



Review

The mystery of mitochondria-ER contact sites in physiology and pathology: A cancer perspective



Ines C.M. Simoes^{a,1}, Giampaolo Morciano^{b,1}, Magdalena Lebiedzinska-Arciszewska^a, Gianluca Aguiari^b, Paolo Pinton^b, Yaiza Potes^{a,2}, Mariusz R. Wieckowski^{a,*,2}

^a Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology of Polish Academy of Sciences, 3 Pasteur Str., 02-093 Warsaw, Poland

^b Department of Medical Sciences, Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy

ARTICLE INFO

Keywords:

Mitochondria-associated membranes (MAM)
Mitochondria
Endoplasmic reticulum
Cancer
Oncogenes
Oncosuppressors

ABSTRACT

Mitochondria-associated membranes (MAM), physical platforms that enable communication between mitochondria and the endoplasmic reticulum (ER), are enriched with many proteins and enzymes involved in several crucial cellular processes, such as calcium (Ca^{2+}) homeostasis, lipid synthesis and trafficking, autophagy and reactive oxygen species (ROS) production. Accumulating studies indicate that tumor suppressors and oncogenes are present at these intimate contacts between mitochondria and the ER, where they influence Ca^{2+} flux between mitochondria and the ER or affect lipid homeostasis at MAM, consequently impacting cell metabolism and cell fate. Understanding these fundamental roles of mitochondria-ER contact sites as important domains for tumor suppressors and oncogenes can support the search for new and more precise anticancer therapies. In the present review, we summarize the current understanding of basic MAM biology, composition and function and discuss the possible role of MAM-resident oncogenes and tumor suppressors.

1. Introduction

Mitochondria-endoplasmic reticulum contacts (MERCs) were initially visualized in the late 1950s and are considered a subfraction of the endoplasmic reticulum (ER) juxtaposed to the outer mitochondrial membrane (OMM) [1]. A subcellular fraction of mitochondria-associated membranes (MAM) was isolated in the early 1990s and originally identified as a site of phospholipid biosynthesis and remodeling [2]. MERCs are quite dynamic structures that do not include fusion of the interacting membranes and thus loss organelle identity. Studies with live cell imaging systems and electron tomography have shown that the ER and mitochondria can be 10–25 nm apart and that from 2% to 5% of the mitochondrial surface area can be wrapped with ER tubules [1,3]. Currently, MAM have been linked to metabolic regulation, calcium (Ca^{2+}) homeostasis, autophagy, aging, senescence and reactive oxygen species (ROS) production. Moreover, the involvement of MAM in inflammation [4], infection with human cytomegalovirus and hepatitis C virus [5,6] and different pathologies, such as Alzheimer's disease [7–11], Parkinson's disease, amyotrophic lateral sclerosis [12–14], obesity [15], type 2 diabetes mellitus [16,17] and GM1-gangliosidosis [18], has been repeatedly documented.

Several groups have comprehensively analyzed the proteome of isolated MAM fractions and reported different numbers of proteins. For example, Zhang et al. identified 991 proteins in the heavy MAM fraction [19], Poston et al. found 1212 proteins in MAM [20], and Hung et al. described up to 1313 nonredundant proteins in MAM [21]. However, Hung et al. [22] and Raturi et al. [23] identified many fewer proteins (68 and 75, respectively), as MAM-specific proteins. In accordance with Poston's characterization and classification, we can classify three groups of proteins detected in the MAM fraction: 1) proteins localized only in MAM (“MAM-resident proteins”), 2) proteins localized in MAM but also present in other cellular compartments (“MAM-enriched proteins”), and 3) proteins transiently present in MAM (“MAM-associated proteins”) [20]. The details of the proteins present in the MAM fraction and their possible functions have been reviewed by Giorgi et al. [24], Schon and Area-Gomez [25], Krols et al. [26], Rimessi et al. [27], Morciano et al. [28], Janikiewicz et al. [29] and, most recently, Fan and Simmen [30].

Recently, accumulating studies have identified important roles of MAM in cancer cell function as well as cancer growth [31]. Non-coincidentally, most oncoproteins (both oncogenes and oncosuppressors) exploit this strategic subcellular compartment to perform

* Corresponding author.

E-mail address: m.wieckowski@nencki.gov.pl (M.R. Wieckowski).

¹ These authors contributed equally.

² These authors share senior authorship.

their roles, most often by interacting with proteins considered to be molecular scaffolds for several molecular pathways. Recent studies have discussed the roles of oncogenes and oncosuppressors in MAM in the modulation of Ca^{2+} homeostasis and ROS production [32]. Indeed, alteration of Ca^{2+} signaling is widely considered a potent contributor to the development of malignant phenotypes; Ca^{2+} signaling essential and continuously rewired in all stages of carcinogenesis. Alterations in these processes as well as changes in the ER-mitochondrial tethering distance and morphology can dramatically disrupt cell function. Thus, we summarize the recently acquired knowledge about MAM functions and MAM-related processes by characterizing these intimate MERCs and their role in cancer.

2. Functions of MAM

2.1. MAM as hubs for lipid metabolism and their implications in cancer

One main feature of oncogenic transformation is the reprogramming of cellular metabolism. Initially described by Otto Warburg and colleagues, the Warburg effect is characterized by increased glucose uptake and subsequent lactate production in cancer cells regardless of O_2 levels and mitochondrial function [33]. As numerous studies have shown, due to their great energy demands for survival and proliferation, cancer cells exhibit increased de novo fatty acid synthesis [34], which is driven mainly by the formation of structural membranes and provides sources of energy and signaling molecules [35].

In cells, lipid synthesis and transport involve specific carrier proteins, membrane contact sites and/or vesicle-mediated pathways. MAM represent an evolutionarily conserved mitochondria-ER contact site [36] with a key role in phospholipid, cholesterol and ceramide synthesis [37]. In fact, MAM have a higher cholesterol and sphingolipid content and are thicker than the contiguous ER membrane [38,39]. Moreover, compared with the contiguous ER membrane, MAM have a different curvature and phospholipid composition, containing raft domains enriched in fatty acids with longer and more saturated acyl chains [40]. Several lipid biosynthetic enzymes could be considered MAM marker proteins because of their specific enrichment in MAM rather than throughout the ER [2]. Examples are enzymes involved in phospholipid synthesis, including phosphatidylserine-1 (PSS1) [41], phosphatidylserine-2 (PSS2) [41], phosphatidylserine decarboxylase (PSD) and phosphatidylethanolamine-*N*-methyltransferase 2 (PEMT2) [37]. Phosphatidylserine (PS) has been shown to be externalized to the external leaflet of the plasma membrane (PM) on cells undergoing apoptosis [42], although in vitro assays showed that this process is not dependent on the levels of PSS1 and PSS2 [43]. During the synthesis of other classes of phospholipids, newly synthesized PS is channeled to the inner mitochondrial membrane (IMM), where PS favors phosphatidylethanolamine (PE) synthesis via PSD [52]. Mitofusin (MFN)2 was recently shown to bind PS and transfers it between the ER and mitochondria. Interestingly, reduced levels of MFN2 were found in cancer cells from different origins [44,45]. Thus, ablation of hepatic MFN2 caused nonalcoholic steatohepatitis that progressed into liver cancer in aging mice [46]. This progression was associated with disrupted PS transfer and, consequently, reduced phospholipid synthesis that led to ER stress, inflammation, fibrosis and, in later stages, cancer [46]. Fig. 1 shows an overview of the lipid-related proteins present in MAM that are involved in cancer.

Both lipogenic and lipolytic pathways were found to be enhanced in cancer cells to allow tumor mass growth [47,48]. Excess lipids are converted to triacylglycerols (TGs) and cholesteryl esters (CE), both of which accumulate in the form of lipid droplets. Several reports have identified increased amounts of lipid droplets in numerous types of tumors, including glioblastoma, leukemia, and breast, pancreatic and colon cancers [49–53]. Moreover, enzymes involved in the synthesis of TGs and ceramide (fatty acid CoA ligase (ACS) 1/4 [54]) and cholesterol (acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1/SOAT1)

[37]) were found to be specifically localized in MAM. Therefore, alterations in the activity of the abovementioned proteins may influence the concentrations of intracellular signaling molecules involved in cancer development. Evidence strongly suggests that the amount of ceramide synthesized at MAM is sufficient to permeabilize the OMM [55], promoting the release of proapoptotic proteins such as apoptosis inducing factor (AIF), cytochrome *c* (cyt *c*) and procaspases into the cytosol in human breast cancer cell lines [56,57]. The role of ceramide as a tumor suppressor lipid is supported by a study in which an inhibitor of ACS1/4 (triacycin C) blocked ceramide production and apoptosis by increasing the content of the antiapoptotic oncogene B-cell lymphoma 2 (BCL-2) [58]. In addition, reports have linked cholesterol metabolism with chemoresistance in breast and pancreatic cancers [59,60]. In a basal state, membrane-bound free cholesterol is converted into soluble CE via ACAT1 localized at MAM and is then incorporated into lipid droplets [61]. Notably, CE were shown to be the major constituent of lipid droplets in cancer cells compared with normal cells [62]. In addition, ACAT1-mediated accumulation of CE is positively correlated with the proliferation and metastasis capacities of cancer cells and is associated with poor prognosis in pancreatic and prostate cancers [63,64]. Studies have shown the activation of phosphatidylinositol-3-kinase (PI3K)/AKT and the mammalian target of rapamycin (mTOR) pathway or the caveolin-1/mitogen-activated protein kinase (MAPK) pathway by ACAT1-induced CE accumulation [65–67]. The role of ACAT1 as a modulator of cancer aggressiveness and chemosensitivity was confirmed by Ayyagari et al., who revealed that inhibition of ACAT1 enhanced apoptosis by increasing caspase 3/7 activation. It was accompanied by decreased the mitochondrial membrane potential, elevated ROS production and increased the expression of tumor suppressor protein p53 (p53) [68].

Recently, caveolin-1, a regulator of cholesterol efflux, was found to be specifically enriched in MAM. Caveolin-1 deficiency led to increased levels of free cholesterol and reduced MAM extension and stability [69] along with impairment of mitochondrial function and susceptibility to apoptosis [70]. However, the role of caveolin-1 is controversial. Recently, Bravo et al. showed that caveolin-1 negatively regulates the remodeling capacity of MAM in response to ER stress by down-regulating the protein kinase A (PKA) signaling pathway and impairing dynamin-related protein 1 (DRP1) phosphorylation, which ultimately enhances cell death in response to ER stress [71].

Additionally, AAA domain-containing 3a (ATAD3a), a mitochondrial protein with unknown function, may play a role in MAM formation by delivering cholesterol from the ER to mitochondria for steroidogenesis [72]. In breast and colon tumor cells, the chaperone glucose-regulated protein 78 (GRP78) cooperates with ATAD3a and stabilizes WASP family member 3 (WASP3), a protein that facilitates actin polymerization, thereby promoting invasion and metastasis [73].

Impaired MAM functionality has been shown to have direct consequences on the deregulation of lipid metabolism, which has implications in different cancers. Since the lipid composition is essential for the structure of ER-mitochondria contact sites, it can also impact Ca^{2+} homeostasis, which in turn may affect ER and mitochondrial function and may be essential in the regulation of apoptotic signaling in tumors.

2.2. Role of Ca^{2+} homeostasis in mitochondria-associated ER membranes and cancer

Ca^{2+} is a highly versatile intracellular messenger. Ca^{2+} homeostasis is essential for many vital physiological responses, including primarily cell proliferation and migration, neuronal excitability, and muscle contraction, and regulates cellular functions, including metabolism and cell fate decisions [74–77]. Moreover, several oncogenic pathways supporting tumor cell growth and survival converge on cellular metabolism and Ca^{2+} signaling [78]. Therefore, expanding the understanding of mitochondrial Ca^{2+} regulation is a current challenge in

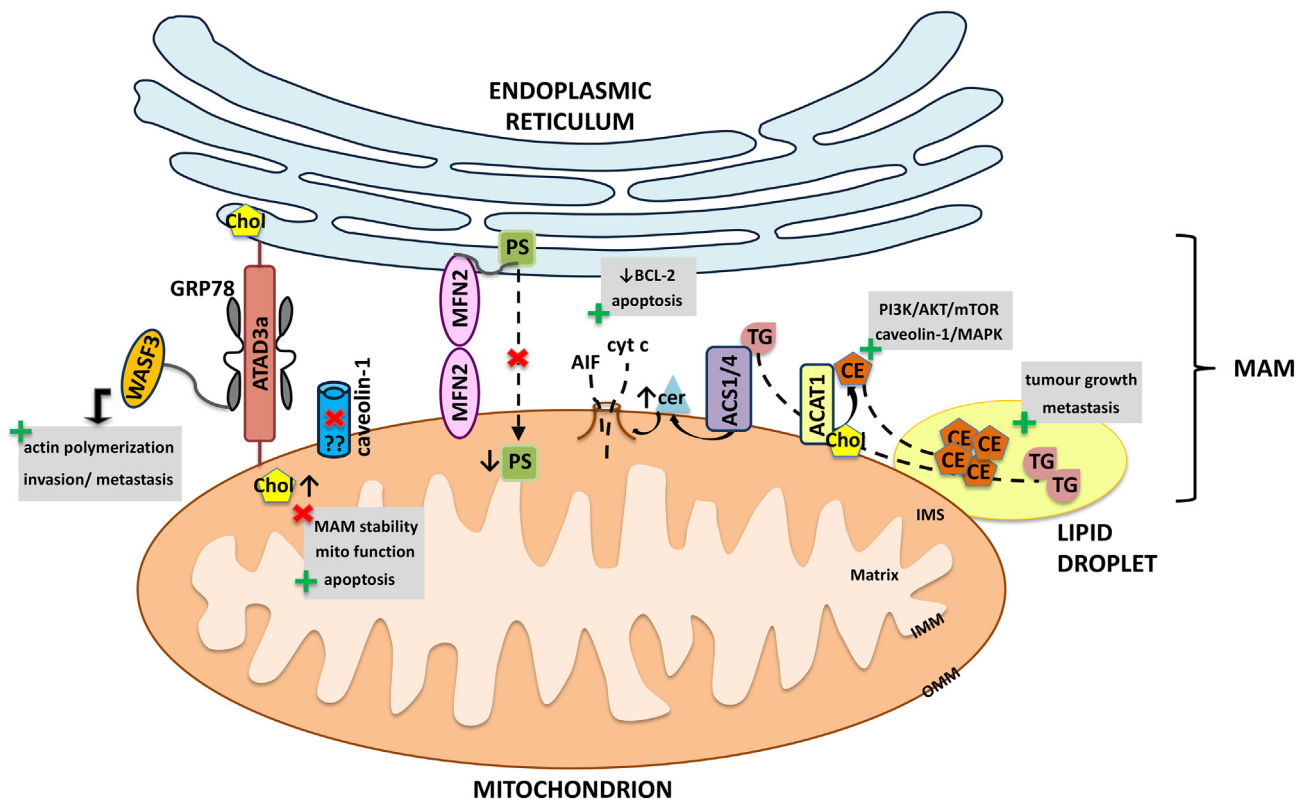


Fig. 1. Schematic representation of MAM with the key proteins involved in lipid metabolism in cancer. In cancer, both lipogenic and phospholipid synthetic pathways are enhanced, and enzymes involved in lipid synthesis are found in MAM. Cholesterol is delivered from the ER to mitochondria through ATAD3a. By interacting with GRP78, this complex stabilizes WASF3, which, facilitates invasion and metastasis by promoting actin polymerization. In the absence of caveolin-1, cholesterol accumulates, leading to a decrease in MAM stability, impairment of mitochondrial function and susceptibility to apoptosis. However, the role of caveolin-1 remains debatable. Reduced MFN2 levels are associated with disruption of PS transfer from the ER to mitochondria, which in turn is associated with liver cancer. Excess fatty acids are converted to TGs and ceramide by ACS1/4, and cholesterol is converted to CE by ACAT1. Then, TGs and CE accumulate in lipid droplets, where they are positively correlated with the capacity of tumors to proliferate and metastasize. Moreover, accumulated ceramide can permeabilize the OMM, promoting the release of proapoptotic factors and thereby favoring apoptosis.

The green “+” symbols indicate induced pathways, while the red “x” symbols indicate affected pathways.

ACAT1 – acyl-coenzyme A:cholesterol acyltransferase-1; ACS1/4 – acyl-coenzyme A:cholesterol acyltransferase-1/4; AIF – apoptosis inducing factor; ATAD3a – WASP family member 3; BCL-2 – B-cell lymphoma 2; CE – cholesteryl esters; chol – cholesterol; cyt c – cytochrome c; GRP78 – glucose-regulated protein 78; IMM – inner mitochondrial membrane; IMS – mitochondrial intermembrane space; MAPK – mitogen-activated protein kinase; MAM – mitochondria-associated endoplasmic reticulum membranes; MFN2 – mitofusin 2; OMM – outer mitochondrial membrane; PI3K/AKT/mTOR – phosphatidylinositol-3-kinase/AKT/mammalian target of rapamycin; PS – phosphatidylserine; TG – triacylglycerols; WASF3 – WASP family member 3.

cancer biomedical research and would lay a good foundation for pinpointing future drug targets.

