Role of Mitochondria-Associated ER Membranes in Calcium Regulation in Cancer-Specific Settings[1]

Giampaolo Morciano[*][†], Saverio Marchi[*], Claudia Morganti[*], Luigi Sbano[*], Mart Bittremieux[‡], Martijn Kerkhofs[‡], Mariangela Corricelli[*], Alberto Danese[*], Agnieszka Karkucinska-Wieckowska[§], Mariusz R. Wieckowski[¶], Geert Bultynck[‡], Carlotta Giorgi[*] and Paolo Pinton[*][†][#]

[*]Department of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology and LTTA center, University of Ferrara, Ferrara, Italy; [†]Cecilia Hospital, GVM Care & Research, E.S.: Health Science Foundation, Cotignola, Italy; [‡]KU Leuven, Lab. Molecular and Cellular Signaling, Dept. Cellular and Molecular Medicine and Leuven Kanker Instituut, Campus Gasthuisberg O&N 1 Box 802, Herestraat 49, 3000 Leuven, Belgium; [§]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland; [¶]Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland; [#]CNR Institute of Cell Biology and Neurobiology, Monterotondo, Italy

Abstract

Mitochondria-associated endoplasmic reticulum (ER) membranes (MAMs) are highly specialized subcellular compartments that are shaped by ER subdomains juxtaposed to mitochondria but are biochemically distinct from pure ER and pure mitochondria. MAMs are enriched in enzymes involved in lipid synthesis and transport, channels for calcium transfer, and proteins with oncogenic/oncosuppressive functions that modulate cell signaling pathways involved in physiological and pathophysiological processes. The term "cancer" denotes a group of disorders that result from uncontrolled cell growth driven by a mixture of genetic and environmental components. Alterations in MAMs are thought to account for the onset as well as the progression and metastasis of cancer and have been a focus of investigation in recent years. In this review, we present the current state of the art regarding MAM-resident proteins and their relevance, alterations, and deregulating functions in different types of cancer from a cell biology and clinical perspective.

Introduction

In the early 1990s, although scientists had experimental proof of the existence of mitochondria-associated membranes (MAMs), they were not aware of the multiple functions of this specialized subcellular compartment in cell physiology and human disease. The unique MAM microdomain between the endoplasmic reticulum (ER) and the mitochondria was initially identified as fraction X [1] after the separation of a crude rat liver mitochondrial preparation. This fraction harbored the specific phospholipid biosynthetic enzyme activity that was present in the crude mitochondrial fraction but absent from the pure mitochondrial fraction. At that time, fraction X was thought to account for the mechanism of action of phospholipid

trafficking between organelles [1,2]. This fraction corresponded to a well-defined region of continuity between donor and acceptor membranes, specifically the mitochondrial and reticular membranes.

Astonishingly, although the MAM microdomain was observed via electron microscopy in the years 1952-1959 as packed zones of ER membranes and mitochondria [3–5], further insights about the microdomain were not revealed for the next 30 years.

Today, we know that ER-mitochondria contact sites are 10- to 25-nm-wide regions [6] (this distance is expected to increase in the rough reticulum) of juxtaposed membranes tethered by proteins, without complete fusion or loss of organelle identity (Figure 1).

These sites have been fully described from many functional points of view, and their roles include i) the regulation of lipid synthesis and transport, serving as the sites where enzymes in lipid synthesis and transport pathways are located [7], both at the ER and mitochondrial membranes (e.g., phosphatidylserine synthase 1-2 [8]), and ii) calcium (Ca^{2+}) transport and signaling [9]. Ca^{2+} is known to be released from the ER through 1,4,5-trisphosphate (IP3) and ryanodine receptors (IP3Rs, RyRs) as a consequence of the functional interaction of agonists on the plasma membrane receptors and the intracellular second messenger IP3; then, Ca^{2+} is taken up into mitochondria in a "quasi-synaptic" manner [10–12] through voltage-dependent anion channels (VDACs) in the outer mitochondrial membrane (OMM) at ER-mitochondria contact sites [13].

Furthermore, mitochondrial Ca^{2+} uptake is facilitated by the highly negative mitochondrial membrane potential and finely tuned by the proteins in the mitochondrial Ca^{2+} uniporter (MCU) complex [14]. The accumulation of Ca^{2+} in the mitochondrial matrix has important implications for several processes, including autophagy, metabolism, and apoptosis [15,16]. In many cell types, a ubiquitous Ca^{2+} signaling mechanism represented by the dynamic variation in free cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_c$) is utilized to sustain multicellular responses, and it is commonly termed "Ca^{2+} oscillations". These intracellular transient and local $[Ca^{2+}]_c$ elevations are generated by Ca^{2+} release channels located either in the ER (like IP3Rs, RyRs, Polycystin-2 [17], and two-pore channels [18]) or in the plasma membrane (Orai channels [19]) and can be propagated inside and through cells [20] by a complex network of Ca^{2+} releasing effectors (like IP3, cADPR, and NAADP) that, individually or in combination, orchestrate the conversion of local $[Ca^{2+}]_c$ signals to global Ca^{2+} oscillations to achieve a well-defined spatiotemporal signaling pattern [21]. Whereas Ca^{2+} oscillations are critical to fuel mitochondrial metabolism, a persistent increase in mitochondrial Ca^{2+} triggers cell death, e.g., through opening of the mitochondrial permeability transition pore (mPTP) [15,16]. Another relevant finding involves the GPX8 protein, a glutathione peroxidase enriched in MAMs, where it selectively regulates Ca^{2+} storage and flux via its transmembrane domain [22]. MAMs also play roles in iii) mitochondrial bioenergetics and iv) mitochondrial morphology and motility [23], in which the close proximity of the organelles regulates the machinery responsible for mitochondrial dynamics. It has been reported that Miro-1, which is anchored to the OMM by its transmembrane domain and protrudes into the cytosol where it
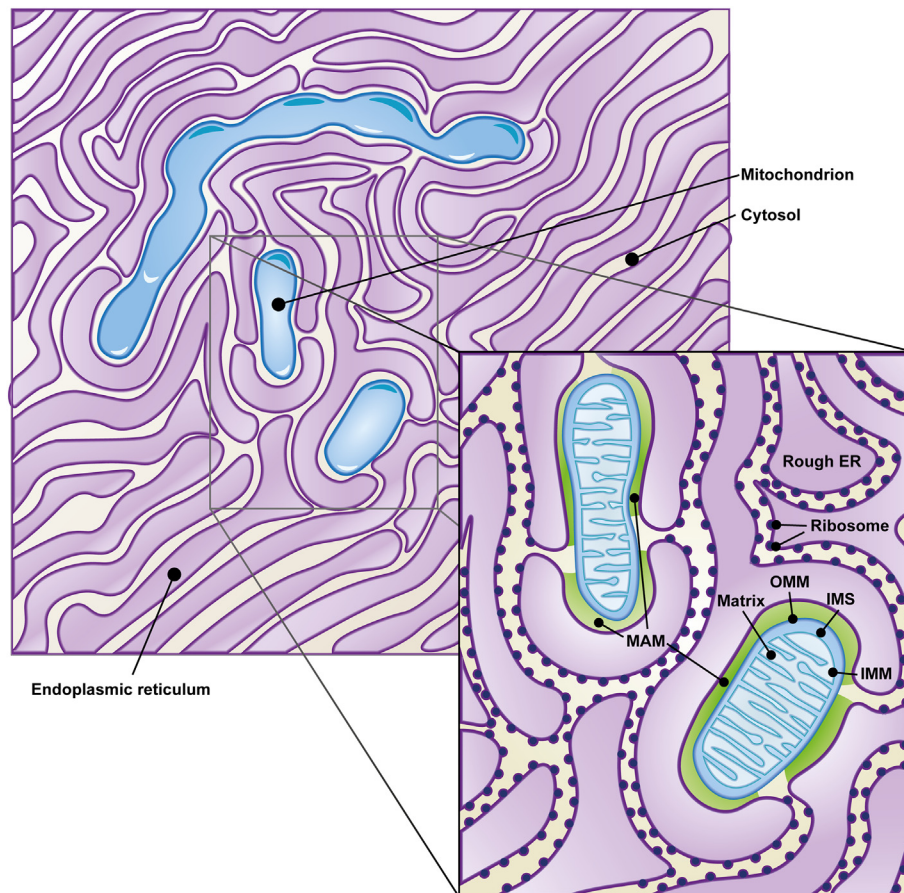

Figure 1. Mitochondria-associated ER membranes. Membranes juxtaposition of both ER and mitochondria organelles in the cytosol gives origin to the highly specialized MAM compartment (green zone in the zoom of the figure), here represented as a cartoon on the basis of a transmission electron microscopy acquisition.

interacts with milton and kinesin proteins [24], organizes mitochondrial movement along microtubules, possibly in a calcium-dependent manner [25]; additionally, Fun14 domain-containing 1 (FUNDC1) together with dynamin-related protein 1 (DRP1) regulates fission and mitophagy under hypoxic conditions (21). MAMs are also reported to be involved in v) inflammation signaling [26] and vi) ER stress [27].

The interaction between the ER and the mitochondria in cancer, which is the focus of this review, has been described in many studies discussing the function of oncogenes and oncosuppressors in the modulation of Ca^{2+} and reactive oxygen species (ROS) transfer at MAMs [28–30]. In particular, the most recent report by Sassano et al. outlines the role of MAMs in cancer growth [31]. Thus, MAMs play a pivotal role in cellular adaptation and cell death pathways, impacting cancer cell function [32].

In this manuscript, we summarize past and recent findings regarding MAM-resident proteins and intracellular calcium modulation, categorized by their investigation in specific cancer types (Table 1). Although we do not rule out the possible engagement of the discussed proteins in other tumor environments, assuming that some mechanisms might apply to multiple cancer types, we collected information on the highest incidence and best-studied cancers, such as breast, lung, and prostate cancer.

Alterations at the ER-Mitochondria Interface in Breast Cancer

Breast cancers (as well as lung cancers) represent one of the most common types of cancer worldwide [33]. As stated in the Introduction, ER-mitochondria contact sites play a crucial role in the onset of cancer, participating in mechanisms involved in rewiring normal cell signaling toward malignancy. In this context, aberrant expression or localization of MAM-resident proteins is widely reported. For instance, in breast cancers, the expression of the stress-activated chaperone sigma-1 receptor (Sig1R), which primarily acts at the ER-mitochondria interface, is higher in metastatic potential cancer cells than in normal tissues [34,35]. The regulatory role of

Sig1R in MAMs in cell survival was defined in a seminal paper by Hayashi and Su [36]. Under basal conditions, Sig1R binds the MAM chaperone BiP/GRP78; however, upon activation of IP3Rs, Sig1R dissociates from BiP and binds IP3R3, thereby stabilizing IP3R3 at the MAMs and enhancing IP3R3-mediated Ca^{2+} fluxes to the mitochondria [36] (Figure 2). Importantly, it has been demonstrated [37] that during conditions of chronic ER stress involving prolonged ER Ca^{2+} depletion, Sig1R translocates from MAMs to the peripheral ER and attenuates cellular damage, thereby preventing cell death [36]. Another mechanism through which Sig1R expression is a critical determinant of cell invasiveness in breast cancer was recently revealed: Sig1R regulates Ca^{2+} homeostasis by forming a functional molecular platform with the calcium-activated K^+ channel SK3 and Orai1, thus driving Ca^{2+} influx and favoring the migration of cancer cells [35]. These findings support the protumorigenic functions of Sig1R, which are related to the regulation of Ca^{2+} dynamics at the ER-mitochondria zone.

