



Cancer: Untethering Mitochondria from the Endoplasmic Reticulum?

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Following the discovery of the mitochondria-associated membrane (MAM) as a hub for lipid metabolism in 1990 and its description as one of the first examples for membrane contact sites at the turn of the century, the past decade has seen the emergence of this structure as a potential regulator of cancer growth and metabolism. The mechanistic basis for this hypothesis is that the MAM accommodates flux of Ca^{2+} from the endoplasmic reticulum (ER) to mitochondria. This flux then determines mitochondrial ATP production, known to be low in many tumors as part of the Warburg effect. However, low mitochondrial Ca^{2+} flux also reduces the propensity of tumor cells to undergo apoptosis, another cancer hallmark. Numerous regulators of this flux have been recently identified as MAM proteins. Not surprisingly, many fall into the groups of tumor suppressors and oncogenes. Given the important role that the MAM could play in cancer, it is expected that proteins mediating its formation are particularly implicated in tumorigenesis. Examples for such proteins are mitofusin-2 and phosphofurin acidic cluster sorting protein 2 that likely act as tumor suppressors. This review discusses how these proteins that mediate or regulate ER–mitochondria tethering are (or are not) promoting or inhibiting tumorigenesis. The emerging picture of MAMs in cancer seems to indicate that in addition to the downregulation of mitochondrial Ca^{2+} import, MAM defects are but one way how cancer cells control mitochondria metabolism and apoptosis.

Keywords: mitochondria-associated membrane, mitochondria-endoplasmic reticulum contacts, mitofusin-2, metabolism, oncoprotein, tumor suppressor

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INTRODUCTION: DISRUPTION OF MITOCHONDRIAL METABOLISM IN CANCER

Lost control of mitochondria metabolism is a central cancer hallmark (1), although not all tumors are characterized by this property. As a consequence, cancer cells frequently rewire their metabolism to rely on glucose even in the presence of oxygen and, thus, reduce their reliance on mitochondria (2). In parallel, tumor cells exhibiting this so-called Warburg phenotype must increase their glycolytic capacity.

Multiple cancer signaling pathways are associated with the glycolytic signature of cancer. For instance, the excessive growth of many solid tumors results in large portions of the tumor mass becoming hypoxic, which subsequently induces production of key glycolysis enzymes *via* the HIF1 α transcription factor (3), including glucose transporters (e.g., GLUT1) or glycolytic enzymes (e.g., phosphofructokinase) (4). Upon their induction, these enzymes shift energy generation away from mitochondria toward glycolysis and glutaminolysis (5–8). This allows tumor cells to accumulate more biomass through increased uptake and metabolism of glucose (2, 9–13). In parallel,

while glycolysis only produces two ATP molecules per glucose molecule, compared to 36 molecules of ATP from the complete oxidation of glucose within mitochondria, glycolysis can still result in higher energy production due to speedier progression of this pathway and higher ATP production per time rate (14). Increased ATP consumption could further accelerate this pathway and could result in almost 100 times faster ATP generation than oxidative phosphorylation (15). Under these conditions, glucose is converted into lactate by conversion of pyruvate through lactate dehydrogenase (LDH) (16, 17). When LDH produces this glycolysis end product, it also replenishes NAD⁺ levels, which act to make the cytosol more oxidizing (18, 19). In tumor cells, however, lactate can also be shuttled to mitochondria, where it can be metabolized to synthesize lipids (20). Secreted leftover lactate contributes to the altered tumor microenvironment by lowering the extracellular pH, activation of the VEGF signaling pathway (21), and driving cell migration (22), to name but a few consequences (23). Together, the increased presence of lactate caused by tumor metabolism critically manipulates multiple metabolic pathways and cell biological mechanisms.

In parallel, HIF1 α can also achieve another characteristic of the Warburg phenotype: the repression of oxidative phosphorylation by cooperating with c-Myc to drive transactivation of pyruvate dehydrogenase kinase 1 (PDK1) and its relatives (24, 25). The induction of this enzyme not only directly reduces mitochondrial oxygen consumption but also further promotes glycolysis by decreasing pyruvate flow to mitochondria, while increasing its conversion to lactate (24, 25). Therefore, the inhibition of PDK1 and related kinases by RNAi or dichloroacetate can potentially rescue some of the metabolic changes in tumor tissue (26).

While it was clear for a long time that cancer mitochondria make less ATP, it had initially been questioned whether the reason for this defect is found within the proteins making up the electron transport chain within mitochondria (27). However, many types of cancer result in a relative depletion of mtDNA, when compared to neighboring tissue (28), as one would expect given the important links between mitochondrial metabolism and cancer. Moreover, numerous mitochondrial enzymes encoded by nuclear or mitochondrial DNA show mutations (29). Specifically, mutations in mtDNA can indirectly affect enzymes of the Krebs cycle, including fumarate hydratase (30), and isocitrate dehydrogenase (31). Moreover, mutations in nuclear-encoded succinate dehydrogenase can by themselves cause paraganglioma (32, 33), potentially from increased ROS production within mitochondria that leads to oxidative damage and eventually transformation (34). Somatic mutations of mtDNA have been discovered in a wide variety of cancers, including colorectal, ovarian, renal, and lung cancers (35–38). Moreover, the depletion of mtDNA by itself can act as tumorigenic *in vitro* as well as *in vivo* (39). Such a loss of mtDNA, as well as mitochondrial mass, can be caused, for instance, by mutations of p53 (40). While an outright loss of mtDNA can sometimes paradoxically reduce mitochondrial ROS production due to arrest of oxidative phosphorylation (40), cancer tissue is normally characterized by increased levels of ROS, due to the rapid growth of tumors (41). Therefore, more frequently, mtDNA mutations accelerate tumor progression *via* increased ROS production that leads to further mutations within

