre Sonveaux.

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http://dx.doi.org/10.1016/j.bbabio.2017.01.003
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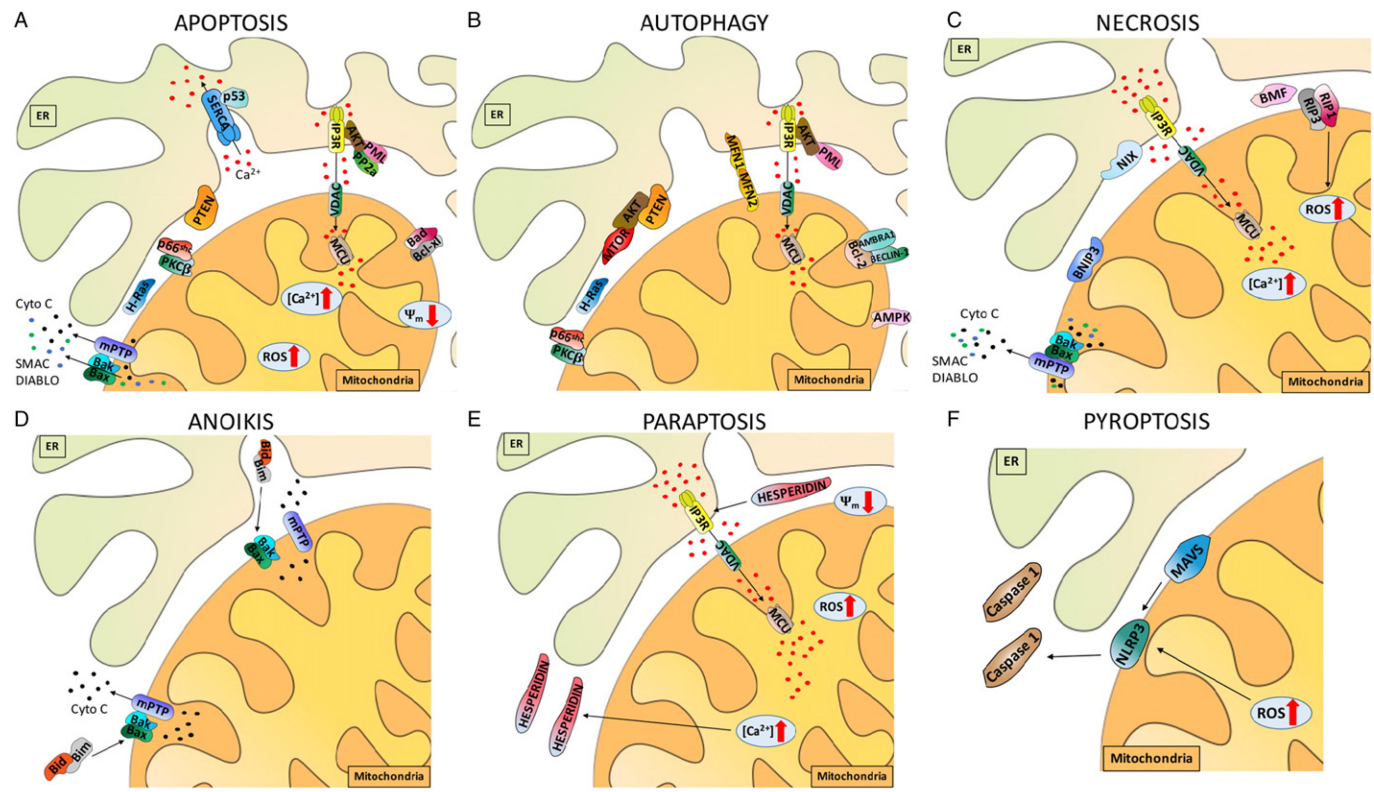

Fig. 1. Summary of the mitochondrial, ER and MAM proteins involved in primary cell death mechanisms. Representation of proteins at the mitochondria-ER interface that play active roles in cell death. Proteins that prevent or promote cell death affect intracellular Ca^{2+} dynamics and homeostasis by binding Ca^{2+} and modulating intracellular Ca^{2+} uptake and release mechanisms. Ca^{2+} overload-induced mitochondrial damage and ROS production display a cause-effect relationship, resulting in a decreased mitochondrial membrane potential (Ψ_m). Calcium channels play essential roles in Ca^{2+} homeostasis, and modifications in their activities are potentially fatal to the cell. SERCA is a Ca^{2+} ATPase that transfers Ca^{2+} from the cytosol to the ER lumen at the expense of ATP hydrolysis. IP3R consists of 4 subunits of approximately 310 kDa each and is essential for efficient Ca^{2+} transfer between the ER and mitochondria. VDAC is the major permeability pathway in the OMM; Ca^{2+} flux across the outer membrane occurs mainly through VDAC. The MCU allows the passage of calcium ions into the mitochondrial matrix. A) MAM proteins involved in apoptosis. The Bcl-2-protein family includes numerous anti-apoptotic (i.e., Bcl-XL) and pro-apoptotic (i.e., Bak, Bax, and Bad) members. H-Ras reduces Ca^{2+} transfer from the ER to mitochondria and blocks the apoptotic program. The oncosuppressor PML regulates Ca^{2+}-dependent apoptosis. PTEN interacts with IP3R to prevent Akt from phosphorylating the receptors. p53 modulates apoptosis by controlling Ca^{2+} flux into the mitochondria. np66Shc and the putative oncogene PKC-β cooperate to preserve the physiological levels of apoptosis and B) autophagy. Autophagy is a self-degradative process that recruits a double membrane-bound vesicle, termed the autophagosome, which then fuses with a lysosome to form an autolysosome. BECLIN1 and AMBRA1 are involved in the initial steps of autophagosome formation. AMPK is an energy sensor that is activated during nutrient deprivation to inhibit the activity of mTOR, a negative regulator of autophagy. The mitochondria-shaping proteins MFN-1/-2 modulate interactions between the mitochondria and ER; their ubiquitination precedes the removal of damaged mitochondria and thus is an early event in autophagy. Concerning necrosis C), recent evidence has implicated Bax, Bmf, BNIP3, and Nix as part of the necrotic program. The kinases RIP1 and RIP3 are key signaling molecules in necrosis and are regulated by caspases and ubiquitination. Anoikis D) involves Bid and Bim, which are activated by the detachment of cells from the ECM and rapidly promote the assembly of Bax–Bak oligomers within the OMM. Hesperidin E) induces paraptosis-like cell death by activating ERK1/2. Pyroptosis F) is induced by NLRP3-dependent caspase-1 activation; MAVS is required for optimal NLRP3 inflammasome activity.

(RIP1) and RIP3 proteins may be involved in this process, as they are speculated to be key signaling molecules involved in necrosis and, in turn, are regulated by caspases and ubiquitination [7–10].

The aforementioned cell death types are considered the main pathways involved in cell death. For a complete description, we must mention atypical cell death modalities, such as anoikis, a particular type of apoptosis induced by the loss of attachment to other cells or matrix. Anoikis involves Bid and Bim, which are activated following the detachment of cells from the extracellular matrix (ECM) and rapidly promote the assembly of Bax–Bak oligomers within the outer mitochondrial membrane (OMM) [11].

Pyroptosis is a cell death mechanism induced by caspase-1 activation, leading to interleukin (IL)-1β and IL-18 release. NLRP3-dependent caspase-1 activation plays a key role in this process. Indeed, mitochondria-associated adapter molecules, MAVS, are required for optimal NLRP3 inflammasome activity [12].

Paraptosis is a type of cell death triggered by the expression of the insulin-like growth factor receptor I [13]. Mitochondrial Ca^{2+} has an extremely important role in hesperidin-induced paraptotic cell death [14].

Other cell death mechanisms that are not yet well characterized include cell death preceded by multinucleation and entosis (a phenomenon that occurs when a cell engulfs one of its live neighboring cells) [15].

All these cell death mechanisms are finely regulated by a complex network of proteins, whose transcription and degradation have profound effects on malignant cancer phenotypes. Some oncogenic mutations disrupt programmed cell death, leading to tumor initiation, progression or metastasis (e.g., mutations in the Bcl-2 protein family deregulate cell death).

Bcl-2 does not behave like a typical oncogene; it promotes cell survival by blocking programmed cell death (i.e., by having a direct effect on endoplasmic reticulum Ca^{2+} handling) instead of disrupting normal proliferation checkpoints [16]. Other examples include p53-like proteins, including TP53 itself, which was the first gene linked to apoptosis. p53 has tumor suppression proprieties, and this gene is mutated in the majority of human tumors [17]. Disruption of the Fas/CD95 receptor pathway, which regulates cell number in the immune system, leads to lymphoproliferative disorders and cancer [18]. Ras activation and phosphatase and tensin homologue (PTEN) loss are common in human tumors; phosphoinositide 3-kinase (PI3K) is activated by Ras and downregulated by the tumor suppressor PTEN.

These mechanisms are only some of the many mechanisms that regulate cell death, all of which induce different morphological phenotypes by regulating or directly controlling the involvement of downstream molecular pathways. In this review, we will focus on Ca^{2+}-dependent cell death mechanisms. Ca^{2+} transients have been implicated in most aspects of cell physiology and play important roles in regulating cell death [19]. In particular, we analyzed the ER and mitochondrial compartments and the intimate interactions that physically occur through the mitochondria-associated ER membranes (MAMs) that play important roles in cellular physiology and participate in the mechanism by which cancer cells resist apoptotic stimuli [20].

2. General concepts, facts, hypothesis and controversy related to Ca^{2+}

The evolution of biological complexity arising from unicellular organisms to pluricellular structures was mediated by the development of dedicated messengers capable of synchronizing the activities of different cells and producing advantageous cooperation. These types of communication required cells to be able to produce an extracellular signal that bound a dedicated receptor on the cell membrane. This messenger, in turn, allowed the transduction of information across the membrane to produce an intracellular second messenger that bound different intracellular ligands after traveling across the cytoplasm and stimulated multiple activities located in different cellular regions.

Considering its availability at the time the first multicellular organisms evolved and its chemical properties, Ca^{2+} became one of the most important (and most studied) intracellular second messengers.

Within cells, the average Ca^{2+} concentration is far above the μM range, but its heterogeneous distribution reflects the importance of its tight regulation. Indeed, Ca^{2+} is present at a high concentrations in the so-called intracellular stores, mainly the endo/sarcoplasmic reticulum (ER/SR) and the Golgi apparatus, where its concentrations range between 300 and 1000 μM [21,22]. In contrast, very low Ca^{2+} concentrations are maintained in the cytoplasm, mitochondrial matrix and peroxisomal lumen, where it is expected to exert signaling effects (ranging from 100 to 500 nM) [23].

In non-excitable cells, binding of an extracellular messenger to a specific G protein-coupled receptor allows the generation of intracellular inositol phosphate 3 (IP3), which binds the IP3 receptor (IP3R) located in the ER membrane. This receptor, an ion channel-linked receptor that opens after binding IP3, leads to the selective passage of Ca^{2+} from the ER lumen to the cytosol. Due to the dramatic difference in the Ca^{2+} concentrations between these two compartments, the opening of IP3R leads to a fast and dramatic increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$), which easily reaches its targets and promotes events such as transcriptional regulation, protein synthesis, metabolic control, hormone secretion, cytoskeletal remodeling, and cell motility. In excitable cells, voltage-gated channels allow the generation of Ca^{2+} signals by importing extracellular Ca^{2+} (usually in the mM range) into the cytosol. These signals, which are reinforced by Ca^{2+}-induced cytoplasmic release of intraluminal ER/SR Ca^{2+}, promote Ca^{2+}-mediated regulation of contractility and vesicle release, supporting the involvement of Ca^{2+} in a plethora of cellular functions [24].

A fascinating aspect of Ca^{2+} signaling that highlights its dramatic importance in the evolution of multicellular organisms is its capacity to regulate cell death. Indeed, multicellular organisms require tight control of the cellular Ca^{2+} concentrations to allow proper tissue homeostasis; loss of this control results in excess proliferation (which may lead to malignancies) or loss of tissue function (neurodegenerative pathologies).

One of the earliest contexts that allowed researchers to comprehend how Ca^{2+} regulates cell death was the activation of the T-cell receptor (TCR) on immature lymphocytes. TCR activation generates IP3 and leads to IP3R opening. Short-term TCR activation has been proposed to lead to short and synchronized Ca^{2+} waves that activate nuclear factor of activated T cells (NFAT), subsequently stimulating IL-2 production and cell survival. In contrast, prolonged TCR activation induces wide and persistent elevation of the cytosolic free calcium concentrations ($[Ca^{2+}]_c$), resulting in apoptosis. These results provide the foundation for the mechanism by which T cells undergo positive selection, which remains one of the prototypical examples of the duality of Ca^{2+} signaling [25].

Many other molecules have been proposed to induce elevated $[Ca^{2+}]_c$ and subsequent apoptosis, including glucocorticoids in thymocytes, angiotensin II in cardiomyocytes, and testosterone in cardiomyocytes, T cells and neurons.

In addition, some pharmacological compounds yield the same result, including compounds that induce ER stress (e.g., thapsigargin [26]) and oxidative stress (e.g., hydrogen peroxide or menadione [27]), as well as prostaglandin intermediates, sphingolipids (e.g., arachidonic acid and c2-ceramide [28]), cisplatin and staurosporine. Indeed, all these compounds share the capacity to induce IP3R-mediated elevations of $[Ca^{2+}]_c$ followed by apoptosis.

Furthermore, the involvement of IP3R in apoptosis has been reported in different cell types through IP3R isoform-specific silencing in response to several apoptotic stimuli [29].

Based on its messenger nature, Ca^{2+} is able to regulate the induction of apoptosis at different sites, presumably to maximize its effect or avoid the possibility that the alteration of one site would compromise message transmission.

Within the cytoplasm, elevated $[Ca^{2+}]$ is able to activate a class of cysteine proteases called calpains. These enzymes are capable of inducing proteolytic activation of caspase-12 that, in turn, induces the cascade activation of caspase-9 and -3, resulting in the execution of the apoptotic program. This program has primarily been associated with ER stress and cisplatin exposure and depends on IP3R [30].

One of the most well-studied Ca^{2+}-induced cell death pathways is the cross-talk between the ER and mitochondria. These compartments communicate through selective signals in regions called MAMs (see following chapter). At these sites, Ca^{2+} released from the ER is directly taken up by the mitochondria through specialized microdomains. The main physiological role of Ca^{2+} uptake involves the control of mitochondrial metabolic activity, as revealed by the ATP production rate. Indeed, the Ca^{2+}-sensitive mitochondrial dehydrogenases (i.e., pyruvate-, α-ketoglutarate- and isocitrate-dehydrogenases) are activated by Ca^{2+}. These three enzymes represent the rate-limiting steps of the Krebs cycle and thus control the supply of electrons into the respiratory chain and the generation of the proton gradient across the inner membrane, which is necessary for Ca^{2+} uptake and ATP production.

In contrast to these physiological parameters, prolonged accumulation of mitochondrial Ca^{2+} may lead to a phenomenon known as the mitochondrial permeability transition (MPT).