According to the most recent findings, Ca^{2+} signaling appears to be an event that is remodulated several times during the malignant transformation pathway of a cell. Indeed, if an initial reduction of mitochondrial Ca^{2+} uptake allows escape from apoptosis, Ca^{2+} fluxes towards the mitochondria via MCU are decisive for tumor growth and metastatic behavior [35,38]. For instance, Tosatto et al. showed that in a set of triple-negative breast cancer cell lines, depletion of MCU impaired cell migration and invasion and hampered tumor progression in MDA-MB-231 xenografts, regulating metastasis through hypoxia-inducible factor 1 (HIF1)–controlled gene reprogramming [38]. Although MCU is not localized at the ER-mitochondria interface, its activity and that of its modulators at the inner mitochondrial membrane finely regulate the cooperativity of Ca^{2+} accumulation inside the matrix (please refer to [39–41] for further details) and could be tuned in a cancer-specific manner [42].

Compared with IP3R isoforms 1 and 2, which are located at the ER membranes, IP3R3 is highly enriched at the MAMs (and is considered a MAM marker [43]), where it conveys Ca^{2+}-mediated

Table 1. Summary of Proteins Discussed in the Review and Their Regulatory Activities at MAMs

Protein	Interactor/Localization	Regulatory Mechanisms	Type of Tumor in Which It Has Been Fully Described
Bap1	IP3R/ER	Ca^{2+} mobilization, apoptosis	Mesothelioma
Bax, Bak	IP3R/ER	ER Ca^{2+} leakage, cell death sensitivity	Hematopoietic, skin, breast, prostate, pancreas
	m	Pore formation in the OMM, mitochondrial dysfunction	
Bcl-2	IP3R/MAMs	ER Ca^{2+} release, cell death resistance	Hematopoietic, lung, breast, prostate
	VDAC1/MAMs	Ca^{2+} passage across OMM, cell death resistance	
Bcl-xL	IP3R/MAMs	ER Ca^{2+} release, energy production, and metabolism	Hematopoietic, prostate, colon
	VDAC1/MAMs	ER-m Ca^{2+} transfer, apoptosis	Hematopoietic
Ero1α	MAMs	Redox homeostasis, ER Ca^{2+} fluxes, immunosuppression	Breast
FATE1	MAMs	ER-m tethering, cancer progression	Colorectal
GRP78	ATAD3/m	WASF3 protein stabilization, cell invasion, and metastasis	Breast, prostate
HK-2	VDAC1/MAMs	Glycolysis	Lung
Mcl-1	VDAC1/MAMs	Mitochondrial Ca^{2+} uptake, cancer cell migration, ROS generation	Lung
	DRP1/MAMs	Mitochondrial dynamics, apoptosis	Cervical
MFN1	m	Mitochondrial dynamics	Prostate
MFN2	MAMs	ER-m tethering	Prostate
NLRP3	MAMs	Inflammation signaling	Breast, prostate, skin, lung
p53	SERCA/MAMs	Regulation of ER Ca^{2+} levels	Almost all
	OSCP/m	Oxidative phosphorylation modulation	
PERK	MAMs	Redox homeostasis, ER-m tethering, tumor initiation	Breast
PML	IP3R/MAMs	ER Ca^{2+} release, cell death	Almost all
PTEN	IP3R/MAMs	Maintenance of IP3R levels, ER-m Ca^{2+} transfers	Lung, prostate, head, stomach, breast, pancreas
K-Ras	MAMs	ER-m Ca^{2+} transfer, cell proliferation, and survival	Several
Sig1R	IP3R/MAMs	ER Ca^{2+} release, cell death	Breast
mTORC2/Akt	IP3R/MAMs	IP3R phosphorylation, ER Ca^{2+} release, apoptosis	Breast, pancreas, prostate

ER, endoplasmic reticulum; *MAMs*, mitochondria associated membranes; *m*, mitochondria.
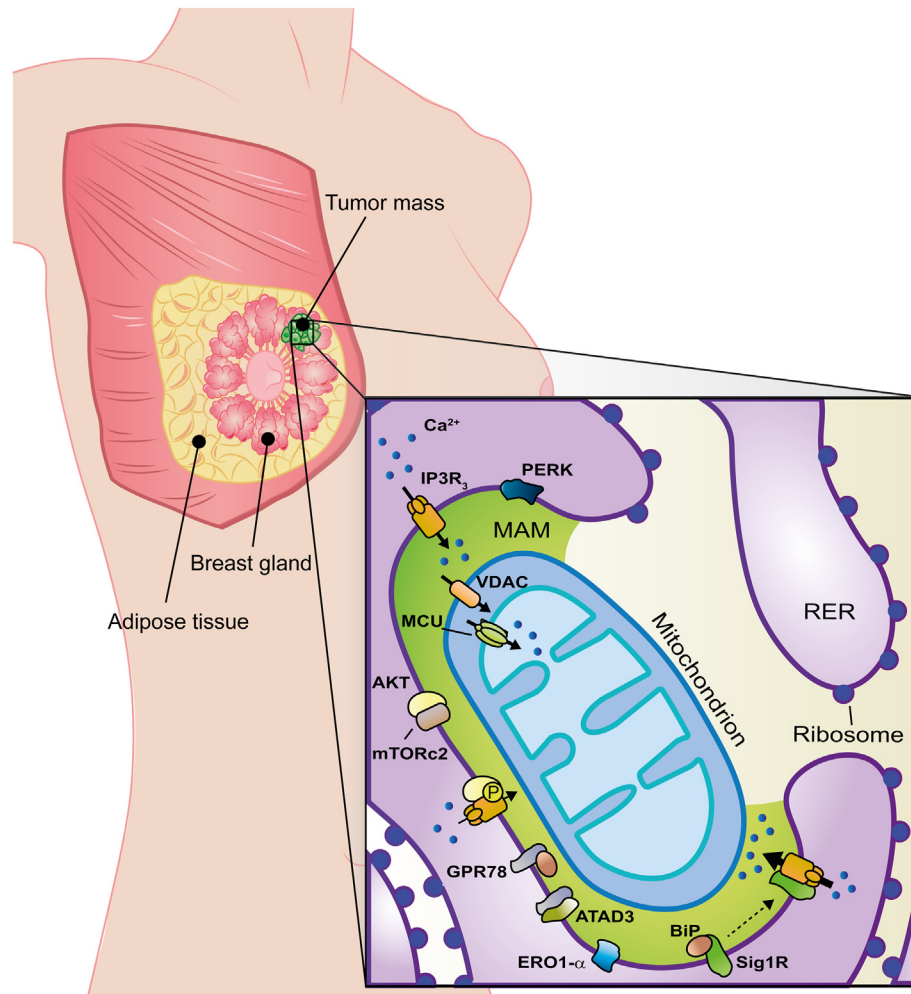
Figure 2. MAM alterations in breast cancer. MAM-resident proteins (green zone) strictly involved in breast cancer onset, progression, and metastasis are shown in the figure. Black arrows highlight calcium homeostasis where their thickness is proportional to the entity of calcium fluxes. See text for further details. Ca^{2+}, calcium; *RER*, rough endoplasmic reticulum.

proapoptotic signals to the mitochondria [44] (Figure 2). This channel is also responsible for the regulation of cellular bioenergetics and metabolism in breast cancer, as its inhibition induces autophagic death [45] and/or mitotic catastrophe in tumorigenic cells, but not in nontumorigenic cells [46,47]. In addition, depletion or pharmacological blocking of this channel increases the level of LC3-II, an autophagy marker, via autophagy protein 5 (Atg5) upregulation and ROS generation, which lead to arrested tumor growth in a related mouse model [45]. These findings correlate with high expression of IP3R3 in human malignant tissues and high concentrations of metabolites in serum samples from patients [48]. In an independent study, inhibition of all IP3Rs using xestospongin B resulted in cell death in cancer cells, without involvement of autophagy. In this case, IP3R inhibition caused a bioenergetics crisis due to halted ER-mitochondrial Ca^{2+} flux [47]. While nontumorigenic cells halt their cell cycle, tumorigenic cells display uncontrolled cell cycle progression, independent of the presence of mitochondrial substrates for anabolic pathways, leading to mitotic catastrophe [32,46,47]. The function of IP3R3 is impacted by a wide range of oncogenes and tumor suppressors that target the receptor [49–51], including the oncogene Akt kinase [52,53]. The PI3K/Akt/mTOR pathway is frequently altered in human breast cancers [54,55]. In 2012, our

group showed that Akt preferentially phosphorylates IP3R3, which reduces ER-mitochondrial Ca^{2+} transfer and inhibits apoptotic responses [56] (Figure 2). These results were based on previous findings indicating the capacity of Akt to phosphorylate IP3Rs at their C-termini [52,53], thereby decreasing Ca^{2+} release and sensitivity to apoptosis [52,57]. Akt is activated at the ER-mitochondria interface, where the mechanistic TOR complex 2 (mTORc2) is located [58], which in turn phosphorylates/activates Akt at position S473 [59]. mTORc2-Akt signaling is fundamental for maintaining proper MAM functionality, and mTORc2 deficiency induces loss of MAM architecture and a wide range of mitochondrial defects [58]. Importantly, in invasive breast cancer specimens, expression of the mTORc2 core component Rictor appears to be significantly upregulated compared with nonmalignant tissues [60]. This change contributes to Akt-dependent tumor progression in HER2-amplified breast cancers [60].

　Due to the role of the MAM region in decoding a wide range of physiological and danger signals, it seems logical that this region would host a large number of molecular chaperones to regulate various intracellular functions. Among these chaperones, the previously cited GRP78 plays a key role in cancer. In both breast and colon tumor cells, GRP78 cooperates with ATAD3a, a

mitochondrial protein with unknown function, to stabilize WASF3, a protein that facilitates actin polymerization, thereby promoting invasion and metastasis [61]. Interestingly, ATAD3 may play a role in ER-mitochondria contact site formation and cholesterol substrate delivery to the mitochondria [62]. Among all organelles inside cells, mitochondria have a singular lipid composition; the presence of phosphatidylglycerol, cardiolipin, and phosphatidylethanolamine confers unique features to mitochondrial membranes. Mitochondria require that a large amount of lipids be imported, and this is allowed by the MAM fractions; accordingly, dysregulation of this pathway or the lipid composition of MAMs has important consequences [63,64]. Since lipid composition and related enzyme activity are essential for the regulation of Ca^{2+} homeostasis, they affect ER-mitochondria contact sites and modify mitochondrial functions [65] and may be essential in regulating apoptotic signaling in tumors. Notably, several papers have reported both enhanced lipogenesis in cancer cells and lipolysis from exogenous fatty acids to allow mass growth [66,67] and, in prostate tumors tissues, alterations in the expression of genes encoding for enzymes designated to produce cholesterol and lipids [68]. Thus, these considerations outline an important picture in which lipid enzyme activities and transport at MAMs are subjected to cancer-specific variations, but further studies are necessary to unveil the exact link among all these actors.

As noted in the Introduction section, MAMs are a molecular platform for the regulation of many oxidoreductase events. In this context, endoplasmic reticulum oxidoreductin 1-α (ERO1-α) is extensively studied because of its enrichment at ER-mitochondria contact sites [69] and its high expression in various types of tumors [70]. Notably, the expression of ERO1-α in breast cancer is associated with a poor prognosis [71]. ERO1-α controls oxidative folding and ER redox homeostasis and regulates ER Ca^{2+} fluxes and consequent mitochondrial Ca^{2+} accumulation [69]. These ERO1-α–mediated functions are key events in the cell death mechanism induced by the procaspase-activating compound-1 (PAC-1), which is able to promote apoptosis in a variety of cancer cell types [72]. Moreover, in triple-negative breast cancer cells, the expression of ERO1-α is positively correlated with that of programmed cell death-ligand 1 (PD-L1), while knockout of ERO1-α results in a significant attenuation of PD-L1–mediated T-cell apoptosis, suggesting a putative role for ERO1-α in tumor-mediated immunosuppression [73].