2.2.1. Interactions between ER Ca^{2+} channels and oncoproteins

Fine tuning of Ca^{2+} oscillations in the ER is associated with the extent of Ca^{2+} -mediated physiological responses [79]. Studies on Ca^{2+} movement across the ER membrane have shown that Ca^{2+} release channels, Ca^{2+} pumps and Ca^{2+} -binding proteins (CaBPs) mediate this process [80,81]. The inositol 1,4,5-triphosphate receptor (IP3R) and ryanodine receptor (RyR) are the primary Ca^{2+} release channels located on the ER membrane. The sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump is localized in the ER membrane and mediates Ca^{2+} uptake into the ER from the cytoplasm at the expense of adenosine triphosphate (ATP) hydrolysis. Many oncosuppressors, including p53, exploit the action of both channels to perform their proapoptotic functions in a Ca^{2+} -dependent manner (Fig. 2). Indeed, a fraction of cellular p53 at MAM interacts with SERCA, exerting transcription-independent effects on Ca^{2+} transfer from the cytosol into the ER lumen; this protein-protein interaction permits an increase in the activity of the ER pump, thus enhancing the level of Ca^{2+} in the ER [82]. The biological significance of these effects is that when apoptosis is stimulated, a larger amount of Ca^{2+} is released via ER-mitochondria

microdomains, and cells become more sensitive to death via the intrinsic apoptosis pathway; the relevance of these findings has been confirmed in vivo [83,84]. The p53 gene is highly subject to mutations in its coding sequence, and these mutations are the main cause of p53 inactivation in human cancers. Consistent with these findings and considering the MAM-resident p53 fraction, the expression of non-nuclear p53^{R175H} and p53^{R273H} mutants, which cannot bind the SERCA pump at the ER, does not restore ER Ca^{2+} homeostasis in p53 knockout (KO) cells; similarly, reintroduction of wild-type p53 (p53wt) restored sensitivity to cell death. The same results were found in the MDA-MB 468 cell line harboring the p53^{R273H} mutant [85]. In addition to regulating the expression of multiple downstream genes, p53 maintains the basal expression of a group of other important tumor suppressor genes, such as phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [86], and this activity was recently reported to be responsible for the marked beneficial anticancer effect of p53.

Similar to p53, breast cancer type 1 susceptibility protein (BRCA1)-associated protein 1 (BAP1) is also a potent tumor suppressor inhibiting carcinogenesis. Indeed, evidence strongly suggests that reduced expression or inactivation of BAP1 promotes the onset of many cancers, such as malignant mesothelioma. Although BAP1 exhibits tumor suppressor activity mainly in the nucleus [87], a large amount of cytosolic

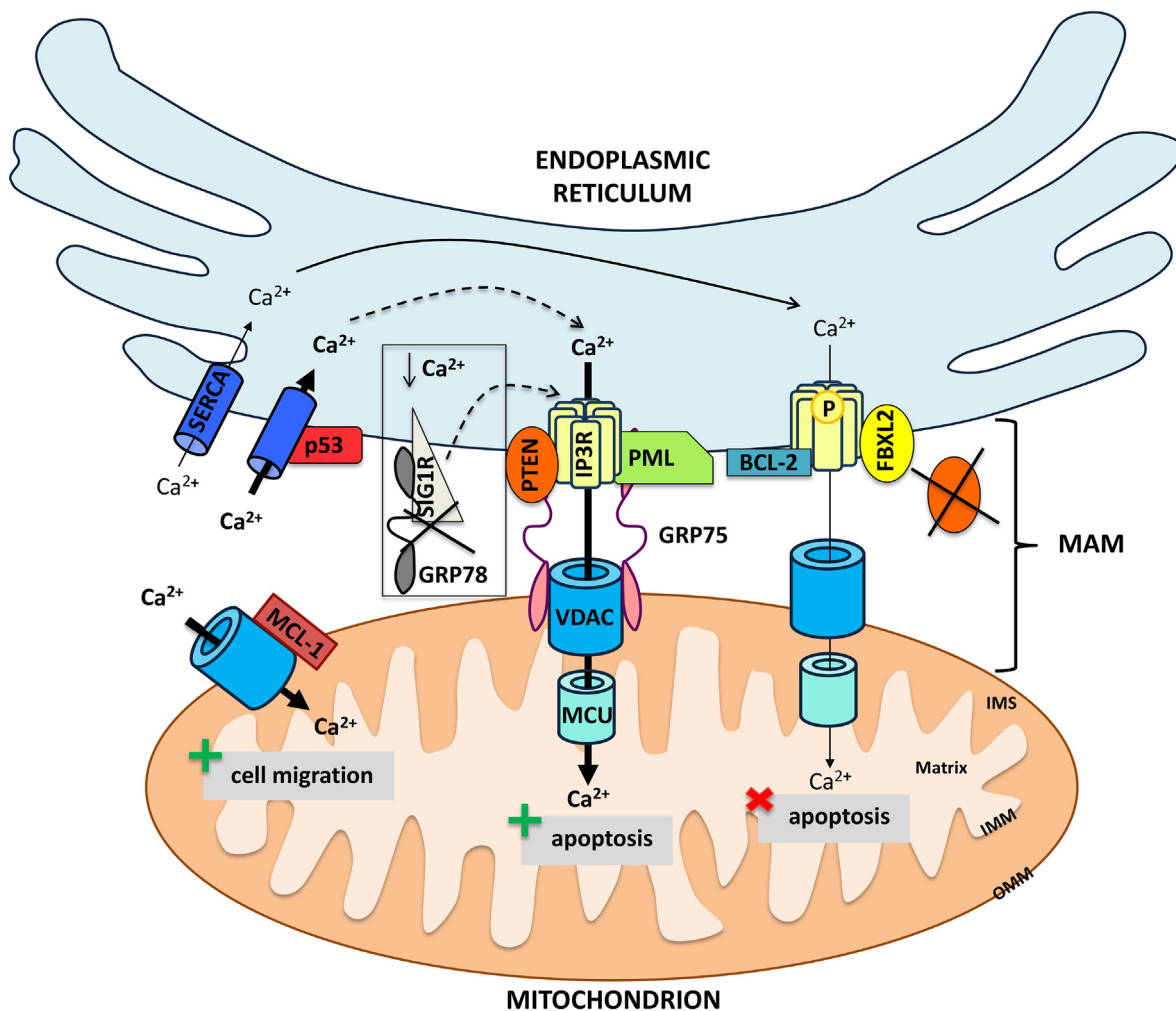


Fig. 2. Ca^{2+} homeostasis alterations at MAM. The most important oncoproteins that can modify Ca^{2+} flux at MAM are shown in this figure. High levels of Ca^{2+} transfer are ensured by functional interactions of p53 with SERCA (from the cytosol to the ER), of both PTEN and PML with IP3R (from the ER to mitochondria via the IP3R/VDAC/GRP75/MCU axis), of SIG1R with IP3R upon dissolution of the SIG1R-GRP78 bond, and of MCL-1 with VDAC1 to support cell migration. Low levels of Ca^{2+} transfer are permitted by functional interactions of either BCL-2 or FBXL2 with IP3R and by silencing of the GRP75 protein. The thick arrows and Ca^{2+} in boldface type denote higher calcium flux; the thin arrows and Ca^{2+} in nonboldface type denote lower Ca^{2+} flux. The green “+” symbols indicate induced pathways, while the red “x” symbols indicate affected pathways. BCL-2 – B-cell lymphoma 2; FBXL2 – F-box and leucine-rich repeat protein 2; GRP75 – glucose-regulated protein 75; GRP78 – glucose-regulated protein 78; IP3R – inositol 1,4,5-triphosphate receptor; IMM – inner mitochondrial membrane; IMS – mitochondrial intermembrane space; MAM – mitochondria-associated endoplasmic reticulum membranes; MCL-1 – myeloid cell leukemia 1; MCU – mitochondrial calcium uniporter; OMM – outer mitochondrial membrane; P – phosphorylation; p53 – tumor suppressor protein p53; PML – promyelocytic leukemia protein; PTEN – phosphatase and tensin homolog deleted on chromosome 10; SERCA – sarco/endoplasmic reticulum Ca^{2+} ATPase; SIG1R – sigma 1 receptor; VDAC – voltage-dependent anion channel.

BAP1 found at the ER (erBAP1) (not shown to be present at MAM) [88] restored the wild-type phenotype upon overexpression. This effect is mediated via the interaction of BAP1 with IP3R3, which is stabilized by the intrinsic deubiquitylating activity of BAP1. This interaction results in the appropriate release of Ca^{2+} from the ER to mitochondria and thus sensitization to apoptosis [88], further supporting the hypothesis that this mechanism is regulated at MAM. A key protein that stabilizes p53 and enhances its function is promyelocytic leukemia protein (PML) [89]. PML exhibits nuclear localization and activity, but it can also be found in the cytoplasm [90].

Indeed, PML is enriched at MAM, where it modulates the apoptotic pathway by interacting with and acting on the IP3R3 channel [91]; specifically, ER-targeted PML (erPML) introduced into PML KO cells permitted optimal Ca^{2+} transfer between the ER and mitochondria upon either agonist administration or apoptotic insult, restoring the Ca^{2+} -dependent apoptotic pathway [91]. Further analysis revealed the following: i) in PML KO cells, the amount of coprecipitated phosphorylated AKT and IP3R3 were higher than those in PML wild-type

cells; ii) dephosphorylation of AKT may occur in a protein phosphatase 2A (PP2A)-dependent manner; and iii) PP2A interacts with PML in nuclear bodies. These findings confirmed the hypothesis that PML forms a complex with IP3R, AKT and PP2A at MAM. Considering this observation, cells lacking PML express phosphorylated AKT and IP3R3 and thus show decreased Ca^{2+} mobilization. Indeed, the presence of PML reduces AKT activity via PP2A recruitment [91]. Additional functions regarding autophagy regulation have been ascribed to PML at MAM; at this location, PML controls autophagosome formation, providing a limiting effect on autophagy induction in cancer cells. Indeed, in the absence of PML, tumor cells can feed themselves by exploiting autophagy as a survival strategy [92].

As evidence that the role of Ca^{2+} at MAM is crucial in cancer, the list of oncoproteins that regulate IP3R channel properties has lengthened; PTEN, BRCA1 and BCL-2 are examples. PTEN counteracts the function of AKT, which acts mainly on the PI3K/AKT/mTOR pathway [93,94]. Endoplasmic reticulum PTEN (erPTEN), enriched at MAM, controls Ca^{2+} flux from the ER to mitochondria, ensuring appropriate

execution of apoptosis when required by the environment. This protein phosphatase is mutated or absent in many human cancers, leading to disruption of Ca^{2+} release from the ER and a consequent decrease in the mitochondrial Ca^{2+} concentration favoring phosphatase-dependent antiapoptotic features [95]. Indeed, *erPTEN* binds IP3R3 and promotes increased Ca^{2+} transfer via dephosphorylation of IP3R3 and AKT [95]. Lipid/protein phosphatase-independent mechanisms that counteract carcinogenesis have been demonstrated both *in vitro* and in xenograft models; in particular, PTEN was reported to compete with F-box and leucine-rich repeat protein 2 (FBXL2), one of the four subunits of the SKP1-cullin-F-box (SCF) ubiquitin protein ligase complex, which functions through phosphorylation-dependent ubiquitination, for binding to IP3R via the IP3 binding domain. By interacting with IP3R3, PTEN promotes the degradation of IP3R3 via the proteasome, thus conferring cellular resistance to apoptosis. Here, the role of *erPTEN* is to compete with FBXL2 for binding to IP3R3 and preventing its degradation [96]. In support of this finding, IP3R3 mutants resistant to proteasomal degradation improve the outcomes of anticancer therapies in mice. Isoform 3 of IP3R is not the only isoform of IP3R targeted for these purposes. BRCA1 actively participates in tumor suppressor activities through its recruitment to the ER during apoptosis, but it is also constitutively localized at MAM, where it interacts with IP3R1, promoting Ca^{2+} release and sensitivity to apoptosis [97]. Another example is the BCL-2 protein, which can participate in functional interactions with all three IP3R isoforms and significantly inhibit Ca^{2+} transfer from the ER to mitochondria. Indeed, overexpression of BCL-2 plays a substantial role in cancer cell survival. The molecular pathway through which BCL-2 confers apoptosis resistance is regulated almost exclusively by the intracellular localization of BCL-2 at the ER and MAM. In 2012, Monaco and colleagues identified one amino acid residue in the BH4 domain of BCL-2 that is essential for the binding of BCL-2 to IP3R. Indeed, mutation of Lys17 to Asp reduces the interaction between BCL-2 and IP3R, consequently restoring Ca^{2+} signaling between the two organelles [98]. Additionally, Förster resonance energy transfer (FRET) measurements have documented the interaction between BCL-2 and IP3R, particularly with subdomain 3a1 of IP3R, a sequence with high homology among known isoforms [99]. Thus, by targeting this site with an interfering peptide and preventing the BCL-2–IP3R interaction, both Ca^{2+} release and apoptosis were restored [99,100]. At MAM, BCL-2 can interact with another type of channel, voltage-dependent anion channel (VDAC) 1 [101,102]. Physiologically, all three VDAC isoforms are channels responsible for Ca^{2+} entry into mitochondria via the OMM, but only VDAC1 plays an important role in apoptosis, permitting a low level of Ca^{2+} transfer [103]. In addition, the N-terminal portion of VDAC1 is responsible for the release of cyt c from mitochondria; in mitochondria, the BCL-2–VDAC1 interaction counteracts the action of cyt c, protecting cells from apoptosis [101].

2.2.2. Other regulators of Ca^{2+} homeostasis at MAM in cancer

Moreover, Ca^{2+} signaling depends largely on the wide variety of CaBPs, which regulate cytosolic Ca^{2+} . Functionally, some of these CaBPs act as Ca^{2+} storage devices, whereas other families of CaBPs act as Ca^{2+} receptors. In recent years, various CaBPs have emerged as important regulators of cell proliferation and differentiation, as well as metastasis and tumor progression in humans. Members of the S100P family of CaBPs have been found to be overexpressed in pancreatic, lung and breast cancers, contributing to tumor progression through Ca^{2+} binding [104]. In particular, the S100A14 and S100A4 proteins, members of the S100 family, are intimately involved in tumorigenesis by promoting cancer cell proliferation [105,106].

Ca^{2+} storage in energized mitochondria was initially described in the 1960s [107]. Since that time, the essential contribution of mitochondrial Ca^{2+} to bioenergetics and the modulation of cytosolic Ca^{2+} oscillations has been firmly established. The molecular basis of mitochondrial Ca^{2+} uptake from the ER is based on the connection between VDAC and IP3R through linkage with glucose-regulated protein

75 (GRP75). Then, Ca^{2+} transport across the IMM down its electrochemical gradient is regulated through the mitochondrial Ca^{2+} uniporter (MCU) [108,109]. Several proteins mediate the regulation of MCU activity, such as mitochondrial Ca^{2+} uptake 1/2 (MIC1/2), MUC regulator 1 (MUCR1) and essential MCU regulator (EMRE) [77,110–112]. Interestingly, depletion of MCU impairs cell migration and invasion in triple-negative breast cancer cell lines and hampers tumor progression in MDA-MB-231 xenografts by regulating metastasis through hypoxia-inducible factor 1 (HIF1)-controlled gene reprogramming [113].

Proteomic analysis of MAM identified several interactions of Ca^{2+} channels with regulators involved in Ca^{2+} dynamics between the ER and mitochondria and confirmed that MAM are central platforms that especially facilitate efficient Ca^{2+} uptake by mitochondria [24,114]. Several multiprotein tethering complexes maintain the close juxtaposition of the ER and mitochondria. GRP75 is considered a key protein at the MAM interface that modulates ER-mitochondria Ca^{2+} signaling through the IP3R-GRP75-VDAC1 complex. GRP75 has been widely studied because it is essential for linking organelles. Recent findings have shown that GRP75 knockdown significantly decreases mitochondrial Ca^{2+} levels and compromises mitochondrial homeostasis [115,116], whereas induction of GRP75 increases VDAC1-IP3R1 and GRP75-IP3R1 protein-protein interactions [17]. However, excessive mitochondrial Ca^{2+} uptake can activate cell death programs by promoting the release of proapoptotic factors such as cyt c, AIF and SMAC/DIABLO from mitochondria [117–119]. Altered Ca^{2+} signaling in MAM is an important hallmark of tumor cells given that it can affect cellular metabolism and resistance to cell death [120]. A reduction in mitochondrial Ca^{2+} uptake may allow cancer cells to escape apoptosis. Moreover, Ca^{2+} flux towards mitochondria via MCU are decisive for tumor growth and metastatic behavior [113,121]. Similarly, GRP75 silencing protects against cell death by preventing mitochondrial Ca^{2+} overload under conditions of cellular stress [122]. IP3R1 and IP3R2 are located at the ER membranes, but the third isoform, IP3R3, is highly enriched at MAM and is also considered a MAM marker [123]. Interestingly, IP3R3 is involved in the regulation of cellular bioenergetics and metabolism in breast cancer. Inhibition of IP3R3 induces autophagic death [124] and/or mitotic catastrophe only in tumorigenic cells [125,126]. AKT preferentially phosphorylates IP3R3 and can bind all three IP3R isoforms in the cytosolic C-terminal consensus domain [127]. This causes a reduction in ER-mitochondrial Ca^{2+} transfer and inhibits apoptotic responses [128]. Moreover, hyperphosphorylation of IP3Rs in tumor cells overexpressing AKT has been reported [129]. In turn, AKT is activated through phosphorylation of both Thr308 by phosphoinositide-dependent kinase 1 (PKD1) [130] and Ser473 by mechanistic TOR complex 2 (mTORC2), which is localized at MAM [131].

A new protein modulator of Ca^{2+} trafficking and apoptotic processes that interacts with GRP75 was recently described. Transglutaminase type 2 (TG2) binds to GRP75 in MAM, contributing to the regulation of ER-mitochondrial Ca^{2+} flux. D'Eletto and colleagues demonstrated that the absence of TG2 enhanced the interaction between IP3R and GRP75 and decreased the number of MERCs [132]. TG2 has been found to be overexpressed in breast cancer and to contribute to cancer cell survival, invasion and motility through activation of the PI3K/AKT survival pathway [133]. Thus, the regulation of GRP75 expression and its connection with ER-mitochondrial Ca^{2+} crosstalk at MAM is a novel protective approach in paradigms of cell death and anticancer therapy.

Sigma 1 receptor (SIG1R) is another protein highly enriched in MAM and involved in Ca^{2+} signaling [134]. SIG1R interacts with GRP78, also called BIP. However, upon depletion of ER Ca^{2+} , SIG1R dissociates from BIP and binds to IP3R, thereby stabilizing IP3R and inducing mitochondrial Ca^{2+} uptake from the ER [134]. Lack of SIG1R mediates the disruption of MERCs, impairing Ca^{2+} signaling and mitochondrial function [135]. In addition, under chronic ER stress

conditions, SIG1R exhibits intrinsic attenuation of apoptosis via its translocation from MAM to the ER to counteract the ER stress response [136,137]. In breast cancers, the expression of SIG1R is higher in cancer cells with metastatic potential [121,138]. Moreover, the protumorigenic functions of SIG1R at MAM are mediated by the formation of a functional molecular platform comprising SIG1R, the small-conductance Ca^{2+} -activated K^+ channel (SK3) and Ca^{2+} release-activated Ca^{2+} modulator 1 (ORAI1), thus driving Ca^{2+} influx and favoring the migration of cancer cells [121].