nuclear and mitochondrial DNA (42). This sets a dangerous cycle in motion that further increases the level of oxidative stress (43, 44). Accordingly, the type II diabetes drug metformin, which reduces both ROS and inhibits complex I (45, 46), reduces the risk of developing cancer (47, 48). This finding suggests that regulation of mitochondrial ROS production is an important point of intervention for the treatment of cancer. In addition, it is also clear that proteins regulating the progression of mitochondrial oxidative phosphorylation and, thus, production of mitochondrial ROS must be found on the list of tumor suppressors and oncoproteins.

ONCOPROTEINS AND TUMOR SUPPRESSORS USE THE ENDOPLASMIC RETICULUM (ER) AS A PLATFORM TO CONTROL MITOCHONDRIA

Recent progress has determined that besides proteins mediating oxidative phosphorylation themselves, regulatory proteins outside mitochondria could determine mitochondrial ROS and tumorigenesis. An attractive location to execute such a function is the mitochondria-associated membrane (MAM) (49). This intracellular signaling hub houses Ca²⁺ exchange between the ER and mitochondria that is required for mitochondrial dehydrogenases and, thus, oxidative phosphorylation (49). Accordingly, cells with blocked active Ca²⁺ release from the ER produce less than half of their normal amount of mitochondrial ATP (50). A need for ER–mitochondria cross talk to fully engage cellular metabolism and energy production had been anticipated in early studies of Bernhard and Rouiller on the regenerating liver, where ER and mitochondria form close appositions in a fasting–feeding-dependent manner (51, 52). Today, altered Ca²⁺ signaling at the MAM is recognized as a hallmark of cancer cells that shifts their metabolism to glycolysis and increases their resistance to cell death (53).

Early studies had identified the MAM as a lipid synthesis platform, where phosphatidylethanolamine (PE) production requires the apposition between the ER and mitochondria (54–56). It is, therefore, not surprising that MAM lipid enzymatic activities are essential for normal Ca²⁺ signaling (57). Critically, the MAM represents a detergent-resistant membrane that forms a locally cholesterol-enriched raft (58, 59). This structure is enriched in the sigma-1 receptor (60), the ER prohibitin-related proteins erlin-1 and erlin-2 (61), the ubiquitin ligase gp78 (62), and the ER oxidoreductase TMX1 (63). Therefore, one way how tumor cells could silence mitochondria metabolism and apoptotic signaling would be by altering ER lipid domain formation that could disrupt normal MAM rafts.

Consistent with such a possibility, a variety of lipid-interfering strategies are currently in development to trigger ER stress-related apoptosis in cancer cells and have been reviewed recently (64). This idea is based on findings that show that cholesterol loading of the ER leads to ER stress and subsequent apoptosis of a variety of cell types (65–67). Mechanistically, this excess cholesterol efficiently blocks ER sarco/ER Ca²⁺-ATPase (SERCA) that pumps Ca²⁺ into the ER, thus resulting in the transfer of Ca²⁺

to mitochondria (68). Similarly, ER lipid saturation, achieved by elevated phosphatidylcholine over PE (69) or palmitate (70), activates the ER stress response and apoptosis *via* the inhibition of SERCA.

However, these mechanisms might all represent a drastic, artificial phenotype that cannot be exploited for cancer therapy and does not operate in the same way within cancer cells. Moreover, their potential links between cancer cell biology and MAM-related mechanisms are currently obscure. Even when restricting to studies on cholesterol and tumor cell function and survival, no clear picture emerges. For example, mitochondria of cancer cells are more susceptible to increases in cholesterol, which tend to trigger ER stress and apoptosis in this system more readily than in normal cells (67, 71). However, cholesterol-lowering drugs such as statins also trigger mitochondria-based apoptosis (72), possibly because cancer mitochondria operate with 2- to 10-fold more cholesterol than mitochondria of normal cells (73). Cholesterol- and PE-rich mitochondria also provide more resistance to Bax-mediated membrane permeabilization (74, 75). We must conclude that cancer cells might be influenced by cholesterol and that altering the lipid balance of cancer cells could affect their MAM rafts, but that a clear outcome of such interventions within cancer cells has not yet emerged. A potential explanation for these observations is that lipid storage at an early stage of cancer changes to lipolysis accompanied by increased cholesterol synthesis in advanced cancer (76, 77). In the context of this review, these observations suggest that the lipid- and cholesterol-dependent structure that is the MAM may undergo tumor stage-specific changes.