RNA-dependent protein kinase (PKR)–like ER kinase (PERK) is a critical ER stress sensor of the unfolded protein response at MAMs [74]. PERK has been identified as a key MAM component for maintaining the ER-mitochondria juxtaposition and ROS-mediated mitochondrial apoptosis [75]. Thus, loss of PERK is expected to cause defects in cell death processes. PERK-dependent signaling is involved in tumor initiation and expansion to preserve redox homeostasis and to promote tumor growth in the MDA-MB-468 and T47D cell lines [76]. Silencing of PERK was shown to reduce tumor growth and restore sensitivity to chemotherapy in resistant tumor xenografts [77]. Moreover, PERK can regulate the translation of angiogenic factors in the development of functional microvessels in tumor cells; thus, it plays a fundamental role in adapting to hypoxic stress and tumor progression [78].

Alterations at the ER-Mitochondria Interface in Hematopoietic Cancers

B-cell lymphoma 2 (Bcl-2) family proteins were originally discovered in the context of hematopoietic and lymphoid systems [79], where antiapoptotic Bcl-2 is upregulated via mechanisms that involve gene translocation and miRNA deregulation [80,81]. It is reported that upregulation of Bcl-2 enables cancer cells to survive despite high expression of proapoptotic Bcl-2-family members, whose levels are elevated by ongoing oncogenic stress [82,83]. For example, in childhood acute lymphoblastic leukemia, apoptosis is avoided in this way in leukemic cells [84].

Because these proteins are principally localized at the mitochondria, ER, and MAMs, their action strongly reflects their intracellular localization. Indeed, antiapoptotic Bcl-2 proteins can suppress ER-mitochondrial Ca^{2+} transfer via different mechanisms [85,86]. Bcl-2 directly targets all three IP3R isoforms, suppressing their Ca^{2+}-flux properties [87–89] and thereby suppressing Ca^{2+} accumulation in the mitochondria. An IP3R-derived peptide corresponding to the Bcl-2-binding site on IP3R1 was able to overcome the ability of Bcl-2 to suppress IP3R-channel function [90]. A cell-permeable variant of this peptide that potentially interferes with the Bcl-2-IP3R interaction at the MAM interface has been proposed to stimulate IP3R-dependent Ca^{2+} elevation and cell death in chronic lymphocytic leukemia and diffuse large B-cell lymphoma models [91]. In contrast, Bcl-2 can sensitize IP3R1 channels to basal IP3, accounting for a decrease in ER Ca^{2+} loading and thus reduced ER-mitochondrial Ca^{2+} transfer [91]. Bcl-2 can also target the N-terminus of the MAM-resident VDAC isoform 1 (VDAC1) [92,93]. Importantly, VDAC1 is the mitochondrial channel responsible for Ca^{2+} passage across the OMM and is particularly involved in mediating proapoptotic Ca^{2+} transfer [94]. Thus, Bcl-2 can suppress proapoptotic Ca^{2+} transfer to the mitochondria by inhibiting VDAC1 [93].

Another apoptosis-inhibiting member of the same family, Bcl-XL [95], is detectable in the MAM compartment [96], where it can target IP3R3. Previous work has indicated that Bcl-XL can promote IP3R-driven Ca^{2+} oscillations [97,98]. Therefore, Bcl-XL enhances Ca^{2+} transfer from the ER to the mitochondria, promoting mitochondrial energy production and cellular metabolism [99]. In addition, via its BH4 domain, Bcl-XL at MAMs can also target VDAC1 [96], and inhibition of VDAC1 by Bcl-XL prevents mitochondrial Ca^{2+} overload and protects against apoptosis [100]. Notably, Bcl-XL has also been reported to enhance VDAC1-mediated Ca^{2+} flux, promoting basal prosurvival Ca^{2+} signaling in particular [101]. Furthermore, the evaluation of myeloid cell leukemia-1 (Mcl-1, whose role in MAMs is indicated later in the text) expression in mantle cell lymphoma revealed that high levels of this protein were related to a highly proliferative state and high-grade morphology [102]. In addition, increased levels of Mcl-1 have been observed in B-cell chronic lymphocytic leukemia and linked to complete remission failure after single-agent therapy [103].

The Bcl-2-associated X protein (Bax) and Bcl-2-homologous antagonist killer (Bak), which are proapoptotic members of Bcl-2 family, function at the ER, where they are involved in preserving Ca^{2+} homeostasis and ensuring proper cell death sensitivity through Ca^{2+} dynamics [104]. Thus, while Bcl-2 overexpression suppresses ER-mitochondrial Ca^{2+} fluxes, Bax overexpression will do the opposite by increasing ER Ca^{2+} loading [105]. Additionally, in T cells, Bax/Bak proteins exert critical roles in antigen-induced proliferation through regulation of IP3R-driven Ca^{2+} dynamics [106]. These proteins also function at the mitochondria, where they initiate mitochondrial dysfunction during apoptosis [104,107]. Thus, Bax and Bak can be considered tumor suppressors, either alone or in cooperation with other alterations. Bax loss-of-function mutations derived from nucleotide insertions/deletions or single amino acid substitutions have been observed in human hematopoietic malignancies [108].

As mentioned above, IP3R/Ca^{2+} channels exhibit a specialized, crucial function in cancer onset and progression. In acute myeloid leukemia, IP3R2 expression is upregulated and associated with dramatically shorter survival [109]. However, the mechanisms linking IP3R2 to adverse clinical events are unknown.

Alterations at the ER-Mitochondria Interface in Lung Cancer

Although Bcl-2 family proteins have been well described in hematopoietic malignancies [79], their mitochondrial control in the MAM platform has mainly been elucidated by studies on solid tumors, such as prostate [110] and lung cancers (Figure 3), both of which are associated with high mortality. The involvement of MAMs in this pathology is not as well documented in the literature as it is for breast cancer, although it can be assumed that the underlying mechanisms are similar. For instance, as noted for hematopoietic cancers, Bcl-2 acts at the contact sites between the ER and the mitochondria [111] to reduce apoptosis by modulating ER Ca^{2+} levels [112,113], but the increase in Bcl-2 expression in lung cancer appears to depend on environmental factors, such as nicotine consumption

[114,115]. Differential expression of Bcl-2-family proteins occurs in non–small cell lung cancer–affected patients and in a related mouse model system [116,117]. Indeed, Bcl-XL [118] and Bcl-2 overexpression has been associated with a poor prognosis [116,117]. However, recent studies on Bcl-2 expression in clinical specimens have provided conflicting data; increased Bcl-2 levels were found to be associated with a better prognosis in lung cancer [119], while there was no correlation with response to anticancer treatments [120]. To exclude possible bias from these studies, further research is recommended.

Mcl-1, another member of the Bcl-2 family, is also overexpressed both in lung cancer cell lines, such as H1299, A549, and non–small cell lung cancer, and in specimens from patients compared with its expression levels in control cell lines and normal adjacent lung tissues [121]. Targeting this protein by reducing its intracellular levels may improve the clinical management of patients [122]. In addition, one study indicated that Mcl-1 promoted lung cancer cell migration by directly interacting with VDAC1, thereby increasing mitochondrial Ca^{2+} uptake and ROS generation [123] (Figure 3). VDAC1-derived peptides that can interfere with the ability of Mcl-1 to bind VDAC1 can counteract lung cancer cell migration.
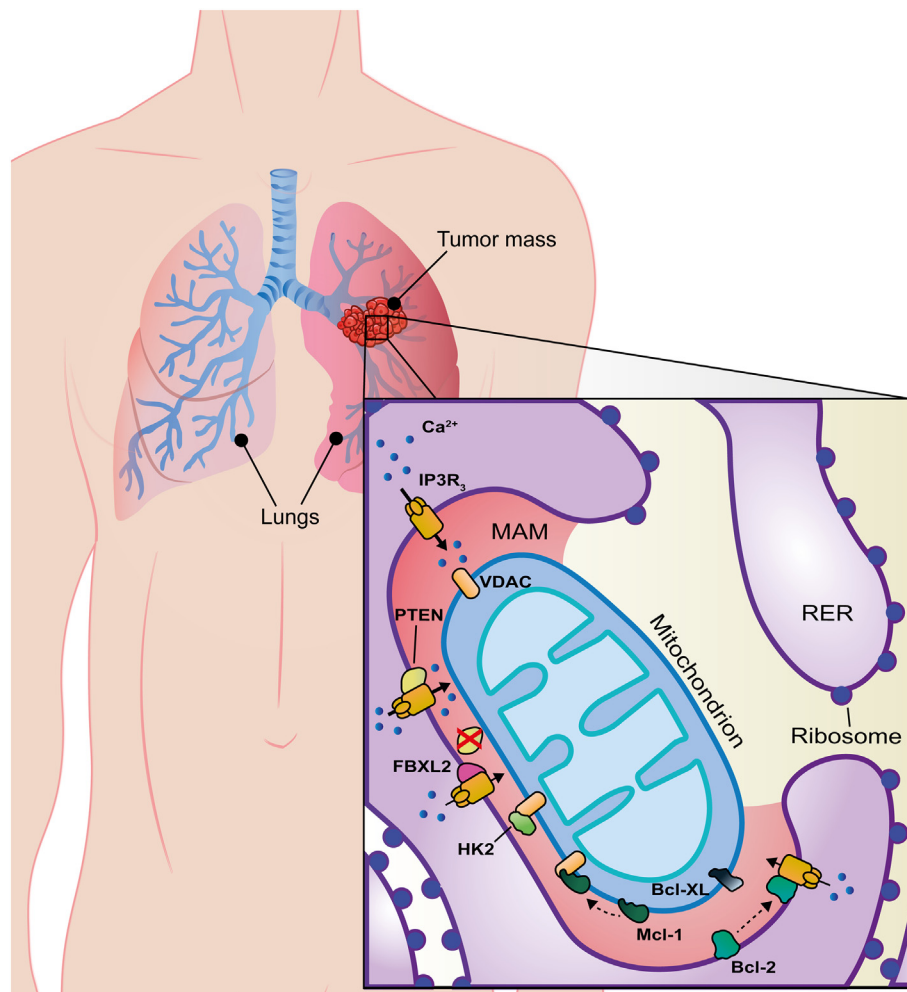

Figure 3. MAM alterations in lung cancer. MAM-resident proteins (red zone) strictly involved in lung cancer onset, progression, and metastasis are shown in the figure. Among all proteins, a novel and complex role for PTEN has been reported; it counteracts FBXL2 binding to promote IP3R3- and Ca^{2+}-mediated apoptosis limiting tumor growth. Indeed, FBXL2 protein binds IP3R3 and targets it for degradation to limit Ca^{2+} influx into mitochondria. Black arrows highlight calcium homeostasis where their thickness is proportional to the entity of calcium fluxes. See text for further details.