One oncogene that comprehensively exploits these features is Ras, which belongs to the family of small GTPases that control multiple pathways involved in cell cycle regulation and cell survival. In 33% of cancers, the Ras oncogene is mutated and sustains tumor growth [139]. Ca^{2+} signaling is a main mechanism through which Ras exerts its antiapoptotic functions; moreover, Ras localizes at PM-ER contact sites and MAM, precisely on the route of intercellular Ca^{2+} flux. During cellular transformation, H-Ras drastically alters Ca^{2+} dynamics by reducing the mitochondrial Ca^{2+} concentration via a pathway mediated by caveolin-1 downregulation and redistribution [140]. On the other hand, K-Ras phosphorylation on Ser181 by protein kinase C (PKC) has been reported to induce rapid dissociation of K-Ras from the PM and its subsequent migration to MAM [141]. Here, the oncogenic K-RasG13D mutation disrupts IP3-induced Ca^{2+} transfer from the ER to mitochondria, mainly due to aberrant expression of SERCA and IP3R subtype proteins compared to that in a cell line with deletion of oncogenic K-Ras. K-RasG13D decreases SERCA and IP3R3 levels and thus decreases ER Ca^{2+} storage and release and increases the levels of type I receptors, which ensure spikes in the Ca^{2+} concentration to support cell proliferation [142]. In confirmation of this finding, deletion of oncogenic K-Ras reverted the cell phenotype by conferring sensitization to Ca^{2+} -dependent apoptosis.

2.2.3. The permeability transition pore complex in cancer

The proteins described above can fine tune intracellular Ca^{2+} flux in a well-defined spatiotemporal manner to ensure the completion of a given biological process—in this case, a prosurvival process. Sustained elevations in mitochondrial Ca^{2+} levels occur under both physiological and pathological conditions and are the main inducer of mitochondrial permeability transition (MPT), an extreme permeabilization of the IMM. MPT is caused by the opening of mitochondrial transmembrane protein complexes named permeability transition pore complexes (PTPCs) and has detrimental effects not only on mitochondria but also on the whole cell [143]. Indeed, loss of the IMM structure causes significant decreases in the mitochondrial membrane potential and ATP production and impairs the osmotic process. Thus, mitochondria swell, and cellular changes leading to the activation of different cell death programs are initiated [144].

The molecular structure of the PTPC is incompletely defined. Currently, only mitochondrial cyclophilin D is considered an important modulator of the PTPC, as both its pharmacological (via treatment with cyclosporine A) and genetic (via generation of KO animals) targeting inhibits pore opening and prevents cell death under mitochondrial Ca^{2+} accumulation [145,146]. The c subunit of Fo-ATP synthase has recently been identified as an essential component for PTPC activity, and many of its features likely explain the downstream inhibition/activation of the PTPC. Indeed, increased expression of the c subunit in cells [147] and its high level in biological fluids [148] predicts excessive cell death. In contrast, the pharmacological targeting [149] or absence of the c subunit [150] desensitizes cells to PTPC opening and protects against cell death. Although it is an important (mega) channel for mitochondrial health, the PTPC has been reported to be dispensable for Ca^{2+} homeostasis under physiological conditions [151].

Oncosuppressors such as those listed above ensure Ca^{2+} transfer between the ER and mitochondria in cooperation with other proteins such as MCU and IP3R phosphorylation to initiate MPT [152–154]. Cancer cells have developed mechanisms to overcome these effects: by

downregulating the expression of oncosuppressors (i.e., PML and PTEN) [91,95]; by expressing antiapoptotic proteins such as BCL-2, which reduces the ER Ca^{2+} content and AKT, which dysregulates Ca^{2+} signaling at MAM; and by H-RAS-induced transformation associated with progressive depletion of intracellular Ca^{2+} —all of these pathways limit the appropriate initiation of MPT [155,156].

Oxidative stress also induces MPT, but analysis of this process in cancer cells reveals a paradox. Cancer cells have higher ROS levels than nontransformed cells [157] but are also more resistant to death. ROS are likely to be equally essential for other pathways, such as proliferation and metastasis [158], and when disadvantageous, ROS can be easily controlled by upregulation of antioxidant enzymes [159,160].

MPT-dependent cell death is closely related to ischemia-reperfusion episodes (i.e., myocardial infarction). Indeed, oxygen deprivation in tissues permits a significant decrease in the pH, associated with increased Na^+ and then Ca^{2+} flux into the cytoplasm and mitochondria [143]. Mitochondrial uptake of Ca^{2+} is amplified because progressive ATP depletion is insufficient for appropriate activation of SERCA to allow Ca^{2+} reuptake into the ER. During ischemia, although PTPC inducers (i.e., inorganic phosphates and a high Ca^{2+} concentration) progressively accumulate, the PTPC remains closed due to the high concentration of H^+ that competes with the Ca^{2+} -binding site for PTPC opening [161]. By contrast, during reperfusion, restoration of a physiological pH in combination with Ca^{2+} overload and oxidative stress triggers PTPC opening and cell death. The same hypoxic environment can be found in solid tumors. Here, the high capacity for glucose uptake and conversion by cancer cells guarantee ATP synthesis through glycolysis, which reduces the concentrations of inorganic phosphates [162,163]; in addition, the formation of lactate from pyruvate lowers the pH, inhibiting appropriate initiation of MPT.

Several interactions between Ca^{2+} channels and regulators that mediate Ca^{2+} -dependent cellular functions in various cancer types are found in MAM. Overall, these reports confirm the importance of this organellar interface in both Ca^{2+} signaling and cancer development and progression.

2.3. Interplay between ROS and Ca^{2+} at MAM in cancer development and suppression

MAM are emerging as platforms for redox signaling given that many cellular redox reactions occur in mitochondria and in the ER. Generally, ROS generation is required, because ROS are signaling molecules that contribute to the maintenance of physiological functions such as diverse metabolic processes and cell growth and differentiation. On the other hand, excessive ROS production induces oxidative stress, which causes cellular damage. ROS have been proposed to play an important role in cancer initiation and progression. Moreover, ROS have been shown to promote anoikis resistance in cancer cells, enabling metastasis [164]. The role of ROS in tumors is still incompletely defined and controversial; indeed, several studies have described ROS as either tumor-promoting or tumor-suppressing agents, and substantial evidence supports both arguments [165]. Interestingly, clinical trials have shown that antioxidant therapy is paradoxically correlated with decreased survival [166]. Another open and interesting question remains regarding the ROS-mediated effect of chemotherapeutics. The review by Yang et al. offers more information about this topic, including the generation of ROS by mitochondria during chemotherapy as well as the responses of cancer cells to chemotherapy based on the induction of oxidative stress [167].

Redox-sensitive proteins are located in different subcellular compartments, including MERCs [168,169]. The dynamic properties of such contact sites allow the exchange of metabolites and proteins involved in ROS homeostasis between the ER and mitochondria. MAM contain a H_2O_2 nanodomain, which is regulated by the cytosolic Ca^{2+} concentration and exerts positive feedback on Ca^{2+} oscillations [170]. Most of the literature indicates that MAM play an essential role in ROS-

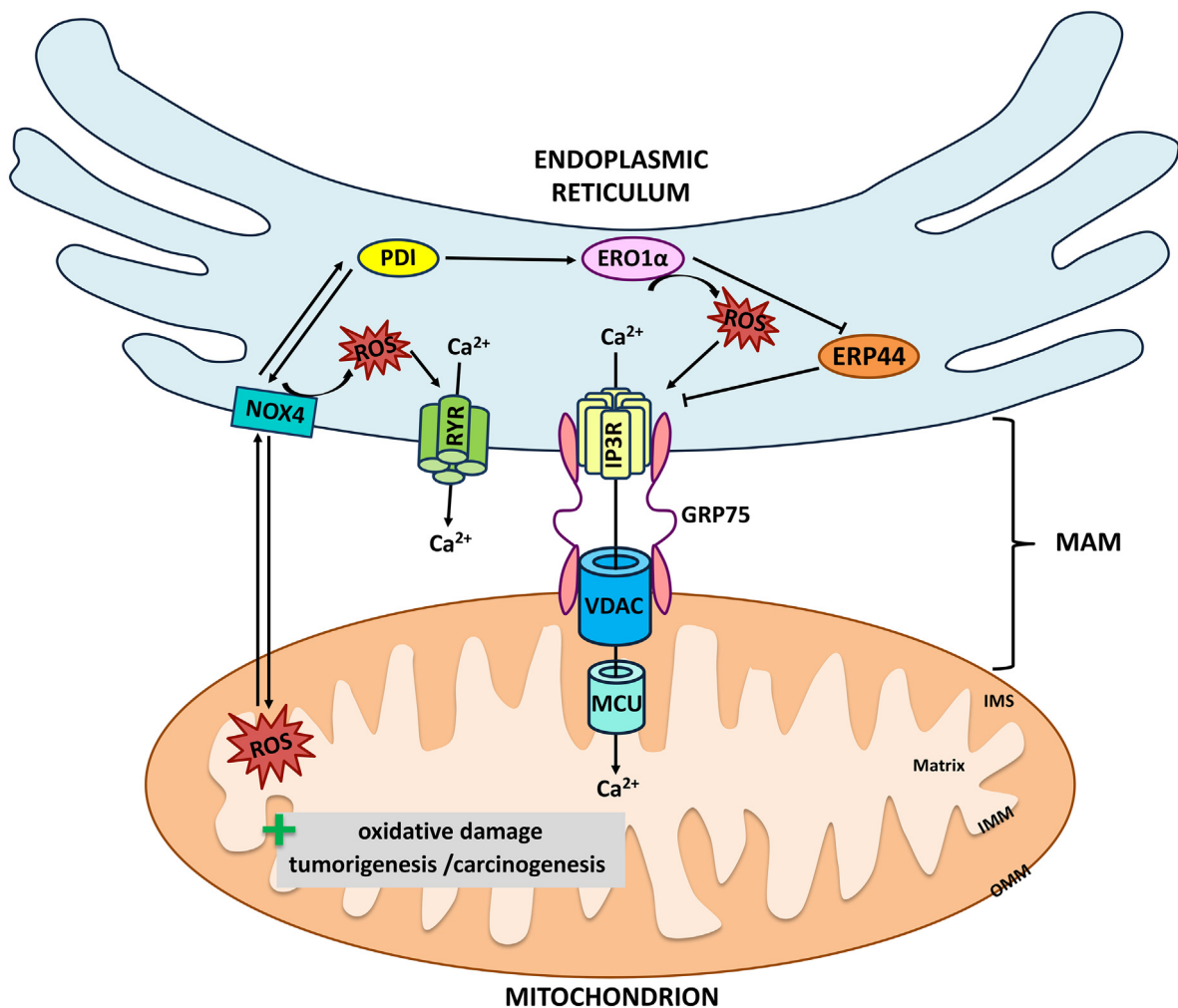


Fig. 3. Global view of reactive ROS signaling in MAM during cancer progression. Cancer-dependent ROS are produced within mitochondria through the electron transport chain and in the endoplasmic reticulum by NOX4 and during oxidation of PDI by ERO1 α . ROS generated by NOX4 induce oxidation of RYR channels, resulting in Ca²⁺ release. ERO1 α -derived ROS can modulate IP3R-GRP75-VDAC-MCU complex activity, thus promoting Ca²⁺ release from the endoplasmic reticulum to mitochondria. Upregulation of ERO1 α compromises the inhibitory action of ERP44 on IP3R activity. The green “+” symbols indicate induced pathways.

ERO1 α – endoplasmic reticulum disulfide oxidase 1 α ; ERP44 – endoplasmic reticulum protein 44; GRP75 – glucose-regulated protein 75; IMM – inner mitochondrial membrane; IMS – mitochondrial intermembrane space; IP3R – inositol 1,4,5-triphosphate receptor; MAM – mitochondria-associated endoplasmic reticulum membranes; MCU – mitochondrial calcium uniporter; NOX4 – nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase 4; OMM – outer mitochondrial membrane; P – phosphorylation; PDI – protein disulfide isomerase; ROS – reactive oxygen species; RYR – ryanodine receptor; VDAC – voltage-dependent anion channel.

mediated cellular signaling in a manner dependent on ROS generation in the ER and mitochondria. Therefore, changes in oxidative stress-responsive signaling through MAM can disrupt Ca²⁺ homeostasis and affect cancer progression (Fig. 3). Accumulating evidence indicates that high levels of ROS mediate the deregulation of ER Ca²⁺ release channels, which results in Ca²⁺ efflux from the ER. RYR function is regulated by the combined action of ER function and mitochondrial ROS production [171,172]. A novel correlation between RYR expression and the tumor grade has recently been described in human breast cancer specimens [173]. Numerous publications have connected mitochondrial-derived ROS with RYR oxidation [174–176]. Oxidative stress leads to RYR channel remodeling due to PKA-mediated phosphorylation at Ser2808, which results in the release of Ca²⁺ and depletion of ER Ca²⁺. In addition, RYR channel function was shown to be affected by nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (NOX). In particular, NOX4 is one of the main NADPH oxidases that controls the redox state of RYR by oxidizing the redox-sensing thiol groups of cysteine residues, inducing Ca²⁺ release [177,178]. Diverse studies have demonstrated that ROS-generating NOX4 acts as a tumor-promoting agent. Increased expression of NOX4 contributes to the

progression and metastasis of human colorectal cancer cells via fine-tuned regulation of ROS and Ca²⁺ levels [179,180]. Another study showed that inhibition of NOX4 attenuates the development of prostate cancer and the progression of metastasis [181,182]. Although RYR responsiveness at MAM has not been demonstrated, previous findings generally support the hypothesis that RYR is regulated at MAM under pathological conditions such as cancer initiation and tumor progression.

The IP3R channel is another potential target of ROS generated by both the ER and mitochondria. Indeed, IP3R regulation by ROS affects MERCs. Numerous studies have reported that IP3R exhibits sensitization to IP3 through the oxidation of thiol groups. This effect can be mediated via different oxidants, such as t-butyl hydroperoxide, diamide and xanthine oxidase [183–185]. In addition, ROS modulate IP3R-mediated Ca²⁺ signaling by facilitating Ca²⁺ release [183]. Thus, oxidative stress plays a key role, as ROS regulate IP3R-mediated Ca²⁺ transfer from the ER into mitochondria. Enrichment of the MAM interface with the endoplasmic reticulum disulfide oxidase 1 α (ERO1 α) protein and protein disulfide isomerase (PDI) also exerts regulatory effects on the IP3R channel. Under chronic ER stress conditions, ERO1 α

is upregulated, leading to excessive H_2O_2 production, which increases the cellular ROS burden [186]. Similarly, the role of ERO1 α as a protein disulfide oxidase that regulates IP3R1 activity has been highlighted. ERO1 α oxidizes the IP3R1 channel, which induces Ca^{2+} release from the ER [187]. ERO1 α , which is enriched at ER-mitochondria contact sites, is also studied in the context of tumors [187]. ERO1 α has been found to be overexpressed in various types of cancers [188]. Notably, expression of ERO1 α in breast cancer is associated with poor prognosis [189]. Seervi and colleagues found that ablation of ERO1 α in multiple cancer cells inhibits both Ca^{2+} release from the ER and apoptosis. This work also demonstrated that ERO1 α -mediated functions are key events in the cell death mechanism induced by procaspase-activating compound-1 (PAC-1), which can promote apoptosis in various cancer cell types [190]. Furthermore, endoplasmic reticulum protein 44 (ERP44), which is located in the ER and MAM, is considered a redox sensor involved in the regulation of IP3R activity through its linkage with this channel [191]. ERP44 inhibits Ca^{2+} release by binding to cysteine residues in the luminal region of IP3R [192]. Thus, when ERO α is upregulated, this oxidase induces the dissociation of ERP44 from IP3R1, resulting in Ca^{2+} transfer from the ER into mitochondria [193]. A recent work showed that ERP44 inhibits lung cancer cell proliferation primarily via IP3R-dependent signaling [194]. Similarly, knockdown of ERP44 has been found to be essential for the inhibition of breast cancer cell growth and survival [195]. The potential regulatory role of ROS production by ERO1 α and PDI proteins in cancer-specific settings based on the above findings supports the importance of MAM in cancer regulation. However, the molecular mechanisms by which ERO1 α and ERP44 contribute to the regulation of tumorigenesis remain unclear. Although more basic research is needed, these members of the PDI family are rapidly emerging as new potential targets for cancer therapies.

The 66-kDa isoform of Src homology/collagen (SHC) adaptor protein (p66Shc) localizes predominantly to both the cytosol and ER [196,197], but a mitochondrial pool has also been reported [198–200]. The precise mitochondrial import mechanism of p66Shc has been disputed for many years, suggesting the presence of p66Shc in MAM [201]. Shc adaptor proteins bind to activated receptor tyrosine kinases (RTKs) in response to various growth factors [202] and participate in the regulation of cellular proliferation and differentiation [203]. The Ser36 residue located in the unique p66Shc collagen homology domain (CH2) is phosphorylated by either protein kinase C β II (PKC β II) [204] or c-Jun N-terminal kinase (JNK) [205] in response to various environmental and intracellular stress stimuli, such as H_2O_2 or Taxol treatment [206], hypoxia/reoxygenation injury [207–210], UV radiation [211], and high-glucose environments [212–214], as well as in response to metabolic stresses [215]. After isomerization by peptidyl-prolyl cis-trans isomerase 1 (PIN1) and dephosphorylation by PP2A, p66Shc is translocated into mitochondria [204]; however, MAM-localized p66Shc may also be able to affect mitochondria. p66Shc induces excessive ROS production and cyt c release from the mitochondrial intermembrane space and initiates the caspase-3-dependent mitochondrial apoptotic pathway [199,216,217]. Since cells lacking p66Shc are more resistant to oxidative stress than those with intact p66Shc, p66Shc KO mice live approximately 30% longer than their wild-type littermates [218].