Induction of the MPT leads to the loss of inner mitochondrial membrane (IMM) permeability, with a rapid breakdown of mitochondrial membrane potential ($\Delta\Psi_{mt}$), loss of ATP, osmotic shock to the organelle and rupture of the OMM [2]. Loss of ATP then decreases ion homeostasis and cell integrity, ultimately resulting in necrosis [31].

This mechanism has been widely investigated in pathological cell death, particularly in cell death associated with ischemia and reperfusion injury.

Most of the current literature is consistent with the notion that the Ca^{2+}-induced MPT is primarily related to necrotic cell death.

Nonetheless, some reports have proposed that the MPT is involved in the regulation of apoptosis. Indeed, the rupture of the OMM during mitochondrial swelling can lead to the release of mitochondrial pro-apoptotic factors, including cytochrome C, apoptosis-inducing factor (AIF), SMAC/DIABLO and EndoG, which are required for the intrinsic apoptosis pathway. Isolated mitochondria exposed to MPT-inducing stimuli are able to induce apoptotic-like morphological rearrangements when mixed with isolated nuclei. In addition, the pro-apoptotic protein Bax can induce the loss of $\Delta\Psi_{mt}$ through a pathway distinct from the Ca^{2+}-inducible, cyclosporin A-sensitive PTP pathway.

Because ATP is a critical component of apoptosis, one could argue that the loss of mitochondrial ATP synthesis due to the MPT would not be permissive to MPT-induced apoptosis. Thus, the MPT may not involve the entire mitochondrial network within the cell, but instead it may appear as "flickering" at the level of a single mitochondrion. This flickering could generate localized and multi-phasic release of pro-apoptotic factors from the mitochondria, leading to apoptosis.

ER-to-mitochondria Ca^{2+} transfer has also been recently linked to type II programmed cell death [32]. Autophagy is usually activated during metabolic energy stress, a condition in which the process promotes the recycling of intracellular contents to produce metabolic intermediates [33]. As mentioned above, mitochondrial Ca^{2+} uptake stimulates ATP production, and blocking this signaling by IP3R knockdown or pharmacological inhibition (i.e., using xestospongin B) stimulates autophagy [34]. This stimulation is mediated by the activation of AMPK, an energy sensor that is activated during nutrient deprivation to inhibit the activity of the mTOR, a negative regulator of autophagy [35]. Recently, contradictory reports have highlighted the complex role of Ca^{2+} in the activation of autophagy. Reports from both Missiroli et al. [36] and Cardenas et al. [37] were focused on the role of autophagy in tumor progression, the former by knockout of the master oncosuppressor PML (promyelocytic leukemia protein) and the latter by pharmacological inhibition and siRNAs targeting the Ca^{2+} machinery. Both reports confirmed the inhibition of ER-to-mitochondria Ca^{2+} transfer, although the authors reported contrasting outcomes related to cell survival. The report from Missiroli et al. [36] proposed that inhibition of Ca^{2+} transfer, a mechanism typical of several pro-tumor conditions (see the following chapters), stimulates pro-survival autophagy, which is only shifted to pro-cell death autophagy when cells were further stressed (i.e., by the administration of chemotherapeutics and pro-autophagic compounds). In contrast, the report from Cardenas et al. indicated that the induction of autophagy was not sufficient to compensate for the energetic crisis in cancer cells, leading to cell death. [37]. Several explanations may justify these different observations, including the differences in experimental procedures, but the most likely explanation is the extent to which Ca^{2+} signaling was inhibited. Indeed, the former report observed a milder Ca^{2+} transfer inhibition that could have resulted in milder autophagic stimulation, leading to a pro-survival state compared with that of the latter report.

Overall, in addition to the many regulatory aspects that should be further investigated, Ca^{2+} is clearly an important intracellular messenger that participates in a complex system of cell functions, with cell death being one of the most relevant functions.

3. Effector system for elevated Ca^{2+} concentrations

A plethora of stimuli influence the increase in the cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) and the release of Ca^{2+} from the ER; therefore, cells are constantly working to maintain the correct concentration gradient.

Under physiological conditions, stimuli generally promote low and transient increases in $[Ca^{2+}]_i$; in contrast, under pathological conditions, variations in $[Ca^{2+}]_i$ induced by these stimuli are pronounced and sustained. In particular, during programmed cell death, especially apoptosis, $[Ca^{2+}]_i$ is dramatically increased. Consequently, the mitochondria take up large amounts of Ca^{2+}, leading to the induction of apoptosis.

These stimuli, termed apoptotic inducers, are physiological (e.g., corticosteroids or nitric oxide enzymes, NOS) or pharmacological (e.g., chemodrugs, such as cisplatin) stimuli. Corticosteroids are widely used to treat cancer and other diseases, such as autoimmunity, by counteracting TCR activation. Interestingly, Ca^{2+} has been shown to be an indispensable factor for T lymphocyte activation and proliferation; moreover, short-term corticosteroid treatments attenuate the TCR-mediated Ca^{2+} elevations necessary for T-cell activation. Prolonged treatments cause thymocyte apoptosis mediated by persistently elevated cytosolic Ca^{2+} levels. However, the exact mechanism of corticosteroid action on Ca^{2+} handling is not well understood. The primary hypothesis is that these hormones inhibit the Src family kinase Lck, which normally regulates IP3R activity [38].

The relationship between Ca^{2+} and hormones is not only restricted to autoimmunity and cancer but is also involved in heart disease. For example, angiotensin activates the apoptotic program by modulating Ca^{2+} signaling. In particular, angiotensin, which is released in response to glucocorticoids and estrogens, generates IP3 and diacylglycerol (DAG) by binding to AT-1 receptor (AT-1R), a G-protein-coupled receptor that activates phospholipase C [39]. As a result, overall Ca^{2+} signaling is activated and apoptosis may be triggered. Interestingly, angiotensin may also function by opening the L-type voltage-dependent Ca^{2+} channel (L-VDCC). In fact, administration of an L-type Ca^{2+} channel blocker inhibits angiotensin-induced apoptosis [40].

Other factors have been shown to be involved in heart disease following alterations in Ca^{2+} signaling. Nitric oxide synthase (NOS) enzymes widely regulate Ca^{2+} homeostasis by inhibiting L-type channel activity and Ca^{2+}-release from the SR. As a result, the apoptotic program is blocked due to a reduction in mitochondrial Ca^{2+} uptake, which prevents mitochondrial fragmentation and cytochrome C release. Overall, the NOS family seems to exert beneficial effects that counteract some pathologies, including ischemia and reperfusion injury. In fact, these enzymes have also been described as the primary causes of several pathologies, particularly cancer. First, alterations in the expression of NOS enzymes have been observed in several human cancers [41]. High NO levels were sufficient to activate anti-apoptotic proteins, such as Akt, Bcl-2 and Ras [42,43]. Interestingly, all these proteins are important mediators of apoptosis, as they regulate Ca^{2+} signaling.

Finally, intracellular Ca^{2+} homeostasis and Ca^{2+} release from the ER may be modulated by various cytotoxic agents. Generally, these compounds are used to promote apoptotic cell death by disrupting Ca^{2+} homeostasis. Notably, most of these compounds are widely used as anticancer treatments. For example, cisplatin, one of the most widely used chemotherapeutic agents, induces Ca^{2+} leakage from the ER, causing a subsequent increase in intracellular Ca^{2+} levels and apoptosis [44].

4. The mitochondrial calcium uniporter (Mcu) complex and the role of its components in tumorigenesis (Fig. 2)

As described above, Ca^{2+} homeostasis is responsible for controlling a vast number of cellular functions. Mitochondrial Ca^{2+} uptake plays an essential role in the maintenance of homeostasis and participates in cellular metabolism, cytosolic Ca^{2+} buffering, secretory functions, cell survival, proliferation, migration and cell death [45]. For many years, mitochondrial Ca^{2+} uptake was ascribed to a single transport mechanism mediated by an individual protein that functions as a uniporter; however, the uniporter was recently shown to be a macromolecular complex consisting of pore-forming and regulatory subunits, rather than a single protein [46].

The pore is physically formed by oligomers of MCU, a protein located in the IMM. MCU has two putative transmembrane domains, with C- and N-terminal domains spanning the mitochondrial matrix [47].

Mitochondrial calcium uptake protein 1 (MICU1) is an important regulatory subunit of the complex; its discovery preceded the identification of MCU [48]. MICU1 performs a gatekeeping function, stabilizing the closed state of the MCU complex and cooperating to allow Ca^{2+} to accumulate inside the mitochondria [49].

MICU2 shares 25% sequence identity with MICU1 and interacts with both MICU1 and MCU. The structure and function of this protein are still subjects of debate [50].

A 10-kDa single-pass membrane protein named efflux-multidrug resistance protein (EMRE) interacts with MICU1 and MCU oligomers. Thus, EMRE acts as a bridge between MICU1 activity and the channel

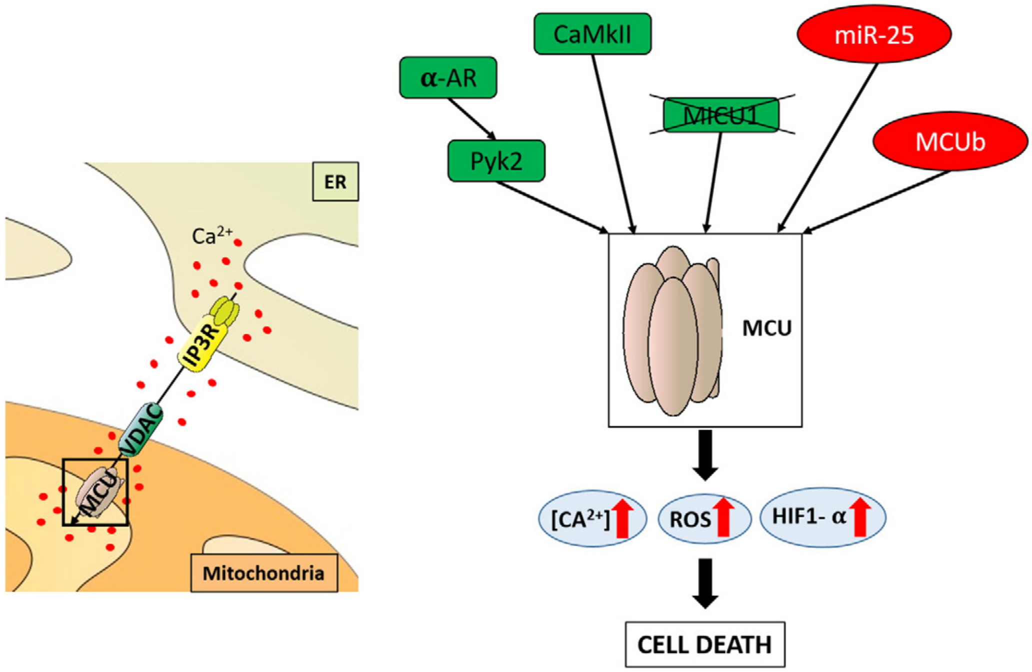

Fig. 2. Proteins that modulate the activity of the MCU complex and cell death. The MCU located in the IMM is responsible for Ca^{2+} uptake. Modulation of MCU complex subunits and function could increase the probability that cells will undergo apoptotic cell death under stress conditions because of the increased basal ROS levels present during mitochondrial Ca^{2+} uptake. Proteins that promote Ca^{2+} entry into the mitochondria are shown in green, and proteins that decrease mitochondrial Ca^{2+} uptake and ROS generation are shown in red. The pathway that activates the MCU complex is inhibited by CaMKII-dependent phosphorylation of the uniporter, and Pyk2 prevents Ca^{2+} overload in the mitochondria, ROS production and subsequent cell death, which are important in tumor progression. MICU1 knockout cells have increased basal ROS levels during mitochondrial Ca^{2+} uptake, leading to apoptotic cell death under stress conditions. In contrast, miR-25 (which decreases MCU expression) and MCUb (which acts as an endogenous dominant-negative isoform of MCU) reduce mitochondrial Ca^{2+} uptake and, consequently, drive resistance to Ca^{2+}-dependent apoptotic death.

proprieties of MCU; its loss drives the reduction of mitochondrial Ca^{2+} uptake to the same extent as MCU depletion [51].

MCUb is an MCU paralogue/isogene that acts as an endogenous dominant-negative isoform [52].

Mitochondrial Ca^{2+} overload has been associated with apoptosis and necrosis in many pathological conditions [17], and as a mitochondrial Ca^{2+} uniporter, MCU has many pathophysiological implications. When a pro-apoptotic stimulus occurs, MCU-expressing cells display an enhanced sensitivity to apoptosis [53]. Moreover, inhibition of the pathway that activates the MCU complex by phosphorylating the uniporter through Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein tyrosine kinase 2 beta (Pyk2) prevents Ca^{2+} overload in mitochondria, reactive oxygen species (ROS) production, and cell death [54]. Therefore, modulation of the expression of MCU complex subunits could improve our understanding of the possible pathogenic role of the uniporter. Below, we discuss its potential tumorigenic, apoptosis-modulating functions.

As shown in our previous study, microRNA-25 (miR-25) is up-regulated in human prostate and colon cancers and targets the MCU gene. miR-25 decreases MCU expression and, consequently, reduces mitochondrial Ca^{2+} uptake and resistance to Ca^{2+}-dependent apoptotic death [55].

Elevated mitochondrial [Ca^{2+}] and ROS accumulation via MCU activity may induce cell death by increasing OMM permeability and the opening of the mitochondrial permeability transition pore (mPTP) [31]. MCU overexpression increases mitochondrial ROS generation and accumulation and, conversely, silences MCU or inhibits its activity through the introduction of the dominant-negative subunit MCUb, decreasing mitochondrial ROS generation induced by various stimuli [56].

MICU1 knockout cells lose their MCU complex gatekeeping function and are highly susceptible to apoptotic cell death under stress conditions because of the increased basal ROS levels during mitochondrial Ca^{2+} uptake [57].

A recent study from the University of Padua highlights a possible role of MCU in the regulation of breast cancer progression via hypoxia inducible factor-1α (HIF-1α). MCU and HIF-1α expression are suggested to be directly related; moreover, HIF-1α-regulated genes are expressed in human breast cancer samples, which is sufficient to consider MCU as a novel marker of cancer progression [58].

5. Roles of other mitochondrial proteins involved in mitochondrial Ca^{2+} homeostasis in tumorigenesis

To date, a consensus supports the theory that the MCU complex is responsible for mitochondrial Ca^{2+} influx. However, other proteins have been proposed to promote mitochondrial Ca^{2+} uptake. The most relevant of these proteins is the leucine zipper-EF hand-containing transmembrane protein 1 channel (LETM1), which seems to function as an electrogenic Ca^{2+}/H^{+} exchanger with a dual role [59]. Indeed, at high mitochondrial [Ca^{2+}] or at low cytosolic pH, LETM1 functions through an extrusion mechanism. In the presence of low mitochondrial [Ca^{2+}], it regulates the entry of this cation [60]. Nevertheless, the true role of LETM1 is not well understood. In fact, many research groups have proposed that LETM1 acts as a mitochondrial K^{+}/H^{+} exchanger [61]. As shown in the study by the Demaurex group using LETM1 and NCLX overexpression and the redox-sensitive probe roGFP, NCLX, but not LETM1, mediates Ca^{2+} extrusion from mitochondria [62]. However, this channel seems to be involved in tumorigenesis. For instance, changes in LETM1 expression have been detected in human malignancies, including triple-negative breast cancer (TNBC) and head and neck squamous cell carcinoma [63,64].