Another protein associated with lung cancer pathology that is expressed at higher levels in cancer cells than in normal tissues [124,125] is hexokinase 2 (HK2). Hexokinases are enzymes that catalyze the first step of glucose metabolism, and they are necessary for tumor initiation and development, as demonstrated in mouse models of KRas-driven lung cancer and ErbB2-driven breast cancer [126]. Following the phosphorylation of HK2 by Akt, HK2 can associate with VDAC1 at the MAM site [127,128] (Figure 3). Here, HK2 phosphorylates glucose using ATP exiting the mitochondria through VDAC1 to generate glucose-6-P and stimulate glycolysis. Thus, HK2 is critical for the Warburg effect in humans, and HK2 depletion restores sensitivity to cell death inducers and oxidative glucose metabolism [129]. Moreover, 2-deoxy-D-glucose, an inhibitor of HK2, has been reported to inhibit human and mouse lung cancer cell growth by inducing cell apoptosis and autophagy. Regarding the Akt protein network, the phosphatase and tensin homologue (PTEN), deleted on chromosome 10, is considered a canonical tumor suppressor, directly counteracting PI3K/Akt/mTOR pathway activity [130,131]. A fraction of this protein is highly enriched at MAM sites, where its function influences Ca^{2+} transfer from the ER to the mitochondria and involves apoptotic behaviors. Depletion of PTEN impairs Ca^{2+} release and lowers Ca^{2+} concentrations in the mitochondria, creating an antiapoptotic environment. Thus, PTEN can interact with IP3Rs and modify Ca^{2+} signaling at MAMs [132]. These findings have many implications for oncology, and PTEN loss of function occurs in many human cancers [133,134] through mutations, deletions, transcriptional silencing, or protein instability. By focusing on the protein phosphatase activity of PTEN (directly implicated in its localization to MAMs), the PTEN^{Y138C} mutation was identified in SCLC, demonstrating that selective loss of protein phosphatase activity decreases cellular PIP3 levels and Akt phosphorylation [135]. Phosphatase-independent mechanisms in which PTEN acts at the molecular level can also occur, as demonstrated recently [136], providing another means of fighting cancer, because PTEN can compete with the E3-ubiquitin ligase F-box protein FBXL2 for IP3R3 binding to limit its degradation. FBXL2-dependent degradation of IP3R3 is increased in cells devoid of PTEN, which results in the inhibition of apoptosis in cells and tumor masses (in lung and prostate cancer as a consequence of reduced Ca^{2+} transfer from ER to mitochondria) [136] (Figure 3). Thus, proper maintenance of IP3R3 protein levels is critical for preventing oncogenesis by enabling tumor-suppressive ER-mitochondria Ca^{2+} transfer.

Alterations at the ER-Mitochondria Interface in Prostate Cancer

In early stages, a high percentage of prostate tumors are dependent on androgens and, thus, sensitive to their ablation, which leads to cell death. These tumors rapidly evolve to an androgen-independent pathological stage that is unavoidable, and therapies must therefore be improved. Experiments in LNCaP cells (androgen-responsive prostate cancer) have shown that Bcl-2 overexpression promotes a high rate of cell replication *in vitro* and tumor growth *in vivo* despite hormone deprivation. However, Bcl-2 depletion via antisense oligonucleotide therapy was found to improve cell death due to cytotoxic agents in the same cell line. In addition, immunohistochemistry analysis of 88 neoplastic prostate adenocarcinoma specimens revealed an increase in the protein levels of Bcl-2, Bcl-XL, and Mcl-1 throughout cancer development [137]. Interestingly, a role in mitochondrial dynamics has

recently been attributed to Mcl-1, which is associated with apoptotic cell death in a Drp-1–dependent manner, and Mcl-1 was found to be enriched at the mitochondria, ER, and MAMs [138].

Involvement of the fusion-fission machinery in apoptosis or cancer development has been observed [139]. Enhancement of mitochondrial fusion by increasing mitochondrial GTPases mitofusin 1 (MFN-1) and mitofusin 2 (MFN-2) levels has been associated with prostate cancer progression [140]. MFN-1 and MFN-2 are essential components of the physical tethering between the ER and mitochondria at the MAM compartment, and they are involved in mitochondrial Ca^{2+} homeostasis (Figure 4).

The tethering role of MFN-2 must be further established. Initially, MFN-2 was chosen as a putative candidate with tethering functions [141,142] for Ca^{2+} transfer because its depletion reduces IP3R-mediated mitochondrial Ca^{2+} uptake [143], leading to a decrease in contact sites [143] in hypothalamic neurons [144]; indeed, MFN-2 appears to be a crucial mediator of the energy balance by acting on the synergy of the mitochondria-ER membrane juxtaposition [144]. The experimental evidence indicating the tethering function of MFN-2 has been questioned and was not shared by Filadi and coworkers, who reported that there is a high percentage of membrane juxtaposition between the ER and MAMs when MFN-2 is silenced; the ablation of MFN-2 initiates Ca^{2+} signaling at contact sites [145] and supports cell death (Figure 4). This hypothesis was confirmed by other independent groups, as referenced in [146,147]. This topic remains controversial, although the localization of MFN-2 and its pivotal roles at MAMs are indisputable.

Finally, the MAM chaperone GRP78, which exhibits Ca^{2+}-binding properties [36], is able to bind the ER antiapoptotic factor clusterin (CLU) during ER stress to facilitate its redistribution at the mitochondria and to minimize the detrimental effects provoked by ER stress, thus inducing prostate cancer cell survival [148].

MAMs in Other Types of Cancer and the Impact of Tumor Suppressors/Oncogenes

In the following paragraphs, the general understanding of and recent discoveries regarding oncogene and tumor suppressor functions at MAMs are summarized. Although oncogenes and tumor suppressors are involved in almost all types of cancer as transcription factors, new transcriptional-independent properties have been revealed in healthy and disease conditions due to their intracellular localization at the ER-mitochondria interface.

i) *p53*

The p53 protein exhibits excellent tumor-suppressive properties and is altered in most human cancers, including colon, breast, lung, bladder, brain, pancreatic, stomach, and esophageal cancer [149]. p53 is a nuclear transcription factor that is activated by a variety of stimuli and subsequently transactivates genes involved in apoptosis, cell cycle regulation, and prevention of cell transformation and cancer progression. Additional p53 activities occur in the cytoplasm, where p53 triggers apoptosis and inhibits autophagy [150], and in the mitochondrial matrix, where it promotes F_1F_O-ATP synthase assembly [151], suggesting an ability to increase oxidative phosphorylation activity by interacting with the oligomycin sensitivity-conferring protein (OSCP) subunit in a transcriptional-independent manner.

Interestingly, a fraction of p53 has also been found to be associated with the ER and MAMs, where it modulates Ca^{2+} homeostasis [152] (Figure 5). In particular, p53 binds and simulates the sarco/ER Ca^{2+}-ATPase (SERCA) pumps at the ER, increasing ER Ca^{2+} levels. As a consequence, during apoptotic stimulation, a greater amount of Ca^{2+} is releasable from the ER versus the mitochondria, promoting mitochondrial Ca^{2+} overload, mPTP opening, release of caspase cofactors, and induction of apoptosis via the intrinsic pathway [153]. In cancer cells, this
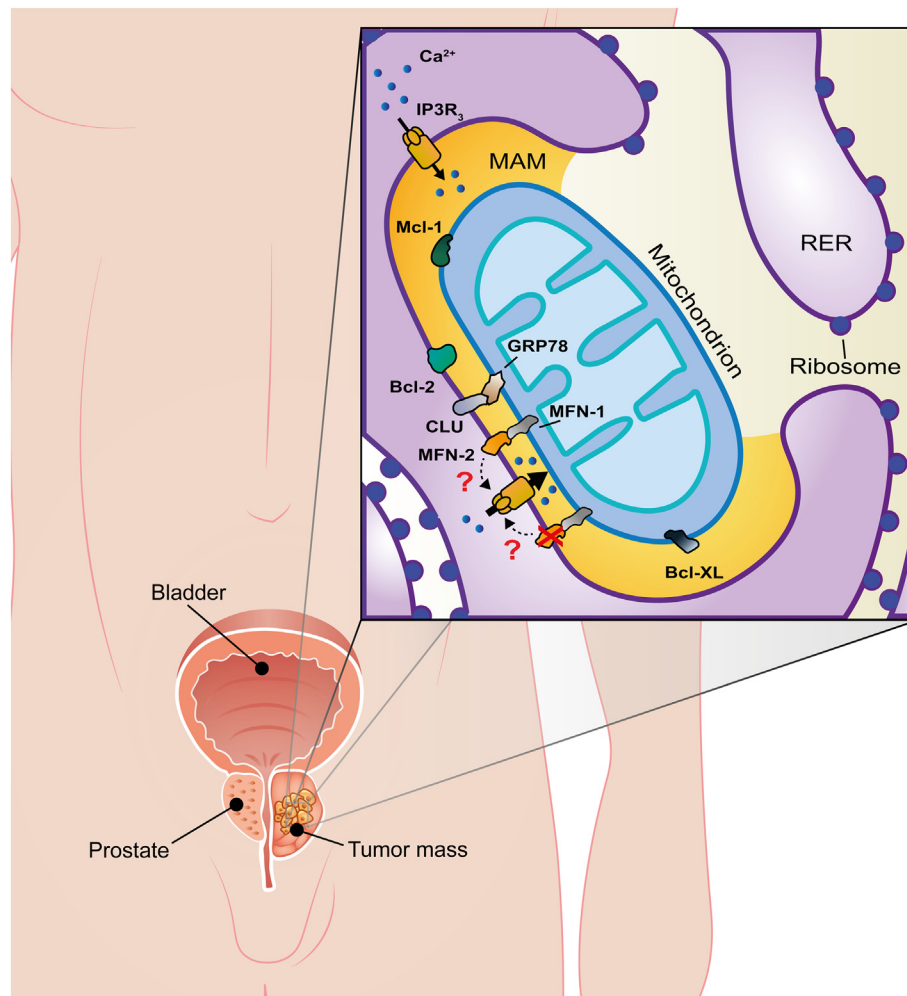
Figure 4. MAM alterations in prostate cancer. MAM-resident proteins (yellow zone) strictly involved in prostate cancer onset, progression. and metastasis are shown in the figure. MFN-2 protein function has been illustrated with quite attention (question marks), as its role at MAMs is far to be established; see text for further details.

mechanism can easily become impaired due to either inactivation of p53 or missense mutations in the coding gene, contributing to disease progression and resistance to chemotherapy [154,155]. Moreover, by combining the dorsal skinfold chamber technique with intravital microscopy, Giorgi et al. elucidated the involvement of p53 in controlling intracellular Ca^{2+} signals and apoptosis in three-dimensional tumor masses in mice. Dysregulation of p53-dependent Ca^{2+} homeostasis led to reduced ER Ca^{2+} release and, consequently, low responsiveness to apoptotic stimulation [156].

ii) *PML and Bap1*
Similar to p53, the promyelocytic leukemia protein (PML) is a potent tumor suppressor that stabilizes the p53 protein and improves its function [157]. PML was originally associated with the pathogenesis of acute promyelocytic leukemia. However, loss of PML has been linked to several human cancers, including prostate, breast, and central nervous system tumors [158]. In addition to its well-characterized nuclear activity, an extranuclear transcription-independent function of PML was identified at MAMs, where it controls cell survival. PML regulates apoptosis in MAMs by modulating Ca^{2+} release through its physical interaction with IP3R3 [159] (Figure 5). Moreover, it was recently demonstrated that the localization of PML at MAMs is fundamental for apoptosis control and autophagy regulation [160] (Figure 5). IP3R3 has emerged as being involved in gastric cancer peritoneal dissemination [161], and its expression is directly associated with the aggressiveness of colorectal carcinomas [162]. Recent findings have provided new insights regarding mesothelioma malignances in which an important tumor suppressor, Bap1, is reduced or inactivated by mutations [163]. ER-localized Bap1

has been shown to bind and stabilize IP3R3, hence modulating Ca^{2+} mobilization in favor of apoptosis. Thus, the depletion of Bap1, along with its nuclear-dependent function, allows cellular transformation and leads to prevalence of mesothelioma onset rather than other type of cancers [163].

iii) *FATE1*
A direct link between cancer progression and alteration of the correct ER-mitochondria distance is presented by the oncoprotein fetal and adult testis-expressed 1 (FATE1). Analysis of The Cancer Genome Atlas colorectal dataset revealed that FATE1 is frequently co-expressed with the ER-resident E3 ligase RNF183, which is correlated with a poor clinical outcome [164], suggesting that these proteins function in human tumors to inhibit cell death. FATE1 localizes at the outer surface of the mitochondria and is associated with MAMs [165]. Moreover, an ER-mitochondria antitethering function has been attributed to FATE1, which is consistent with a decrease in mitochondrial Ca^{2+} uptake and cell survival [165] (Figure 5). FATE1 protects cells from apoptosis induced by mitotane, a compound that promotes the accumulation of toxic cholesterol esters and triggers ER stress by inhibiting the MAM-resident enzyme SOAT1 [166]. Most importantly, high FATE1 expression is an indicator of poor prognosis in adrenocortical carcinoma patients [165].