Oncogenesis can be an effect of improper receptor signaling and oxidative damage to cellular components; thus, both pathways involving p66Shc can play a crucial role in this process (Fig. 4). p66Shc not only increases ROS production but also indirectly controls the expression of superoxide dismutase 2 (SOD2) and catalase by interacting with the forkhead box O3 (FOXO3a) and p53 proteins [219]. Interestingly, p53, a known oncogenic protein, also regulates p66Shc-dependent apoptosis [220]. The intense inflammation in hepatocellular carcinoma contributes to p66Shc upregulation, which correlates with poor clinical prognosis for patients. In addition, knockdown of p66Shc inhibits cancer cell proliferation and tumor growth. In a hepatocellular carcinoma model, the pro-oxidant function of p66Shc was associated with

activation of the signal transducer and activator of transcription 3 (STAT3) protein [221]. Many studies have shown the importance of p66Shc in the development and progression of prostate cancer, as it can be modulated by steroid hormones [222]. 5 α -Dihydrotestosterone induces p66Shc Ser36 phosphorylation and promotes its translocation to mitochondria, with a subsequent increase in ROS production due to its interaction with cyt c [222]. A more recent study showed that a further increase in the pro-oxidant activity of p66Shc leads to the transformation of androgen-sensitive cells into prostate cancer cells and to the progression of castration-resistant prostate cancer, which has no effective treatment [223]. In the most advanced and invasive stage of the disease, a high level of p66Shc was associated with migration of the transformed cells [224,225]. In different experimental models of breast cancer, the protein level of p66Shc was positively correlated with metastasis [226,227]. However, this correlation was mostly related to the adaptor function of p66Shc in the cytosolic fraction and was dependent on activation of growth factor receptors [228].

In several cellular models of lung cancer, both the adaptor and pro-oxidant pathways seemed to play a substantial role [96,229,230]. The most recent reports, as well as older data, show that MAM-localized p66Shc is involved in many intracellular signaling pathways associated with the oxidative stress response, proliferation and differentiation; thus, it seems reasonable that p66Shc should also be considered an oncoprotein. Collectively, these reports confirm that p66Shc is an important factor and should be considered in cancer diagnosis and as a therapeutic target for cancer.

2.4. Other functions of MAM in cancer

The mitochondrial fusion-fission machinery provides an important link among ER-mitochondria tethering, Ca^{2+} homeostasis and tumors. Mitochondria move along the cytoskeleton and frequently divide and fuse in response to different stimuli; this behavior has been observed and associated with cancer development [231]. Interestingly, enhancement of mitochondrial fusion by increasing MFN1/2 levels has been associated with prostate cancer progression [232]. In particular, the role of OMM-embedded MFN2, which is also localized at MAM, remains controversial. Scorrano and colleagues observed that a reduction in IP3R-mediated mitochondrial Ca^{2+} uptake by cells upon MFN2 silencing was caused by disruption of MERCs [233,234]; however, this evidence was not confirmed by Filadi et al., who ascribed an increase in membrane juxtaposition between the two organelles and subsequent upregulation of the Ca^{2+} signaling cascade to MFN2 depletion [235] in support of cell death. This process remains a popular topic due to the strategic MFN2 localization in MAM and its role in proapoptotic and antiproliferative signaling in cancer diseases [236,237]. A brief overview of the MAM-related proteins discussed in this section is presented in Fig. 5.

Another important protein involved in mitochondrial dynamics is DRP1, which is a key mediator of mitochondrial fission and plays a role in mitochondrial apoptosis. During mitochondrial apoptosis, Drp1 interacts directly with BCL-2-associated X protein (BAX), inducing its translocation from the cytosol to mitochondria, thereby mediating mitochondrial fragmentation and inhibition of osteosarcoma cell growth in response to cancer therapy [238]. Additionally, Drp1 undergoes mitochondrial anchored RING-finger containing protein (MAPL)/MUL1-dependent small ubiquitin-like modifier 1 (SUMO)ylation, which allows the stabilization of ER/mitochondrial contact sites and subsequently results in mitochondrial constriction, Ca^{2+} flux and cyt c release into the cytosol [239]. In fact, along with proapoptotic proteins, DRP1 is indicted to be a primary player in the BH3 mimetic-mediated cyt c pathway [240]. However, the role of DRP1 in cancer remains controversial. Recent studies have shown that DRP1 ablation decreases the invasion and migration rates of lung cancer cells [241] and suppresses pancreatic tumor growth [242], while DRP1 overexpression is linked with proliferation and metastasis in lung adenocarcinoma [241].

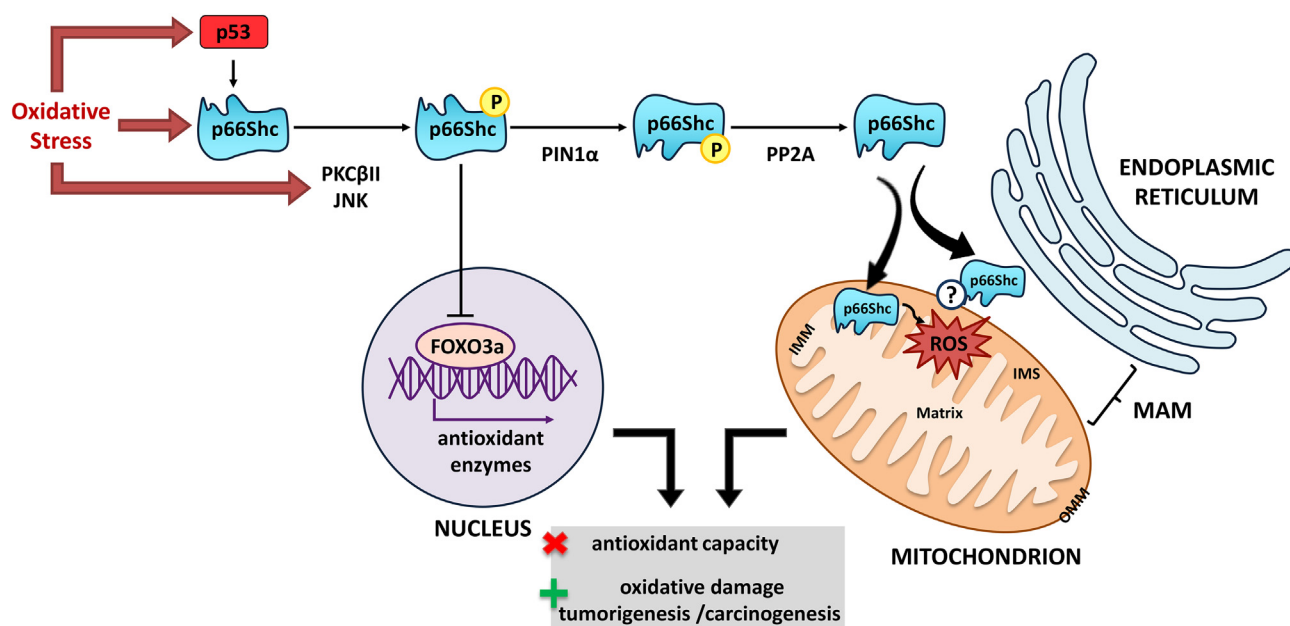


Fig. 4. Implication of the p66Shc-associated pathway in oncogenic processes. The stress-activated pro-oxidant function of p66Shc depends on the proapoptotic activity of the p53 protein and is amplified by inactivation of the FOXO3a transcription factor, which results in inhibited biosynthesis of antioxidant enzymes (such as SOD2). Stress-activated kinases such as PKCβII and JNK phosphorylate p66Shc, which initiates modifications indispensable for its translocation to mitochondria and/or MAM. In mitochondria, p66Shc triggers excessive ROS production by interacting with cyt c, although the precise mechanism in MAM remains unclear. Together with the downregulated antioxidant defense, mitochondrial ROS accumulation leads to oxidative damage and cellular malfunction, which can contribute to the initiation of oncogenic processes.

The green “+” symbols indicate induced pathways, while the red “x” symbols indicate affected pathways.

FOXO3a – forkhead box O3 transcription factor; IMM – inner mitochondrial membrane; IMS – mitochondrial intermembrane space; JNK – c-Jun N-terminal kinase; MAM – mitochondria-associated endoplasmic reticulum membranes; OMM – outer mitochondrial membrane; p53 – tumor suppressor protein p53; p66Shc – p66 isoform of Src homology/collagen (SHC) adaptor protein; PIN1 – peptidyl-prolyl cis-trans isomerase 1; PKCβII – phosphorylated by protein kinase Cβ II; PP2A – protein phosphatase 2A; ROS – reactive oxygen species.

Furthermore, DRP1 is reported to interact with the antiapoptotic isoform of myeloid cell leukemia 1 (MCL-1L) at the OMM. The MCL-1L protein belongs to the BCL-2 family and is enriched in the ER and MAM [243]. In the absence of MCL-1L (e.g., when its expression is lowered by anticancer therapies), DRP1 cannot bind to MCL-1L and ensure physiological mitochondrial fragmentation, thus producing a persistent mitochondrial hyperfusion state that propagates apoptotic waves [243]. MCL-1 is overexpressed in lung cancer cell lines, such as H1299 and A549, in non-small cell lung cancer cell lines, and in specimens from patients [244]. Reducing intracellular levels of MCL-1 may improve the clinical management of patients [245]. Interestingly, Huang et al. proposed that MCL-1 promotes lung cancer cell migration by interacting directly with VDAC1, probably by increasing Ca^{2+} uptake via mitochondrial ROS generation [246].

Moreover, the ER-mitochondria tethering protein complex FIS-BAP31 in MAM serves as a platform for the activation of apoptotic signaling at the ER by recruiting procaspase 8 [247]. This complex regulates Ca^{2+} signaling and comprises fission protein 1 (FIS1) and B-cell receptor-associated protein 31 (BAP31) [247]. BAP31 is involved in protein quality control and Fas-mediated apoptosis by recruiting initiator caspases [248,249]. Fas-mediated apoptosis is initiated by FIS1, which, via its OMM localization, induces an apoptotic signal to the ER. Then, FIS1 binds to the integral membrane chaperone protein BAP31, establishing the FIS1-BAP31 complex and facilitating BAP31 cleavage into p20BAP31 by caspase 8 [250,251]. Caspase-mediated cleavage promotes mitochondria-ER crosstalk through Ca^{2+} -dependent signaling. This signaling pathway generates a feedback loop by transferring Ca^{2+} from the ER to mitochondria, which activates mitochondria-mediated apoptosis [247]. Under these conditions, MAM accommodate marked mitochondrial Ca^{2+} uptake from the ER to promote cell death.

Additionally, BAP31 has been implicated in the interaction with

phosphofurin acidic cluster sorting 2 protein (PACS2), a multifunctional protein involved in the communication between the ER and mitochondria by controlling their juxtaposition. Therefore, in the absence of PACS2, mitochondrial fragmentation occurs in a BAP31-dependent manner [252]. In the presence of apoptotic stimuli, PACS2 induces the translocation of BH3 interacting-domain death agonist (BID) to mitochondria, thereby inducing the apoptotic cascade that culminates in cell death [252].

Despite these findings, additional studies are needed to better understand and clarify the mechanisms underlying the involvement of MAM in the abovementioned pathways.

3. Conclusions

Biochemical and fluorescence microscopy approaches have permitted the isolation and investigation of MAM in many pathophysiological contexts. Here, accumulating findings indicate that several tumor suppressors and oncogenes, as well as other proteins closely related to the oncogenic process, are present in the contact sites between mitochondria and the ER, where they can influence MAM homeostasis during cancer transformation. MAM homeostasis involves the architectural remodeling of MAM in terms of both spatial rearrangement and changes in the expression of channel proteins, such as those influencing lipid trafficking and Ca^{2+} flux between mitochondria and the ER, and/or the relocation of their direct regulators and interactors. Indeed, the percentage of mitochondrial membranes closely juxtaposed to the ER increases and decreases depending on many determinants. This dynamic creates a continuously evolving network that consequently impacts cell metabolism, bioenergetics and almost all aspects of tumor development, such as the Warburg effect itself, proliferation, migration, dedifferentiation and metastasis. Evidence reporting the significant

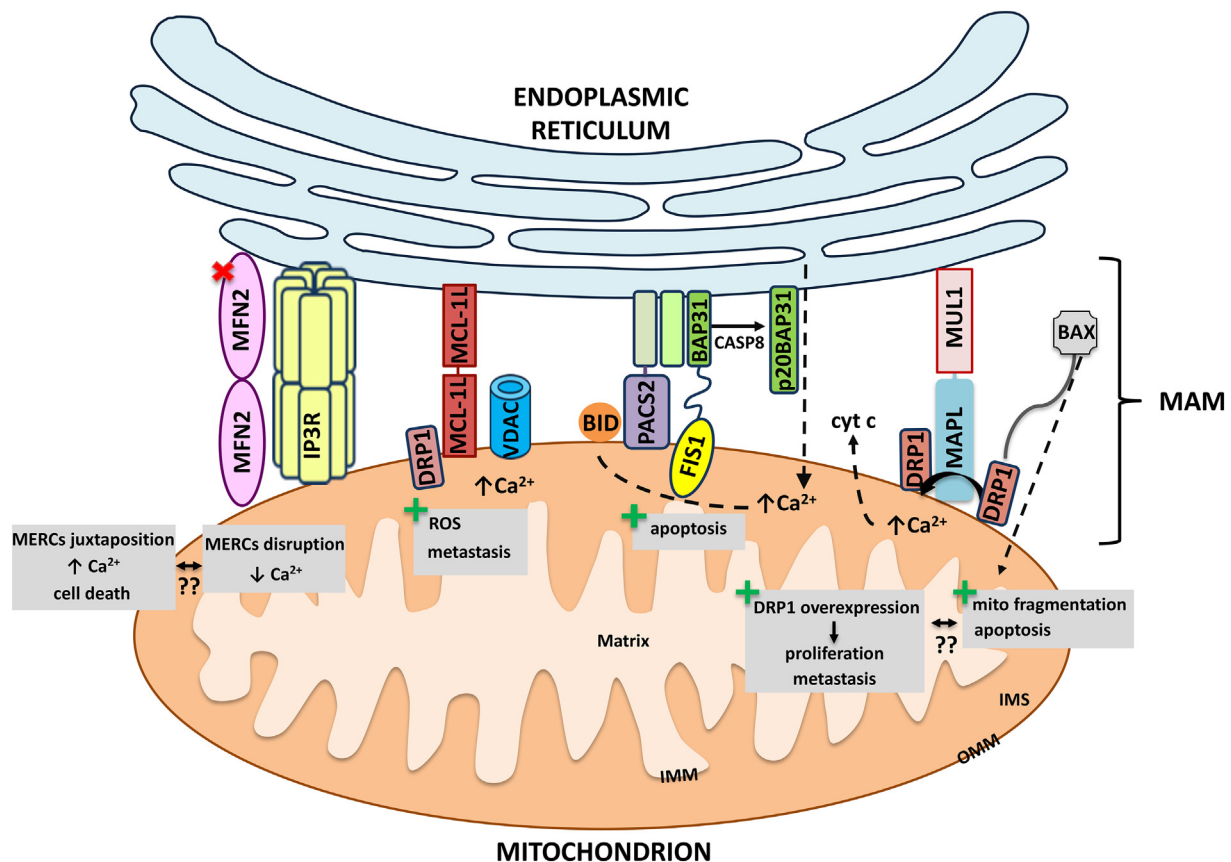


Fig. 5. Global view of other MAM-related proteins involved in cancer. Mitochondrial fusion and fission events have been implicated in cancer. Augmented levels of MFN1/2 support an increase in fusion events during cancer progression. However, the role of MFN2 in MERCs is controversial, because MFN2 silencing has been linked to MERCs disruption and decreased Ca^{2+} levels or to MERCs juxtapposition and increased Ca^{2+} levels by distinct authors. Another protein implicated in cancer is DRP1. In the presence of MCL-1L, DRP1 can bind to MCL-1L, which increases Ca^{2+} uptake and mitochondrial ROS production by interacting with VDAC1, thereby promoting cancer cell invasion. DRP1 can also interact with BAX, causing its translocation to mitochondria, thereby mediating mitochondrial fragmentation and apoptosis. DRP1 can undergo MAPL/MUL1-mediated SUMOylation, which allows mitochondrial constriction, Ca^{2+} flux and cyt c release into the cytosol. The role of DRP1 in apoptosis is opposed by some data showing that DRP1 overexpression increases the rates of cancer proliferation and metastasis. In addition, FIS1 plays a role in mediating apoptosis. Formation of the FIS1-BAP31 complex enables BAP31 cleavage into p20BAP31, which activates ER-mitochondrial Ca^{2+} crosstalk. Increased flux of Ca^{2+} into mitochondria activates cell death programs. This apoptotic stimulus induces PACS2 to translocate BID to mitochondria, which may enhance the activation of the apoptotic cascade.

The green “+” symbols indicate induced pathways, while the red “x” symbols indicate affected pathways.

BAP31 – B-cell receptor-associated protein 31; BAX – BCL-2-associated X protein; BID – BH3 interacting-domain death agonist; Ca^{2+} – calcium; CASP8 – caspase 8; cyt c – cytochrome c; DRP1 – dynamamin-related protein 1; FIS1 – fission protein 1; IMM – inner mitochondrial membrane; IMS – mitochondrial intermembrane space; IP3R – inositol 1,4,5-triphosphate receptor; MAM – mitochondria-associated endoplasmic reticulum membranes; MAPL – mitochondrial anchored RING-finger containing protein; MERCs – mitochondria-endoplasmic reticulum contacts; MCL-1L – myeloid cell leukemia 1; MFN2 – mitofusin 2; mito – mitochondria; OMM – outer mitochondrial membrane; PACS2 – phosphofurin acidic cluster sorting 2 protein; ROS – reactive oxygen species; VDAC – voltage-dependent anion channel.

increase in MERCs in tumor cells with cancer stem cell properties compared to other cell phenotypes, as well as the strong relationship of the profoundly altered expression (and function) of IP3R3 and MCU with invasion, metastasis and decreased 5-year survival, underscores the importance of considering MAM homeostasis in the design of clinical strategies and treatment opportunities. Consistent with this observation, cells with invasive potential (i.e., MDA-MB231 cells) are characterized by elevated Ca^{2+} flux, which is essential for the Warburg effect. In other cancer types, such as colon cancer, MAM-resident proteins (i.e., the chaperones GRP78 and ATAD3a) facilitate invasion and metastasis.