Nevertheless, consistent with altered lipid and cholesterol content of cancer mitochondria (73), ER-mitochondria tethering might be different at least in a subset of cancers. This hypothesis has been put forward over 60 years ago by Howatson and Ham (78), who observed reduced numbers of mitochondria and of ER-mitochondria contacts in liver cancer. These observations anticipated follow-up observations, which also detected lower amounts of mitochondria in tumor tissue in multiple instances (79–81). Moreover, despite (or maybe because of) their potentially increased distance from intracellular Ca^{2+} sources, some tumor cell mitochondria have an increased Ca^{2+} uptake capacity compared to mitochondria from normal tissue when examined as isolated entities *in vitro* (82, 83). This property is also reflected in the upregulation of mitochondrial Ca^{2+} uniporters (MCUs) in breast cancer cells (84). Potentially, however, MCU is also a target of miR-25 that can reduce its amount in cancer cells (85). While these findings suggest more research is needed to assess the role of MCU in cancer, they also demonstrate that tumor mitochondria have different Ca^{2+} handling. Moreover, the end result of both observations for cancer mitochondria could actually be the same. While some tumor mitochondria may import less Ca^{2+} *via* reduced amounts of MCU, others may show increased Ca^{2+} uptake capacity from a compensation for decreased Ca^{2+} availability in the tumor cell, maybe due to defective ER-mitochondria tethering. Generally speaking, these findings also indicate that cancer cells undertake massive remodeling of upstream signaling mechanisms that result in reduced mitochondrial ATP output in cancer cells, as postulated by Warburg (2). Importantly, as an end

result, this remodeling may very well end up making the residual power generation within mitochondria essential (86).

Consistent with such a modulated ER-mitochondria Ca^{2+} flux, MAM-localized oncoproteins and tumor suppressors indeed interact with Ca^{2+} handling proteins and modulate their activity. Consistent with this hypothesis, the tumor suppressors and oncoproteins p53 (87), the phosphatase tensin homolog (PTEN) (88), the kinase Akt (89), breast/ovarian cancer susceptibility gene 1 (BRCA1) (90), and the promyelocytic leukemia (PML) protein (91, 92) all localize to mitochondria or to ER-mitochondria contacts. Here, they exert their cancer-regulating activities by interacting with Ca^{2+} -handling proteins and either boost ER-mitochondria Ca^{2+} flux (tumor suppressors) or inhibit it (oncoproteins), for details see **Figure 1**.

For instance, p53 is enriched on the MAM, where it interacts with the ER Ca^{2+} pump SERCA and promotes ER-mitochondria Ca^{2+} cross talk not only to induce apoptosis but also to promote ATP production needed within the ER (93). Thus, the tumor suppressor p53 acts to beneficially facilitate existing mechanisms and, thus, overall improves mitochondrial functioning. In addition, it appears that these p53 functions depend on mitochondrial ROS production, since ROS induce p53 enrichment on mitochondria, where it can promote the opening of the permeability transition pore (PTP) (94) upon a stabilizing interaction with Hsp90 (95). This finding provides another connection between mitochondrial Ca^{2+} signaling, oxidative phosphorylation, and p53, but the interaction of this tumor suppressor with the mitochondrial proton gradient and Ca^{2+} signaling goes further. p53 can also influence mitochondria function *via* direct interaction with the F_1F_0 -ATPase to promote respiration and reduce ROS production (96). Interestingly, this function could directly link p53 to the control of mitochondrial permeability transition, since the F_1F_0 -ATPase or parts of it are the most likely candidates for forming the mitochondrial PTP (97–99). Given that hexokinase II localizes to the PTP (100), from where it increases the use of glucose (101), p53 and hexokinase II may oppose each other in the control of tumor cell growth, as is indeed the case in castration-resistant prostate cancer (102). Interestingly, hexokinase II binding to voltage-dependent anion channel (VDAC) increases in parallel with cholesterol loading of mitochondria, thus providing additional evidence that cancer-associated alterations of MAM and mitochondria properties shift cellular energy generation to glycolysis (73). All of these MAM-associated functions depict p53 as a factor that would beneficially control mitochondrial oxidative phosphorylation: not only as a gatekeeper, which would promote ER-mitochondria Ca^{2+} flux, but also as a chaperone, which can make mitochondrial ATP production more efficient and which can arrest mitochondrial ATP production in the case of excessive ROS production.