iv) *Mitochondrial fission factors*
Fission factors are also mitochondrial regulators of cell death, thus representing pivotal components of apoptosis signaling pathways [167]. Downregulation of a fission 1 homologue (Fis1) and Drp1 was demonstrated to reduce apoptosis [168].
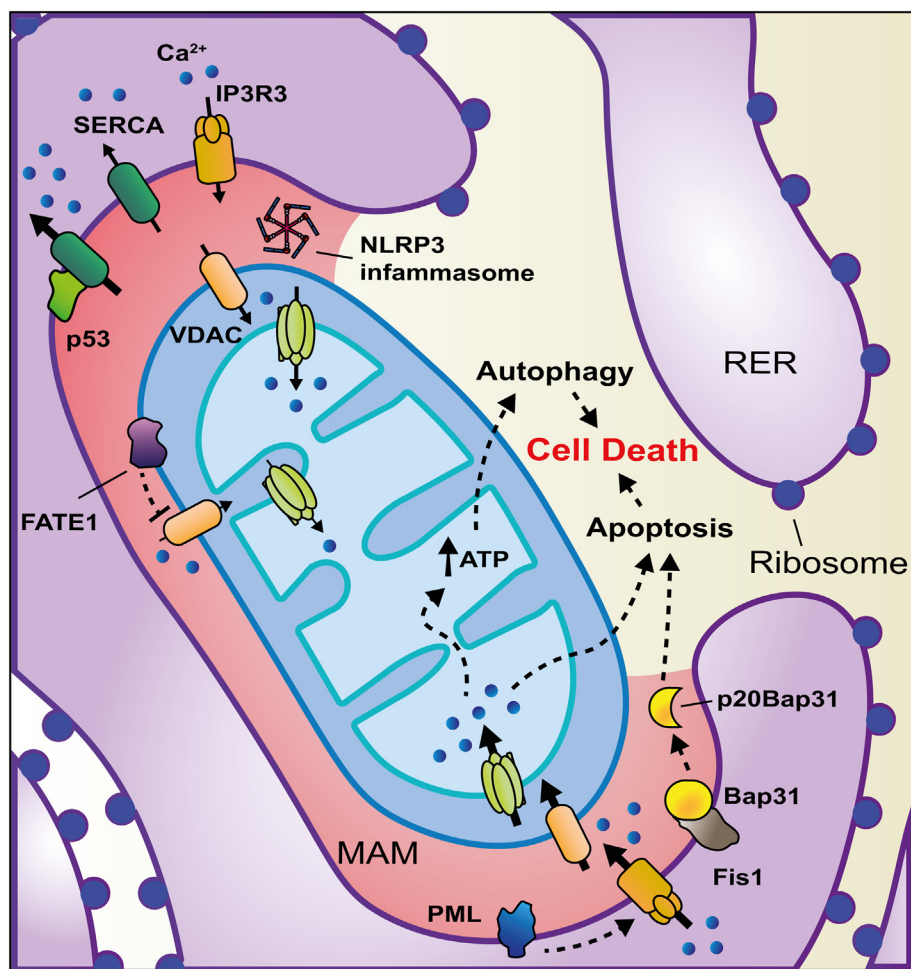
Figure 5. MAM alterations and other types of cancer. Proteins with key functions (see text for details) in a wide range of tumors are represented in the figure. A pink zone between the mitochondrion and the ER outlines MAM subcellular compartment. *ATP*, adenosine triphosphate.

Interestingly, Fis1 is responsible for the formation of a platform at MAM sites, which triggers a feedback loop by releasing Ca^{2+} from the ER, which stimulates mitochondria-mediated apoptosis. Specifically, Fis1 interacts with Bap31 at the ER, creating a bridge that enhances the cleavage of Bap31 in its proapoptotic form, p20Bap31 [169]. Thus, alterations of Fis1 contribute to tumor development. It has been reported that Fis1 is overexpressed in oncolytic cell tumors [170], and Fis1 deletion can drive the selection of compensatory mutations, resulting in defective growth control and cell death regulation, which are characteristics of human tumor cells [171].

v) *NLRP3 inflammasome*

Although inflammation is not the focus of this review, we would like to briefly refer to the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome due to its crucial association with cancer onset. NLRP3 is a multimeric complex that induces innate inflammatory responses with apoptosis-associated speck-like protein containing a CARD complex (ASC), which recruits procaspase-1. Procaspase-1 is then cleaved to caspase-1, which converts pro–IL-1β and pro–IL-18 to the active forms that are responsible for additional recruitment of other inflammatory cells [172]. Interestingly, a considerable fraction of NLRP3 is associated with MAMs following inflammasome activation, suggesting that NLRP3 checks and modulates mitochondrial activity [173]. Cancer-associated inflammatory responses are directly involved in cancer biology, including tumor initiation, progression, metastasis, and ttreatment. For example, NLRP3 is markedly upregulated in macrophages in pancreatic ductal adenocarcinoma, where NLRP3 directs the polarization of tumor-associated macrophages and, hence, controls immunogenic or tolerogenic CD4+ T-cell differentiation and CD8+ T-cell

activation [174]. Furthermore, in NLRP3 KO mice, inflammasome components have been shown to exacerbate liver colorectal cancer metastatic growth [175]. Moreover, the activation of the NLRP3 inflammasome and the expression and secretion of active IL-1β in melanomas cause disease progression [176].

Oncogenic Ras Signaling

Ras proteins, which belong to the family of small GTPases controlling cell proliferation, cell cycling, and cell survival, are frequently deregulated in several types of human cancers [177]. One of the family members K-Ras has recently been found to engage in cross talk with Ca^{2+} signaling and to impact ER-mitochondrial Ca^{2+} transfer [178]. By comparing two isogenic colorectal cancer cell lines, one expressing mutated oncogenic K-Ras $^{G13D/wild-type}$ and one in which the oncogenic allele was deleted (K-Ras$^{-/wild-type}$), it was found that the presence of oncogenic K-Ras ^{G13D} suppressed IP3-induced Ca^{2+} release due to a decrease in ER Ca^{2+} store contents. These functional aberrations in Ca^{2+} signaling could be linked to remodeling of the expression of ER Ca^{2+} transport systems, revealing that K-Ras ^{G13D}–expressing cells express less SERCA2b and switch their IP3R-isoform expression profile compared with K-Ras ^{G13D}– deleted cells. In particular, cells with oncogenic K-Ras display a decrease in IP3R3 expression levels and an increase in IP3R1 expression levels, establishing a decrease in susceptibility to apoptotic

stimuli and an increase in the ability to generate prosurvival Ca^{2+} oscillations that sustain cell proliferation. As such, upon the deletion of K-Ras, IP3R3 expression is elevated, and the likelihood of IP3R3 accumulating in MAMs is therefore also increased and is correlated with restoration of ER-mitochondrial Ca^{2+} transfer and apoptosis sensitivity. Hence, beyond the direct regulation of oncogenes and tumor suppressors of Ca^{2+}-transport systems in MAMs, it is clear that these genes can also impact the expression levels and, thus, the overall number of channels available for the MAM compartment, affecting ER-mitochondrial Ca^{2+} transfer and cell death susceptibility (or other cancer-related hallmarks).

Conclusions

An increasing number of studies have identified important roles of MAMs in cancer processes, although further study is required to completely elucidate the molecular mechanisms involved. Changes in the ER-mitochondrial tethering distance and morphology have dramatic effects on the health of a cell, which communicates with the "outside world" via lipids, Ca^{2+}, ROS, and the exchange of other mediators among organelles. There are likely to be many ER-mitochondria contact sites, leading to many unanswered questions. For example, how will increased knowledge of MAMs impact human studies and clinical therapies? Are there any properties of MAM-resident proteins that could be attributed to one specific type of cancer rather than another? Are there different types of MAMs with specific functions and protein populations? In addition to Ca^{2+}, are there other crucial mediators that could modulate the plasticity and function of MAMs in the cell? Are lipid synthesis and transfer at ER-mitochondria membrane contact sites involved in cancer, and what are the underlying mechanisms? If the proteins that localize or relocalize to MAMs vary, how do these various proteins regulate their localization, and under what specific condition are they active?

References

[1] Vance JE (1990). Phospholipid synthesis in a membrane fraction associated with mitochondria. *J Biol Chem* **265**(13), 7248–7256.

[2] Rusinol AE, Cui Z, Chen MH, and Vance JE (1994). 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**(44), 27494–27502.

[3] Copeland DE and Dalton AJ (1959). An association between mitochondria and the endoplasmic reticulum in cells of the pseudobranch gland of a teleost. *J Biophys Biochem Cytol.* **5**(3), 393–396.

[4] Bernhard W, Haguenau F, Gautier A, and Oberling C (1952). Submicroscopical structure of cytoplasmic basophils in the liver, pancreas and salivary gland; study of ultrafine slices by electron microscope. *Z Zellforsch Mikrosk Anat* **37**(3), 281–300.

[5] Bernhard W and Rouiller C (1956). Close topographical relationship between mitochondria and ergastoplasm of liver cells in a definite phase of cellular activity. *J Biophys Biochem Cytol* **2**(4 Suppl), 73–78.

[6] Csordas G, Renken C, Varnai P, Walter L, Weaver D, and Buttle KF, et al (2006). Structural and functional features and significance of the physical linkage between ER and mitochondria. *J Cell Biol* **174**(7), 915–921.

[7] Vance JE (2015). Phospholipid synthesis and transport in mammalian cells. *Traffic* **16**(1), 1–18.

[8] Stone SJ and Vance JE (2000). Phosphatidylserine synthase-1 and -2 are localized to mitochondria-associated membranes. *J Biol Chem* **275**(44), 34534–34540.

[9] Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, and Cavagna D, et al (2006). Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. *J Cell Biol* **175**(6), 901–911.

[10] Naon D and Scorrano L (2014). At the right distance: ER-mitochondria juxtaposition in cell life and death. *Biochim Biophys Acta* **1843**(10), 2184–2194.

[11] Csordas G, Thomas AP, and Hajnoczky G (1999). Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J* **18**(1), 96–108.

[12] Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, and Lifshitz LM, et al (1998). Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. *Science* **280**(5370), 1763–1766.

[13] De Pinto VD and Palmieri F (1992). Transmembrane arrangement of mitochondrial porin or voltage-dependent anion channel (VDAC). *J Bioenerg Biomembr* **24**(1), 21–26.

[14] Marchi S and Pinton P (2014). The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications. *J Physiol* **592**(5), 829–839.

[15] Bonora M, Morganti C, Morciano G, Pedriali G, Lebiedzinska-Arciszewska M, and Aquila G, et al (2017). Mitochondrial permeability transition involves dissociation of F1FO ATP synthase dimers and C-ring conformation. *EMBO Rep* **18**(7), 1077–1089.

[16] Morciano G, Giorgi C, Bonora M, Punzetti S, Pavasini R, and Wieckowski MR, et al (2015). Molecular identity of the mitochondrial permeability transition pore and its role in ischemia-reperfusion injury. *J Mol Cell Cardiol* **78**, 142–153.

[17] Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, and Witzgall R, et al (2002). Polycystin-2 is an intracellular calcium release channel. *Nat Cell Biol* **4**(3), 191–197.

[18] Zhu MX, Ma J, Parrington J, Calcraft PJ, Galione A, and Evans AM (2010). Calcium signaling via two-pore channels: local or global, that is the question. *Am J Physiol Cell Physiol* **298**(3), C430–41.

[19] Frischauf I, Schindl R, Derler I, Bergsmann J, Fahrner M, and Romanin C (2008). The STIM/Orai coupling machinery. *Channels (Austin)* **2**(4), 261–268.

[20] Leybaert L and Sanderson MJ (2012). Intercellular Ca(2+) waves: mechanisms and function. *Physiol Rev* **92**(3), 1359–1392.