An important feature of MAM in cancer cells is their ability to modulate the lipid composition of organelle membranes. Lipid synthesis and transfer occur in membranes, and increased amounts of lipids are required for tumor growth. Indeed, alterations in mitochondrial lipids (i.e., increased percentages of cholesterol, TGs and sphingolipids) are seen in several kinds of tumors, and these alterations in turn modify Ca^{2+} homeostasis and sensitization to different types of cell death. This

observation highlights the importance of these findings as an additional novel perspective for therapeutic strategies to induce alternative cell death programs to overcome apoptosis resistance.

Although the usefulness of managing all these pathways has been demonstrated, much remains to be done to understand how and in what manner actual clinical therapies may benefit from this manipulation. For example, can MAM signaling make a difference in the new era of personalized therapy either linked to a specific type of cancer, related to the onset of a mutation or specific for the sex of the individual affected? Sex differences in MAM have partially been analyzed in cardiovascular diseases, where female hearts are subjected to lower Ca^{2+} flux towards mitochondria than male hearts, resulting in decreased injury upon ischemia [253]. Moreover, the presence/absence of hormones such as estrogen may modify channel proteins involved in Ca^{2+} signaling, such as SERCA [254]. Whether sex may modify MAM architecture (and protein expression) in cancer is still unknown. Overall, specific types of cancer can be represented by selective settings of MAM-resident proteins that distinguish that cancer from others. Can we

identify different types of MAM organelles stratified by different functions and protein expression patterns? Indeed, proteins may shuttle to and from MAM in response to specific stimuli.

Can we study MAM homeostasis in vivo by establishing new mouse models? Indeed, the actual efforts have aimed to study the proteome of transgenic mice to compare a given gene involved in cancer to that gene in control mice via mass spectrometry-based quantitative analysis. This approach could be useful to understand whether MAM can control the type of cell death or if they can be considered an ideal target for pathogens to impair inflammasome activation during infection.

Are there other structural regulators of ER-mitochondria tethering? This topic is important to expand, because it intersects with additional pathways, such as mitochondrial fission. Recent studies suggest that MERCs are established at constriction sites. Proteins involved in mitochondrial division (i.e., DRP1) may be recruited to these sites and impact mitochondrial morphology and Ca^{2+} signaling in cancer. Understanding the fundamental roles of MERCs as important domains for tumor suppressor and oncogene localization and determining an exact answer to each of these questions will facilitate the search for new and more precise anticancer therapies.

Funding

This research was funded by the Polish National Science Centre grant UMO-2018/29/B/NZ1/00589 to Y.P. and M.R.W. Moreover, M.R.W. and I.C.M.S. gratefully acknowledge financial support for this research from the FOIE GRAS and mtFOIE GRAS projects. These projects received funding from the European Union's Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 722619 (FOIE GRAS) and Grant Agreement No. 734719 (mtFOIE GRAS). M.L.-A. was funded by a Polish National Science Centre grant (UMO-2015/17/D/NZ1/00030). P.P. is grateful to Camilla degli Scrovegni for continuous support. Moreover, P.P. is supported by the Italian Association for Cancer Research (AIRC, IG-23670), Telethon Foundation (GGP11139B), Progetti di Rilevante Interesse Nazionale (PRIN, 2017 E5L5P3) and local funds from the University of Ferrara.

CRedit authorship contribution statement

Ines C.M. Simoes: Writing - original draft, Writing - review & editing. **Giampaolo Morciano:** Writing - original draft, Writing - review & editing. **Magdalena Lebedzinska-Arciszewska:** Writing - original draft, Writing - review & editing. **Gianluca Aguiari:** Writing - review & editing. **Paolo Pinton:** Writing - original draft, Writing - review & editing. **Yaiza Potes:** Writing - original draft, Writing - review & editing. **Mariusz R. Wieckowski:** Writing - original draft, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare no conflicts of interest.

References

- [1] J.D. Lever, J.B. Chappell, Mitochondria isolated from rat brown adipose tissue and liver, *J. Biophys. Biochem. Cytol.* 4 (1958) 287–290.
- [2] J.E. Vance, Phospholipid synthesis in a membrane fraction associated with mitochondria, *J. Biol. Chem.* 265 (1990) 7248–7256.
- [3] Y. Wu, C. Whiteus, C.S. Xu, K.J. Hayworth, R.J. Weinberg, H.F. Hess, P. De Camilli, Contacts between the endoplasmic reticulum and other membranes in neurons, *Proc. Natl. Acad. Sci. U. S. A.* 114 (2017) E4859–E4867.
- [4] S. Missiroli, S. Patergnani, N. Caroccia, G. Pedriali, M. Perrone, M. Previati, M.R. Wieckowski, C. Giorgi, Mitochondria-associated membranes (MAM) and inflammation, *Cell Death Dis.* 9 (2018) 329.
- [5] S.M. Horner, H.M. Liu, H.S. Park, J. Briley, M. Gale Jr., Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 14590–14595.
- [6] C.D. Williamson, A.M. Colberg-Poley, Access of viral proteins to mitochondria via mitochondria-associated membranes, *Rev. Med. Virol.* 19 (2009) 147–164.
- [7] E. Area-Gomez, E.A. Schon, On the pathogenesis of Alzheimer's disease: the MAM hypothesis, *FASEB J.* 31 (2017) 864–867.
- [8] E. Area-Gomez, E.A. Schon, Mitochondria-associated ER membranes and Alzheimer disease, *Curr. Opin. Genet. Dev.* 38 (2016) 90–96.
- [9] M.D. Tambini, M. Pera, E. Kanter, H. Yang, C. Guardia-Laguarta, D. Holtzman, D. Sulzer, E. Area-Gomez, E.A. Schon, ApoE4 upregulates the activity of mitochondria-associated ER membranes, *EMBO Rep.* 17 (2016) 27–36.
- [10] 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, Upregulated function of mitochondria-associated ER membranes in Alzheimer disease, *EMBO J.* 31 (2012) 4106–4123.
- [11] E. Area-Gomez, A.J. de Groof, I. Boldogh, T.D. Bird, G.E. Gibson, C.M. Koehler, W.H. Yu, K.E. Duff, M.P. Yaffe, L.A. Pon, E.A. Schon, Presenilins are enriched in endoplasmic reticulum membranes associated with mitochondria, *Am. J. Pathol.* 175 (2009) 1810–1816.
- [12] S. Watanabe, H. Ilieva, H. Tamada, H. Nomura, O. Komine, F. Endo, S. Jin, P. Mancias, H. Kiyama, K. Yamana, Mitochondria-associated membrane collapse is a common pathomechanism in SIGMAR1- and SOD1-linked ALS, *EMBO Mol. Med.* 8 (2016) 1421–1437.
- [13] R. Stoica, S. Paillusson, P. Gomez-Suaga, J.C. Mitchell, D.H. Lau, E.H. Gray, R.M. Sancho, G. Vizcay-Barrena, K.J. De Vos, C.E. Shaw, D.P. Hanger, W. Noble, C.C. Miller, ALS/FTD-associated FUS activates GSK-3beta to disrupt the VAPB-PTPIP51 interaction and ER-mitochondria associations, *EMBO Rep.* 17 (2016) 1326–1342.
- [14] R. Stoica, K.J. De Vos, S. Paillusson, S. Mueller, R.M. Sancho, K.F. Lau, G. Vizcay-Barrena, W.L. Lin, Y.F. Xu, J. Lewis, D.W. Dickson, L. Petrucelli, J.C. Mitchell, C.E. Shaw, C.C. Miller, ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43, *Nat. Commun.* 5 (2014) 3996.
- [15] A.P. Arruda, B.M. Pers, G. Parlakgul, E. Guney, K. Inouye, G.S. Hotamisligil, Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity, *Nat. Med.* 20 (2014) 1427–1435.
- [16] E. Tubbs, J. Rieusset, Metabolic signaling functions of ER-mitochondria contact sites: role in metabolic diseases, *J. Mol. Endocrinol.* 58 (2017) R87–R106.
- [17] E. Tubbs, P. Theurey, G. Vial, N. Bendridi, A. Bravard, M.A. Chauvin, J. Ji-Cao, F. Zoulim, B. Bartosch, M. Ovide, H. Vidal, J. Rieusset, Mitochondria-associated endoplasmic reticulum membrane (MAM) integrity is required for insulin signaling and is implicated in hepatic insulin resistance, *Diabetes* 63 (2014) 3279–3294.
- [18] R. Sano, I. Annunziata, A. Patterson, S. Moshiah, 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.
- [19] A. Zhang, C.D. Williamson, D.S. Wong, M.D. Bullough, K.J. Brown, Y. Hathout, A.M. Colberg-Poley, Quantitative proteomic analyses of human cytomegalovirus-induced restructuring of endoplasmic reticulum-mitochondrial contacts at late times of infection, *Mol. Cell Proteomics* 10 (2011) (M111 009936).
- [20] 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.
- [21] J.H. Ma, S. Shen, J.J. Wang, Z. He, A. Poon, J. Li, J. Qu, S.X. Zhang, Comparative proteomic analysis of the mitochondria-associated ER membrane (MAM) in a long-term type 2 diabetic rodent model, *Sci. Rep.* 7 (2017) 2062.
- [22] V. Hung, S.S. Lam, N.D. Udeshi, T. Svinikina, G. Guzman, V.K. Mootha, S.A. Carr, A.Y. Ting, Proteomic mapping of cytosol-facing outer mitochondrial and ER membranes in living human cells by proximity biotinylation, *Elife* 6 (2017).
- [23] 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.
- [24] C. Giorgi, S. Missiroli, S. Patergnani, J. Duszyński, M.R. Wieckowski, P. Pinton, Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications, *Antioxid. Redox Signal.* 22 (2015) 995–1019.
- [25] E.A. Schon, E. Area-Gomez, Mitochondria-associated ER membranes in Alzheimer disease, *Mol. Cell. Neurosci.* 55 (2013) 26–36.
- [26] M. Krols, G. van Isterdael, B. Asselbergh, A. Kremer, S. Lippens, V. Timmerman, S. Janssens, Mitochondria-associated membranes as hubs for neurodegeneration, *Acta Neuropathol.* 131 (2016) 505–523.
- [27] A. Rimessi, G. Pedriali, B. Vezzani, A. Tarocco, S. Marchi, M.R. Wieckowski, C. Giorgi, P. Pinton, Interorganellar calcium signaling in the regulation of cell metabolism: a cancer perspective, *Semin. Cell Dev. Biol.* 98 (2020) 167–180.
- [28] G. Morciano, S. Marchi, C. Morganti, L. Sbrano, M. Bittremieux, M. Kerkhofs, M. Corricelli, A. Danese, A. Karkucinska-Wieckowska, M.R. Wieckowski, G. Bultynck, C. Giorgi, P. Pinton, Role of mitochondria-associated ER membranes in calcium regulation in cancer-specific settings, *Neoplasia* 20 (2018) 510–523.
- [29] J. Janikiewicz, J. Szymanski, D. Malinska, P. Patalas-Krawczyk, B. Michalska, J. Duszyński, C. Giorgi, M. Bonora, A. Dobrzyn, M.R. Wieckowski, Mitochondria-associated membranes in aging and senescence: structure, function, and dynamics, *Cell Death Dis.* 9 (2018) 332.
- [30] Y. Fan, T. Simmen, Mechanistic connections between endoplasmic reticulum (ER) redox control and mitochondrial metabolism, *Cells* 8 (2019).
- [31] M.L. Sassano, A.R. van Vliet, P. Agostinis, Mitochondria-associated membranes as networking platforms and regulators of cancer cell fate, *Front. Oncol.* 7 (2017) 174.