Under physiological conditions, mitochondrial Ca^{2+} influx must be equal to mitochondrial Ca^{2+} efflux. This action is principally achieved by the Na^{+}-Ca^{2+}-Li^{+} exchanger (NCLX), which exchanges 3 Na^{+} ions for 1 Ca^{2+} ion [65]. This flux is primarily achieved because the mitochondrial Na^{+} concentration is less than the cytosolic Na^{+} concentration. This Na^{+} gradient, coupled with the large negative mitochondrial membrane potential, provides a huge driving force for Ca^{2+} extrusion. Despite its importance in Ca^{2+} extrusion, the actual link between this exchanger and tumorigenesis has not been well described. Nevertheless, the mitochondrial Ca^{2+} levels have been increased with the benzodiazepine CGP37157, a specific inhibitor of NCLX, to promote subsequent mitochondrial damage and to induce apoptosis [66]; interestingly, these effects have also been observed in a prostate cancer cell line [67].

As reported above, mitochondrial Ca^{2+} is primarily transported into the matrix by MCU. However, Ca^{2+} must pass through the OMM. The voltage-dependent anion channel (VDAC) exerts this function. VDAC is a large channel (2.5–3 nm) that represents the primary permeability pathway through which solutes enter the OMM [68]. The VDAC channel has often been referred to as a "general diffusion pore," although this appellation is not accurate. In fact, VDAC finely regulates several cellular processes, particularly apoptosis, due to its capacity to allow the "passage" of Ca^{2+} and thus to amplify or diminish the apoptotic response. In the 2012 study by the Van Remmen group, mitochondrial superoxide release occurred through VDAC [69].

Because the absence of apoptosis is recognized as one of the hallmarks of cancer, the mPTP may play a pivotal role in cancer.

Accordingly, the role of the mPTP in cell death has been investigated in several cancer types, including colon cancer, osteosarcomas and leukemia. However, despite the great potential of this target as a cancer treatment, the use of mPTP modulators during tumorigenesis has not shown great efficacy, likely because the molecular structure of mPTP is not well understood and not all of its components have been identified. The C subunit of mitochondrial F1/FO ATP synthase was recently shown to be a fundamental regulator of mPTP activity [70-72]. Indeed, upon its pharmacological inhibition, the induction of the MPT by Ca^{2+} is inhibited. Thus, this mPTP member may be a novel target for promising new anti-cancer therapies [73].

6. Oncogene- and oncosuppressor-mediated modulation of mitochondrial Ca^{2+} homeostasis

As reported above, only a few proteins regulate the proper influx and efflux of Ca^{2+} from the mitochondria. In contrast, several proteins regulate Ca^{2+} flux towards this organelle with the ultimate purpose of activating/inhibiting apoptosis. Not surprisingly, most of these proteins are oncogenes and tumor suppressors.

Historically, the proto-oncogene Bcl-2 was the first protein to be identified as an anti-apoptotic protein capable of preventing apoptosis in a Ca^{2+}-dependent manner [28,74]. For example, alterations of Bcl-2 function have been identified in several leukemias and carcinomas [75]. Bcl-2 is a member of the large Bcl-2-protein family, which contains numerous anti-apoptotic and pro-apoptotic members [76]. Notably, the anti-apoptotic protein Bcl-XL, which is deregulated in several cancer types, blocks the apoptosis pathway by neutralizing pro-apoptotic Bcl-2 members, such as Bak, Bax, Bid and Bim [77]. In addition, Bcl-XL exerts its anti-apoptotic functions by regulating the activity of Ca^{2+} channels, including IP3Rs and VDAC isoforms [78].

Furthermore, the protein kinase B (PKB)/Akt protein regulates Ca^{2+} flux inside the mitochondria. In fact, this well-known oncogene reduces Ca^{2+} release from the ER by modulating IP3R activity, thus protecting cells from apoptotic stimuli [79]. Furthermore, the PKB/Akt signaling pathway is regulated by PTEN [80]. Notably, PTEN is a tumor suppressor whose expression is lost in a plethora of human malignancies (e.g., breast and prostate cancer). A lack of PTEN increases PKB/Akt activity, which in turn regulates the reduction of mitochondrial Ca^{2+} accumulation due to a decrease in Ca^{2+} release from the ER and interferes with the apoptotic machinery [81].

In addition to its transcriptional role, the tumor suppressor p53 modulates apoptosis by controlling Ca^{2+} flux into the mitochondria. In fact, p53 was shown to cooperate with sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps at the ER-MAM compartment [17]. As a result, the activity of these pumps increases to increase the amount of Ca^{2+} released from the ER. Thus, mitochondria are overloaded with Ca^{2+}, and the apoptotic program is triggered. However, p53 not only cooperates with SERCA pumps at the MAM level but also acts as a bridge to maintain the correct PML localization at ER-mitochondria sites [36]. Notably, the oncosuppressor PML, which is deregulated in several human cancers [82,83], regulates Ca^{2+}-dependent apoptosis [84]. In the absence of p53, PML is no longer localized at the juxtapositions between mitochondria and MAMs, and its pro-apoptotic functions are lost. In addition, as shown in the study by Missiroli et al. [36], PML-p53-dependent regulation of mitochondrial homeostasis is also a crucial element in the autophagic pathway, thus highlighting the possibility of creating pioneering therapeutic strategies against malignancies characterized by the absence/mutations of PML and p53.

Likewise, p66Shc and the putative oncogene protein kinase C-β (PKC-β) cooperate to preserve the physiological levels of apoptosis and autophagy. Indeed, upon activation by PKC-β, p66Shc becomes localized to the mitochondrial compartment, where it influences apoptosis and autophagy by regulating the mitochondrial Ca^{2+} levels and bioenergetics [85,86].

Ca^{2+}-dependent apoptosis may also be regulated by at least two members of the Ras family. In fact, altered expression of K- and H-Ras is sufficient to reduce Ca^{2+} transfer from the ER to the mitochondria, thus blocking the apoptotic program [87]. Remarkably, this oncogene is mutated in 33% of cancers, including pancreatic, colorectal and lung cancers [88].

7. MAMs: structure and composition

Specific organization of the intracellular organelles enables direct communication between various compartments within the cell. Among the different direct interactions or "close contacts" between cellular organelles, MAMs have recently attracted the attention of many researchers, as represented by the growing number of publications describing the important roles of MAMs in physiology and pathology. MAMs consist of regions of the ER involved in direct interactions with the mitochondria. However, proteins from other cellular compartments have also been found in MAMs, suggesting that MAMs also form close contacts with other intracellular structures in addition to the ER. For instance, plasma membrane (PM) proteins are observed in MAMs, indicating the presence of close contacts between the mitochondria and the PM [89]. According to numerous studies, mitochondria-ER contact sites are dynamic structures. However, because we are able to isolate these structures, the interactions between these membranes are strong, and they are not destroyed during isolation procedures. Plasma membrane-associated membranes (PAMs) [90] and ER-mitochondria encounter structures (ERMES) [91] are other examples of the physical and functional contacts that are isolated during cellular subfractionation.

Most researchers believe that the first evidence showing that the mitochondria and ER are closely positioned at some regions comes from the early 1970s in the studies by Franke and Kartenbeck [92] and Morre et al. [93]. However, the first reports on the direct association between the mitochondria and ER date back to as early as the late 1950s [94]. Almost simultaneously with the aforementioned observations from the 1970s, Lewis and Tata [95] observed that a fraction of the ER was observed in low-speed centrifugation pellets containing the mitochondrial fraction during subfractionation of rat liver homogenates. Based on this observation, we acknowledge this paper as the first report to describe a MAM isolation procedure. Almost twenty years later, in the early 1990s, the Vance group made great progress in the MAM field by presenting a detailed protocol describing the isolation of pure MAM fractions in a series of articles published in J. Biol. Chem. [96], which was improved upon by Meier et al. [97] ten years later. Over the years, the MAM isolation method was improved and optimized to enable the isolation of MAMs from different animal tissues and cell cultures [98]. The existence of the MAM fraction is not an exclusive characteristic of mammalian cells; close interactions between mitochondria and the ER have also been described in yeast [99]. Interestingly, similar contacts between mitochondria and the ER have been described for chloroplasts and the ER in plants [100].

In addition to the development of more refined protocols for isolating pure MAM fractions, the list of proteins present at mitochondria-ER contact sites increases every year. Although many proteins localized at the MAM have been identified, we have not determined which proteins can be used as universal MAM markers because some MAM proteins are only present in certain organs, tissues or cell types. Another problem is the observation that no protein is exclusively localized to the MAM fraction. Instead, the localization of a specific protein at the MAM is only appropriately termed as enriched because these proteins are also present in other cellular compartments. Regarding the molecular composition of the MAM fraction, an article by Poston et al. [89] presents a detailed proteomic analysis of the MAM. These authors detected and classified approximately 1200 proteins from the MAM fraction isolated from a mouse brain and confirmed that the MAM fraction contains proteins characteristic of the PM and the Golgi apparatus (24% and 6%, respectively, of the total proteins detected in MAMs) [89]. Detailed, systematic lists of proteins present in the MAM fraction grouped based on their function have been presented by several groups, including Schon and Area-Gomez [101], Poston et al. [89], Vance [102], Marchi et al. [103], Patergnani et al. [104], Raturi and Simmen [105], and Giorgi et al. [20]. Based on the long list of proteins found in the MAM fraction or that translocate to the MAMs under certain conditions, MAMs seem to play important roles in various processes. Originally, the MAM fraction was considered important for lipid synthesis and trafficking (long-chain fatty acid coenzyme A ligase-1 (FACL-1) and -4, phosphatidylserine synthase-1 (PSS-1) and -2, serine active site containing 1 (SERAC1), fatty acid transport protein 4 (FATP4), acyl-CoA desaturase, phosphatidylethanolamine N-methyltransferase 2 (PEMT2) and many other proteins present in MAM involved in this process are reviewed in [101]) and Ca^{2+} handling (e.g., IP3R, ryanodine receptor, sigma-1 receptor (SIG1R), and promyelocytic leukemia protein (PML)). MAMs were later linked to the modulation of mitochondrial morphology (mitochondria-shaping proteins and chaperone proteins (MFN-1 and -2)), apoptosis (Bcl-2, hematopoietic cell-specific Lyn substrate1 (HCLS1)-binding protein 3 (HS1BP3)), mitochondrial contact site formation (VDAC and adenine nucleotide translocase (ANT)), protein folding (calnexin (CNX)) and sorting (phosphofurin acidic cluster sorting protein 2 (PACS-2)), ER stress (glucose-regulated protein 75-kDa (GRP75) and endoplasmic reticulum resident protein 44 (ERp44)), inflammation (inflammasome components: NALP3, adaptor ASC and thioredoxin interacting protein (TXNIP)), autophagy (pre-autophagosome/autophagosome markers (ATG14 and ATG5), and p66Shc) and the cellular response to oxidative stress (p66Shc protein, an Ero1α). The presence of these important proteins involved in crucial cellular processes explains why alterations in MAM composition are related to the pathogenesis of different disorders [105], including type-2 diabetes (mTOR complex 2 (mTORC2) and MAM-associated Akt), and several neuronal-based diseases, such as Parkinson's disease and Huntington's disease, and neurodegenerative diseases, such as schizophrenia, dementia and seizures. Moreover, MAMs have been proposed to be involved in familial Alzheimer's disease (FAD) [106,107] and GM1-gangliosidosis [108].

8. MAM proteins modulate Ca^{2+} homeostasis, cell death and tumorigenesis (Table 1)

The ER and mitochondrial networks control different aspects of cellular metabolism, and, through their both dynamic and close interactions, are also involved in the transmission of physiological and pathological Ca^{2+} and ROS signals directly from the ER to the mitochondria.

Table 1
MAM proteins involved in tumorigenesis and tumor progression. A = amplification; M = mutation; D = deletion.

Protein	Gene expression in cancer	Relation to Ca^{2+}	References
Akt	Pancreas (A, D), Breast (M), Prostate (A)	Inhibition of Ca^{2+} release from the ER	[79,124]
AMBRA1	Breast (A), Prostate (A, M)	Involved in regulating TPC-dependent calcium release	[5]
AMPK	Skin (M), Prostate (A), Pancreas (A, D)	Chronic calcium exposure decreases AMPK activity	[35]
Bad	Prostate (A), Pancreas (A, D), Uterus (A, D)	Sensitizes the mitochondria to Ca^{2+}, making them more susceptible to Ca^{2+} release from the ER	[125]
Bak	Breast (A), Prostate (A), Skin (A, M)	Regulate calcium leakage from the endoplasmic reticulum	[126]
Bax	Prostate (A), Pancreas (A, D), Breast (A), Stomach (M)	Regulate calcium leakage from the endoplasmic reticulum	[126]
Bcl-XL	Uterus (A, M), Breast (A), Prostate (A), Colon (A), Nervous System (D, M)	Acts on Bax inhibitor-1 (BI-1) to increase Ca^{2+} leakage from the ER, with BI-1 acting as a Ca^{2+} channel or as an IP3R sensitizer	[74,127]
Bcl-2	B-cell (M), Central Nervous System (A, M), Pancreas (D), Breast (A)	Induction of Ca^{2+} release from the ER	[74]
Bid	Prostate (A), Nervous System (A)	Regulates calcium concentrations and homeostasis in the ER	[74]
Bim	Prostate (A, M, D), Breast (A, M), Uterus (M, A)	Bim-deficient cells exhibit severe defects in calcium release	[8,75,128]
BECLIN1	Prostate (A), Breast (A)	Activated by increased $[Ca^{2+}]_i$ and may induce autophagy	[5]
BMF	Uterus (B, M, A), Prostate (A),	Supports Bim in some cell death processes	[8]
BNIP3	Prostate (A, D), Pancreas (A, D)	Induces atypical cell death with features of both apoptosis and necrosis	[7]
MAVS	Breast (A), Prostate (A), Ovary (A, D), Stomach (M, A)	Lead to defects in mitochondrial calcium	[12]
MFN-1	Lung (A, M), Ovary (A), Esophagus (A, M), Breast (A), Head (A, M)	Important as a mediator of mitochondrial fusion	[129,130]
MFN-2	Pancreas (A, D), Esophagus (M, A, D), Prostate (M, A, D)	Facilitates calcium cross-talk between the ER and mitochondria	[129,130]
mTOR	Skin (M), Uterus (M, A), Prostate (M, A, D)	Intracellular Ca^{2+} signaling is a crucial component in the canonical mTOR-dependent autophagy pathway	[33,35]
NIX	Prostate (D), Ovary (D, A)	Increase ER/SR calcium stores in cardiac myocytes	[9]
NLRP3	Breast (A), Prostate (A), Skin (M), Lung (M, A)	Localization of PML at ER/MAM contact sites is required for its pro-apoptotic activity via a calcium (Ca^{2+})-mediated pathway	[12]
PKC-β	Breast (A), Prostate (A), Lung (M), Skin (M)	Binds Ca^{2+} through its C2 domain	[131]
PML	Prostate (M), Colon (M), Breast (A), Lung (M, A), Uterus (A, M)	Regulates apoptosis in the ER by modulating calcium release, negative regulator of Akt	[36,83,84]
PP2a	Prostate (A), Central Nervous System (M), Pancreas (A, D)	Regulates calcium transients in cardiomyocytes	[106,132]
PTEN	Uterus (M), Prostate (M, D), Head (M, D), Stomach (M), Breast (A, M), Pancreas (M)	Regulates Ca^{2+} release via IP3R3	[80,81]
p53	Almost all	Interacts with the C-terminal portion of the SERCA pump, increasing ER Ca^{2+} loading	[17,19,35]
p66	Prostate (A), Breast (A), Esophagus (A, M, D)	Involved in the cellular response to oxidative stress	[85,121]
HRAS	Pancreas (A, D), Breast (A), Urinary Tract (M), Head (M)	Regulation of Ca^{2+} signaling	[87]
RIP1	Skin (A), Prostate (A), Breast (A)	Phosphorylated by increased cytoplasmic calcium concentrations	[133]
RIP3	Skin (M), Prostate (A)	Mediates oxidative stress through CaMKII	[10]

Thus, their contact sites are considered specialized microdomains for the transfer of Ca^{2+} signals. Ca^{2+} ions released from the ER by IP3Rs cross the OMM [2], which is freely permeable through VDACs, move to the IMM and accumulate in the matrix via the MCU complex in a process mediated by local Ca^{2+} sites of accumulation, which overcome the apparent low Ca^{2+} affinity of the MCU [109]. Nevertheless, if excessive Ca^{2+} influx occurs, pro-apoptotic factors, such SMAC/DIABLO, cytochrome C, and AIF may be released into the cytosol, resulting in apoptosis triggered by the opening of the mPTP [110,111].