An important question that cell biologists are currently trying to answer is whether other mitochondrial regulatory proteins could fulfill similar roles to the ones described above for p53, PTEN, Akt, BRCA1, and PML. Given the characteristic mechanisms that these proteins use to influence mitochondrial metabolism and apoptosis regulation, such proteins should influence mitochondrial ROS and ATP production, likely *via* influencing the availability of Ca^{2+} within mitochondria. While

Protein/Complex	Tumor suppressor evidence	Oncoprotein evidence
p53	-During stress, p53 becomes enriched at the MAM. Here it binds SERCA and changes its oxidative state, leading to greater Ca ²⁺ influx into mitochondria and subsequent apoptosis (Giorgi <i>et al.</i> , 2015) -ROS induces mitochondrial enrichment, where it can promote permeability transition pore opening (Vaseva <i>et al.</i> , 2012, Alexandrova <i>et al.</i> , 2015) -p53 interacts with and activates the F ₁ F ₀ -ATPase and reduces ROS production (Bergeaud <i>et al.</i> , 2013)	
PTEN	-PTEN silencing reduces sensitivity to Ca ²⁺ mediated apoptosis since PTEN is found at the MAM and can interact with IP3Rs to regulate Ca ²⁺ flux into mitochondria (Bononi <i>et al.</i> , 2013)	
Akt		-mTORC2 and Akt localize to the MAM, where mTORC2 activates Akt to phosphorylate IP3R, PACS-2 and hexokinase 2. Apart from regulating apoptosis by regulating IP3R phosphorylation, and hence activity, Akt-mediated phosphorylation of Hexokinase 2 has been proposed to be responsible for increased glycolysis in cancer. (Betz <i>et al.</i> , 2013)
BRCA1	-BRCA1 is found at the ER, but becomes enriched during apoptotic signaling, where it binds to IP3R and sensitizes it to its ligand, IP3, thereby sensitizing cells for Ca ²⁺ mediated apoptosis (Hedgepeth <i>et al.</i> , 2015)	
PML	-PML is found in the MAM, where it can form complexes with Akt, PP2a (protein phosphatase 2 a) and IP3R, both regulators of IP3R activity, and therefore modulate Ca ²⁺ mediated apoptosis (Giorgi <i>et al.</i> , 2010) -PML is normally found in MAMs and represses autophagy. Under stress, its relocalization out of the MAM is required for autophagy induction. In cancer, its absence allows increased autophagy levels to provide nutrients to the tumor. (Missiroli <i>et al.</i> , 2016)	
Mitofusin-2	-Mitofusin-2 silencing protects against several kinds of apoptosis triggers (Guo <i>et al.</i> , 2007) -Lower Mitofusin-2 staining correlates with higher tumor size and stage (Wang <i>et al.</i> , 2015b) -Mitofusin-2 silencing reduces ER-Ca ²⁺ flux and therefore protects against apoptosis (Wang <i>et al.</i> , 2015b, Wu <i>et al.</i> , 2016) -Mitofusin-2 upregulation decreases tumor growth and metastasis <i>in vivo</i> and <i>in vitro</i> (Zhou <i>et al.</i> , 2016) -Mitofusin-2 sensitizes breast cancer cells to apoptosis and represses proliferation (Ma <i>et al.</i> , 2015)	-Mitofusin-2 silencing suppresses cell proliferation, likely by regulating autophagy (Ding <i>et al.</i> , 2015) -Mitofusin-2 silencing reduces glycolysis and oxygen consumption (Ding <i>et al.</i> , 2015)
PACS-2	-PACS-2 is a hotspot for genomic instability in colorectal cancer (Anderson <i>et al.</i> , 2001)	
Nogo-B/reticulon-4B	-Small cell lung cancer and Adult T-cell leukemia/lymphoma (ATLL) have low Nogo-B levels. (Li <i>et al.</i> , 2001), Shimakage <i>et al.</i> , 2006) -Overexpression of Nogo-B in cancer cells restores apoptosis sensitivity (Li <i>et al.</i> , 2001) -Nogo-A/B downregulation maybe a marker for melanoma progression since it is downregulated in metastatic melanomas as opposed to primary tumors (Calik <i>et al.</i> , 2016)	
PERK	- Mitofusin-2 interacts with and inhibits PERK at the MAM. Mitofusin-2 knockout, PERK knockdown cells are resistant to apoptosis (van Vliet & Agostinis, 2016) -New evidence suggests PERK might act as a tumor suppressor in complete knockouts (Pyltel <i>et al.</i> , 2016)	-Historically, tumor cells expressing PERK have been shown to exhibit accelerated tumor progression (Feis & Koumenis, 2006) -PERK promotes angiogenesis and cytoprotective autophagy in tumors, and in general, chemoresistance (Bu & Djehli, 2016) - New evidence suggests PERK haplo-insufficiency in tumors promotes tumorigenesis (Pyltel <i>et al.</i> , 2016)
ARCSome (BAP31 and Fis1)	-Downregulation of Fis1 in tumors results in increased apoptosis resistance (Wang <i>et al.</i> , 2012)	-Targeting overexpressed BAP31 in melanoma has anti-tumor activity (Yu <i>et al.</i> , 2015)
IP3R, Grp75, VDAC		-ER Ca ²⁺ transfer to mitochondria is essential in cancerous cells (Cardenas <i>et al.</i> , 2016) -VDAC is expressed highly in tumors (Shoshan-Barmatz <i>et al.</i> , 2015) and can serve as a marker for tumor progression (Pernemalm <i>et al.</i> , 2013) -Grp75 overexpression increases tumor aggressiveness (Wadhawa <i>et al.</i> , 2006) -Metastatic liver has higher Grp75 levels (Yi <i>et al.</i> , 2008) -Grp75 sequesters and inactivates p53 in some cancer cell lines (Merrick <i>et al.</i> , 1996)
PTPIP51, VAPB		-PTPIP51 is upregulated in glioblastoma and may serve as marker for tumor progression (Petri <i>et al.</i> , 2015, Petri <i>et al.</i> , 2011) -VAPB promotes breast tumor growth potentially due to increased Akt activity (Rao <i>et al.</i> , 2012)