[21] Wu W, Lin C, Wu K, Jiang L, Wang X, and Li W, et al (2016). FUNDC1 regulates mitochondrial dynamics at the ER-mitochondrial contact site under hypoxic conditions. *EMBO J* **35**(13), 1368–1384.

[22] Yoboue ED, Rimessi A, Anelli T, Pinton P, and Sitia R (2017). Regulation of calcium fluxes by GPX8, a type-II transmembrane peroxidase enriched at the mitochondria-associated endoplasmic reticulum membrane. *Antioxid Redox Signal* **27**(9), 583–595.

[23] Rowland AA and Voeltz GK (2012). Endoplasmic reticulum-mitochondria contacts: function of the junction. *Nat Rev Mol Cell Biol* **13**(10), 607–625.

[24] Glater EE, Megeath LJ, Stowers RS, and Schwarz TL (2006). Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. *J Cell Biol* **173**(4), 545–557.

[25] Wang X and Schwarz TL (2009). The mechanism of Ca2+ -dependent regulation of kinesin-mediated mitochondrial motility. *Cell* **136**(1), 163–174.

[26] Horner SM, Liu HM, Park HS, Briley J, and Gale Jr M (2011). 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**(35), 14590–14595.

[27] Haile Y, Deng X, Ortiz-Sandoval C, Tahbaz N, Janowicz A, and Lu JQ, et al (2017). Rab32 connects ER stress to mitochondrial defects in multiple sclerosis. *J Neuroinflammation* **14**(1), 19.

[28] Gutierrez T and Simmen T (2014). Endoplasmic reticulum chaperones and oxidoreductases: critical regulators of tumor cell survival and immunorecognition. *Front Oncol* **4**, 291.

[29] Gutierrez T and Simmen T (2017). Endoplasmic reticulum chaperones tweak the mitochondrial calcium rheostat to control metabolism and cell death. *Cell Calcium* **70**, 64–75.

[30] Booth DM, Enyedi B, Geiszt M, Varnai P, and Hajnoczky G (2016). Redox nanodomains are induced by and control calcium signaling at the ER-mitochondrial interface. *Mol Cell* **63**(2), 240–248.

[31] Sassano ML, van Vliet AR, and Agostinis P (2017). Mitochondria-associated membranes as networking platforms and regulators of cancer cell fate. *Front Oncol* **7**, 174.

[32] Ivanova H, Kerkhofs M, La Rovere RM, and Bultynck G (2017). Endoplasmic reticulum-mitochondrial Ca2+ fluxes underlying cancer cell survival. *Front Oncol* **7**, 70.

[33] Barrdahl M, Rudolph A, Hopper JL, Southey MC, Broeks A, and Fasching PA, et al (2017). Gene-environment interactions involving functional variants: Results from the Breast Cancer Association Consortium. *Int J Cancer* **141**(9), 1830–1840.

[34] Aydar E, Onganer P, Perrett R, Djamgoz MB, and Palmer CP (2006). The expression and functional characterization of sigma (sigma) 1 receptors in breast cancer cell lines. *Cancer Lett* **242**(2), 245–257.

[35] Gueguinou M, Crottes D, Chantome A, Rapetti-Mauss R, Potier-Cartereau M, and Clarysse L, et al (2017). The SigmaR1 chaperone drives breast and colorectal cancer cell migration by tuning SK3-dependent Ca2+ homeostasis. *Oncogene* **36**(25), 3640–3647.

[36] Hayashi T and Su TP (2007). Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* **131**(3), 596–610.

[37] Hayashi T and Su TP (2003). Intracellular dynamics of sigma-1 receptors (sigma (1) binding sites) in NG108-15 cells. *J Pharmacol Exp Ther* **306**(2), 726–733.

[38] Tosatto A, Sommaggio R, Kummerow C, Bentham RB, Blacker TS, and Berecz T, et al (2016). The mitochondrial calcium uniporter regulates breast cancer progression via HIF-1alpha. *EMBO Mol Med* **8**(5), 569–585.

[39] Kamer KJ, Grabarek Z, and Mootha VK (2017). High-affinity cooperative Ca2+ binding by MICU1-MICU2 serves as an on-off switch for the uniporter. *EMBO Rep* **18**(8), 1397–1411.

[40] Paillard M, Csordas G, Szanda G, Golenar T, Debattisti V, and Bartok A, et al (2017). Tissue-specific mitochondrial decoding of cytoplasmic Ca2+ signals is controlled by the stoichiometry of MICU1/2 and MCU. *Cell Rep* **18**(10), 2291–2300.

[41] Patron M, Checchetto V, Raffaello A, Teardo E, Vecellio Reane D, and Mantoan M, et al (2014). MICU1 and MICU2 finely tune the mitochondrial Ca2+ uniporter by exerting opposite effects on MCU activity. *Mol Cell* **53**(5), 726–737.

[42] Marchi S, Lupini L, Patergnani S, Rimessi A, Missiroli S, and Bonora M, et al (2013). Downregulation of the mitochondrial calcium uniporter by cancer-related miR-25. *Curr Biol* **23**(1), 58–63.

[43] Wieckowski MR, Giorgi C, Lebiedzinska M, Duszynski J, and Pinton P (2009). Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells. *Nat Protoc* **4**(11), 1582–1590.

[44] Mendes CC, Gomes DA, Thompson M, Souto NC, Goes TS, and Goes AM, et al (2005). The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca2+ signals into mitochondria. *J Biol Chem* **280**(49), 40892–40900.

[45] Singh A, Chagtoo M, Tiwari S, George N, Chakravarti B, and Khan S, et al (2017). Inhibition of inositol 1, 4, 5-trisphosphate receptor induce breast cancer cell death through deregulated autophagy and cellular bioenergetics. *J Cell Biochem* **118**(8), 2333–2346.

[46] Bultynck G (2016). Onco-IP3Rs feed cancerous cravings for mitochondrial Ca (2.). *Trends Biochem Sci* **41**(5), 390–393.

[47] Cardenas C, Muller M, McNeal A, Lovy A, Jana F, and Bustos G, et al (2016). Selective vulnerability of cancer cells by inhibition of Ca(2+) transfer from endoplasmic reticulum to mitochondria. *Cell Rep* **15**(1), 219–220.

[48] Singh A, Sharma RK, Chagtoo M, Agarwal G, George N, and Sinha N, et al (2017). 1H NMR metabolomics reveals association of high expression of inositol 1, 4, 5 trisphosphate receptor and metabolites in breast cancer patients. *PLoS One* **12**(1)e0169330.

[49] Marchi S, Patergnani S, Missiroli S, Morciano G, Rimessi A, and Wieckowski MR, et al (2017). Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. *Cell Calcium* **69**, 62–72.

[50] Vervliet T, Clerix E, Seitaj B, Ivanova H, Monaco G, and Bultynck G (2017). Modulation of Ca2+ signaling by anti-apoptotic B-cell lymphoma 2 proteins at the endoplasmic reticulum-mitochondrial interface. *Front Oncol* **7**, 75.

[51] Bittremieux M, Parys JB, Pinton P, and Bultynck G (2016). ER functions of oncogenes and tumor suppressors: modulators of intracellular Ca(2+) signaling. *Biochim Biophys Acta* **1863**(6 Pt B), 1364–1378.

[52] Khan MT, Wagner II L, Yule DI, Bhanumathy C, and Joseph SK (2006). Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors. *J Biol Chem* **281**(6), 3731–3737.

[53] Szado T, Vanderheyden V, Parys JB, De Smedt H, Rietdorf K, and Kotelevets L, et al (2008). 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**(7), 2427–2432.

[54] Gonzalez-Angulo AM, Ferrer-Lozano J, Stemke-Hale K, Sahin A, Liu S, and Barrera JA, et al (2011). PI3K pathway mutations and PTEN levels in primary and metastatic breast cancer. *Mol Cancer Ther* **10**(6), 1093–1101.

[55] Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, and Davies M, et al (2008). An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* **68**(15), 6084–6091.

[56] Marchi S, Marinello M, Bononi A, Bonora M, Giorgi C, and Rimessi A, et al (2012). Selective modulation of subtype III IP(3)R by Akt regulates ER Ca(2) (+) release and apoptosis. *Cell Death Dis* **3**e304.

[57] Marchi S, Rimessi A, Giorgi C, Baldini C, Ferroni L, and Rizzuto R, et al (2008). Akt kinase reducing endoplasmic reticulum Ca2+ release protects cells from Ca2+-dependent apoptotic stimuli. *Biochem Biophys Res Commun* **375**(4), 501–505.

[58] Betz C, Stracka D, Prescianotto-Baschong C, Frieden M, Demaurex N, and Hall MN (2013). Feature Article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. *Proc Natl Acad Sci U S A* **110**(31), 12526–12534.

[59] Sarbassov DD, Guertin DA, Ali SM, and Sabatini DM (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**(5712), 1098–1101.

[60] Morrison Joly M, Hicks DJ, Jones B, Sanchez V, Estrada MV, and Young C, et al (2016). Rictor/mTORC2 drives progression and therapeutic resistance of HER2-amplified breast cancers. *Cancer Res* **76**(16), 4752–4764.

[61] Teng Y, Ren X, Li H, Shull A, Kim J, and Cowell JK (2016). Mitochondrial ATAD3A combines with GRP78 to regulate the WASF3 metastasis-promoting protein. *Oncogene* **35**(3), 333–343.

[62] Issop L, Fan J, Lee S, Rone MB, Basu K, and Mui J, et al (2015). Mitochondria-associated membrane formation in hormone-stimulated Leydig cell steroidogenesis: role of ATAD3. *Endocrinology* **156**(1), 334–345.

[63] van Vliet AR, Verfaillie T, and Agostinis P (2014). New functions of mitochondria associated membranes in cellular signaling. *Biochim Biophys Acta* **1843**(10), 2253–2262.

[64] Szymanski J, Janikiewicz J, Michalska B, Patalas-Krawczyk P, Perrone M, and Ziolkowski W, et al (2017). Interaction of mitochondria with the endoplasmic reticulum and plasma membrane in calcium homeostasis, lipid trafficking and mitochondrial structure. *Int J Mol Sci* **18**(7).

[65] Vance JE (2014). MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond. *Biochim Biophys Acta* **1841**(4), 595–609.

[66] Zaidi N, Lupien L, Kuemmerle NB, Kinlaw WB, Swinnen JV, and Smans K (2013). Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids. *Prog Lipid Res* **52**(4), 585–589.

[67] Kuemmerle NB, Rysman E, Lombardo PS, Flanagan AJ, Lipe BC, and Wells WA, et al (2011). Lipoprotein lipase links dietary fat to solid tumor cell proliferation. *Mol Cancer Ther* **10**(3), 427–436.

[68] Ettinger SL, Sobel R, Whitmore TG, Akbari M, Bradley DR, and Gleave ME, et al (2004). Dysregulation of sterol response element-binding proteins and downstream effectors in prostate cancer during progression to androgen independence. *Cancer Res* **64**(6), 2212–2221.

[69] Anelli T, Bergamelli L, Margittai E, Rimessi A, Fagioli C, and Malgaroli A, et al (2012). Ero1alpha regulates Ca(2+) fluxes at the endoplasmic reticulum-mitochondria interface (MAM). *Antioxid Redox Signal* **16**(10), 1077–1087.

[70] Kakihana T, Nagata K, and Sitia R (2012). Peroxides and peroxidases in the endoplasmic reticulum: integrating redox homeostasis and oxidative folding. *Antioxid Redox Signal* **16**(8), 763–771.

[71] Kutomi G, Tamura Y, Tanaka T, Kajiwara T, Kukita K, and Ohmura T, et al (2013). Human endoplasmic reticulum oxidoreductin 1-alpha is a novel predictor for poor prognosis of breast cancer. *Cancer Sci* **104**(8), 1091–1096.