MAMs represent platforms for the anchoring of many pro- and anti-apoptotic factors involved in tumor regulation.

This role was previously defined for the serine/threonine kinase Akt [112], which is physically and functionally linked to both the ER and mitochondria. Akt phosphorylates numerous proteins, including members of the Bcl-2 family (i.e., Bad and Bax), to activate its anti-apoptotic function, and hexokinase 2 (HK2), which phosphorylates VDAC1 to prevent Ca^{2+}-dependent opening of the mPTP and the release of pro-apoptotic proteins [113].

Bcl-2 is an oncoprotein that has a central role in the regulation of apoptosis because of its capacity to delay or block the programmed death pathway in different cell types. Bcl-2 overexpresssion reduces the steady state Ca^{2+} levels within the ER, resulting in reduced mitochondrial fragmentation and the initiation of apoptosis [28]. Bax and Bak are members of the Bcl-2 family that control apoptosis from the ER and mitochondria in response to Ca^{2+} stimulation [114].

As shown in a recent study by our group, myeloid cell leukemia protein 1-long isoform (Mcl-1L) is highly expressed in human malignancies and is localized to the mitochondrial membrane. This localization is consistent with its role in the control of key mitochondrial events during apoptosis to counteract the activity of the pro-apoptotic proteins Bak and Bax [115].

The tumor suppressor PTEN, one of the most commonly lost or mutated tumor suppressor genes in human cancers, also localizes to MAMs. PTEN is a dual-specific phosphatase for lipids and proteins that exhibits enhanced ER localization during Ca^{2+}-dependent apoptosis. In this case, PTEN interacts with IP3R3 to counteract Akt-mediated phosphorylation of the receptors and induce a subsequent increase in Ca^{2+} transfer from the ER to the mitochondria to trigger apoptosis in a protein phosphatase-dependent manner [81].

IP3R3 is the favorite target of another important tumor suppressor, PML, which is localized to the nucleus and cytosol and is associated with the ER and MAMs [84]. Indeed, PML forms a multiprotein complex with IP3R3, Akt and the protein phosphatase PP2a to modulate the apoptotic pathway triggered by Ca^{2+} accumulation in the matrix. The suppression of this protein blocks PP2a localization at MAMs, which in turn does not prevent Akt-mediated IP3R3 phosphorylation. Therefore, IP3R3 hyperphosphorylation inhibits Ca^{2+} transfer from the ER to the mitochondria, thereby inhibiting apoptosis [116].

Based on recent studies, fetal and adult testis expressed 1 (FATE1), a cancer-testis antigen that has a role in regulating the ER-mitochondria distance and Ca^{2+} uptake by the mitochondria, [117] inhibits pro-apoptotic signaling in many cancer cell lines by destabilizing BIK, a pro-apoptotic protein [118].

The interactions between the mitochondria and the ER are modulated by different proteins, including the mitochondria-shaping proteins MFN-1/-2 (mitofusin-1/-2). In particular, the absence of MFN-2 changes the morphology of the ER and decreases the interactions between the mitochondria and ER by 40%, causing altered transfer of Ca^{2+} signals to the mitochondria [119].

Moreover, the mitochondria and the ER are the major sites of ROS production, and many regulators of the oxidative state of the cell are localized at the MAM [120]. p66Shc plays a key role in this process, as it is a cytosolic protein involved in the cellular response to oxidative stress and tumorigenicity that has recently been identified at mitochondria-ER association sites. The increase in p66Shc induced by elevated intracellular ROS levels is, in turn, triggered by steroid-induced signaling, which promotes cell proliferation in prostate cancer cells [121]. Instead, the oxidative cellular environment promotes the phosphorylation of p66Shc at Ser36 that migrates to the mitochondrial matrix after being recognized by the prolyl-isomerase Pin1 and dephosphorylated by phosphatase 2A. Here, the protein perturbs mitochondrial function that ultimately leads to apoptosis [85]. Based on other data from the Lebiedzinska group, a significant portion of p66Shc is also present in MAM fractions and its levels increase in an age-dependent manner [122].

Moreover, according to the recent findings by Anelli et al. [123], Ero1α, a quality controller of oxidative protein folding in the ER, is enriched in MAMs and regulates Ca^{2+} flux. The levels of redox-active Ero1α impact Ca^{2+} storage and IP3R-dependent flux. Its silencing inhibits Ca^{2+} re-uptake by the mitochondria, likely by modifying MCU activity. The overexpression of redox-active Ero1α increases passive Ca^{2+} efflux from the ER, thus reducing the $[Ca^{2+}]_{ER}$ and mitochondrial Ca^{2+} fluxes in response to IP3 agonists.

All these proteins cooperate to define a complex picture where mitochondrial Ca^{2+} signals are crucial initiators of apoptosis.

9. Conclusions

Ca^{2+} plays a pivotal role in many biological systems and is of great interest for its potential implications in human malignancies. The interaction between the mitochondria and the ER Ca^{2+} store plays an essential role in allowing these organelles to effectively and rapidly respond to cellular Ca^{2+} signals. ER-mitochondria contact sites, also known as MAMs, are involved in Ca^{2+} homeostasis and participate in cholesterol and phospholipid metabolism and other mitochondrial functions and dynamics. Since the role of MAMs has been revealed, their relevance in disease has become apparent. Altered mitochondrial-ER contacts can deregulate Ca^{2+} signaling, which results in alterations in metabolism and cell death. The study of Ca^{2+} homeostasis and these functional domains has become critical, particularly in relation to cell death and cancer onset and regulation. All these findings highlight the fundamental activity of MAMs, which act as hotspot domains in cancer onset and progression, although their roles in regulating cell death are only partially understood. Therefore, we must expand our knowledge about the composition, function, and regulation of MAMs and how protein networks cooperate to control communication within this organelle to search for new cancer treatments.

Conflict of interest statement

The authors report no relationships that could be construed as a conflict of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

P.P. is grateful to Camilla degli Scrovegni for providing continuous support. P.P. is supported by: Telethon (GGP15219/B), the Italian Association for Cancer Research (AIRC) (IG- 18624), the Italian Cystic Fibrosis Research Foundation (19/2014), the Italian Ministry of Education, University and Research (COFIN no. 20129JLHSY_002, FIRB no. RBAP11FXBC_002, and Futuro in Ricerca no. RBFR10EGVP_001), local funds from the University of Ferrara and the Italian Ministry of Health. C.G is supported by AIRC (MFAG-13521), the Italian Ministry of Health, Cariplo and local funds from the University of Ferrara. S.P. is supported by a research fellowship FISM—Fondazione Italiana Sclerosi Multipla—cod. 2014/B/3. MRW is supported by the Polish National Science Centre grant (UMO-2014/15/B/NZ1/00490).

References

[1] L. Galluzzi, J.M. Bravo-San Pedro, I. Vitale, S.A. Aaronson, J.M. Abrams, D. Adam, E.S. Alnemri, L. Altucci, D. Andrews, M. Annicchiarico-Petruzzelli, E.H. Baehrecke, N.G. Bazan, M.J. Bertrand, K. Bianchi, M.V. Blagosklonny, K. Blomgren, C. Borner, D.E. Bredesen, C. Brenner, M. Campanella, E. Candi, F. Cecconi, F.K. Chan, N.S. Chandel, E.H. Cheng, J.E. Chipuk, J.A. Cidlowski, A. Ciechanover, T.M. Dawson, V.L. Dawson, V. De Laurenzi, R. De Maria, K.M. Debatin, N. Di Daniele, V.M. Dixit, B.D. Dynlacht, W.S. El-Deiry, G.M. Fimia, R.A. Flavell, S. Fulda, C. Garrido, M.L. Gougeon, D.R. Green, H. Gronemeyer, G. Hajnoczky, J.M. Hardwick, M.O. Hengartner, H. Ichijo, B. Joseph, P.J. Jost, T. Kaufmann, O. Kepp, D.J. Klionsky, R.A. Knight, S. Kumar, J.J. Lemasters, B. Levine, A. Linkermann, S.A. Lipton, R.A. Lockshin, C. Lopez-Otin, E. Lugli, F. Madeo, W. Malorni, J.C. Marine, S.J. Martin, J.C. Martinou, J.P. Medema, P. Meier, S. Melino, N. Mizushima, U. Moll, C. Munoz-Pinedo, G. Nunez, A. Oberst, T. Panaretakis, J.M. Penninger, M.E. Peter, M. Piacentini, P. Pinton, J.H. Prehn, H. Puthalakath, G.A. Rabinovich, K.S. Ravichandran, R. Rizzuto, C.M. Rodrigues, D.C. Rubinsztein, T. Rudel, Y. Shi, H.U. Simon, B.R. Stockwell, G. Szabadkai, S.W. Tait, H.L. Tang, N. Tavernarakis, Y. Tsujimoto, T. Vanden Berghe, P. Vandenabeele, A. Villunger, E.F. Wagner, H. Walczak, E. White, W.G. Wood, J. Yuan, Z. Zakeri, B. Zhivotovsky, G. Melino, G. Kroemer, Essential versus accessory aspects of cell death: recommendations of the NCCD 2015, Cell Death Differ. 22 (2015) 58–73.

[2] G. Kroemer, L. Galluzzi, P. Vandenabeele, J. Abrams, E.S. Alnemri, E.H. Baehrecke, M.V. Blagosklonny, W.S. El-Deiry, P. Golstein, D.R. Green, M. Hengartner, R.A. Knight, S. Kumar, S.A. Lipton, W. Malorni, G. Nunez, M.E. Peter, J. Tschopp, J. Yuan, M. Piacentini, B. Zhivotovsky, G. Melino, D. Nomenclature committee on cell, classification of cell death: recommendations of the nomenclature committee on cell death, Cell Death Differ. 16 (2009) (2009) 3–11.

[3] J.F. Curtin, T.G. Cotter, Apoptosis: historical perspectives, Essays Biochem. 39 (2003) 1–10.

[4] K. Wang, D.J. Klionsky, Mitochondria removal by autophagy, Autophagy 7 (2011) 297–300.

[5] M. Yazdankhah, S. Farioli-Vecchioli, A.B. Tonchev, A. Stoykova, F. Cecconi, The autophagy regulators Ambra1 and Beclin 1 are required for adult neurogenesis in the brain subventricular zone, Cell Death Dis. 5 (2014), e1403. .

[6] P. Golstein, G. Kroemer, Cell death by necrosis: towards a molecular definition, Trends Biochem. Sci. 32 (2007) 37–43.

[7] R.M. Graham, J.W. Thompson, J. Wei, N.H. Bishopric, K.A. Webster, Regulation of Bnip3 death pathways by calcium, phosphorylation, and hypoxia-reoxygenation, Antioxid. Redox Signal. 9 (2007) 1309–1315.

[8] J.D. Pinon, V. Labi, A. Egle, A. Villunger, Bim and Bmf in tissue homeostasis and malignant disease, Oncogene 27 (Suppl. 1) (2008) S41–S52.

[9] J. Zhang, P.A. Ney, Role of BNIP3 and NIX in cell death, autophagy, and mitophagy, Cell Death Differ. 16 (2009) 939–946.

[10] T. Zhang, Y. Zhang, M. Cui, L. Jin, Y. Wang, F. Lv, Y. Liu, W. Zheng, H. Shang, J. Zhang, M. Zhang, H. Wu, J. Guo, X. Zhang, X. Hu, C.M. Cao, R.P. Xiao, CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis, Nat. Med. 22 (2016) 175–182.

[11] S. Yoshino, T. Hara, H.J. Nakaoka, A. Kanamori, Y. Murakami, M. Seiki, T. Sakamoto, The ERK signaling target RNF126 regulates anoikis resistance in cancer cells by changing the mitochondrial metabolic flux, Cell Discov. 2 (2016) 16019.

[12] T. Prochnicki, M.S. Mangan, E. Latz, Recent insights into the molecular mechanisms of the NLRP3 inflammasome activation, F1000Res. 5 (2016) 1469.

[13] S. Sperandio, I. de Belle, D.E. Bredesen, An alternative, nonapoptotic form of programmed cell death, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 14376–14381.

[14] S. Yumnam, G.E. Hong, S. Raha, V.V. Saralamma, H.J. Lee, W.S. Lee, E.H. Kim, G.S. Kim, Mitochondrial dysfunction and Ca(2+) overload contributes to hesperidin induced paraptosis in hepatoblastoma cells, HepG2, J. Cell. Physiol. 231 (2016) 1261–1268.

[15] M. Overholtzer, A.A. Mailleux, G. Mouneimne, G. Normand, S.J. Schnitt, R.W. King, E.S. Cibas, J.S. Brugge, A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion, Cell 131 (2007) 966–979.

[16] P. Pinton, R. Rizzuto, Bcl-2 and Ca^{2+} homeostasis in the endoplasmic reticulum, Cell Death Differ. 13 (2006) 1409–1418.

[17] C. Giorgi, M. Bonora, G. Sorrentino, S. Missiroli, F. Poletti, J.M. Suski, F. Galindo Ramirez, R. Rizzuto, F. Di Virgilio, E. Zito, P.P. Pandolfi, M.R. Wieckowski, F. Mammano, G. Del Sal, P. Pinton, p53 at the endoplasmic reticulum regulates apoptosis in a Ca^{2+}-dependent manner, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 1779–1784.