FIGURE 1 | Description of key evidence on the role of mitochondria-associated membrane (MAM) tethering factors as tumor suppressors or oncoproteins.

recent reviews have provided outstanding global overviews of this hypothesis that we recommend the reader to consult as well (53, 103, 104), our review will specialize on the most central subcategory of proteins regulating the availability of Ca²⁺ within mitochondria. These are the proteinaceous tethers between the ER and mitochondria. While the identity of such proteinaceous tethers is currently much better understood in the yeast model system (105), where the ER–mitochondria encounter structure (ERMES) and ER membrane protein complex (EMC) are known or implicated in tethering the two organelles (106, 107), respectively, numerous proteins have been implicated in the formation or regulation of ER–mitochondria tethers in human cells (108). We will discuss these tether protein complexes as well as tethering regulators below. The current knowledge about ER–mitochondria tethers in cancer is summarized in **Figures 1** and **2**.

Mitofusin-2

Mitofusins are a pair of GTPases that promote mitochondrial fusion (109). Mitofusins also determine ER–mitochondria apposition through a variety of proposed mechanisms. The most recent findings about their role for ER–mitochondria contacts suggest that they determine the outer mitochondrial membrane (OMM) protein composition (110). Through this function, mitofusins determine the surface properties of mitochondria, which could impact the interaction of mitochondria with the ER. Indeed, confirming this hypothesis, the expression balance between the two mitofusins regulates the relative apposition

between mitochondria and the rough and smooth ER (rER/sER). Specifically, mitofusin-1 appears to inhibit the formation of sER–mitochondria contacts, whereas mitofusin-2 appears to interfere with the formation of rER–mitochondria contacts in cells with increased mitofusin-1 levels (111, 112). Currently, it is unclear whether this effect is *via* a direct regulation of contact formation between the two subpopulations of the ER with mitochondria, or whether the influence of the mitofusins on mitochondrial OMM proteins could explain these findings. In the latter scenario, protein subdomains on the OMM could mediate contact formation preferentially with the rER or sER. Regardless, these two findings clearly indicate that mitofusins determine the interaction between the ER and mitochondria. However, the exact role and the actual consequences of the mitofusins for this interorganellar interaction are currently being hotly debated.

In the case of mitofusin-2, the role for ER–mitochondria tethering extends beyond the regulation of the proportion between sER and rER–mitochondria contact formation, since the Scorrano lab had identified this mitochondrial GTPase as globally critical for MAM formation in mammalian cells (113). This role of mitofusin-2 in ER–mitochondria tethering was identified *via* a loss of FRET signal from two distinct ER–mitochondria proximity indicator probes (114, 115), by a reduction of fluorescence signal overlap between mitochondrial RFP and ER YFP, by a reduced mitochondrial uptake of IP₃R-released ER Ca²⁺ (113), by reduced numbers of ER tubules in the proximity of mitochondria on electron micrographs (116),