[72] Seervi M, Sobhan PK, Joseph J, Ann Mathew K, and Santhoshkumar TR (2013). 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**e968.

[73] Tanaka T, Kutomi G, Kajiwara T, Kukita K, Kochin V, and Kanaseki T, et al (2017). Cancer-associated oxidoreductase ERO1-alpha promotes immune escape through up-regulation of PD-L1 in human breast cancer. *Oncotarget* **8** (15), 24706–24718.

[74] van Vliet AR, Giordano F, Gerlo S, Segura I, Van Eygen S, and Molenberghs G, et al (2017). The ER stress sensor PERK coordinates ER-plasma membrane contact site formation through interaction with filamin-A and F-actin remodeling. *Mol Cell* **65**(5), 885–899 e6.

[75] Verfaillie T, Rubio N, Garg AD, Bultynck G, Rizzuto R, and Decuypere JP, et al (2012). PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ* **19**(11), 1880–1891.

[76] Bobrovnikova-Marjon E, Grigoriadou C, Pytel D, Zhang F, Ye J, and Koumenis C, et al (2010). PERK promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage. *Oncogene* **29**(27), 3881–3895.

[77] Salaroglio IC, Panada E, Moiso E, Buondonno I, Provero P, and Rubinstein M, et al (2017). PERK induces resistance to cell death elicited by endoplasmic reticulum stress and chemotherapy. *Mol Cancer* **16**(1), 91.

[78] Blais JD, Addison CL, Edge R, Falls T, Zhao H, and Wary K, et al (2006). Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. *Mol Cell Biol* **26**(24), 9517–9532.

[79] Reed JC (2008). Bcl-2-family proteins and hematologic malignancies: history and future prospects. *Blood* **111**(7), 3322–3330.

[80] Balatti V, Pekarky Y, Rizzotto L, and Croce CM (2013). miR deregulation in CLL. *Adv Exp Med Biol* **792**, 309–325.

[81] Crisan D (1996). BCL-2 gene rearrangements in lymphoid malignancies. *Clin Lab Med* **16**(1), 23–47.

[82] Brunelle JK and Letai A (2009). Control of mitochondrial apoptosis by the Bcl-2 family. *J Cell Sci* **122**(Pt 4), 437–441.

[83] Davids MS and Letai A (2012). Targeting the B-cell lymphoma/leukemia 2 family in cancer. *J Clin Oncol* **30**(25), 3127–3135.

[84] Coustan-Smith E, Kitanaka A, Pui CH, McNinch L, Evans WE, and Raimondi SC, et al (1996). Clinical relevance of BCL-2 overexpression in childhood acute lymphoblastic leukemia. *Blood* **87**(3), 1140–1146.

[85] Akl H, Vervloessem T, Kiviluoto S, Bittremieux M, Parys JB, and De Smedt H, et al (2014). A dual role for the anti-apoptotic Bcl-2 protein in cancer: mitochondria versus endoplasmic reticulum. *Biochim Biophys Acta* **1843**(10), 2240–2252.

[86] Vervliet T, Parys JB, and Bultynck G (2016). Bcl-2 proteins and calcium signaling: complexity beneath the surface. *Oncogene* **35**(39), 5079–5092.

[87] Monaco G, Decrock E, Akl H, Ponsaerts R, Vervliet T, and Luyten T, et al (2012). Selective regulation of IP3-receptor-mediated Ca2+ signaling and apoptosis by the BH4 domain of Bcl-2 versus Bcl-Xl. *Cell Death Differ* **19**(2), 295–309.

[88] Rong YP, Aromolaran AS, Bultynck G, Zhong F, Li X, and McColl K, et al (2008). Targeting Bcl-2-IP3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals. *Mol Cell* **31**(2), 255–265.

[89] Rong YP, Bultynck G, Aromolaran AS, Zhong F, Parys JB, and De Smedt H, et al (2009). 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**(34), 14397–14402.

[90] Zhong F, Harr MW, Bultynck G, Monaco G, Parys JB, and De Smedt H, et al (2011). Induction of Ca(2)+-driven apoptosis in chronic lymphocytic leukemia cells by peptide-mediated disruption of Bcl-2-IP3 receptor interaction. *Blood* **117**(10), 2924–2934.

[91] Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, and Pozzan T, et al (2005). Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc Natl Acad Sci U S A* **102**(1), 105–110.

[92] Abu-Hamad S, Arbel N, Calo D, Arzoine L, Israelson A, and Keinan N, et al (2009). The VDAC1 N-terminus is essential both for apoptosis and the protective effect of anti-apoptotic proteins. *J Cell Sci* **122**(Pt 11), 1906–1916.

[93] Arbel N and Shoshan-Barmatz V (2010). Voltage-dependent anion channel 1-based peptides interact with Bcl-2 to prevent antiapoptotic activity. *J Biol Chem* **285**(9), 6053–6062.

[94] De Stefani D, Bononi A, Romagnoli A, Messina A, De Pinto V, and Pinton P, et al (2012). VDAC1 selectively transfers apoptotic Ca2+ signals to mitochondria. *Cell Death Differ* **19**(2), 267–273.

[95] Zamzami N, Brenner C, Marzo I, Susin SA, and Kroemer G (1998). Subcellular and submitochondrial mode of action of Bcl-2-like oncoproteins. *Oncogene* **16** (17), 2265–2282.

[96] Monaco G, Decrock E, Arbel N, van Vliet AR, La Rovere RM, and De Smedt H, et al (2015). The BH4 domain of anti-apoptotic Bcl-XL, but not that of the related Bcl-2, limits the voltage-dependent anion channel 1 (VDAC1)-mediated transfer of pro-apoptotic Ca2+ signals to mitochondria. *J Biol Chem* **290**(14), 9150–9161.

[97] Li C, Wang X, Vais H, Thompson CB, Foskett JK, and White C (2007). Apoptosis regulation by Bcl-x(L) modulation of mammalian inositol 1,4,5-trisphosphate receptor channel isoform gating. *Proc Natl Acad Sci U S A* **104** (30), 12565–12570.

[98] White C, Li C, Yang J, Petrenko NB, Madesh M, and Thompson CB, et al (2005). The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. *Nat Cell Biol* **7**(10), 1021–1028.

[99] Williams A, Hayashi T, Wolozny D, Yin B, Su TC, and Betenbaugh MJ, et al (2016). The non-apoptotic action of bcl-xL: regulating Ca(2+) signaling and bioenergetics at the ER-mitochondrion interface. *J Bioenerg Biomembr* **48**(3), 211–225.

[100] Arbel N, Ben-Hail D, and Shoshan-Barmatz V (2012). Mediation of the antiapoptotic activity of Bcl-xL protein upon interaction with VDAC1 protein. *J Biol Chem* **287**(27), 23152–23161.

[101] Huang H, Hu X, Eno CO, Zhao G, Li C, and White C (2013). An interaction between Bcl-xL and the voltage-dependent anion channel (VDAC) promotes mitochondrial Ca2+ uptake. *J Biol Chem* **288**(27), 19870–19881.

[102] Khoury JD, Medeiros LJ, Rassidakis GZ, McDonnell TJ, Abruzzo LV, and Lai R (2003). Expression of Mcl-1 in mantle cell lymphoma is associated with high-grade morphology, a high proliferative state, and p53 overexpression. *J Pathol* **199**(1), 90–97.

[103] Kitada S, Andersen J, Akar S, Zapata JM, Takayama S, and Krajewski S, et al (1998). Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with In vitro and In vivo chemoresponses. *Blood* **91**(9), 3379–3389.

[104] Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, and Pozzan T, et al (2003). BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science* **300**(5616), 135–139.

[105] Chami M, Prandini A, Campanella M, Pinton P, Szabadkai G, and Reed JC, et al (2004). Bcl-2 and Bax exert opposing effects on Ca2+ signaling, which do not depend on their putative pore-forming region. *J Biol Chem* **279**(52), 54581–54589.

[106] Jones RG, Bui T, White C, Madesh M, Krawczyk CM, and Lindsten T, et al (2007). The proapoptotic factors Bax and Bak regulate T Cell proliferation through control of endoplasmic reticulum Ca(2+) homeostasis. *Immunity* **27** (2), 268–280.

[107] Mikhailov V, Mikhailova M, Degenhardt K, Venkatachalam MA, White E, and Saikumar P (2003). Association of Bax and Bak homo-oligomers in mitochondria. Bax requirement for Bak reorganization and cytochrome c release. *J Biol Chem* **278**(7), 5367–5376.

[108] Meijerink JP, Mensink EJ, Wang K, Sedlak TW, Sloetjes AW, and de Witte T, et al (1998). Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. *Blood* **91**(8), 2991–2997.

[109] Shi JL, Fu L, and Wang WD (2015). High expression of inositol 1,4,5-trisphosphate receptor, type 2 (ITPR2) as a novel biomarker for worse prognosis in cytogenetically normal acute myeloid leukemia. *Oncotarget* **6**(7), 5299–5309.

[110] Chaudhary KS, Abel PD, and Lalani EN (1999). Role of the Bcl-2 gene family in prostate cancer progression and its implications for therapeutic intervention. *Environ Health Perspect* **107**(Suppl 1), 49–57.

[111] Pinton P and Rizzuto R (2006). Bcl-2 and Ca2+ homeostasis in the endoplasmic reticulum. *Cell Death Differ* **13**(8), 1409–1418.

[112] Lam M, Dubyak G, Chen L, Nunez G, Miesfeld RL, and Distelhorst CW (1994). Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca2+ fluxes. *Proc Natl Acad Sci U S A* **91**(14), 6569–6573.

[113] Pinton P, Ferrari D, Magalhaes P, Schulze-Osthoff K, Di Virgilio F, and Pozzan T, et al (2000). Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx in Bcl-2-overexpressing cells. *J Cell Biol* **148**(5), 857–862.

[114] Martin B, Paesmans M, Berghmans T, Branle F, Ghisdal L, and Mascaux C, et al (2003). Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer* **89**(1), 55–64.

[115] Nishioka T, Luo LY, Shen L, He H, Mariyannis A, and Dai W, et al (2014). Nicotine increases the resistance of lung cancer cells to cisplatin through enhancing Bcl-2 stability. *Br J Cancer* **110**(7), 1785–1792.

[116] Karczmarek-Borowska B, Filip A, Wojcierowski J, Smolen A, Korobowicz E, and Korszen-Pilecka I, et al (2006). Estimation of prognostic value of Bcl-xL gene expression in non–small cell lung cancer. *Lung Cancer* **51**(1), 61–69.

[117] Malkinson AM (1998). Molecular comparison of human and mouse pulmonary adenocarcinomas. *Exp Lung Res* **24**(4), 541–555.

[118] Park D, Magis AT, Li R, Owonikoko TK, Sica GL, and Sun SY, et al (2013). Novel small-molecule inhibitors of Bcl-XL to treat lung cancer. *Cancer Res* **73** (17), 5485–5496.

[119] Krug LM, Miller VA, Filippa DA, Venkatraman E, Ng KK, and Kris MG (2003). Bcl-2 and bax expression in advanced non–small cell lung cancer: lack of correlation with chemotherapy response or survival in patients treated with docetaxel plus vinorelbine. *Lung Cancer* **39**(2), 139–143.

[120] Brooks KR, To KJoshi MB, Conlon DH, Herndon II JE, and D'Amico TA, et al (2003). Measurement of chemoresistance markers in patients with stage III non–small cell lung cancer: a novel approach for patient selection. *Ann Thorac Surg* **76**(1), 187–193 discussion 93.

[121] Song L, Coppola D, Livingston S, Cress D, and Haura EB (2005). Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non–small cell lung cancer cells. *Cancer Biol Ther* **4**(3), 267–276.

[122] Zhang H, Guttikonda S, Roberts L, Uziel T, Semizarov D, and Elmore SW, et al (2011). Mcl-1 is critical for survival in a subgroup of non–small-cell lung cancer cell lines. *Oncogene* **30**(16), 1963–1968.