- [32] T. Gutierrez, T. Simmen, Endoplasmic reticulum chaperones and oxidoreductases: critical regulators of tumor cell survival and immunorecognition, *Front. Oncol.* 4 (2014) 291.
- [33] O. Warburg, On the origin of cancer cells, *Science* 123 (1956) 309–314.
- [34] G. Medes, A. Thomas, S. Weinhouse, Metabolism of neoplastic tissue. IV. A study of lipid synthesis in neoplastic tissue slices in vitro, *Cancer Res.* 13 (1953) 27–29.
- [35] J.V. Swinnen, F. Vanderhoydonc, A.A. Elgamal, M. Eelen, I. Vercaeren, S. Joniau, H. Van Poppel, L. Baert, K. Goossens, W. Heyns, G. Verhoeven, Selective activation of the fatty acid synthesis pathway in human prostate cancer, *Int. J. Cancer* 88 (2000) 176–179.
- [36] G. Achleitner, B. Gaigg, A. Krasser, E. Kainersdorfer, S.D. Kohlwein, A. Perktold, G. Zellnig, G. Daum, Association between the endoplasmic reticulum and mitochondria of yeast facilitates interorganelle transport of phospholipids through membrane contact, *Eur. J. Biochem.* 264 (1999) 545–553.
- [37] 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.
- [38] J.P. Monteiro, P.J. Oliveira, A.S. Jurado, Mitochondrial membrane lipid remodeling in pathophysiology: a new target for diet and therapeutic interventions, *Prog. Lipid Res.* 52 (2013) 513–528.
- [39] D.T. Browman, M.E. Resek, L.D. Zajchowski, S.M. Robbins, Erlin-1 and erlin-2 are novel members of the prohibitin family of proteins that define lipid-raft-like domains of the ER, *J. Cell Sci.* 119 (2006) 3149–3160.
- [40] A. Chaudhuri, A. Chattopadhyay, Transbilayer organization of membrane cholesterol at low concentrations: implications in health and disease, *Biochim. Biophys. Acta* 1808 (2011) 19–25.
- [41] S.J. Stone, J.E. Vance, Phosphatidylserine synthase-1 and -2 are localized to mitochondria-associated membranes, *J. Biol. Chem.* 275 (2000) 34534–34540.
- [42] S.H. Lee, X.W. Meng, K.S. Flatten, D.A. Loegering, S.H. Kaufmann, Phosphatidylserine exposure during apoptosis reflects bidirectional trafficking between plasma membrane and cytoplasm, *Cell Death Differ.* 20 (2013) 64–76.
- [43] P.A. Grandmaison, T.S. Nanowski, J.E. Vance, Externalization of phosphatidylserine during apoptosis does not specifically require either isoform of phosphatidylserine synthase, *Biochim. Biophys. Acta* 1636 (2004) 1–11.
- [44] X. Cheng, D. Zhou, J. Wei, J. Lin, Cell-cycle arrest at G2/M and proliferation inhibition by adenovirus-expressed mitofusin-2 gene in human colorectal cancer cell lines, *Neoplasma* 60 (2013) 620–626.
- [45] J. Rehman, H.J. Zhang, P.T. Toth, Y. Zhang, G. Marsboom, Z. Hong, R. Salgia, A.N. Husain, C. Wietholt, S.L. Archer, Inhibition of mitochondrial fission prevents cell cycle progression in lung cancer, *FASEB J.* 26 (2012) 2175–2186.
- [46] M.I. Hernandez-Alvarez, D. Sebastian, S. Vives, S. Ivanova, P. Bartocioni, P. Kakimoto, N. Plana, S.R. Veiga, V. Hernandez, N. Vasconcelos, G. Peddinti, A. Adrover, M. Jove, R. Pamplona, I. Gordaliza-Alaguero, E. Calvo, N. Cabre, R. Castro, A. Kuzmanic, M. Boutant, D. Sala, T. Hyotylainen, M. Oresic, J. Fort, E. Errasti-Murugarren, C.M.P. Rodrigues, M. Orozco, J. Joven, C. Canto, M. Palacin, S. Fernandez-Veledo, J. Vendrell, A. Zorzano, Deficient endoplasmic reticulum-mitochondrial phosphatidylserine transfer causes liver disease, *Cell* 177 (2019) 881–895 (e817).
- [47] N. Zaidi, L. Lupien, N.B. Kuemmerle, W.B. Kinlaw, J.V. Swinnen, K. Smans, Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids, *Prog. Lipid Res.* 52 (2013) 585–589.
- [48] N.B. Kuemmerle, E. Rysman, P.S. Lombardo, A.J. Flanagan, B.C. Lipe, W.A. Wells, J.R. Pettus, H.M. Froehlich, V.A. Memoli, P.M. Morganeli, J.V. Swinnen, L.A. Timmerman, L. Chaychi, C.J. Fricano, B.L. Eisenberg, W.B. Coleman, W.B. Kinlaw, Lipoprotein lipase links dietary fat to solid tumor cell proliferation, *Mol. Cancer Ther.* 10 (2011) 427–436.
- [49] F. Geng, D. Guo, Lipid droplets, potential biomarker and metabolic target in glioblastoma, *Intern. Med. Rev. (Wash. D. C.)* 3 (2017).
- [50] M.M. Gaida, C. Mayer, U. Dapunt, S. Stegmaier, P. Schirmacher, G.H. Wabnitz, G.M. Hansch, Expression of the bitter receptor T2R38 in pancreatic cancer: localization in lipid droplets and activation by a bacteria-derived quorum-sensing molecule, *Oncotarget* 7 (2016) 12623–12632.
- [51] M.F. Mulas, C. Abete, D. Pulisci, A. Pani, B. Massidda, S. Dessi, A. Mandas, Cholesterol esters as growth regulators of lymphocytic leukaemia cells, *Cell Prolif.* 44 (2011) 360–371.
- [52] C.J. Antalis, T. Arnold, T. Rasool, B. Lee, K.K. Buhman, R.A. Siddiqui, High ACAT1 expression in estrogen receptor negative basal-like breast cancer cells is associated with LDL-induced proliferation, *Breast Cancer Res. Treat.* 122 (2010) 661–670.
- [53] M.T. Accioly, P. Pacheco, C.M. Maya-Monteiro, N. Carrossini, B.K. Robbs, S.S. Oliveira, C. Kaufmann, J.A. Morgado-Diaz, P.T. Bozza, J.P. Viola, Lipid bodies are reservoirs of cyclooxygenase-2 and sites of prostaglandin-E2 synthesis in colon cancer cells, *Cancer Res.* 68 (2008) 1732–1740.
- [54] T.M. Lewin, J.H. Kim, D.A. Granger, J.E. Vance, R.A. Coleman, Acyl-CoA synthetase isoforms 1, 4, and 5 are present in different subcellular membranes in rat liver and can be inhibited independently, *J. Biol. Chem.* 276 (2001) 24674–24679.
- [55] J. Stiban, L. Caputo, M. Colombini, Ceramide synthesis in the endoplasmic reticulum can permeabilize mitochondria to proapoptotic proteins, *J. Lipid Res.* 49 (2008) 625–634.
- [56] H.S. Vethakanraj, T.A. Babu, G.B. Sudarsanan, P.K. Duraisamy, S. Ashok Kumar, Targeting ceramide metabolic pathway induces apoptosis in human breast cancer cell lines, *Biochem. Biophys. Res. Commun.* 464 (2015) 833–839.
- [57] M. Flowers, G. Fabrias, A. Delgado, J. Casas, J.L. Abad, M.C. Cabot, C6-ceramide and targeted inhibition of acid ceramidase induce synergistic decreases in breast cancer cell growth, *Breast Cancer Res. Treat.* 133 (2012) 447–458.
- [58] R.A. Coleman, T.M. Lewin, C.G. Van Horn, M.R. Gonzalez-Baro, Do long-chain acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? *J. Nutr.* 132 (2002) 2123–2126.
- [59] S. Hulstsch, M. Kankainen, L. Paavolainen, R.M. Kovanen, E. Ikonen, S. Kangaspeska, V. Pietiainen, O. Kallioniemi, Association of tamoxifen resistance and lipid reprogramming in breast cancer, *BMC Cancer* 18 (2018) 850.
- [60] J. Li, X. Qu, J. Tian, J.T. Zhang, J.X. Cheng, Cholesterol esterification inhibition and gemcitabine synergistically suppress pancreatic ductal adenocarcinoma proliferation, *PLoS One* 13 (2018) e0193318.
- [61] L. Puglielli, G. Konopka, E. Pack-Chung, L.A. Ingano, O. Berezovska, B.T. Hyman, T.Y. Chang, R.E. Tanzi, D.M. Kovacs, Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid beta-peptide, *Nat. Cell Biol.* 3 (2001) 905–912.
- [62] S. Koizume, Y. Miyagi, Lipid droplets: a key cellular organelle associated with cancer cell survival under normoxia and hypoxia, *Int. J. Mol. Sci.* 17 (2016).
- [63] K.H. Stopsack, T.A. Gerke, O. Andren, S.O. Andersson, E.L. Giovannucci, L.A. Mucci, J.R. Rider, Cholesterol uptake and regulation in high-grade and lethal prostate cancers, *Carcinogenesis* 38 (2017) 806–811.
- [64] J. Li, D. Gu, S.S. Lee, B. Song, S. Bandyopadhyay, S. Chen, S.F. Konieczny, T.L. Ratliff, X. Liu, J. Xie, J.X. Cheng, Abrogating cholesterol esterification suppresses growth and metastasis of pancreatic cancer, *Oncogene* 35 (2016) 6378–6388.
- [65] T. Ohmoto, K. Nishitsuji, N. Yoshitani, M. Mizuguchi, Y. Yanagisawa, H. Saito, N. Sakashita, K604, a specific acylCoA: cholesterol acyltransferase 1 inhibitor, suppresses proliferation of U251MG glioblastoma cells, *Mol. Med. Rep.* 12 (2015) 6037–6042.
- [66] M.P. Kim, G.E. Gallick, Gemcitabine resistance in pancreatic cancer: picking the key players, *Clin. Cancer Res.* 14 (2008) 1284–1285.
- [67] M. Liscovitch, Y. Lavie, Multidrug resistance: a role for cholesterol efflux pathways? *Trends Biochem. Sci.* 25 (2000) 530–534.
- [68] V.N. Ayyagari, X. Wang, P.L. Diaz-Sylvester, K. Groesch, L. Brard, Assessment of acyl-CoA cholesterol acyltransferase (ACAT-1) role in ovarian cancer progression-an in vitro study, *PLoS One* 15 (2020) e0228024.
- [69] A. Sala-Vila, I. Navarro-Lerida, M. Sanchez-Alvarez, M. Bosch, C. Calvo, J.A. Lopez, E. Calvo, C. Ferguson, M. Giacomello, A. Serafini, L. Scorrano, J.A. Enriquez, J. Balsinde, R.G. Parton, J. Vazquez, A. Pol, M.A. Del Pozo, Interplay between hepatic mitochondria-associated membranes, lipid metabolism and caveolin-1 in mice, *Sci. Rep.* 6 (2016) 27351.
- [70] M. Bosch, M. Mari, A. Herms, A. Fernandez, A. Fajardo, A. Kassan, A. Giralt, A. Colell, D. Balmora, E. Barbero, E. Gonzalez-Moreno, N. Matias, F. Tebar, J. Balsinde, M. Camps, C. Enrich, S.P. Gross, C. Garcia-Ruiz, E. Perez-Navarro, J.C. Fernandez-Checa, A. Pol, Caveolin-1 deficiency causes cholesterol-dependent mitochondrial dysfunction and apoptotic susceptibility, *Curr. Biol.* 21 (2011) 681–686.
- [71] R. Bravo-Sagua, V. Parra, C. Ortiz-Sandoval, M. Navarro-Marquez, A.E. Rodriguez, N. Diaz-Valdivia, C. Sanhueza, C. Lopez-Crisosto, N. Tahbaz, B.A. Rothermel, J.A. Hill, M. Cifuentes, T. Simmen, A.F.G. Quest, S. Lavandero, Caveolin-1 impairs PKA-DRP1-mediated remodelling of ER-mitochondria communication during the early phase of ER stress, *Cell Death Differ.* 26 (2019) 1195–1212.
- [72] L. Issop, J. Fan, S. Lee, M.B. Rone, K. Basu, J. Mui, V. Papadopoulos, Mitochondria-associated membrane formation in hormone-stimulated Leydig cell steroidogenesis: role of ATAD3, *Endocrinology* 156 (2015) 334–345.
- [73] Y. Teng, X. Ren, H. Li, A. Shull, J. Kim, J.K. Cowell, Mitochondrial ATAD3A combines with GRP78 to regulate the WASF3 metastasis-promoting protein, *Oncogene* 35 (2016) 333–343.
- [74] M.N. Lee, H.S. Hwang, S.H. Oh, A. Roshanzadeh, J.W. Kim, J.H. Song, E.S. Kim, J.T. Koh, Elevated extracellular calcium ions promote proliferation and migration of mesenchymal stem cells via increasing osteopontin expression, *Exp. Mol. Med.* 50 (2018) 142.
- [75] C.H. Cho, J.S. Woo, C.F. Perez, E.H. Lee, A focus on extracellular Ca(2+) entry into skeletal muscle, *Exp. Mol. Med.* 49 (2017) e378.
- [76] B. Lu, Q. Zhang, H. Wang, Y. Wang, M. Nakayama, D. Ren, Extracellular calcium controls background current and neuronal excitability via an UNC79-UNC80-NALCN cation channel complex, *Neuron* 68 (2010) 488–499.
- [77] P. Pinton, S. Leo, M.R. Wieckowski, G. Di Benedetto, R. Rizzuto, Long-term modulation of mitochondrial Ca2+ signals by protein kinase C isozymes, *J. Cell Biol.* 165 (2004) 223–232.
- [78] C. Cardenas, P. Pinton, G. Bultynck, Editorial: inter-organelle calcium communication in cancer, *Front. Oncol.* 8 (2018) 14.
- [79] G. Dupont, L. Combettes, Fine tuning of cytosolic Ca(2+) oscillations, *F1000Res* 5 (2016).
- [80] M. Yanez, J. Gil-Longo, M. Campos-Toimil, Calcium binding proteins, *Adv. Exp. Med. Biol.* 740 (2012) 461–482.
- [81] C.W. Taylor, D.L. Prole, T. Rahman, Ca(2+) channels on the move, *Biochemistry* 48 (2009) 12062–12080.
- [82] 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. Mammato, G. Del Sal, P. Pinton, p53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 1779–1784.
- [83] C. Giorgi, M. Bonora, S. Missiroli, F. Poletti, F.G. Ramirez, G. Morciano, C. Morganti, P.P. Pandolfi, F. Mammato, P. Pinton, Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling, *Oncotarget* 6 (2015) 1435–1445.
- [84] F. Talos, O. Petrenko, P. Mena, U.M. Moll, Mitochondrially targeted p53 has tumor suppressor activities in vivo, *Cancer Res.* 65 (2005) 9971–9981.
- [85] C. Giorgi, M. Bonora, S. Missiroli, C. Morganti, G. Morciano, M.R. Wieckowski,

- P. Pinton, Alterations in mitochondrial and endoplasmic reticulum signaling by p53 mutants, *Front. Oncol.* 6 (2016) 42.
- [86] K. Pappas, J. Xu, S. Zairis, L. Resnick-Silverman, F. Abate, N. Steinbach, S. Ozturk, L.H. Saal, T. Su, P. Cheung, H. Schmidt, T.A. Aaronson, H. Hibshoosh, J. Manfredi, R. Rabadan, R. Parsons, p53 maintains baseline expression of multiple tumor suppressor genes, *Mol. Cancer Res.* 15 (2017) 1051–1062.
- [87] K.H. Ventii, N.S. Devi, K.L. Friedrich, T.A. Chernova, M. Tighiouart, E.G. Van Meir, K.D. Wilkinson, BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization, *Cancer Res.* 68 (2008) 6953–6962.
- [88] A. Bononi, C. Giorgi, S. Patergnani, D. Larson, K. Verbruggen, M. Tanji, L. Pellegrini, V. Signorato, F. Olivetto, S. Pastorino, M. Nasu, A. Napolitano, G. Gaudino, P. Morris, G. Sakamoto, L.K. Ferris, A. Danese, A. Raimondi, C. Tacchetti, S. Kuchay, H.I. Pass, E.B. Affar, H. Yang, P. Pinton, M. Carbone, BAP1 regulates IP3R3-mediated Ca(2+) flux to mitochondria suppressing cell transformation, *Nature* 546 (2017) 549–553.
- [89] R. Bernardi, P.P. Scaglioni, S. Bergmann, H.F. Horn, K.H. Vousden, P.P. Pandolfi, PML regulates p53 stability by sequestering Mdm2 to the nucleolus, *Nat. Cell Biol.* 6 (2004) 665–672.
- [90] H.K. Lin, S. Bergmann, P.P. Pandolfi, Cytoplasmic PML function in TGF-beta signaling, *Nature* 431 (2004) 205–211.
- [91] C. Giorgi, K. Ito, H.K. Lin, C. Santangelo, M.R. Wieckowski, M. Lebedzinska, 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.
- [92] S. Missirolu, 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.
- [93] L. Salmena, A. Carracedo, P.P. Pandolfi, Tenets of PTEN tumor suppression, *Cell* 133 (2008) 403–414.
- [94] V. Stambolic, A. Suzuki, J.L. de la Pompa, G.M. Brothers, C. Mirtsos, T. Sasaki, J. Ruland, J.M. Penninger, D.P. Siderovski, T.W. Mak, Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN, *Cell* 95 (1998) 29–39.
- [95] A. Bononi, M. Bonora, S. Marchi, S. Missirolu, F. Poletti, C. Giorgi, P.P. Pandolfi, P. Pinton, Identification of PTEN at the ER and MAM and its regulation of Ca(2+) signaling and apoptosis in a protein phosphatase-dependent manner, *Cell Death Differ.* 20 (2013) 1631–1643.
- [96] S. Kuchay, C. Giorgi, D. Simoneschi, J. Pagan, S. Missirolu, A. Saraf, L. Florens, M.P. Washburn, A. Collazo-Lorduy, M. Castillo-Martin, C. Cordon-Cardo, S.M. Sebti, P. Pinton, M. Pagano, PTEN counteracts FBXL2 to promote IP3R3- and Ca(2+) mediated apoptosis limiting tumour growth, *Nature* 546 (2017) 554–558.
- [97] S.C. Hedgepeth, M.I. Garcia, L.E. Wagner 2nd, A.M. Rodriguez, S.V. Chintapalli, R.R. Snyder, G.D. Hankins, B.R. Henderson, K.M. Brodie, D.I. Yule, D.B. van Rossum, D. Boehning, The BRCA1 tumor suppressor binds to inositol 1,4,5-trisphosphate receptors to stimulate apoptotic calcium release, *J. Biol. Chem.* 290 (2015) 7304–7313.
- [98] G. Monaco, E. Decrock, H. Akl, R. Ponsaerts, T. Vervliet, T. Luyten, M. De Maeyer, L. Missiaen, C.W. Distelhorst, H. De Smedt, J.B. Parys, L. Leybaert, G. Bultynck, Selective regulation of IP3-receptor-mediated Ca2+ signaling and apoptosis by the BH4 domain of Bcl-2 versus Bcl-Xl, *Cell Death Differ.* 19 (2012) 295–309.
- [99] Y.P. Rong, A.S. Aromolaran, G. Bultynck, F. Zhong, X. Li, K. McColl, S. Matsuyama, S. Herlitze, H.L. Roderick, M.D. Bootman, G.A. Mignery, J.B. Parys, H. De Smedt, C.W. Distelhorst, Targeting Bcl-2-IP3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals, *Mol. Cell* 31 (2008) 255–265.
- [100] Y.P. Rong, G. Bultynck, A.S. Aromolaran, F. Zhong, J.B. Parys, H. De Smedt, G.A. Mignery, H.L. Roderick, M.D. Bootman, C.W. Distelhorst, The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP3 receptor, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 14397–14402.
- [101] N. Arbel, V. Shoshan-Barmatz, Voltage-dependent anion channel 1-based peptides interact with Bcl-2 to prevent antiapoptotic activity, *J. Biol. Chem.* 285 (2010) 6053–6062.
- [102] S. Abu-Hamad, N. Arbel, D. Calo, L. Arzoine, A. Israelson, N. Keinan, R. Ben-Romano, O. Friedman, V. Shoshan-Barmatz, The VDACL1 N-terminus is essential both for apoptosis and the protective effect of anti-apoptotic proteins, *J. Cell Sci.* 122 (2009) 1906–1916.
- [103] D. De Stefani, A. Bononi, A. Romagnoli, A. Messina, V. De Pinto, P. Pinton, R. Rizzuto, VDACL1 selectively transfers apoptotic Ca2+ signals to mitochondria, *Cell Death Differ.* 19 (2012) 267–273.
- [104] H. Jiang, H. Hu, X. Tong, Q. Jiang, H. Zhu, S. Zhang, Calcium-binding protein S100P and cancer: mechanisms and clinical relevance, *J. Cancer Res. Clin. Oncol.* 138 (2012) 1–9.
- [105] C. Xu, H. Chen, X. Wang, J. Gao, Y. Che, Y. Li, F. Ding, A. Luo, S. Zhang, Z. Liu, S100A14, a member of the EF-hand calcium-binding proteins, is overexpressed in breast cancer and acts as a modulator of HER2 signaling, *J. Biol. Chem.* 289 (2014) 827–837.
- [106] S.K. Mishra, H.R. Siddique, M. Saleem, S100A4 calcium-binding protein is key player in tumor progression and metastasis: preclinical and clinical evidence, *Cancer Metastasis Rev.* 31 (2012) 163–172.
- [107] H.F. Deluca, G.W. Engstrom, Calcium uptake by rat kidney mitochondria, *Proc. Natl. Acad. Sci. U. S. A.* 47 (1961) 1744–1750.
- [108] S. Marchi, P. Pinton, The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications, *J. Physiol.* 592 (2014) 829–839.
- [109] C. Giorgi, S. Marchi, P. Pinton, The machineries, regulation and cellular functions of mitochondrial calcium, *Nat. Rev. Mol. Cell Biol.* 19 (2018) 713–730.
- [110] 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.
- [111] K. Mallilankaraman, C. Cardenas, P.J. Doonan, H.C. Chandramoorthy, K.M. Irrinki, T. Golenar, G. Cogsdas, P. Madireddi, J. Yang, M. Muller, R. Miller, J.E. Kolesar, J. Molgo, B. Kaufman, G. Hajnoczky, J.K. Foskett, M. Madesh, MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism, *Nat. Cell Biol.* 14 (2012) 1336–1343.
- [112] M.R. Alam, L.N. Groschner, W. Parichatnond, L. Kuo, A.I. Bondarenko, R. Rost, M. Waldeck-Weiermair, R. Malli, W.F. Graier, Mitochondrial Ca2+ uptake 1 (MICU1) and mitochondrial Ca2+ uniporter (MCU) contribute to metabolism-secretion coupling in clonal pancreatic beta-cells, *J. Biol. Chem.* 287 (2012) 34445–34454.
- [113] A. Tosatto, R. Sommaggio, C. Kummerow, R.B. Benthman, T.S. Blacker, T. Berecz, M.R. Duchan, 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.
- [114] C. Giorgi, A. Danese, S. Missirolu, S. Patergnani, P. Pinton, Calcium dynamics as a machine for decoding signals, *Trends Cell Biol.* 28 (2018) 258–273.
- [115] H. Xu, N. Guan, Y.L. Ren, Q.J. Wei, Y.H. Tao, G.S. Yang, X.Y. Liu, D.F. Bu, Y. Zhang, S.N. Zhu, IP3R-Grp75-VDACL1-MCU calcium regulation axis antagonists protect podocytes from apoptosis and decrease proteinuria in an Adriamycin nephropathy rat model, *BMC Nephrol.* 19 (2018) 140.
- [116] X. Yang, Y. Li, L. Zheng, X. He, Y. Luo, K. Huang, W. Xu, Glucose-regulated protein 75 in foodborne disease models induces renal tubular necrosis, *Food Chem. Toxicol.* 133 (2019) 110720.
- [117] A. Andreyev, P. Tamrakar, R.E. Rosenthal, G. Fiskum, Calcium uptake and cytochrome c release from normal and ischemic brain mitochondria, *Neurochem. Int.* 117 (2018) 15–22.
- [118] 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.
- [119] P.S. Vosler, D. Sun, S. Wang, Y. Gao, D.B. Kintner, A.P. Signore, G. Cao, J. Chen, Calcium dysregulation induces apoptosis-inducing factor release: cross-talk between PARP-1- and calpain-signaling pathways, *Exp. Neurol.* 218 (2009) 213–220.
- [120] 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.
- [121] M. Gueguinou, D. Crottes, A. Chantome, R. Rapetti-Mauss, M. Potier-Cartreau, L. Clarysse, A. Girault, Y. Fourbon, P. Jezequel, C. Guerin-Charbonnel, G. Fromont, P. Martin, B. Pellissier, R. Schiappa, E. Chamorey, O. Mignen, A. Uguen, F. Borgese, C. Vandier, O. Soriani, The SigmaR1 chaperone drives breast and colorectal cancer cell migration by tuning SK3-dependent Ca(2+) homeostasis, *Oncogene* 36 (2017) 3640–3647.
- [122] B. Honrath, I. Metz, N. Bendridi, J. Rieusset, C. Culmsee, A.M. Dolga, Glucose-regulated protein 75 determines ER-mitochondrial coupling and sensitivity to oxidative stress in neuronal cells, *Cell Death Discov.* 3 (2017) 17076.
- [123] M.R. Wieckowski, C. Giorgi, M. Lebedzinska, J. Duszynski, P. Pinton, Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells, *Nat. Protoc.* 4 (2009) 1582–1590.
- [124] A. Singh, M. Chagtoo, S. Tiwari, N. George, B. Chakravarti, S. Khan, S. Lakshmi, M.M. Godbole, Inhibition of inositol 1, 4, 5-trisphosphate receptor induce breast cancer cell death through deregulated autophagy and cellular bioenergetics, *J. Cell. Biochem.* 118 (2017) 2333–2346.
- [125] G. Bultynck, Onco-IP3Rs feed cancerous cravings for mitochondrial Ca(2+), *Trends Biochem. Sci.* 41 (2016) 390–393.
- [126] 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.* 15 (2016) 219–220.
- [127] M.T. Khan, L. Wagner 2nd, D.I. Yule, C. Bhanumathy, S.K. Joseph, Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors, *J. Biol. Chem.* 281 (2006) 3731–3737.
- [128] S. Marchi, M. Marinello, A. Bononi, M. Bonora, C. Giorgi, A. Rimessi, P. Pinton, Selective modulation of subtype III IP(3)R by Akt regulates ER Ca(2+) release and apoptosis, *Cell Death Dis.* 3 (2012) e304.
- [129] T. Szado, V. Vanderheyden, J.B. Parys, H. De Smedt, K. Rietdorf, L. Kotelevets, E. Chastre, F. Khan, U. Landegren, O. Soderberg, M.D. Bootman, H.L. Roderick, Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca2+ release and apoptosis, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 2427–2432.
- [130] L. Stephens, K. Anderson, D. Stokoe, H. Erdjument-Bromage, G.F. Painter, A.B. Holmes, P.R. Gaffney, C.B. Reese, F. McCormick, P. Tempst, J. Coadwell, P.T. Hawkins, Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B, *Science* 279 (1998) 710–714.
- [131] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, *Science* 307 (2005) 1098–1101.
- [132] M. D'Elletto, F. Rossin, L. Occhigrossi, M.G. Farrace, D. Faccenda, R. Desai, S. Marchi, G. Refolo, L. Falasca, M. Antonielli, F. Ciccocanti, G.M. Fimia, P. Pinton, M. Campanella, M. Piacentini, Transglutaminase type 2 regulates ER-mitochondria contact sites by interacting with GRP75, *Cell Rep.* 25 (2018) 3573–3581 (e3574).
- [133] L.S. Mangala, J.Y. Fok, I.R. Zorrilla-Calancha, A. Verma, K. Mehta, Tissue transglutaminase expression promotes cell attachment, invasion and survival in breast