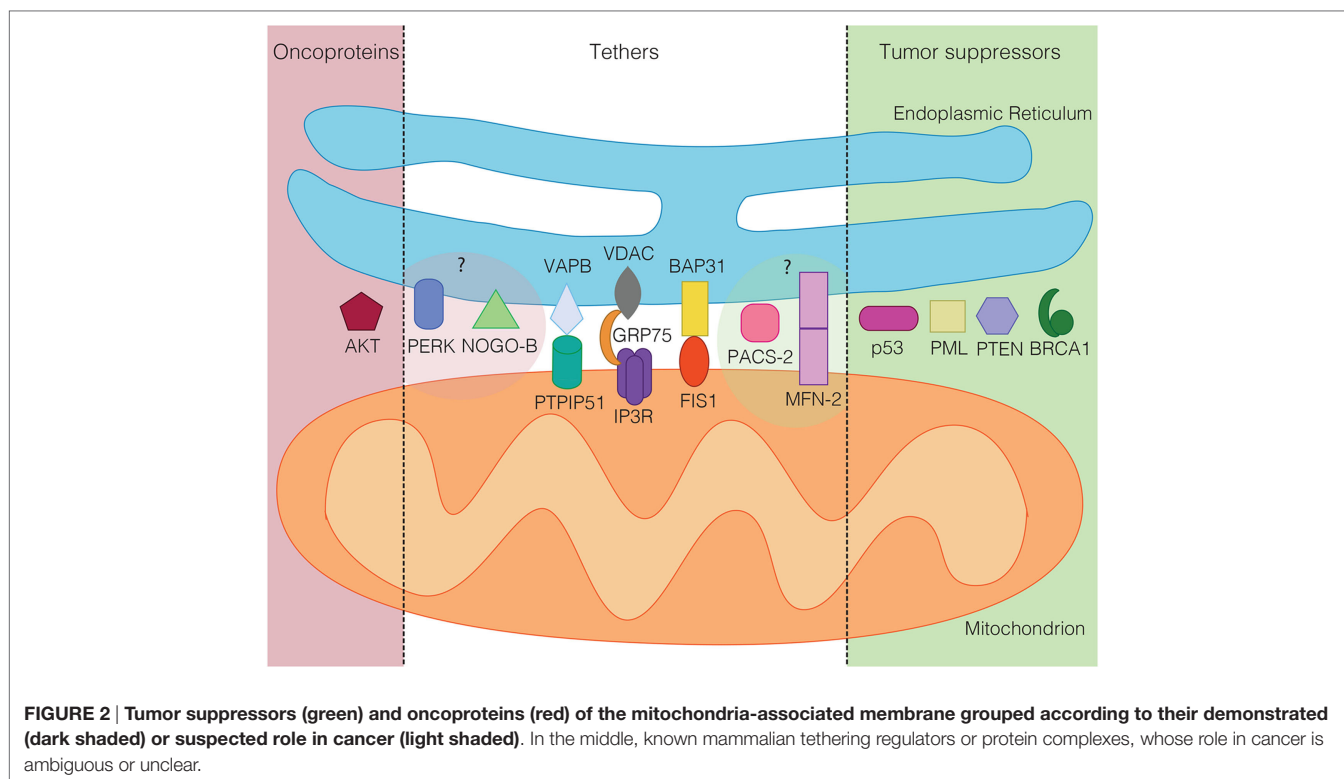


FIGURE 2 | Tumor suppressors (green) and oncoproteins (red) of the mitochondria-associated membrane grouped according to their demonstrated (dark shaded) or suspected role in cancer (light shaded). In the middle, known mammalian tethering regulators or protein complexes, whose role in cancer is ambiguous or unclear.

by increased resistance to ER stress-mediated apoptosis (117), and by a reduction of a coefficient that measures the extent of close ER contacts relative to the total mitochondria surface (115). However, these results did not unequivocally determine whether mitofusin-2 is an actual tether or whether it simply controls tethering. Moreover, despite these multiple results from many experimental approaches suggesting mitofusin-2 is an ER-mitochondria tether, these findings have been challenged by studies that measured the actual distance of ER-mitochondria contacts *via* electron microscopy and found a decrease in ER-mitochondria contacts in *Mfn2*^{-/-} cells (118, 119). Moreover, etoposide- and ceramide-mediated apoptosis proceeds faster in *Mfn2* knockdown cells (119). As discussed by others and us recently (105, 108), multiple hypotheses could explain these discrepant findings. In our opinion, a compelling observation has been made recently by the Scorrano lab, which identified cellular culture conditions as critical for the role of mitofusin-2 for the formation of MAMs (115). This explanation would be an extension of the observations that mitofusin-2 knockout cells exhibit ER stress and that ER stress increases MAM contact formation (120, 121). Regardless of these outstanding questions, it is undisputed by all researchers of the field that mitofusins, and particularly mitofusin-2, are important regulators of MAM contacts. However, further research will have to determine the biogenesis and consequences of the reported phenotypes.

A role in ER-mitochondria contact formation raises the possibility that mitofusin-2 could also play a role as an oncoprotein or tumor suppressor. Since normal apoptosis progression requires a functional MAM, understanding its role in cancer

may provide clues as to what function mitofusin-2 performs for the MAM. Indeed, and consistent with a role of mitofusin-2 as a MAM promoter, cancer cells with high levels of mitofusin-2 are more susceptible for apoptosis and more competent for ER-mitochondria Ca²⁺ flux (122–124). Further demonstrating the tumor-suppressive role of mitofusin-2, a panel of hepatocellular carcinoma (HCC) showed significant downregulation of mitofusin-2 and correlated with worse overall survival (125). Accordingly, mitofusin-2 mRNA is targeted by miR-761 in HCC tissues. The upregulation of mitofusin-2 *via* inhibiting miR-761 decreased tumor growth and metastasis both *in vivo* and *in vitro* (126). Similar findings have been reported from breast cancer cells, where the ectopic expression of mitofusin-2 leads to pro-apoptotic and antiproliferative signaling (127). Consistent with these findings, mitofusin-2 knockdown leads to reduced respiration, presumably due to blocked ER-mitochondria Ca²⁺ flux, but also reduces glycolysis, thus reducing overall ATP levels in HeLa cells (128). Together, these observations indicate that mitofusin-2 is a factor in cancer that typically results as reduced or absent in the cancer scenario (Figures 1 and 2). While some of the findings may turn out to be cell-type specific, these findings are more consistent with a role of mitofusin-2 as a MAM promoter and a tumor suppressor.