[123] Huang H, Shah K, Bradbury NA, Li C, and White C (2014). 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**e1482.

[124] Mathupala SP, Rempel A, and Pedersen PL (2001). Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. *J Biol Chem* **276**(46), 43407–43412.

[125] Shinohara Y, Yamamoto K, Kogure K, Ichihara J, and Terada H (1994). Steady state transcript levels of the type II hexokinase and type 1 glucose transporter in human tumor cell lines. *Cancer Lett* **82**(1), 27–32.

[126] Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, and Wheaton W, et al (2013). Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Cancer Cell* **24**(2), 213–228.

[127] Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, and Hay N (2001). Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. *Genes Dev* **15**(11), 1406–1418.

[128] Miyamoto S, Murphy AN, and Brown JH (2008). Akt mediates mitochondrial protection in cardiomyocytes through phosphorylation of mitochondrial hexokinase-II. *Cell Death Differ* **15**(3), 521–529.

[129] Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, and Cairns R, et al (2011). Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. *J Exp Med* **208**(2), 313–326.

[130] Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, and Sasaki T, et al (1998). Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* **95**(1), 29–39.

[131] Salmena L, Carracedo A, and Pandolfi PP (2008). Tenets of PTEN tumor suppression. *Cell* **133**(3), 403–414.

[132] Bononi A, Bonora M, Marchi S, Missiroli S, Poletti F, and Giorgi C, et al (2013). Identification of PTEN at the ER and MAMs and its regulation of Ca(2+) signaling and apoptosis in a protein phosphatase-dependent manner. *Cell Death Differ* **20**(12), 1631–1643.

[133] Chalhoub N and Baker SJ (2009). PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol* **4**, 127–150.

[134] Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, and Hemmings BA, et al (1998). The lipid phosphatase activity of PTEN is critical for its tumor supressor function. *Proc Natl Acad Sci U S A* **95**(23), 13513–13518.

[135] Tibarewal P, Zilidis G, Spinelli L, Schurch N, Maccario H, and Gray A, et al (2012). PTEN protein phosphatase activity correlates with control of gene expression and invasion, a tumor-suppressing phenotype, but not with AKT activity. *Sci Signal* **5**(213), ra18.

[136] Kuchay S, Giorgi C, Simoneschi D, Pagan J, Missiroli S, and Saraf A, et al (2017). PTEN counteracts FBXL2 to promote IP3R3- and Ca2+-mediated apoptosis limiting tumour growth. *Nature* **546**(7659), 554–558.

[137] Krajewska M, Krajewski S, Epstein JI, Shabaik A, Sauvageot J, and Song K, et al (1996). Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. *Am J Pathol* **148**(5), 1567–1576.

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

[139] Morciano G, Pedrali G, Sbano L, Iannitti T, Giorgi C, and Pinton P (2016). Intersection of mitochondrial fission and fusion machinery with apoptotic pathways: role of Mcl-1. *Biol Cell* **108**(10), 279–293.

[140] Philley JV, Kannan A, Qin W, Sauter ER, Ikebe M, and Hertweck KL, et al (2016). Complex-I alteration and enhanced mitochondrial fusion are associated with prostate cancer progression. *J Cell Physiol* **231**(6), 1364–1374.

[141] Klecker T, Bockler S, and Westermann B (2014). Making connections: interorganelle contacts orchestrate mitochondrial behavior. *Trends Cell Biol* **24**(9), 537–545.

[142] Raturi A and Simmen T (2013). Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). *Biochim Biophys Acta* **1833**(1), 213–224.

[143] de Brito OM and Scorrano L (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456**(7222), 605–610.

[144] Schneeberger M, Dietrich MO, Sebastian D, Imbernon M, Castano C, and Garcia A, et al (2013). Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance. *Cell* **155**(1), 172–187.

[145] Filadi R, Greotti E, Turacchio G, Luini A, Pozzan T, and Pizzo P (2015). Mitofusin 2 ablation increases endoplasmic reticulum-mitochondria coupling. *Proc Natl Acad Sci U S A* **112**(17), E2174-1.

[146] Cosson P, Marchetti A, Ravazzola M, and Orci L (2012). Mitofusin-2 independent juxtaposition of endoplasmic reticulum and mitochondria: an ultrastructural study. *PLoS One* **7**(9)e46293.

[147] Wang PT, Garcin PO, Fu M, Masoudi M, St-Pierre P, and Pante N, et al (2015). Distinct mechanisms controlling rough and smooth endoplasmic reticulum contacts with mitochondria. *J Cell Sci* **128**(15), 2759–2765.

[148] Li N, Zoubeidi A, Beraldi E, and Gleave ME (2013). GRP78 regulates clusterin stability, retrotranslocation and mitochondrial localization under ER stress in prostate cancer. *Oncogene* **32**(15), 1933–1942.

[149] Vogelstein B, Lane D, and Levine AJ (2000). Surfing the p53 network. *Nature* **408**(6810), 307–310.

[150] Green DR and Kroemer G (2009). Cytoplasmic functions of the tumour suppressor p53. *Nature* **458**(7242), 1127–1130.

[151] Bergeaud M, Mathieu L, Guillaume A, Moll UM, Mignotte B, and Le Floch N, et al (2013). Mitochondrial p53 mediates a transcription-independent regulation of cell respiration and interacts with the mitochondrial F(1)F0-ATP synthase. *Cell Cycle* **12**(17), 2781–2793.

[152] Giorgi C, Bonora M, Sorrentino G, Missiroli S, Poletti F, and Suski JM, et al (2015). p53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner. *Proc Natl Acad Sci U S A* **112**(6), 1779–1784.

[153] Pinton P, Ferrari D, Rapizzi E, Di Virgilio F, Pozzan T, and Rizzuto R (2001). The Ca2+ concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. *EMBO J* **20**(11), 2690–2701.

[154] Giorgi C, Bonora M, Missiroli S, Morganti C, Morciano G, and Wieckowski MR, et al (2016). Alterations in mitochondrial and endoplasmic reticulum signaling by p53 mutants. *Front Oncol* **6**, 42.

[155] Bittremieux M and Bultynck G (2015). p53 and Ca(2+) signaling from the endoplasmic reticulum: partners in anti-cancer therapies. *Oncoscience* **2**(3), 233–238.

[156] Giorgi C, Bonora M, Missiroli S, Poletti F, Ramirez FG, and Morciano G, et al (2015). Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling. *Oncotarget* **6**(3), 1435–1445.

[157] Bernardi R, Scaglioni PP, Bergmann S, Horn HF, Vousden KH, and Pandolfi PP (2004). PML regulates p53 stability by sequestering Mdm2 to the nucleolus. *Nat Cell Biol* **6**(7), 665–672.

[158] Gurrieri C, Capodieci P, Bernardi R, Scaglioni PP, Nafa K, and Rush LJ, et al (2004). Loss of the tumor suppressor PML in human cancers of multiple histologic origins. *J Natl Cancer Inst* **96**(4), 269–279.

[159] Giorgi C, Ito K, Lin HK, Santangelo C, Wieckowski MR, and Lebiedzinska M, et al (2010). PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. *Science* **330**(6008), 1247–1251.

[160] Missiroli S, Bonora M, Patergnani S, Poletti F, Perrone M, and Gafa R, et al (2016). PML at mitochondria-associated membranes is critical for the repression of autophagy and cancer development. *Cell Rep* **16**(9), 2415–2427.

[161] Sakakura C, Hagiwara A, Fukuda K, Shimomura K, Takagi T, and Kin S, et al (2003). Possible involvement of inositol 1,4,5-trisphosphate receptor type 3

(IP3R3) in the peritoneal dissemination of gastric cancers. *Anticancer Res* **23** (5A), 3691–3697.

[162] Shibao K, Fiedler MJ, Nagata J, Minagawa N, Hirata K, and Nakayama Y, et al (2010). The type III inositol 1,4,5-trisphosphate receptor is associated with aggressiveness of colorectal carcinoma. *Cell Calcium* **48**(6), 315–323.

[163] Bononi A, Giorgi C, Patergnani S, Larson D, Verbruggen K, and Tanji M, et al (2017). BAP1 regulates IP3R3-mediated Ca2+ flux to mitochondria suppressing cell transformation. *Nature* **546**(7659), 549–553.

[164] Maxfield KE, Taus PJ, Corcoran K, Wooten J, Macion J, and Zhou Y, et al (2015). Comprehensive functional characterization of cancer-testis antigens defines obligate participation in multiple hallmarks of cancer. *Nat Commun* **6**, 8840.

[165] Doghman-Bouguerra M, Granatiero V, Sbiera S, Sbiera I, Lacas-Gervais S, and Brau F, et al (2016). FATE1 antagonizes calcium- and drug-induced apoptosis by uncoupling ER and mitochondria. *EMBO Rep* **17**(9), 1264–1280.

[166] Sbiera S, Leich E, Liebisch G, Sbiera I, Schirbel A, and Wiemer L, et al (2015). Mitotane inhibits sterol-O-acyl transferase 1 triggering lipid-mediated endoplasmic reticulum stress and apoptosis in adrenocortical carcinoma cells. *Endocrinology* **156**(11), 3895–3908.

[167] Youle RJ and Karbowski M (2005). Mitochondrial fission in apoptosis. *Nat Rev Mol Cell Biol* **6**(8), 657–663.

[168] Lee YJ, Jeong SY, Karbowski M, Smith CL, and Youle RJ (2004). Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol Biol Cell* **15**(11), 5001–5011.

[169] Iwasawa R, Mahul-Mellier AL, Datler C, Pazarentzos E, and Grimm S (2011). Fis1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction. *EMBO J* **30**(3), 556–568.

[170] Ferreira-da-Silva A, Valacca C, Rios E, Populo H, Soares P, and Sobrinho-Simoes M, et al (2015). Mitochondrial dynamics protein Drp1 is overexpressed in oncocytic thyroid tumors and regulates cancer cell migration. *PLoS One* **10**(3)e0122308.

[171] Cheng WC, Teng X, Park HK, Tucker CM, Dunham MJ, and Hardwick JM (2008). Fis1 deficiency selects for compensatory mutations responsible for cell death and growth control defects. *Cell Death Differ* **15**(12), 1838–1846.

[172] Gross O, Thomas CJ, Guarda G, and Tschopp J (2011). The inflammasome: an integrated view. *Immunol Rev* **243**(1), 136–151.

[173] Zhou R, Yazdi AS, Menu P, and Tschopp J (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**(7329), 221–225.

[174] Daley D, Mani VR, Mohan N, Akkad N, Pandian G, and Savadkar S, et al (2017). NLRP3 signaling drives macrophage-induced adaptive immune suppression in pancreatic carcinoma. *J Exp Med* **214**(6), 1711–1724.

[175] Dupaul-Chicoine J, Arabzadeh A, Dagenais M, Douglas T, Champagne C, and Morizot A, et al (2015). The Nlrp3 inflammasome suppresses colorectal cancer metastatic growth in the liver by promoting natural killer cell tumoricidal activity. *Immunity* **43**(4), 751–763.

[176] Okamoto M, Liu W, Luo Y, Tanaka A, Cai X, and Norris DA, et al (2010). Constitutively active inflammasome in human melanoma cells mediating autoinflammation via caspase-1 processing and secretion of interleukin-1beta. *J Biol Chem* **285**(9), 6477–6488.

[177] Downward J (2003). Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* **3**(1), 11–22.

[178] Pierro C, Cook SJ, Foets TC, Bootman MD, and Roderick HL (2014). Oncogenic K-Ras suppresses IP(3)-dependent Ca(2)(+) release through remodelling of the isoform composition of IP(3)Rs and ER luminal Ca(2)(+) levels in colorectal cancer cell lines. *J Cell Sci* **127**(Pt 7), 1607–1619.