- cancer cells, *Oncogene* 26 (2007) 2459–2470.
- [134] T. Hayashi, T.P. Su, Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival, *Cell* 131 (2007) 596–610.
- [135] N. Bernard-Marissal, J.J. Medard, H. Azzedine, R. Chraat, Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMARI loss of function mediated motor neuron degeneration, *Brain* 138 (2015) 875–890.
- [136] T. Mitsuda, T. Omi, H. Tanimukai, Y. Sakagami, S. Tagami, M. Okochi, T. Kudo, M. Takeda, Sigma-1Rs are upregulated via PERK/eIF2alpha/ATF4 pathway and execute protective function in ER stress, *Biochem. Biophys. Res. Commun.* 415 (2011) 519–525.
- [137] T. Hayashi, T.P. Su, Intracellular dynamics of sigma-1 receptors (sigma(1) binding sites) in NG108-15 cells, *J. Pharmacol. Exp. Ther.* 306 (2003) 726–733.
- [138] E. Aydar, P. Onganer, R. Perret, M.B. Djamgoz, C.P. Palmer, The expression and functional characterization of sigma (sigma) 1 receptors in breast cancer cell lines, *Cancer Lett.* 242 (2006) 245–257.
- [139] G.A. Hobbs, C.J. Der, K.L. Rossman, RAS isoforms and mutations in cancer at a glance, *J. Cell Sci.* 129 (2016) 1287–1292.
- [140] 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.
- [141] T.G. Bivona, S.E. Quatela, B.O. Bodemann, I.M. Ahearn, M.J. Soskis, A. Mor, J. Miura, H.H. Wiener, L. Wright, S.G. Saba, D. Yim, A. Fein, I. Perez de Castro, 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. Tsubimoto, 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.
- [145] A.C. Schinzel, O. Takeuchi, Z. Huang, J.K. Fisher, Z. Zhou, J. Rubens, C. Hetz, N.N. Daniai, M.A. Moskowitz, S.J. Korsmeyer, Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 12005–12010.
- [146] E. Basso, L. Fante, J. Fowlkes, V. Petronilli, M.A. Forte, P. Bernardi, Properties of the permeability transition pore in mitochondria devoid of cyclophilin D, *J. Biol. Chem.* 280 (2005) 18558–18561.
- [147] M. Bonora, A. Bononi, E. De Marchi, C. Giorgi, M. Lebedzinska, 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.
- [148] G. Campo, G. Morciano, R. Pavasini, M. Bonora, L. Sbrano, S. Biscaglia, M. Bovolenta, M. Pinotti, S. Punzetti, P. Rizzo, G. Aquila, C. Giorgi, R. Ferrari, P. Pinton, Fo ATP synthase C subunit serum levels in patients with ST-segment elevation myocardial infarction: preliminary findings, *Int. J. Cardiol.* 221 (2016) 993–997.
- [149] G. Morciano, D. Preti, G. Pedriali, G. Aquila, S. Missiroli, A. Fantinati, N. Carocchia, S. Pacifico, M. Bonora, A. Talarico, C. Morganti, P. Rizzo, R. Ferrari, M.R. Wieckowski, G. Campo, C. Giorgi, C. Trapella, P. Pinton, Discovery of novel 1,3,8-triazaspiro[4.5]decane derivatives that target the c subunit of F1/FO-adenosine triphosphate (ATP) synthase for the treatment of reperfusion damage in myocardial infarction, *J. Med. Chem.* 61 (2018) 7131–7143.
- [150] M.A. Neginskaya, M.E. Solesio, E.V. Berezhnaya, G.F. Amodeo, N. Mnatsakanyan, E.A. Jonas, E.V. Pavlov, ATP synthase C-subunit-deficient mitochondria have a small cyclosporine A-sensitive channel, but lack the permeability transition pore, *Cell Rep.* 26 (2019) 11–17 (e12).
- [151] E. De Marchi, M. Bonora, C. Giorgi, P. Pinton, The mitochondrial permeability transition pore is a dispensable element for mitochondrial calcium efflux, *Cell Calcium* 56 (2014) 1–13.
- [152] M. Bonora, P. Pinton, The mitochondrial permeability transition pore and cancer: molecular mechanisms involved in cell death, *Front. Oncol.* 4 (2014) 302.
- [153] A.V. Vaseva, N.D. Marchenko, K. Ji, S.E. Tsirka, S. Holzmann, U.M. Moll, p53 opens the mitochondrial permeability transition pore to trigger necrosis, *Cell* 149 (2012) 1536–1548.
- [154] I. Marzo, C. Brenner, N. Zamzami, S.A. Susin, G. Beutner, D. Brdiczka, R. Remy, Z.H. Xie, J.C. Reed, G. Kroemer, The permeability transition pore complex: a target for apoptosis regulation by caspases and bcl-2-related proteins, *J. Exp. Med.* 187 (1998) 1261–1271.
- [155] J. Kang, S. Pervaiz, Crosstalk between Bcl-2 family and Ras family small GTPases: potential cell fate regulation? *Front. Oncol.* 2 (2012) 206.
- [156] C. Brenner, H. Cadiou, H.L. Vieira, N. Zamzami, I. Marzo, Z. Xie, B. Leber, D. Andrews, H. Duclouhier, J.C. Reed, G. Kroemer, Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator, *Oncogene* 19 (2000) 329–336.
- [157] C. Wang, Z. Wang, W. Liu, Z. Ai, ROS-generating oxidase NOX1 promotes the self-renewal activity of CD133+ thyroid cancer cells through activation of the Akt signaling, *Cancer Lett.* 447 (2019) 154–163.
- [158] S.C. Gupta, D. Hevia, S. Patchva, B. Park, W. Koh, B.B. Aggarwal, Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy, *Antioxid. Redox Signal.* 16 (2012) 1295–1322.
- [159] J.E. Biaglow, R.A. Miller, The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy, *Cancer Biol. Ther.* 4 (2005) 6–13.
- [160] K. Dvorakova, C.M. Payne, M.E. Tome, M.M. Briehl, M.A. Vasquez, C.N. Waltmire, A. Coon, R.T. Dorr, Molecular and cellular characterization of imexon-resistant RPMI8226/1 myeloma cells, *Mol. Cancer Ther.* 1 (2002) 185–195.
- [161] A.P. Halestrap, P.M. Kerr, S. Javadov, K.Y. Woodfield, Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart, *Biochim. Biophys. Acta* 1366 (1998) 79–94.
- [162] C.C. Hsu, L.M. Tseng, H.C. Lee, Role of mitochondrial dysfunction in cancer progression, *Exp. Biol. Med. (Maywood)* 241 (2016) 1281–1295.
- [163] O. Warburg, F. Wind, E. Negelein, The metabolism of tumors in the body, *J. Gen. Physiol.* 8 (1927) 519–530.
- [164] S. Douma, T. Van Laar, J. Zevenhoven, R. Meuwissen, E. Van Garderen, D.S. Peeper, Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB, *Nature* 430 (2004) 1034–1039.
- [165] L. He, T. He, S. Farrar, L. Ji, T. Liu, X. Ma, Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species, *Cell. Physiol. Biochem.* 44 (2017) 532–553.
- [166] M. Goodman, R.M. Bostick, O. Kucuk, D.P. Jones, Clinical trials of antioxidant as cancer prevention agents: past, present, and future, *Free Radic. Biol. Med.* 51 (2011) 1068–1084.
- [167] H. Yang, R.M. Villani, H. Wang, M.J. Simpson, M.S. Roberts, M. Tang, X. Liang, The role of cellular reactive oxygen species in cancer chemotherapy, *J. Exp. Clin. Cancer Res.* 37 (2018) 266.
- [168] E.D. Yoboue, A. Rimessi, T. Anelli, P. Pinton, R. Sitia, Regulation of calcium fluxes by GPX8, a type-II transmembrane peroxidase enriched at the mitochondria-associated endoplasmic reticulum membrane, *Antioxid. Redox Signal.* 27 (2017) 583–595.
- [169] S.Y. Gilady, M. Bui, E.M. Lynes, M.D. Benson, R. Watts, J.E. Vance, T. Simmen, Ero1alpha requires oxidizing and nonoxidizing conditions to localize to the mitochondria-associated membrane (MAM), *Cell Stress Chaperones* 15 (2010) 619–629.
- [170] D.M. Booth, B. Enyedi, M. Geiszt, P. Varnai, G. Hajnoczky, Redox nanodomains are induced by and control calcium signaling at the ER-mitochondrial interface, *Mol. Cell* 63 (2016) 240–248.
- [171] Y. Potes, Z. Perez-Martinez, J.C. Bermejo-Millo, A. Rubio-Gonzalez, M. Fernandez-Fernandez, M. Bermudez, J.M. Arche, J.J. Solano, J.A. Boga, M. Olivan, B. Caballero, I. Vega-Naredo, A. Coto-Montes, Overweight in the elderly induces a switch in energy metabolism that undermines muscle integrity, *Aging Dis.* 10 (2019) 217–230.
- [172] D.C. Andersson, M.J. Betzenhauser, S. Reiken, A.C. Meli, A. Umanskaya, W. Xie, T. Shiomi, R. Zalk, A. Lacampagne, A.R. Marks, Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging, *Cell Metab.* 14 (2011) 196–207.
- [173] M. Abdul, S. Ramlal, N. Hoosein, Ryanodine receptor expression correlates with tumor grade in breast cancer, *Pathol. Oncol. Res.* 14 (2008) 157–160.
- [174] L.L. Cooper, W. Li, Y. Lu, J. Centracchio, R. Terentyeva, G. Koren, D. Terentyev, Redox modification of ryanodine receptors by mitochondria-derived reactive oxygen species contributes to aberrant Ca2+ handling in ageing rabbit hearts, *J. Physiol.* 591 (2013) 5895–5911.
- [175] E. Bovo, S.L. Lipsius, A.V. Zima, Reactive oxygen species contribute to the development of arrhythmogenic Ca(2)(+) waves during beta-adrenergic receptor stimulation in rabbit cardiomyocytes, *J. Physiol.* 590 (2012) 3291–3304.
- [176] H.T. Ho, S.C. Stevens, R. Terentyeva, C.A. Carnes, D. Terentyev, S. Gyorke, Arrhythmogenic adverse effects of cardiac glycosides are mediated by redox modification of ryanodine receptors, *J. Physiol.* 589 (2011) 4697–4708.
- [177] B.L. Prosser, C.W. Ward, W.J. Lederer, X-ROS signaling: rapid mechano-chemo transduction in heart, *Science* 333 (2011) 1440–1445.
- [178] Q.A. Sun, D.T. Hess, L. Nogueira, S. Yong, D.E. Bowles, J. Eu, K.R. Laurita, G. Meissner, J.S. Stamler, Oxygen-coupled redox regulation of the skeletal muscle ryanodine receptor-Ca2+ release channel by NADPH oxidase 4, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 16098–16103.
- [179] X.L. Lin, L. Yang, S.W. Fu, W.F. Lin, Y.J. Gao, H.Y. Chen, Z.Z. Ge, Overexpression of NOX4 predicts poor prognosis and promotes tumor progression in human colorectal cancer, *Oncotarget* 8 (2017) 33586–33600.
- [180] N. Hempel, M. Trebak, Crosstalk between calcium and reactive oxygen species signaling in cancer, *Cell Calcium* 63 (2017) 70–96.