[18] M.G. de Bielke, L. Perez, J. Yancoski, J.B. Oliveira, S. Danielian, FAS haploinsufficiency caused by extracellular missense mutations underlying autoimmune lymphoproliferative syndrome, J. Clin. Immunol. 35 (2015) 769–776.

[19] C. Giorgi, M. Bonora, S. Missiroli, F. Poletti, F.G. Ramirez, G. Morciano, C. Morganti, P.P. Pandolfi, F. Mammano, P. Pinton, Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling, Oncotarget 6 (2015) 1435–1445.

[20] C. Giorgi, S. Missiroli, S. Patergnani, J. Duszynski, M.R. Wieckowski, P. Pinton, Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications, Antioxid. Redox Signal. 22 (2015) 995–1019.

[21] P. Pinton, T. Pozzan, R. Rizzuto, The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store, with functional properties distinct from those of the endoplasmic reticulum, EMBO J. 17 (1998) 5298–5308.

[22] M. Bonora, C. Giorgi, A. Bononi, S. Marchi, S. Patergnani, A. Rimessi, R. Rizzuto, P. Pinton, Subcellular calcium measurements in mammalian cells using jellyfish photoprotein aequorin-based probes, Nat. Protoc. 8 (2013) 2105–2118.

[23] R. Rizzuto, A.W. Simpson, M. Brini, T. Pozzan, Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin, Nature 358 (1992) 325–327.

[24] E. Carafoli, The fateful encounter of mitochondria with calcium: how did it happen? Biochim. Biophys. Acta 1797 (2010) 595–606.

[25] R.S. Lewis, Calcium signaling mechanisms in T lymphocytes, Annu. Rev. Immunol. 19 (2001) 497–521.

[26] E. Szegezdi, U. Fitzgerald, A. Samali, Caspase-12 and ER-stress-mediated apoptosis: the story so far, Ann. N. Y. Acad. Sci. 1010 (2003) 186–194.

[27] H.K. Baumgartner, J.V. Gerasimenko, C. Thorne, P. Ferdek, T. Pozzan, A.V. Tepikin, O.H. Petersen, R. Sutton, A.J. Watson, O.V. Gerasimenko, Calcium elevation in mitochondria is the main Ca^{2+} requirement for mitochondrial permeability transition pore (mPTP) opening, J. Biol. Chem. 284 (2009) 20796–20803.

[28] P. Pinton, D. Ferrari, E. Rapizzi, F. Di Virgilio, T. Pozzan, R. Rizzuto, The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action, EMBO J. 20 (2001) 2690–2701.

[29] H. Ivanova, T. Vervliet, L. Missiaen, J.B. Parys, H. De Smedt, G. Bultynck, Inositol 1,4,5-trisphosphate receptor-isoform diversity in cell death and survival, Biochim. Biophys. Acta 1843 (2014) 2164–2183.

[30] T. Nakagawa, J. Yuan, Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis, J. Cell Biol. 150 (2000) 887–894.

[31] M. Bonora, M.R. Wieckowski, C. Chinopoulos, O. Kepp, G. Kroemer, L. Galluzzi, P. Pinton, Molecular mechanisms of cell death: central implication of ATP synthase in mitochondrial permeability transition, Oncogene 34 (2015) 1475–1486.

[32] A. Criollo, J.M. Vicencio, E. Tasdemir, M.C. Maiuri, S. Lavandero, G. Kroemer, The inositol trisphosphate receptor in the control of autophagy, Autophagy 3 (2007) 350–353.

[33] D.J. Klionsky, K. Abdelmohsen, A. Abe, M.J. Abedin, H. Abeliovich, A. Acevedo Arozena, H. Adachi, C.M. Adams, P.D. Adams, K. Adeli, P.J. Adhihetty, S.G. Adler, G. Agam, R. Agarwal, M.K. Aghi, M. Agnello, P. Agostinis, P.V. Aguilar, J. Aguirre-Ghiso, E.M. Airoldi, S. Ait-Si-Ali, T. Akematsu, E.T. Akporiaye, M. Al-Rubeai, G.M. Albaiceta, C. Albanese, D. Albani, M.L. Albert, J. Aldudo, H. Algul, M. Alirezaei, I. Alloza, A. Almasan, M. Almonte-Beceril, E.S. Alnemri, C. Alonso, N. Altan-Bonnet, D.C. Altieri, S. Alvarez, L. Alvarez-Erviti, S. Alves, G. Amadoro, A. Amano, C. Amantini, S. Ambrosio, I. Amelio, A.O. Amer, M. Amessou, A. Amon, Z. An, F.A. Anania, S.U. Andersen, U.P. Andley, C.K. Andreadi, N. Andrieu-Abadie, A. Anel, D.K. Ann, S. Anoopkumar-Dukie, M. Antonioli, H. Aoki, N. Apostolova, S. Aquila, K. Aquilano, K. Araki, E. Arama, A. Aranda, J. Araya, A. Arcaro, E. Arias, H. Arimoto, A.R. Ariosa, J.L. Armstrong, T. Arnould, I. Arsov, K. Asanuma, V. Askanas, E. Asselin, R. Atarashi, S.S. Atherton, J.D. Atkin, L.D. Attardi, P. Auberger, G. Auburger, L. Aurelian, R. Autelli, L. Avagliano, M.L. Avantaggiati, L. Avrahami, S. Awale, N. Azad, T. Bachetti, J.M. Backer, D.H. Bae, J.S. Bae, O.N. Bae, S.H. Bae, E.H. Baehrecke, S.H. Baek, S. Baghdiguian, A. Bagniewska-Zadworna, H. Bai, J. Bai, X.Y. Bai, Y. Bailly, K.N. Balaji, W. Balduini, A. Ballabio, R. Balzan, R. Banerjee, G. Banhegyi, H. Bao, B. Barbeau, M.D. Barrachina, E. Barreiro, B. Bartel, A. Bartolome, D.C. Bassham, M.T. Bassi, R.C. Bast Jr., A. Basu, M.T. Batista, H. Batoko, M. Battino, K. Bauckman, B.L. Baumgarner, K.U. Bayer, R. Beale, J.F. Beaulieu, G.R. Beck Jr., C. Becker, J.D. Beckham, P.A. Bedard, P.J. Bednarski, T.J. Begley, C. Behl, C. Behrends, G.M. Behrens, K.E. Behrns, E. Bejarano, A. Belaid, F. Belleudi, G. Benard, G. Berchem, D. Bergamaschi, M. Bergami, B. Berkhout, L. Berliocchi, A. Bernard, M. Bernard, F. Bernassola, A. Bertolotti, A.S. Bess, S. Besteiro, S. Bettuzzi, S. Bhalla, S. Bhattacharyya, S.K. Bhutia, C. Biagosch, M.W. Bianchi, M. Biard-Piechaczyk, V. Billes, C. Bincoletto, B. Bingol, S.W. Bird, M. Bitoun, I. Bjedov, C. Blackstone, L. Blanc, G.A. Blanco, H.K. Blomhoff, E. Boada-Romero, S. Bockler, M. Boes, K. Boesze-Battaglia, L.H. Boise, A. Bolino, A. Boman, P. Bonaldo, M. Bordi, J. Bosch, L.M. Botana, J. Botti, G. Bou, M. Bouche, M. Bouchecareilh, M.J. Boucher, M.E. Boulton, S.G. Bouret, P. Boya, M. Boyer-Guittaut, P.V. Bozhkov, N. Brady, V.M. Braga, C. Brancolini, G.H. Braus, J.M. Bravo-San Pedro, L.A. Brennan, E.H. Bresnick, P. Brest, D. Bridges, M.A. Bringer, M. Brini, G.C. Brito, B. Brodin, P.S. Brookes, E.J. Brown, K. Brown, H.E. Broxmeyer, A. Bruhat, P.C. Brum, J.H. Brumell, N. Brunetti-Pierri, R.J. Bryson-Richardson, S. Buch, A.M. Buchan, H. Budak, D.V. Bulavin, S.J. Bultman, G. Bultynck, V. Bumbasirevic, Y. Burelle, R.E. Burke, M. Burmeister, P. Butikofer, L. Caberlotto, K. Cadwell, M. Cahova, D. Cai, J. Cai, Q. Cai, S. Calatayud, N. Camougrand, M. Campanella, G.R. Campbell, M. Campbell, S. Campello, R. Candau, I. Caniggia, L. Cantoni, L. Cao, A.B. Caplan, M. Caraglia, C. Cardinali, S.M. Cardoso, J.S. Carew, L.A. Carleton, C.R. Carlin, S. Carloni, S.R. Carlsson, D. Carmona-Gutierrez, L.A. Carneiro, O. Carnevali, S. Carra, A. Carrier, B. Carroll, C. Casas, J. Casas, G. Cassinelli, P. Castets, S. Castro-Obregon, G. Cavallini, I. Ceccherini, F. Cecconi, A.I. Cederbaum, V. Cena, S. Cenci, C. Cerella, D. Cervia, S. Cetrullo, H. Chaachouay, H.J. Chae, A.S. Chagin, C.Y. Chai, G. Chakrabarti, G. Chamilos, E.Y. Chan, M.T. Chan, D. Chandra, P. Chandra, C.P. Chang, R.C. Chang, T.Y. Chang, J.C. Chatham, S. Chatterjee, S. Chauhan, Y. Che, M.E. Cheetham, R. Cheluvappa, C.J. Chen, G. Chen, G.C. Chen, G. Chen, H. Chen, J.W. Chen, J.K. Chen, M. Chen, M. Chen, P. Chen, Q. Chen, Q. Chen, S.D. Chen, S. Chen, S.S. Chen, W. Chen, W.J. Chen, W.Q. Chen, W. Chen, X. Chen, Y.H. Chen, Y.G. Chen, Y. Chen, Y. Chen, Y. Chen, Y.J. Chen, Y.Q. Chen, Y. Chen, Z. Chen, Z. Chen, A. Cheng, C.H. Cheng, H. Cheng, H. Cheong, S. Cherry, J. Chesney, C.H. Cheung, E. Chevet, H.C. Chi, S.G. Chi, F. Chiacchiera, H.L. Chiang, R. Chiarelli, M. Chiariello, M. Chieppa, L.S. Chin, M. Chiong, G.N. Chiu, D.H. Cho, S.G. Cho, W.C. Cho, Y.Y. Cho, Y.S. Cho, A.M. Choi, E.J. Choi, E.K. Choi, J. Choi, M.E. Choi, S.I. Choi, T.F. Chou, S. Chouaib, D. Choubey, V. Choubey, K.C. Chow, K. Chowdhury, C.T. Chu, T.H. Chuang, T. Chun, H. Chung, T. Chung, Y.L. Chung, Y.J. Chwae, V. Cianfanelli, R. Ciarcia, I.A. Ciechomska, M.R. Ciriolo, M. Cirone, S. Claerhout, M.J. Clague, J. Claria, P.G. Clarke, R. Clarke, E. Clementi, C. Cleyrat, M. Cnop, E.M. Coccia, T. Cocco, P. Codogno, J. Coers, E.E. Cohen, D. Colecchia, L. Coletto, N.S. Coll, E. Colucci-Guyon, S. Comincini, M. Condello, K.L. Cook, G.H. Coombs, C.D. Cooper, J.M. Cooper, I. Coppens, M.T. Corasaniti, M. Corazzari, R. Corbalan, E. Corcelle-Termeau, M.D. Cordero, C. Corral-Ramos, O. Corti, A. Cossarizza, P. Costelli, S. Costes, S.L.