Phosphofurin Acidic Cluster Sorting Protein 2 (PACS-2)

About 10 years ago, the cytosolic PACS-2 was identified as a homolog of the previously identified PACS-1 (129, 130). Unlike its closely related sister protein PACS-1 that

regulates trafficking at the level of the trans-Golgi network and endosomes (131), PACS-2 determines the interaction between the ER and mitochondria, consistent with its partial localization to these organelles (130). Besides targeting of Bid to mitochondria and other functions described elsewhere (132), PACS-2 is required for the proper formation of the MAM (57, 133).

Here, PACS-2 acts as a MAM tethering regulator, but likely does not function as a MAM tether on its own. PACS-2 knockdown or knockout, nevertheless, interferes with several key MAM functions. For instance, PACS-2 knockdown detaches the ER from mitochondria, as seen by light and electron microscopy (130). This and other activities of PACS-2 depend on its serine 437 residue, which promotes active PACS-2 (134). Moreover, the phosphorylation of this site by Akt is a prerequisite to maintain MAM formation and is downstream of mammalian target of rapamycin complex 2 (mTORC2) (89). mTORC2/Akt-mediated phosphorylation of PACS-2 maintains proper Ca^{2+} availability for mitochondria, needed for mitochondria metabolism (50), but also apoptosis progression (135). From this insight, it makes, perfect sense that in a cancer scenario, PACS-2 is a hot spot of chromosome instability, as indeed observed in colorectal cancer (136), possibly in a stage-dependent manner (137). Therefore, similar to the better-characterized mitofusin-2, it is expected that PACS-2 acts as a tumor suppressor (Figures 1 and 2), whose absence would be indeed expected to lead to ER–mitochondria uncoupling, but this has not been determined at this point. No information is currently available about cancer-associated mutations in PACS-2, but it is clear that the regulatory serine 437 residue would correspond to a prime candidate.

Nogo-B/Reticulon-4B

Like mitofusin-2 and PACS-2, Nogo-B/reticulon-4B is a structural regulator of the ER, promoting the formation of tubular ER (138). Compared to the highly related Nogo-A that is restricted to neuronal cells, Nogo-B is expressed ubiquitously (139). Upon overexpression of this protein, the proportion of tubular ER increases over sheet-like ER (138). A Nogo-B overexpression could occur, for instance, during ER stress or hypoxia that leads to increased reticulon-4 expression dependent on the ER transcription factor ATF6 (140). Interestingly in the context of this review, increased Nogo-B expression associated with hypoxia increases the distance between the ER and mitochondria, suggesting that Nogo-B acts as an inhibitor of ER–mitochondria tethering (141). Nogo-B is not the only reticulon that localizes to the MAM and whose overexpression modulates ER–mitochondria contact formation: the same has been reported for reticulon-1C, although its activity seems to be opposite (142).

Again, like in the case of mitofusin-2 and PACS-2, the question arises as to what is the functional basis of a role for Nogo-B in regulating the apposition between the ER and mitochondria. It appears that a common pattern is emerging, where ER- and mitochondria-associated factors that determine their respective membrane composition or shape also increase or decrease organellar apposition. This is again confirmed upon knockout of Nogo-B. In this scenario, ER tubulation is lost and the diameter

of ER structures increases (143). Apparently contradicting a role as a MAM inhibitor, cells lacking Nogo-B are resistant to apoptosis, which normally depends on ER–mitochondria Ca^{2+} flux. While this finding could suggest that the role of Nogo-B is less clear than anticipated, this effect could also depend on a role of Nogo-B on the apposition between the ER and the plasma membrane: Nogo-B-deficient cells exhibit decreased store-operated Ca^{2+} entry, which suggests that this reticulon acts to increase contacts between the ER and the plasma membrane (143). This observation raises the important issue that ER tethering factors could promote apposition in the case of contacts with one organelle, but decrease apposition in the case of contacts with other organelles.

If our hypothesis were correct that ER–mitochondria tethering antagonizes tumor progression, then we would expect to find expression of a MAM-inhibitory Nogo-B to be high in cancer. However, the first paper linking Nogo-B to cancer found this to be the opposite (144): ectopic expression of Nogo-B restores apoptosis susceptibility in cancer cells, and small cell lung cancer was found to exhibit low levels of Nogo-B. Similarly, low levels of Nogo-B were found in leukemia and lymphoma (145), as well as in metastatic malignant melanoma (146). Along these lines, it is possible to see the effect of Ras transformation that results in cleavage of Nogo-B as a disruption of its MAM-regulating activities (147). Together, these observations are more consistent with a role of Nogo-B in promoting cell death.

While these findings may suggest that MAM tethering cannot be unequivocally tied to tumor suppression, additional cancer-relevant functions could complicate the role in cancer for Nogo-B. Besides the previously mentioned role for ER–plasma membrane apposition by Nogo-B, such functions have indeed been detected in the case of Nogo-A, the Nogo form expressed in the central nervous system that acts to promote MAM formation. In addition to regulating the MAM, Nogo-A downregulates Rho signaling in neuronal cells and thus inhibits migration of glioma cells (148). Nogo-A also stabilizes the receptor tyrosine kinases ErbB3 and ErbB4 through the sequestration of their ubiquitin ligase Nrdp1 within ER tubules. This then results in an increase in proliferative signaling upon Nogo-A overexpression (149). Such secondary functions likely preclude a clear, logical connection of Nogo-B between its published role in MAM suppression and its activities as a tumor suppressor as well.