- [181] T. Mazumdar, J. DeVecchio, T. Shi, J. Jones, A. Agyeman, J.A. Houghton, Hedgehog signaling drives cellular survival in human colon carcinoma cells, *Cancer Res.* 71 (2011) 1092–1102.
- [182] N. Sampson, E. Brunner, A. Weber, M. Pühr, G. Schafer, C. Szyndralewicz, H. Klocker, Inhibition of Nox4-dependent ROS signaling attenuates prostate fibroblast activation and abrogates stromal-mediated protumorigenic interactions, *Int. J. Cancer* 143 (2018) 383–395.
- [183] S. Bansaghi, T. Golenar, M. Madesh, G. Csordas, S. RamachandraRao, K. Sharma, D.I. Yule, S.K. Joseph, G. Hajnoczky, Isoform- and species-specific control of inositol 1,4,5-trisphosphate (IP3) receptors by reactive oxygen species, *J. Biol. Chem.* 289 (2014) 8170–8181.
- [184] J.T. Lock, W.G. Sinkins, W.P. Schilling, Effect of protein S-glutathionylation on Ca²⁺ homeostasis in cultured aortic endothelial cells, *Am. J. Physiol. Heart Circ. Physiol.* 300 (2011) H493–H506.
- [185] G.S. Bird, G.M. Burgess, J.W. Putney Jr., Sulfhydryl reagents and cAMP-dependent kinase increase the sensitivity of the inositol 1,4,5-trisphosphate receptor in hepatocytes, *J. Biol. Chem.* 268 (1993) 17917–17923.
- [186] E. Zito, ERO1: A protein disulfide oxidase and H₂O₂ producer, *Free Radic. Biol. Med.* 83 (2015) 299–304.
- [187] 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.
- [188] W.G. Zoller, H. Hermans, J.R. Bogner, D. Hahn, M. Middeke, Duplex sonography in the diagnosis of renovascular hypertension, *Klin. Wochenschr.* 68 (1990) 830–834.
- [189] G. Kutomi, Y. Tamura, T. Tanaka, T. Kajiwara, K. Kukita, T. Ohmura, H. Shima, T. Takamaru, F. Satomi, Y. Suzuki, T. Torigoe, N. Sato, K. Hirata, Human endoplasmic reticulum oxidoreductin 1-alpha is a novel predictor for poor prognosis of breast cancer, *Cancer Sci.* 104 (2013) 1091–1096.
- [190] M. Seervi, P.K. Sobhan, J. Joseph, K. Ann Mathew, T.R. Santhoshkumar, ERO1alpha-dependent endoplasmic reticulum-mitochondrial calcium flux contributes to ER stress and mitochondrial permeabilization by procaspase-activating compound-1 (PAC-1), *Cell Death Dis.* 4 (2013) e968.
- [191] K. Mikoshiba, The IP3 receptor/Ca²⁺ channel and its cellular function, *Biochem. Soc. Symp.* (2007) 9–22.
- [192] K. Mikoshiba, IP3 receptor/Ca²⁺ channel: from discovery to new signaling concepts, *J. Neurochem.* 102 (2007) 1426–1446.
- [193] D.Y. Wang, C. Abbasi, S. El-Rass, J.Y. Li, F. Dawood, K. Naito, P. Sharma, N. Bousette, S. Singh, P.H. Backx, B. Cox, X.Y. Wen, P.P. Liu, A.O. Gramolini, Endoplasmic reticulum resident protein 44 (ERp44) deficiency in mice and zebrafish leads to cardiac developmental and functional defects, *J. Am. Heart Assoc.* 3 (2014) e001018.
- [194] X. Huang, M. Jin, Y.X. Chen, J. Wang, K. Zhai, Y. Chang, Q. Yuan, K.T. Yao, G. Ji, ERP44 inhibits human lung cancer cell migration mainly via IP3R2, *Aging (Albany NY)* 8 (2016) 1276–1286.
- [195] R. Wise, S. Duhachek-Muggy, Y. Qi, M. Zolkiewski, A. Zolkiewska, Protein disulfide isomerases in the endoplasmic reticulum promote anchorage-independent growth of breast cancer cells, *Breast Cancer Res. Treat.* 157 (2016) 241–252.
- [196] K. Sato, M. Kimoto, M. Kakumoto, D. Horiuchi, T. Iwasaki, A.A. Tokmakov, Y. Fukami, Adaptor protein Shc undergoes translocation and mediates up-regulation of the tyrosine kinase c-Src in EGF-stimulated A431 cells, *Genes Cells* 5 (2000) 749–764.
- [197] L.V. Lotti, L. Lanfrancone, E. Migliaccio, C. Zompetta, G. Pelicci, A.E. Salcini, B. Falini, P.G. Pelicci, M.R. Tortori, Shc proteins are localized on endoplasmic reticulum membranes and are redistributed after tyrosine kinase receptor activation, *Mol. Cell. Biol.* 16 (1996) 1946–1954.
- [198] M. Giorgio, E. Migliaccio, F. Orsini, D. Paolucci, M. Moroni, C. Contursi, G. Pelliccia, L. Luzi, S. Minucci, M. Maricaccio, P. Pinton, R. Rizzuto, P. Bernardi, F. Paolucci, P.G. Pelicci, Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis, *Cell* 122 (2005) 221–233.
- [199] F. Orsini, E. Migliaccio, M. Moroni, C. Contursi, V.A. Raker, D. Piccini, I. Martin-Padura, G. Pelliccia, M. Trinei, M. Bono, C. Puri, C. Tacchetti, M. Ferrini, R. Mannucci, I. Nicoletti, L. Lanfrancone, M. Giorgio, P.G. Pelicci, The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential, *J. Biol. Chem.* 279 (2004) 25689–25695.
- [200] A. Ventura, M. MacCarana, V.A. Raker, P.G. Pelicci, A cryptic targeting signal induces isoform-specific localization of p46Shc to mitochondria, *J. Biol. Chem.* 279 (2004) 2299–2306.
- [201] M. Lebedzinska, 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.
- [202] E. Migliaccio, S. Mele, A.E. Salcini, G. Pelicci, K.M. Lai, G. Superti-Furga, T. Pawson, P.P. Di Fiore, L. Lanfrancone, P.G. Pelicci, Opposite effects of the p52shc/p46shc and p66shc splicing isoforms on the EGF receptor-MAP kinase-*fos* signalling pathway, *EMBO J.* 16 (1997) 706–716.
- [203] K.S. Ravichandran, Signaling via Shc family adapter proteins, *Oncogene* 20 (2001) 6322–6330.
- [204] 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.
- [205] S. Khalid, A. Drasche, M. Thurner, M.I. Ashraf, F. Fresser, G. Baier, L. Kremser, H. Lindner, J. Troppmair, c-Jun N-terminal kinase (JNK) phosphorylation of serine 36 is critical for p66Shc activation, *Sci. Rep.* 6 (2016) 20930.
- [206] C.P. Yang, S.B. Horwitz, Taxol mediates serine phosphorylation of the 66-kDa Shc isoform, *Cancer Res.* 60 (2000) 5171–5178.
- [207] M. Zhu, J. Ding, H. Jiang, L. Kong, Z. Sun, J. Chen, C. Miao, Propofol ameliorates endothelial inflammation induced by hypoxia/reoxygenation in human umbilical vein endothelial cells: role of phosphatase A2, *Vasc. Pharmacol.* 73 (2015) 149–157.
- [208] R.D. Spescha, J. Klohs, A. Semerano, G. Giacalone, R.S. Derungs, M.F. Reiner, D. Rodriguez Gutierrez, N. Mendez-Carmona, M. Glanzmann, G. Savarese, N. Krinkel, A. Akhmedov, S. Keller, P. Mocharla, M.R. Kaufmann, R.H. Wenger, J. Vogel, L. Kulic, R.M. Nitsch, J.H. Beer, L. Peruzzotti-Jametti, M. Sessa, T.F. Luscher, G.G. Camici, Post-ischaemic silencing of p66Shc reduces ischaemia/reperfusion brain injury and its expression correlates to clinical outcome in stroke, *Eur. Heart J.* 36 (2015) 1590–1600.
- [209] S. Haga, K. Terui, M. Fukai, Y. Oikawa, K. Irani, H. Furukawa, S. Todo, M. Ozaki, Preventing hypoxia/reoxygenation damage to hepatocytes by p66(shc) ablation: up-regulation of anti-oxidant and anti-apoptotic proteins, *J. Hepatol.* 48 (2008) 422–432.
- [210] G. Bianchi, C. Di Giulio, C. Rapino, M. Rapino, A. Antonucci, A. Cataldi, p53 and p66 proteins compete for hypoxia-inducible factor 1 alpha stabilization in young and old rat hearts exposed to intermittent hypoxia, *Gerontology* 52 (2006) 17–23.
- [211] S. Le, T.J. Connors, A.C. Maroney, c-Jun N-terminal kinase specifically phosphorylates p66ShcA at serine 36 in response to ultraviolet irradiation, *J. Biol. Chem.* 276 (2001) 48332–48336.
- [212] Y.S. Cheng, J. Chao, C. Chen, L.L. Lv, Y.C. Han, B.C. Liu, The PKCbeta-p66shc-NADPH oxidase pathway plays a crucial role in diabetic nephropathy, *J. Pharm. Pharmacol.* 71 (2019) 338–347.
- [213] P. Song, S. Yang, L. Xiao, X. Xu, C. Tang, Y. Yang, M. Ma, J. Zhu, F. Liu, L. Sun, PKCdelta promotes high glucose induced renal tubular oxidative damage via regulating activation and translocation of p66Shc, *Oxidative Med. Cell. Longev.* 2014 (2014) 746531.
- [214] C.V. Diogo, J.M. Suski, M. Lebedzinska, A. Karkucinska-Wieckowska, A. Wojtala, M. Pronicki, J. Duszynski, P. Pinton, P. Portincasa, P.J. Oliveira, M.R. Wieckowski, Cardiac mitochondrial dysfunction during hyperglycemia—the role of oxidative stress and p66Shc signaling, *Int. J. Biochem. Cell Biol.* 45 (2013) 114–122.
- [215] E. De Marchi, F. Baldassari, A. Bononi, M.R. Wieckowski, P. Pinton, Oxidative stress in cardiovascular diseases and obesity: role of p66Shc and protein kinase C, *Oxidative Med. Cell. Longev.* 2013 (2013) 564961.
- [216] W. Shan, L. Gao, W. Zeng, Y. Hu, G. Wang, M. Li, J. Zhou, X. Ma, X. Tian, J. Yao, Activation of the SIRT1/p66shc antiapoptosis pathway via carnosis acid-induced inhibition of miR-34a protects rats against nonalcoholic fatty liver disease, *Cell Death Dis.* 6 (2015) e1833.
- [217] P. Yang, Z. Zhao, E.A. Reece, Activation of oxidative stress signaling that is implicated in apoptosis with a mouse model of diabetic embryopathy, *Am. J. Obstet. Gynecol.* 198 (130) (2008) e131–e137.
- [218] E. Migliaccio, M. Giorgio, S. Mele, G. Pelicci, P. Reboldi, P.P. Pandolfi, L. Lanfrancone, P.G. Pelicci, The p66shc adaptor protein controls oxidative stress response and life span in mammals, *Nature* 402 (1999) 309–313.
- [219] S. Nemoto, T. Finkel, Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway, *Science* 295 (2002) 2450–2452.
- [220] V. Gambino, G. De Michele, O. Venezia, P. Migliaccio, V. Dall'Olio, L. Bernard, S.P. Minardi, M.A. Della Fazio, D. Bartoli, G. Servillo, M. Alcalay, L. Luzi, M. Giorgio, H. Scrabble, P.G. Pelicci, E. Migliaccio, Oxidative stress activates a specific p53 transcriptional response that regulates cellular senescence and aging, *Aging Cell* 12 (2013) 435–445.
- [221] P. Huang, X. Feng, Z. Zhao, B. Yang, T. Fang, M. Guo, J. Xia, p66Shc promotes HCC progression in the tumor microenvironment via STAT3 signaling, *Exp. Cell Res.* 383 (2019) 111550.
- [222] S. Veeramani, T.C. Yuan, F.F. Lin, M.F. Lin, Mitochondrial redox signaling by p66Shc is involved in regulating androgenic growth stimulation of human prostate cancer cells, *Oncogene* 27 (2008) 5057–5068.
- [223] D.R. Miller, M.A. Ingersoll, A. Chatterjee, B. Baker, S. Shrishrimal, E.A. Kosmacek, Y. Zhu, P.W. Cheng, R.E. Oberley-Deegan, M.F. Lin, p66Shc protein through a redox mechanism enhances the progression of prostate cancer cells towards castration-resistance, *Free Radic. Biol. Med.* 139 (2019) 24–34.
- [224] F.A. Khanday, T. Yamamori, I. Mattagajasingh, Z. Zhang, A. Bugayenko, A. Naqvi, L. Santhanam, N. Nabi, K. Kasuno, B.W. Day, K. Irani, Rac1 leads to phosphorylation-dependent increase in stability of the p66shc adaptor protein: role in Rac1-induced oxidative stress, *Mol. Cell* 17 (2006) 122–129.
- [225] M.A. Ingersoll, Y.W. Chou, J.S. Lin, T.C. Yuan, D.R. Miller, Y. Xie, Y. Tu, R.E. Oberley-Deegan, S.K. Batra, M.F. Lin, p66Shc regulates migration of castration-resistant prostate cancer cells, *Cell. Signal.* 46 (2018) 1–14.
- [226] P.A. Davol, R. Bagdasaryan, G.J. Elfenbein, A.L. Maizel, A.R. Frackelton Jr., Shc proteins are strong, independent prognostic markers for both node-negative and node-positive primary breast cancer, *Cancer Res.* 63 (2003) 6772–6783.
- [227] J.G. Jackson, T. Yoneda, G.M. Clark, D. Yee, Elevated levels of p66 Shc are found in breast cancer cell lines and primary tumors with high metastatic potential, *Clin. Cancer Res.* 6 (2000) 1135–1139.
- [228] E. Haines, C. Saucier, A. Claign, The adaptor proteins p66Shc and Grb2 regulate the activation of the GTPases ARF1 and ARF6 in invasive breast cancer cells, *J. Biol. Chem.* 289 (2014) 5687–5703.
- [229] Z. Zheng, J. Yang, D. Zhao, D. Gao, X. Yan, Z. Yao, Z. Liu, Z. Ma, Downregulated adaptor protein p66(Shc) mitigates autophagy process by low nutrient and enhances apoptotic resistance in human lung adenocarcinoma A549 cells, *FEBS J.* 280 (2013) 4522–4530.

- [230] L.V. Kharin, V.A. Dorozhkin, R.P. Kharina, Allergic reaction to catgut, *Vestn. Khir. Im. I. I. Grek.* 109 (1972) 113–114.
- [231] G. Morciano, G. Pedriali, L. Sbrano, T. Iannitti, C. Giorgi, P. Pinton, Intersection of mitochondrial fission and fusion machinery with apoptotic pathways: role of Mcl-1, *Biol. Cell.* 108 (2016) 279–293.
- [232] J.V. Phillely, A. Kannan, W. Qin, E.R. Sauter, M. Ikebe, K.L. Hertweck, D.A. Troyer, O.J. Semmes, S. Dasgupta, Complex-I alteration and enhanced mitochondrial fusion are associated with prostate cancer progression, *J. Cell. Physiol.* 231 (2016) 1364–1374.
- [233] O.M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria, *Nature* 456 (2008) 605–610.
- [234] D. Naon, M. Zaninello, M. Giacomello, T. Varanita, F. Grespi, S. Lakshminarayanan, A. Serafini, M. Semenzato, S. Herkenne, M.I. Hernandez-Alvarez, A. Zorzano, D. De Stefani, G.W. Dorn 2nd, L. Scorrano, Critical reappraisal confirms that Mitofusin 2 is an endoplasmic reticulum-mitochondria tether, *Proc. Natl. Acad. Sci. U. S. A.* 113 (2016) 11249–11254.
- [235] R. Filadi, E. Greotti, G. Turacchio, A. Luini, T. Pozzan, P. Pizzo, Mitofusin 2 ablation increases endoplasmic reticulum-mitochondria coupling, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) E2174–E2181.
- [236] G. Pang, Q. Xie, J. Yao, Mitofusin 2 inhibits bladder cancer cell proliferation and invasion via the Wnt/beta-catenin pathway, *Oncol. Lett.* 18 (2019) 2434–2442.
- [237] J.E. Vance, S.J. Stone, J.R. Faust, Abnormalities in mitochondria-associated membranes and phospholipid biosynthetic enzymes in the mnd/mnd mouse model of neuronal ceroid lipofuscinosis, *Biochim. Biophys. Acta* 1344 (1997) 286–299.
- [238] J.H. Yen, H.S. Huang, C.J. Chuang, S.T. Huang, Activation of dynamin-related protein 1 - dependent mitochondria fragmentation and suppression of osteosarcoma by cryptotanshinone, *J. Exp. Clin. Cancer Res.* 38 (2019) 42.
- [239] J. Prudent, R. Zunino, A. Sugiura, S. Mattie, G.C. Shore, H.M. McBride, MAPL SUMOylation of Drp1 stabilizes an ER/mitochondrial platform required for cell death, *Mol. Cell* 59 (2015) 941–955.
- [240] M. Milani, A.J. Beckett, A. Al-Zeebeey, X. Luo, I.A. Prior, G.M. Cohen, S. Varadarajan, DRP-1 functions independently of mitochondrial structural perturbations to facilitate BH3 mimetic-mediated apoptosis, *Cell Death Discov.* 5 (2019) 117.
- [241] J.T. Ma, X.Y. Zhang, R. Cao, L. Sun, W. Jing, J.Z. Zhao, S.L. Zhang, L.T. Huang, C.B. Han, Effects of dynamin-related protein 1 regulated mitochondrial dynamic changes on invasion and metastasis of lung cancer cells, *J. Cancer* 10 (2019) 4045–4053.
- [242] S. Nagdas, J.A. Kashatus, A. Nascimento, S.S. Hussain, R.E. Trainor, S.R. Pollock, S.J. Adair, A.D. Michaels, H. Sesaki, E.B. Stelow, T.W. Bauer, D.F. Kashatus, Drp1 promotes KRas-driven metabolic changes to drive pancreatic tumor growth, *Cell Rep.* 28 (2019) 1845–1859 (e1845).
- [243] 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.
- [244] L. Song, D. Coppola, S. Livingston, D. Cress, E.B. Haura, Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non-small cell lung cancer cells, *Cancer Biol. Ther.* 4 (2005) 267–276.
- [245] H. Zhang, S. Guttikonda, L. Roberts, T. Uziel, D. Semizarov, S.W. Elmore, J.D. Levenson, L.T. Lam, Mcl-1 is critical for survival in a subgroup of non-small-cell lung cancer cell lines, *Oncogene* 30 (2011) 1963–1968.
- [246] H. Huang, K. Shah, N.A. Bradbury, C. Li, C. White, Mcl-1 promotes lung cancer cell migration by directly interacting with VDAC to increase mitochondrial Ca²⁺ uptake and reactive oxygen species generation, *Cell Death Dis.* 5 (2014) e1482.
- [247] R. Iwasawa, A.L. Mahul-Mellier, C. Datler, E. Pazarentzos, S. Grimm, Fis1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction, *EMBO J.* 30 (2011) 556–568.
- [248] Y. Wakana, S. Takai, K. Nakajima, K. Tani, A. Yamamoto, P. Watson, D.J. Stephens, H.P. Hauri, M. Tagaya, Bap31 is an itinerant protein that moves between the peripheral endoplasmic reticulum (ER) and a juxtannuclear compartment related to ER-associated degradation, *Mol. Biol. Cell* 19 (2008) 1825–1836.
- [249] M. Nguyen, D.G. Breckenridge, A. Ducret, G.C. Shore, Caspase-resistant BAP31 inhibits fas-mediated apoptotic membrane fragmentation and release of cytochrome c from mitochondria, *Mol. Cell. Biol.* 20 (2000) 6731–6740.
- [250] D. Chandra, G. Choy, X. Deng, B. Bhatia, P. Daniel, D.G. Tang, Association of active caspase 8 with the mitochondrial membrane during apoptosis: potential roles in cleaving BAP31 and caspase 3 and mediating mitochondrion-endoplasmic reticulum cross talk in etoposide-induced cell death, *Mol. Cell. Biol.* 24 (2004) 6592–6607.
- [251] D.G. Breckenridge, M. Stojanovic, R.C. Marcellus, G.C. Shore, Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome c release to the cytosol, *J. Cell Biol.* 160 (2003) 1115–1127.
- [252] T. Simmen, J.E. Aslan, A.D. Blagoveshchenskaya, L. Thomas, L. Wan, Y. Xiang, S.F. Feliciangeli, C.H. Hung, C.M. Crump, G. Thomas, PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis, *EMBO J.* 24 (2005) 717–729.
- [253] Y. Arieli, H. Gursahani, M.M. Eaton, L.A. Hernandez, S. Schaefer, Gender modulation of Ca²⁺ uptake in cardiac mitochondria, *J. Mol. Cell. Cardiol.* 37 (2004) 507–513.
- [254] S. Li, A.A. Gupte, The role of estrogen in cardiac metabolism and diastolic function, *Methodist Debakey Cardiovasc. J.* 13 (2017) 4–8.