Cotman, A. Coto-Montes, S. Cottet, E. Couve, L.R. Covey, L.A. Cowart, J.S. Cox, F.P. Coxon, C.B. Coyne, M.S. Cragg, R.J. Craven, T. Crepaldi, J.L. Crespo, A. Criollo, V. Crippa, M.T. Cruz, A.M. Cuervo, J.M. Cuezva, T. Cui, P.R. Cutillas, M.J. Czaja, M.F. Czyzyk-Krzeska, R.K. Dagda, U. Dahmen, C. Dai, W. Dai, Y. Dai, K.N. Dalby, L. Dalla Valle, G. Dalmasso, M. D'Amelio, M. Damme, A. Darfeuille-Michaud, C. Dargemont, V.M. Darley-Usmar, S. Dasarathy, B. Dasgupta, S. Dash, C.R. Dass, H.M. Davey, L.M. Davids, D. Davila, R.J. Davis, T.M. Dawson, V.L. Dawson, P. Daza, J. de Belleroche, P. de Figueiredo, R.C. de Figueiredo, J. de la Fuente, L. De Martino, A. De Matteis, G.R. De Meyer, A. De Milito, M. De Santi, W. de Souza, V. De Tata, D. De Zio, J. Debnath, R. Dechant, J.P. Decuypere, S. Deegan, B. Dehay, B. Del Bello, D.P. Del Re, R. Delage-Mourroux, L.M. Delbridge, L. Deldicque, E. Delorme-Axford, Y. Deng, J. Dengjel, M. Denizot, P. Dent, C.J. Der, V. Deretic, B. Derrien, E. Deutsch, T.P. Devarenne, R.J. Devenish, S. Di Bartolomeo, N. Di Daniele, F. Di Domenico, A. Di Nardo, S. Di Paola, A. Di Pietro, L. Di Renzo, A. DiAntonio, G. Diaz-Araya, I. Diaz-Laviada, M.T. Diaz-Meco, J. Diaz-Nido, C.A. Dickey, R.C. Dickson, M. Diederich, P. Digard, I. Dikic, S.P. Dinesh-Kumar, C. Ding, W.X. Ding, Z. Ding, L. Dini, J.H. Distler, A. Diwan, M. Djavaheri-Mergny, K. Dmytruk, R.C. Dobson, V. Doetsch, K. Dokladny, S. Dokudovskaya, M. Donadelli, X.C. Dong, X. Dong, Z. Dong, T.M. Donohue Jr., K.S. Doran, G. D'Orazi, G.W. Dorn 2nd, V. Dosenko, S. Dridi, L. Drucker, J. Du, L.L. Du, L. Du, A. du Toit, P. Dua, L. Duan, P. Duann, V.K. Dubey, M.R. Duchen, M.A. Duchosal, H. Duez, I. Dugail, V.I. Dumit, M.C. Duncan, E.A. Dunlop, W.A. Dunn Jr., N. Dupont, L. Dupuis, R.V. Duran, T.M. Durcan, S. Duvezin-Caubet, U. Duvvuri, V. Eapen, D. Ebrahimi-Fakhari, A. Echard, L. Eckhart, C.L. Edelstein, A.L. Edinger, L. Eichinger, T. Eisenberg, A. Eisenberg-Lerner, N.T. Eissa, W.S. El-Deiry, V. El-Khoury, Z. Elazar, H. Eldar-Finkelman, C.J. Elliott, E. Emanuele, U. Emmenegger, N. Engedal, A.M. Engelbrecht, S. Engelender, J.M. Enserink, R. Erdmann, J. Erenpreisa, R. Eri, J.L. Eriksen, A. Erman, R. Escalante, E.L. Eskelinen, L. Espert, L. Esteban-Martinez, T.J. Evans, M. Fabri, G. Fabrias, C. Fabrizi, A. Facchiano, N.J. Faergeman, A. Faggioni, W.D. Fairlie, C. Fan, D. Fan, J. Fan, S. Fang, M. Fanto, A. Fanzani, T. Farkas, M. Faure, F.B. Favier, H. Fearnhead, M. Federici, E. Fei, T.C. Felizardo, H. Feng, Y. Feng, Y. Feng, T.A. Ferguson, A.F. Fernandez, M.G. Fernandez-Barrena, J.C. Fernandez-Checa, A. Fernandez-Lopez, M.E. Fernandez-Zapico, O. Feron, E. Ferraro, C.V. Ferreira-Halder, L. Fesus, R. Feuer, F.C. Fiesel, E.C. Filippi-Chiela, G. Filomeni, G.M. Fimia, J.H. Fingert, S. Finkbeiner, T. Finkel, F. Fiorito, P.B. Fisher, M. Flajolet, F. Flamigni, O. Florey, S. Florio, R.A. Floto, M. Folini, C. Follo, E.A. Fon, F. Fornai, F. Fortunato, A. Fraldi, R. Franco, A. Francois, A. Francois, L.B. Frankel, I.D. Fraser, N. Frey, D.G. Freyssenet, C. Frezza, S.L. Friedman, D.E. Frigo, D. Fu, J.M. Fuentes, J. Fueyo, Y. Fujitani, Y. Fujiwara, M. Fujiya, M. Fukuda, S. Fulda, C. Fusco, B. Gabryel, M. Gaestel, P. Gailly, M. Gajewska, S. Galadari, G. Galili, I. Galindo, M.F. Galindo, G. Galliciotti, L. Galluzzi, L. Galluzzi, V. Galy, N. Gammoh, S. Gandy, A.K. Ganesan, S. Ganesan, I.G. Ganley, M. Gannage, F.B. Gao, F. Gao, J.X. Gao, L. Garcia Nannig, E. Garcia Vescovi, M. Garcia-Macia, C. Garcia-Ruiz, A.D. Garg, P.K. Garg, R. Gargini, N.C. Gassen, D. Gatica, E. Gatti, J. Gavard, E. Gavathiotis, L. Ge, P. Ge, S. Ge, P.W. Gean, V. Gelmetti, A.A. Genazzani, J. Geng, P. Genschik, L. Gerner, J.E. Gestwicki, D.A. Gewirtz, S. Ghavami, E. Ghigo, D. Ghosh, A.M. Giammarioli, F. Giampieri, C. Giampietri, A. Giatromanolaki, D.J. Gibbings, L. Gibellini, S.B. Gibson, V. Ginet, A. Giordano, F. Giorgini, E. Giovannetti, S.E. Girardin, S. Gispert, S. Giuliano, C.L. Gladson, A. Glavic, M. Gleave, N. Godefroy, R.M. Gogal Jr., K. Gokulan, G.H. Goldman, D. Goletti, M.S. Goligorsky, A.V. Gomes, L.C. Gomes, H. Gomez, C. Gomez-Manzano, R. Gomez-Sanchez, D.A. Goncalves, E. Goncu, Q. Gong, C. Gongora, C.B. Gonzalez, P. Gonzalez-Alegre, P. Gonzalez-Cabo, R.A. Gonzalez-Polo, I.S. Goping, C. Gorbea, N.V. Gorbunov, D.R. Goring, A.M. Gorman, S.M. Gorski, S. Goruppi, S. Goto-Yamada, C. Gotor, R.A. Gottlieb, I. Gozes, D. Gozuacik, Y. Graba, M. Graef, G.E. Granato, G.D. Grant, S. Grant, G.L. Gravina, D.R. Green, A. Greenhough, M.T. Greenwood, B. Grimaldi, F. Gros, C. Grose, J.F. Groulx, F. Gruber, P. Grumati, T. Grune, J.L. Guan, K.L. Guan, B. Guerra, C. Guillen, K. Gulshan, J. Gunst, C. Guo, L. Guo, M. Guo, W. Guo, X.G. Guo, A.A. Gust, A.B. Gustafsson, E. Gutierrez, M.G. Gutierrez, H.S. Gwak, A. Haas, J.E. Haber, S. Hadano, M. Hagedorn, D.R. Hahn, A.J. Halayko, A. Hamacher-Brady, K. Hamada, A. Hamai, A. Hamann, M. Hamasaki, I. Hamer, Q. Hamid, E.M. Hammond, F. Han, W. Han, J.T. Handa, J.A. Hanover, M. Hansen, M. Harada, L. Harhaji-Trajkovic, J.W. Harper, A.H. Harrath, A.L. Harris, J. Harris, U. Hasler, P. Hasselblatt, K. Hasui, R.G. Hawley, T.S. Hawley, C. He, C.Y. He, F. He, G. He, R.R. He, X.H. He, Y.W. He, Y.Y. He, J.K. Heath, M.J. Hebert, R.A. Heinzen, G.V. Helgason, M. Hensel, E.P. Henske, C. Her, P.K. Herman, A. Hernandez, C. Hernandez, S. Hernandez-Tiedra, C. Hetz, P.R. Hiesinger, K. Higaki, S. Hilfiker, B.G. Hill, J.A. Hill, W.D. Hill, K. Hino, D. Hofius, P. Hofman, G.U. Hoglinger, J. Hohfeld, M.K. Holz, Y. Hong, D.A. Hood, J.J. Hoozemans, T. Hoppe, C. Hsu, C.Y. Hsu, L.C. Hsu, D. Hu, G. Hu, H.M. Hu, H. Hu, M.C. Hu, Y.C. Hu, Z.W. Hu, F. Hua, Y. Hua, C. Huang, H.L. Huang, K.H. Huang, K.Y. Huang, S. Huang, S. Huang, W.P. Huang, Y.R. Huang, Y. Huang, Y. Huang, T.B. Huber, P. Huebbe, W.K. Huh, J.J. Hulmi, G.M. Hur, J.H. Hurley, Z. Husak, S.N. Hussain, S. Hussain, J.J. Hwang, S. Hwang, T.I. Hwang, A. Ichihara, Y. Imai, C. Imbriano, M. Inomata, T. Into, V. Iovane, J.L. Iovanna, R.V. Iozzo, N.Y. Ip, J.E. Irazoqui, P. Iribarren, Y. Isaka, A.J. Isakovic, H. Ischiropoulos, J.S. Isenberg, M. Ishaq, H. Ishida, I. Ishii, J.E. Ishmael, C. Isidoro, K. Isobe, E. Isono, S. Issazadeh-Navikas, K. Itahana, E. Itakura, A.I. Ivanov, A.K. Iyer, J.M. Izquierdo, Y. Izumi, V. Izzo, M. Jaattela, N. Jaber, D.J. Jackson, W.T. Jackson, T.G. Jacob, T.S. Jacques, C. Jagannath, A. Jain, N.R. Jana, B.K. Jang, A. Jani, B. Janji, P.R. Jannig, P.J. Jansson, S. Jean, M. Jendrach, J.H. Jeon, N. Jessen, E.B. Jeung, K. Jia, L. Jia, H. Jiang, H. Jiang, L. Jiang, T. Jiang, X. Jiang, X. Jiang, X. Jiang, Y. Jiang, Y. Jiang, A. Jimenez, C. Jin, H. Jin, L. Jin, M. Jin, S. Jin, U.K. Jinwal, E.K. Jo, T. Johansen, D.E. Johnson, G.V. Johnson, J.D. Johnson, E. Jonasch, C. Jones, L.A. Joosten, J. Jordan, A.M. Joseph, B. Joseph, A.M. Joubert, D. Ju, J. Ju, H.F. Juan, K. Juenemann, G. Juhasz, H.S. Jung, J.U. Jung, Y.K. Jung, H. Jungbluth, M.J. Justice, B. Jutten, N.O. Kaakoush, K. Kaarniranta, A. Kaasik, T. Kabuta, B. Kaeffer, K. Kagedal, A. Kahana, S. Kajimura, O. Kakhlon, M. Kalia, D.V. Kalvakolanu, Y. Kamada, K. Kambas, V.O. Kaminskyy, H.H. Kampinga, M. Kandouz, C. Kang, R. Kang, T.C. Kang, T. Kanki, T.D. Kanneganti, H. Kanno, A.G. Kanthasamy, M. Kantorow, M. Kaparakis-Liaskos, O. Kapuy, V. Karantza, M.R. Karim, P. Karmakar, A. Kaser, S. Kaushik, T. Kawula, A.M. Kaynar, P.Y. Ke, Z.J. Ke, J.H. Kehrl, K.E. Keller, J.K. Kemper, A.K. Kenworthy, O. Kepp, A. Kern, S. Kesari, D. Kessel, R. Ketteler, C. Kettelhut Ido, B. Khambu, M.M. Khan, V.K. Khandelwal, S. Khare, J.G. Kiang, A.A. Kiger, A. Kihara, A.L. Kim, C.H. Kim, D.R. Kim, D.H. Kim, E.K. Kim, H.Y. Kim, H.R. Kim, J.S. Kim, J.H. Kim, J.C. Kim, J.H. Kim, K.W. Kim, M.D. Kim, M.M. Kim, P.K. Kim, S.W. Kim, S.Y. Kim, Y.S. Kim, Y. Kim, A. Kimchi, A.C. Kimmelman, T. Kimura, J.S. King, K. Kirkegaard, V. Kirkin, L.A. Kirshenbaum, S. Kishi, Y. Kitajima, K. Kitamoto, Y. Kitaoka, K. Kitazato, R.A. Kley, W.T. Klimecki, M. Klinkenberg, J. Klucken, H. Knaevelsrud, E. Knecht, L. Knuppertz, J.L. Ko, S. Kobayashi, J.C. Koch, C. Koechlin-Ramonatxo, U. Koenig, Y.H. Koh, K. Kohler, S.D. Kohlwein, M. Koike, M. Komatsu, E. Kominami, D. Kong, H.J. Kong, E.G. Konstantakou, B.T. Kopp, T. Korcsmaros, L. Korhonen, V.I. Korolchuk, N.V. Koshkina, Y. Kou, M.I. Koukourakis, C. Koumenis, A.L. Kovacs, T. Kovacs, W.J. Kovacs, D. Koya, C. Kraft, D. Krainc, H. Kramer, T. Kravic-Stevovic, W. Krek, C. Kretz-Remy, R. Krick, M. Krishnamurthy, J. Kriston-Vizi, G. Kroemer, M.C. Kruer, R. Kruger, N.T. Ktistakis, K. Kuchitsu, C. Kuhn, A.P. Kumar, A. Kumar, A. Kumar, D. Kumar, D. Kumar, R. Kumar, S. Kumar, M. Kundu, H.J. Kung, A. Kuno, S.H. Kuo, J. Kuret, T. Kurz, T. Kwok, T.K. Kwon, Y.T. Kwon, I. Kyrmizi, A.R. La Spada, F. Lafont, T. Lahm, A. Lakkaraju, T. Lam, T. Lamark, S. Lancel, T.H. Landowski, D.J. Lane, J.D. Lane, C. Lanzi, P. Lapaquette, L.R. Lapierre, J. Laporte, J. Laukkarinen, G.W. Laurie, S. Lavandero, L. Lavie, M.J. LaVoie, B.Y. 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Torriglia, M.R. Torrisi, C. Tournier, R. Towns, V. Trajkovic, L.H. Travassos, G. Triola, D.N. Tripathi, D. Trisciuoglio, R. Troncoso, I.P. Trougakos, A.C. Truttmann, K.J. Tsai, M.P. Tschan, Y.H. Tseng, T. Tsukuba, A. Tsung, A.S. Tsvetkov, S. Tu, H.Y. Tuan, M. Tucci, D.A. Tumbarello, B. Turk, V. Turk, R.F. Turner, A.A. Tveita, S.C. Tyagi, M. Ubukata, Y. Uchiyama, A. Udelnow, T. Ueno, M. Umekawa, R. Umemiya-Shirafuji, B.R. Underwood, C. Ungermann, R.P. Ureshino, R. Ushioda, V.N. Uversky, N.L. Uzcategui, T. Vaccari, M.I. Vaccaro, L. Vachova, H. Vakifahmetoglu-Norberg, R. Valdor, E.M. Valente, F. Vallette, A.M. Valverde, G. Van den Berghe, L. Van Den Bosch, G.R. van den Brink, F.G. van der Goot, I.J. van der Klei, L.J. van der Laan, W.G. van Doorn, M. van Egmond, K.L. van Golen, L. Van Kaer, M. van Lookeren Campagne, P. Vandenabeele, W. Vandenberghe, I. Vanhorebeek, I. Varela-Nieto, M.H. Vasconcelos, R. Vasko, D.G. Vavvas, I. Vega-Naredo, G. Velasco, A.D. Velentzas, P.D. Velentzas, T. Vellai, E. Vellenga, M.H. Vendelbo, K. Venkatachalam, N. Ventura, S. Ventura, P.S. Veras, M. Verdier, B.G. Vertessy, A. Viale, M. Vidal, H.L. Vieira, R.D. Vierstra, N. Vigneswaran, N. Vij, M. Vila, M. Villar, V.H. Villar, J. Villarroya, C. Vindis, G. Viola, M.T. Viscomi, G. Vitale, D.T. Vogl, O.V. Voitsekhovskaja, C. von Haefen, K. von Schwarzenberg, D.E. Voth, V. Vouret-Craviari, K. Vuori, J.M. Vyas, C. Waeber, C.L. Walker, M.J. Walker, J. Walter, L. Wan, X. Wan, B. Wang, C. Wang, C.Y. Wang, C. Wang, C. Wang, C. Wang, D. Wang, F. Wang, F. Wang, G. Wang, H.J. Wang, H. Wang, H.G. Wang, H. Wang, H.D. Wang, J. Wang, J. Wang, M. Wang, M.Q. Wang, P.Y. Wang, P. Wang, R.C. Wang, S. Wang, T.F. Wang, X. Wang, X.J. Wang, X.W. Wang, X. Wang, X. Wang, Y. Wang, Y. Wang, Y. Wang, Y.J. Wang, Y. Wang, Y. Wang, Y.T. Wang, Y. Wang, Z.N. Wang, P. Wappner, C. Ward, D.M. Ward, G. Warnes, H. Watada, Y. Watanabe, K. Watase, T.E. Weaver, C.D. Weekes, J. Wei, T. Weide, C.C. Weihl, G. Weindl, S.N. Weis, L. Wen, X. Wen, Y. Wen, B. Westermann, C.M. Weyand, A.R. White, E. White, J.L. Whitton, A.J. Whitworth, J. Wiels, F. Wild, M.E. Wildenberg, T. Wileman, D.S. Wilkinson, S. Wilkinson, D. Willbold, C. Williams, K. Williams, P.R. Williamson, K.F. Winklhofer, S.S. Witkin, S.E. Wohlgemuth, T. Wollert, E.J. Wolvetang, E. Wong, G.W. Wong, R.W. Wong, V.K. Wong, E.A. Woodcock, K.L. Wright, C. Wu, D. Wu, G.S. Wu, J. Wu, J. Wu, M. Wu, M. Wu, S. Wu, W.K. Wu, Y. Wu, Z. Wu, C.P. Xavier, R.J. Xavier, G.X. Xia, T. Xia, W. Xia, Y. Xia, H. Xiao, J. Xiao, S. Xiao, W. Xiao, C.M. Xie, Z. Xie, Z. Xie, M. Xilouri, Y. Xiong, C. Xu, C. Xu, F. Xu, H. Xu, H. Xu, J. Xu, J. Xu, J. Xu, L. Xu, X. Xu, Y. Xu, Y. Xu, Z.X. Xu, Z. Xu, Y. Xue, T. Yamada, A. Yamamoto, K. Yamanaka, S. Yamashina, S. Yamashiro, B. Yan, B. Yan, X. Yan, Z. Yan, Y. Yanagi, D.S. Yang, J.M. Yang, L. Yang, M. Yang, P.M. Yang, P. Yang, Q. Yang, W. Yang, W.Y. Yang, X. Yang, Y. Yang, Y. Yang, Z. Yang, Z. Yang, M.C. Yao, P.J. Yao, X. Yao, Z. Yao, Z. Yao, L.S. Yasui, M. Ye, B. Yedvobnick, B. Yeganeh, E.S. Yeh, P.L. Yeyati, F. Yi, L. Yi, X.M. Yin, C.K. Yip, Y.M. Yoo, Y.H. Yoo, S.Y. Yoon, K. Yoshida, T. Yoshimori, K.H. Young, H. Yu, J.J. Yu, J.T. Yu, J. Yu, L. Yu, W.H. Yu, X.F. Yu, Z. Yu, J. Yuan, Z.M. Yuan, B.Y. Yue, J. Yue, Z. Yue, D.N. Zacks, E. Zacksenhaus, N. Zaffaroni, T. Zaglia, Z. Zakeri, V. Zecchini, J. Zeng, M. Zeng, Q. Zeng, A.S. Zervos, D.D. Zhang, F. Zhang, G. Zhang, G.C. Zhang, H. Zhang, H. Zhang, H. Zhang, H. Zhang, J. Zhang, J. Zhang, J. Zhang, J. Zhang, J.P. Zhang, L. Zhang, L. Zhang, L. Zhang, L. Zhang, M.Y. Zhang, X. Zhang, X.D. Zhang, Y. Zhang, Y. Zhang, Y. Zhang, Y. Zhang, Y. Zhang, M. Zhao, W.L. Zhao, X. Zhao, Y.G. Zhao, Y. Zhao, Y. Zhao, Y.X. Zhao, Z. Zhao, Z.J. Zhao, D. Zheng, X.L. Zheng, X. Zheng, B. Zhivotovsky, Q. Zhong, G.Z. Zhou, G. Zhou, H. Zhou, S.F. Zhou, X.J. Zhou, H. Zhu, H. Zhu, W.G. Zhu, W. Zhu, X.F. Zhu, Y. Zhu, S.M. Zhuang, X. Zhuang, E. Ziparo, C.E. Zois, T. Zoladek, W.X. Zong, A. Zorzano, S.M. Zughaier, Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition), Autophagy 12 (2016) 1–222.

[34] C. Cardenas, R.A. Miller, I. Smith, T. Bui, J. Molgo, M. Muller, H. Vais, K.H. Cheung, J. Yang, I. Parker, C.B. Thompson, M.J. Birnbaum, K.R. Hallows, J.K. Foskett, Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca^{2+} transfer to mitochondria, Cell 142 (2010) 270–283.

[35] D.R. Green, L. Galluzzi, G. Kroemer, Cell biology. Metabolic control of cell death, Science 345 (2014) 1250256.

[36] S. Missiroli, M. Bonora, S. Patergnani, F. Poletti, M. Perrone, R. Gafa, E. Magri, A. Raimondi, G. Lanza, C. Tacchetti, G. Kroemer, P.P. Pandolfi, P. Pinton, C. Giorgi, PML at mitochondria-associated membranes is critical for the repression of autophagy and cancer development, Cell Rep. 16 (2016) 2415–2427.

[37] C. Cardenas, M. Muller, A. McNeal, A. Lovy, F. Jana, G. Bustos, F. Urra, N. Smith, J. Molgo, J.A. Diehl, T.W. Ridky, J.K. Foskett, Selective vulnerability of cancer cells by inhibition of Ca(2+) transfer from endoplasmic reticulum to mitochondria, Cell Rep. 14 (2016) 2313–2324.

[38] M.W. Harr, Y. Rong, M.D. Bootman, H.L. Roderick, C.W. Distelhorst, Glucocorticoid-mediated inhibition of Lck modulates the pattern of T cell receptor-induced calcium signals by down-regulating inositol 1,4,5-trisphosphate receptors, J. Biol. Chem. 284 (2009) 31860–31871.

[39] P. Aranguiz-Urroz, D. Soto, A. Contreras, R. Troncoso, M. Chiong, J. Montenegro, D. Venegas, C. Smolic, P. Ayala, W.G. Thomas, S. Lavandero, G. Diaz-Araya, Differential participation of angiotensin II type 1 and 2 receptors in the regulation of cardiac cell death triggered by angiotensin II, Am. J. Hypertens. 22 (2009) 569–576.

[40] G. Bkaily, N. El-Bizri, M. Nader, K.M. Hazzouri, J. Riopel, D. Jacques, D. Regoli, P. D'Orleans-Juste, F. Gobeil Jr., L. Avedanian, Angiotensin II induced increase in frequency of cytosolic and nuclear calcium waves of heart cells via activation of AT1 and AT2 receptors, Peptides 26 (2005) 1418–1426.

[41] H. Vahora, M.A. Khan, U. Alalami, A. Hussain, The potential role of nitric oxide in halting cancer progression through chemoprevention, J. Cancer Prev. 21 (2016) 1–12.

[42] S. Huerta-Yepez, S. Baritaki, G. Baay-Guzman, M.A. Hernandez-Luna, A. Hernandez-Cueto, M.I. Vega, B. Bonavida, Contribution of either YY1 or BclXL-induced inhibition by the NO-donor DETANONOate in the reversal of drug resistance, both in vitro and in vivo. YY1 and BclXL are overexpressed in prostate cancer, Nitric Oxide Biol. Chem. 29 (2013) 17–24.

[43] M. Yamaguchi, The anti-apoptotic effect of regucalcin is mediated through multisignaling pathways, Apoptosis 18 (2013) 1145–1153.

[44] A.M. Florea, D. Busselberg, Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects, Cancer 3 (2011) 1351–1371.
[45] S. Marchi, P. Pinton, Alterations of calcium homeostasis in cancer cells, Curr. Opin. Pharmacol. 29 (2016) 1–6.
[46] S. Marchi, P. Pinton, The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications, J. Physiol. 592 (2014) 829–839.
[47] J.M. Baughman, F. Perocchi, H.S. Girgis, M. Plovanich, C.A. Belcher-Timme, Y. Sancak, X.R. Bao, L. Strittmatter, O. Goldberger, R.L. Bogorad, V. Koteliansky, V.K. Mootha, Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter, Nature 476 (2011) 341–345.
[48] F. Perocchi, V.M. Gohil, H.S. Girgis, X.R. Bao, J.E. McCombs, A.E. Palmer, V.K. Mootha, MICU1 encodes a mitochondrial EF hand protein required for Ca(2+) uptake, Nature 467 (2010) 291–296.
[49] K. Mallilankaraman, P. Doonan, C. Cardenas, H.C. Chandramoorthy, M. Muller, R. Miller, N.E. Hoffman, R.K. Gandhirajan, J. Molgo, M.J. Birnbaum, B.S. Rothberg, D.O. Mak, J.K. Foskett, M. Madesh, MICU1 is an essential gatekeeper for MCU-mediated mitochondrial Ca(2+) uptake that regulates cell survival, Cell 151 (2012) 630–644.
[50] M. Patron, V. Checchetto, A. Raffaello, E. Teardo, D. Vecellio Reane, M. Mantoan, V. Granatiero, I. Szabo, D. De Stefani, R. Rizzuto, MICU1 and MICU2 finely tune the mitochondrial Ca^{2+} uniporter by exerting opposite effects on MCU activity, Mol. Cell 53 (2014) 726–737.
[51] Y. Sancak, A.L. Markhard, T. Kitami, E. Kovacs-Bogdan, K.J. Kamer, N.D. Udeshi, S.A. Carr, D. Chaudhuri, D.E. Clapham, A.A. Li, S.E. Calvo, O. Goldberger, V.K. Mootha, EMRE is an essential component of the mitochondrial calcium uniporter complex, Science 342 (2013) 1379–1382.
[52] A. Raffaello, D. De Stefani, D. Sabbadin, E. Teardo, G. Merli, A. Picard, V. Checchetto, S. Moro, I. Szabo, R. Rizzuto, The mitochondrial calcium uniporter is a multimer that can include a dominant-negative pore-forming subunit, EMBO J. 32 (2013) 2362–2376.
[53] D. De Stefani, A. Raffaello, E. Teardo, I. Szabo, R. Rizzuto, A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter, Nature 476 (2011) 336–340.
[54] M. Giacomello, I. Drago, P. Pizzo, T. Pozzan, Mitochondrial Ca^{2+} as a key regulator of cell life and death, Cell Death Differ. 14 (2007) 1267–1274.
[55] S. Marchi, L. Lupini, S. Patergnani, A. Rimessi, S. Missiroli, M. Bonora, A. Bononi, F. Corra, C. Giorgi, E. De Marchi, F. Poletti, R. Gafa, G. Lanza, M. Negrini, R. Rizzuto, P. Pinton, Downregulation of the mitochondrial calcium uniporter by cancer-related miR-25, Curr. Biol. 23 (2013) 58–63.
[56] Y. Liao, Y. Hao, H. Chen, Q. He, Z. Yuan, J. Cheng, Mitochondrial calcium uniporter protein MCU is involved in oxidative stress-induced cell death, Protein Cell 6 (2015) 434–442.
[57] A.N. Antony, M. Paillard, C. Moffat, E. Juskeviciute, J. Correnti, B. Bolon, E. Rubin, G. Csordas, E.L. Seifert, J.B. Hoek, G. Hajnoczky, MICU1 regulation of mitochondrial Ca(2+) uptake dictates survival and tissue regeneration, Nat. Commun. 7 (2016) 10955.
[58] A. Tosatto, R. Sommaggio, C. Kummerow, R.B. Bentham, T.S. Blacker, T. Berecz, M.R. Duchen, A. Rosato, I. Bogeski, G. Szabadkai, R. Rizzuto, C. Mammucari, The mitochondrial calcium uniporter regulates breast cancer progression via HIF-1alpha, EMBO Mol. Med. 8 (2016) 569–585.
[59] M.F. Tsai, D. Jiang, L. Zhao, D. Clapham, C. Miller, Functional reconstitution of the mitochondrial Ca^{2+}/H^{+} antiporter Letm1, J. Gen. Physiol. 143 (2014) 67–73.
[60] D. Jiang, L. Zhao, D.E. Clapham, Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^{+} antiporter, Science 326 (2009) 144–147.
[61] E. Froschauer, K. Nowikovsky, R.J. Schweyen, Electroneutral K^{+}/H^{+} exchange in mitochondrial membrane vesicles involves Yol027/Letm1 proteins, Biochim. Biophys. Acta 1711 (2005) 41–48.
[62] U. De Marchi, J. Santo-Domingo, C. Castelbou, I. Sekler, A. Wiederkehr, N. Demaurex, NCLX protein, but not LETM1, mediates mitochondrial Ca^{2+} extrusion, thereby limiting Ca^{2+}-induced NAD(P)H production and modulating matrix redox state, J. Biol. Chem. 289 (2014) 20377–20385.
[63] N. Li, Y. Zheng, C. Xuan, Z. Lin, L. Piao, S. Liu, LETM1 overexpression is correlated with the clinical features and survival outcome of breast cancer, Int. J. Clin. Exp. Pathol. 8 (2015) 12893–12900.
[64] C.A. Wang, Q. Liu, Y. Chen, S. Liu, J. Xu, X. Cui, Y. Zhang, L. Piao, Clinical implication of leucine zipper/EF hand-containing transmembrane-1 overexpression in the prognosis of triple-negative breast cancer, Exp. Mol. Pathol. 98 (2015) 254–259.
[65] R. Palty, W.F. Silverman, M. Hershfinkel, T. Caporale, S.L. Sensi, J. Parnis, C. Nolte, D. Fishman, V. Shoshan-Barmatz, S. Herrmann, D. Khananshvili, I. Sekler, NCLX is an essential component of mitochondrial Na^{+}/Ca^{2+} exchange, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 436–441.
[66] M. Montero, M.T. Alonso, E. Carnicero, I. Cuchillo-Ibanez, A. Albillos, A.G. Garcia, J. Garcia-Sancho, J. Alvarez, Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca^{2+} transients that modulate secretion, Nat. Cell Biol. 2 (2000) 57–61.
[67] I. Kaddour-Djebbar, V. Lakshmikanthan, R.B. Shirley, Y. Ma, R.W. Lewis, M.V. Kumar, Therapeutic advantage of combining calcium channel blockers and TRAIL in prostate cancer, Mol. Cancer Ther. 5 (2006) 1958–1966.
[68] M. Colombini, The VDAC channel: molecular basis for selectivity, Biochim. Biophys. Acta 1863 (2016) 2498–2502.
[69] M.S. Lustgarten, A. Bhattacharya, F.L. Muller, Y.C. Jang, T. Shimizu, T. Shirasawa, A. Richardson, H. Van Remmen, Complex I generated, mitochondrial matrix-directed superoxide is released from the mitochondria through voltage dependent anion channels, Biochem. Biophys. Res. Commun. 422 (2012) 515–521.
[70] M. Bonora, A. Bononi, E. De Marchi, C. Giorgi, M. Lebiedzinska, S. Marchi, S. Patergnani, A. Rimessi, J.M. Suski, A. Wojtala, M.R. Wieckowski, G. Kroemer, L. Galluzzi, P. Pinton, Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition, Cell Cycle 12 (2013) 674–683.
[71] K.N. Alavian, G. Beutner, E. Lazrove, S. Sacchetti, H.A. Park, P. Licznerski, H. Li, P. Nabili, K. Hockensmith, M. Graham, G.A. Porter Jr., E.A. Jonas, An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 10580–10585.
[72] V. Giorgio, S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, G.D. Glick, V. Petronilli, M. Zoratti, I. Szabo, G. Lippe, P. Bernardi, Dimers of mitochondrial ATP synthase form the permeability transition pore, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 5887–5892.
[73] M. Bonora, P. Pinton, The mitochondrial permeability transition pore and cancer: molecular mechanisms involved in cell death, Front. Oncol. 4 (2014) 302.
[74] P. Pinton, D. Ferrari, P. Magalhaes, K. Schulze-Osthoff, F. Di Virgilio, T. Pozzan, R. Rizzuto, Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx in Bcl-2-overexpressing cells, J. Cell Biol. 148 (2000) 857–862.
[75] J.C. Daniel, W.R. Smythe, The role of Bcl-2 family members in non-small cell lung cancer, Semin. Thorac. Cardiovasc. Surg. 16 (2004) 19–27.
[76] M. Bittremieux, J.B. Parys, P. Pinton, G. Bultynck, ER functions of oncogenes and tumor suppressors: modulators of intracellular Ca(2+) signaling, Biochim. Biophys. Acta 1863 (2016) 1364–1378.
[77] J.K. Brunelle, A. Letai, Control of mitochondrial apoptosis by the Bcl-2 family, J. Cell Sci. 122 (2009) 437–441.
[78] H. Huang, X. Hu, C.O. Eno, G. Zhao, C. Li, C. White, An interaction between Bcl-xL and the voltage-dependent anion channel (VDAC) promotes mitochondrial Ca^{2+} uptake, J. Biol. Chem. 288 (2013) 19870–19881.
[79] S. Marchi, A. Rimessi, C. Giorgi, C. Baldini, L. Ferroni, R. Rizzuto, P. Pinton, Akt kinase reducing endoplasmic reticulum Ca^{2+} release protects cells from Ca^{2+}-dependent apoptotic stimuli, Biochem. Biophys. Res. Commun. 375 (2008) 501–505.
[80] A. Di Cristofano, P.P. Pandolfi, The multiple roles of PTEN in tumor suppression, Cell 100 (2000) 387–390.
[81] A. Bononi, M. Bonora, S. Marchi, S. Missiroli, F. Poletti, C. Giorgi, P.P. Pandolfi, P. Pinton, Identification of PTEN at the ER and MAMs and its regulation of Ca(2+) signaling and apoptosis in a protein phosphatase-dependent manner, Cell Death Differ. 20 (2013) 1631–1643.
[82] S. Patergnani, C. Giorgi, S. Maniero, S. Missiroli, P. Maniscalco, I. Bononi, F. Martini, G. Cavallesco, M. Tognon, P. Pinton, The endoplasmic reticulum mitochondrial calcium cross talk is downregulated in malignant pleural mesothelioma cells and plays a critical role in apoptosis inhibition, Oncotarget 6 (2015) 23427–23444.
[83] P. Plevova, J. Bouchal, M. Fiuraskova, L. Foretova, M. Navratilova, J. Zapletalova, R. Curik, O. Kubala, J. Prokop, Z. Kolar, PML protein expression in hereditary and sporadic breast cancer, Neoplasma 54 (2007) 263–268.
[84] C. Giorgi, K. Ito, H.K. Lin, C. Santangelo, M.R. Wieckowski, M. Lebiedzinska, A. Bononi, M. Bonora, J. Duszynski, R. Bernardi, R. Rizzuto, C. Tacchetti, P. Pinton, P.P. Pandolfi, PML regulates apoptosis at endoplasmic reticulum by modulating calcium release, Science 330 (2010) 1247–1251.
[85] P. Pinton, A. Rimessi, S. Marchi, F. Orsini, E. Migliaccio, M. Giorgio, C. Contursi, S. Minucci, F. Mantovani, M.R. Wieckowski, G. Del Sal, P.G. Pelicci, R. Rizzuto, Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc, Science 315 (2007) 659–663.
[86] S. Patergnani, S. Marchi, A. Rimessi, M. Bonora, C. Giorgi, K.D. Mehta, P. Pinton, PRKCB/protein kinase C, beta and the mitochondrial axis as key regulators of autophagy, Autophagy 9 (2013) 1367–1385.
[87] A. Rimessi, S. Marchi, S. Patergnani, P. Pinton, H-Ras-driven tumoral maintenance is sustained through caveolin-1-dependent alterations in calcium signaling, Oncogene 33 (2014) 2329–2340.
[88] A.E. Karnoub, R.A. Weinberg, Ras oncogenes: split personalities, Nat. Rev. Mol. Cell Biol. 9 (2008) 517–531.
[89] C.N. Poston, S.C. Krishnan, C.R. Bazemore-Walker, In-depth proteomic analysis of mammalian mitochondria-associated membranes (MAM), J. Proteome 79 (2013) 219–230.
[90] J.M. Suski, M. Lebiedzinska, A. Wojtala, J. Duszynski, C. Giorgi, P. Pinton, M.R. Wieckowski, Isolation of plasma membrane-associated membranes from rat liver, Nat. Protoc. 9 (2014) 312–322.
[91] B. Kornmann, The molecular hug between the ER and the mitochondria, Curr. Opin. Cell Biol. 25 (2013) 443–448.
[92] W.W. Franke, J. Kartenbeck, Outer mitochondrial membrane continuous with endoplasmic reticulum, Protoplasma 73 (1971) 35–41.
[93] D.J. Morre, W.D. Merritt, C.A. Lembi, Connections between mitochondria and endoplasmic reticulum in rat liver and onion stem, Protoplasma 73 (1971) 43–49.
[94] D.E. Copeland, A.J. Dalton, An association between mitochondria and the endoplasmic reticulum in cells of the pseudobranch gland of a teleost, J. Biophys. Biochem. Cytol. 5 (1959) 393–396.
[95] J.A. Lewis, J.R. Tata, A rapidly sedimenting fraction of rat liver endoplasmic reticulum, J. Cell Sci. 13 (1973) 447–459.
[96] A.E. Rusinol, Z. Cui, M.H. Chen, J.E. Vance, A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins, J. Biol. Chem. 269 (1994) 27494–27502.
[97] P.J. Meier, M.A. Spycher, U.A. Meyer, Isolation and characterization of rough endoplasmic reticulum associated with mitochondria from normal rat liver, Biochim. Biophys. Acta 646 (1981) 283–297.
[98] M.R. Wieckowski, C. Giorgi, M. Lebiedzinska, J. Duszynski, P. Pinton, Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells, Nat. Protoc. 4 (2009) 1582–1590.
[99] M.M. Schumacher, J.Y. Choi, D.R. Voelker, Phosphatidylserine transport to the mitochondria is regulated by ubiquitination, J. Biol. Chem. 277 (2002) 51033–51042.

[100] Z. Wang, C. Benning, Chloroplast lipid synthesis and lipid trafficking through ER-plastid membrane contact sites, Biochem. Soc. Trans. 40 (2012) 457–463.

[101] E.A. Schon, E. Area-Gomez, Mitochondria-associated ER membranes in Alzheimer disease, Mol. Cell. Neurosci. 55 (2013) 26–36.

[102] J.E. Vance, MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond, Biochim. Biophys. Acta 1841 (2014) 595–609.

[103] S. Marchi, C. Giorgi, M. Oparka, J. Duszynski, M.R. Wieckowski, P. Pinton, Oncogenic and oncosuppressive signal transduction at mitochondria-associated endoplasmic reticulum membranes, Mol. Cell. Oncol. 1 (2014), e956469. .

[104] S. Patergnani, J.M. Suski, C. Agnoletto, A. Bononi, M. Bonora, E. De Marchi, C. Giorgi, S. Marchi, S. Missiroli, F. Poletti, A. Rimessi, J. Duszynski, M.R. Wieckowski, P. Pinton, Calcium signaling around mitochondria associated membranes (MAMs), Cell Commun. Signal 9 (2011) 19.

[105] A. Raturi, T. Simmen, Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM), Biochim. Biophys. Acta 1833 (2013) 213–224.

[106] E. Area-Gomez, M. Del Carmen Lara Castillo, M.D. Tambini, C. Guardia-Laguarta, A.J. de Groof, M. Madra, J. Ikenouchi, M. Umeda, T.D. Bird, S.L. Sturley, E.A. Schon, Up-regulated function of mitochondria-associated ER membranes in Alzheimer disease, EMBO J. 31 (2012) 4106–4123.

[107] E. Zampese, C. Fasolato, T. Pozzan, P. Pizzo, Presenilin-2 modulation of ER-mitochondria interactions: FAD mutations, mechanisms and pathological consequences, Commun. Integr. Biol. 4 (2011) 357–360.

[108] R. Sano, I. Annunziata, A. Patterson, S. Moshiach, E. Gomero, J. Opferman, M. Forte, A. d'Azzo, GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca(2+)-dependent mitochondrial apoptosis, Mol. Cell 36 (2009) 500–511.

[109] D. De Stefani, A. Bononi, A. Romagnoli, A. Messina, V. De Pinto, P. Pinton, R. Rizzuto, VDAC1 selectively transfers apoptotic Ca^{2+} signals to mitochondria, Cell Death Differ. 19 (2012) 267–273.

[110] M. Bonora, J.M. Bravo-San Pedro, G. Kroemer, L. Galluzzi, P. Pinton, Novel insights into the mitochondrial permeability transition, Cell Cycle 13 (2014) 2666–2670.

[111] C. Giorgi, F. Baldassari, A. Bononi, M. Bonora, E. De Marchi, S. Marchi, S. Missiroli, S. Patergnani, A. Rimessi, J.M. Suski, M.R. Wieckowski, P. Pinton, Mitochondrial Ca(2+) and apoptosis, Cell Calcium 52 (2012) 36–43.

[112] C.H. Chan, U. Jo, A. Kohrman, A.H. Rezaeian, P.C. Chou, C. Logothetis, H.K. Lin, Post-translational regulation of Akt in human cancer, Cell Biosci. 4 (2014) 59.

[113] D.J. Roberts, V.P. Tan-Sah, J.M. Smith, S. Miyamoto, Akt phosphorylates HK-II at Thr-473 and increases mitochondrial HK-II association to protect cardiomyocytes, J. Biol. Chem. 288 (2013) 23798–23806.

[114] L.K. Nutt, J. Chandra, A. Pataer, B. Fang, J.A. Roth, S.G. Swisher, R.G. O'Neil, D.J. McConkey, Bax-mediated Ca^{2+} mobilization promotes cytochrome c release during apoptosis, J. Biol. Chem. 277 (2002) 20301–20308.

[115] G. Morciano, C. Giorgi, D. Balestra, S. Marchi, D. Perrone, M. Pinotti, P. Pinton, Mcl-1 involvement in mitochondrial dynamics is associated with apoptotic cell death, Mol. Biol. Cell 27 (2016) 20–34.

[116] P. Pinton, C. Giorgi, P.P. Pandolfi, The role of PML in the control of apoptotic cell fate: a new key player at ER-mitochondria sites, Cell Death Differ. 18 (2011) 1450–1456.

[117] M. Doghman-Bouguerra, V. Granatiero, S. Sbiera, I. Sbiera, S. Lacas-Gervais, F. Brau, M. Fassnacht, R. Rizzuto, E. Lalli, FATE1 antagonizes calcium- and drug-induced apoptosis by uncoupling ER and mitochondria, EMBO Rep. 17 (2016) 1264–1280.

[118] K.E. Maxfield, P.J. Taus, K. Corcoran, J. Wooten, J. Macion, Y. Zhou, M. Borromeo, R.K. Kollipara, J. Yan, Y. Xie, X.J. Xie, A.W. Whitehurst, Comprehensive functional characterization of cancer-testis antigens defines obligate participation in multiple hallmarks of cancer, Nat. Commun. 6 (2015) 8840.

[119] O.M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria, Nature 456 (2008) 605–610.

[120] S. Marchi, C. Giorgi, J.M. Suski, C. Agnoletto, A. Bononi, M. Bonora, E. De Marchi, S. Missiroli, S. Patergnani, F. Poletti, A. Rimessi, J. Duszynski, M.R. Wieckowski, P. Pinton, Mitochondria-ros crosstalk in the control of cell death and aging, J. Signal Transduct. 2012 (2012) 329635.

[121] M. Rajendran, P. Thomes, L. Zhang, S. Veeramani, M.F. Lin, p66Shc–a longevity redox protein in human prostate cancer progression and metastasis: p66Shc in cancer progression and metastasis, Cancer Metastasis Rev. 29 (2010) 207–222.

[122] M. Lebiedzinska, J. Duszynski, R. Rizzuto, P. Pinton, M.R. Wieckowski, Age-related changes in levels of p66Shc and serine 36-phosphorylated p66Shc in organs and mouse tissues, Arch. Biochem. Biophys. 486 (2009) 73–80.

[123] T. Anelli, L. Bergamelli, E. Margittai, A. Rimessi, C. Fagioli, A. Malgaroli, P. Pinton, M. Ripamonti, R. Rizzuto, R. Sitia, Ero1alpha regulates Ca(2+) fluxes at the endoplasmic reticulum-mitochondria interface (MAM), Antioxid. Redox Signal. 16 (2012) 1077–1087.

[124] M.J. Zinda, M.A. Johnson, J.D. Paul, C. Horn, B.W. Konicek, Z.H. Lu, G. Sandusky, J.E. Thomas, B.L. Neubauer, M.T. Lai, J.R. Graff, AKT-1, -2, and -3 are expressed in both normal and tumor tissues of the lung, breast, prostate, and colon, Clin. Cancer Res. 7 (2001) 2475–2479.

[125] S.S. Roy, M. Madesh, E. Davies, B. Antonsson, N. Danial, G. Hajnoczky, Bad targets the permeability transition pore independent of Bax or Bak to switch between Ca^{2+}-dependent cell survival and death, Mol. Cell 33 (2009) 377–388.

[126] S.A. Oakes, L. Scorrano, J.T. Opferman, M.C. Bassik, M. Nishino, T. Pozzan, S.J. Korsmeyer, Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 105–110.

[127] B. Bonneau, J. Prudent, N. Popgeorgiev, G. Gillet, Non-apoptotic roles of Bcl-2 family: the calcium connection, Biochim. Biophys. Acta 1833 (2013) 1755–1765.

[128] M.W. Ludwinski, J. Sun, B. Hilliard, S. Gong, F. Xue, R.J. Carmody, J. DeVirgiliis, Y.H. Chen, Critical roles of Bim in T cell activation and T cell-mediated autoimmune inflammation in mice, J. Clin. Invest. 119 (2009) 1706–1713.

[129] G.W. Dorn 2nd, M. Song, K. Walsh, Functional implications of mitofusin 2-mediated mitochondrial-SR tethering, J. Mol. Cell. Cardiol. 78 (2015) 123–128.

[130] M. Schneeberger, M.O. Dietrich, D. Sebastian, M. Imbernon, C. Castano, A. Garcia, Y. Esteban, A. Gonzalez-Franquesa, I.C. Rodriguez, A. Bortolozzi, P.M. Garcia-Roves, R. Gomis, R. Nogueiras, T.L. Horvath, A. Zorzano, M. Claret, Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance, Cell 155 (2013) 172–187.

[131] S.C. Kohout, S. Corbalan-Garcia, A. Torrecillas, J.C. Gomez-Fernandez, J.J. Falke, C2 domains of protein kinase C isoforms alpha, beta, and gamma: activation parameters and calcium stoichiometries of the membrane-bound state, Biochemistry 41 (2002) 11411–11424.

[132] M. Lei, X. Wang, Y. Ke, R.J. Solaro, Regulation of Ca(2+) transient by PP2A in normal and failing heart, Front. Physiol. 6 (2015) 13.

[133] M. Nomura, A. Ueno, K. Saga, M. Fukuzawa, Y. Kaneda, Accumulation of cytosolic calcium induces necroptotic cell death in human neuroblastoma, Cancer Res. 74 (2014) 1056–1066.