Protein Kinase RNA-Like ER Kinase (PERK)

A more recently discovered tethering factor is the ER kinase PERK (150). While PERK is more commonly known as the kinase that phosphorylates eukaryotic initiation factor 2 α and thus blocks translation of ER proteins under ER stress conditions (151), PERK also localizes to the MAM, where it promotes the apposition between the ER and mitochondria. Accordingly, PERK knockout cells exhibit a MAM that is less tight and show resistance to apoptosis inducers (152). Interestingly, these functions of PERK at the MAM are accompanied by its interaction with mitofusin-2 (117). A general role of PERK in the functioning

of membrane contact sites is confirmed by its role in the formation of ER contacts with the plasma membrane (153).

From these functions, and if restricting a cancer role to its function on the MAM, we would predict that PERK, like PACS-2 and mitofusin-2, should act as a tumor suppressor. However, historically, PERK-expressing tumor cells have been found to have a growth advantage (154). This finding is based on the role of PERK in the unfolded protein response, where it protects cells against oxidative stress originating from the ER (155). Despite the induction of the pro-apoptotic transcription factor CHOP, PERK tends to elicit a tumor-promoting function due to its role in increasing angiogenesis as well as resistance to chemotherapy (156). Hence, the picture of PERK in cancer might be complex. Consistent with this idea and as expected from its ambiguous role in ER stress and MAM tethering, more recently it has become clear that PERK can promote both tumor progression and suppression (157).

Multimeric Mammalian MAM Tethering Complexes

While the yeast model system has shown that its MAM relies on multimeric, ER- and mitochondria-localized protein complexes [ERMES and EMC (105)], these complexes either do not exist in mammalian cells (ERMES) or their functioning in tethering is currently unknown (EMC). Nevertheless, the set of proteins mediating the tethering of the ER to mitochondria is expected to comprise multimeric protein complexes that localize to both the ER and mitochondria in mammalian cells as well. Indeed, a couple of multimeric MAM tethering complexes have been proposed to exist over the past decade. One such protein complex is the ARCosome that is formed when ER-localized BAP31 interacts with mitochondrial Fis1 (158). Interestingly, the ARCosome undergoes modulation upon cell stress, which results in its association with caspase-8. This interaction alters the function of the ARCosome, which then becomes involved in mitochondrial fission through formation of the p20 fragment of BAP31 (159). This suggests the ARCosome could be central in pro-apoptotic roles of the MAM, suggesting that cancer is characterized by its absence or disruption.

However, not much is known about components of the ARCosome and cancer. A recent publication suggests that BAP31 is overexpressed in malignant melanoma (160). While this finding apparently contradicts our expectations, it might result in altered pro-apoptotic signaling of the ARCosome. More aligned with the idea that the ARCosome would suppress tumor growth, miR-484 downregulates Fis1 in cancer, associated with increased cancer resistance (161).

Another ER-mitochondria protein complex consists in the association between IP₃Rs, the voltage-gated anion channel (VDAC), and the OMM chaperone Grp75 (162). Within this complex, VDAC (163) and Grp75 (162) act to boost ER-mitochondria Ca²⁺ flux. However, the exact importance of this complex for the formation and maintenance of the MAM is not known, since IP₃R triple knockout cells do not show an altered MAM (121). Moreover, it is not known whether deletion or overexpression of any member of this complex modulates MAM formation.

Nevertheless, the transfer of Ca²⁺ from the ER to mitochondria accommodated by IP₃Rs and VDAC is typically low in cancer cells, but essential (164). Not surprisingly, the members of this protein complex exhibit multiple connections to cancer, and all are important regulators of cell survival and cell death.

VDAC is an important control point not only for the influx of Ca²⁺ into mitochondria but also for the efflux of pro-apoptotic molecules and, thus, controls both mitochondria metabolism and cell death. Typically, VDAC is highly expressed in tumor tissue (165), and its expression level has been proposed to correlate so much with poor prognosis to be a candidate biomarker (166). Grp75 is also called mortalin, due to its antiproliferative effects (167). In cancer, however, Grp75 appears to act tumor-promoting, since its expression increases upon liver cancer metastasis (168) and overexpression of Grp75 increases the aggressiveness of a variety of tumor cell lines (169). Here, like in the case of VDAC and PERK, additional, MAM-unrelated functions may lead to a complex readout of the role of Grp75 in cancer. One such example may be that Grp75 can sequester and inactivate p53 (170).

A more recently described ER-mitochondria tethering complex is based on the OMM protein PTPIP51 and the ER vesicle-associated membrane protein-associated protein B (VAPB) that spans the ER membrane. Indicative of its role in ER-mitochondria tethering, depleting its components disrupts mitochondrial Ca²⁺ import (171). PTPIP51 is known to be upregulated in glioblastoma, a role which may depend on the function of PTPIP51 as a promoter of growth factor signaling (172, 173). Similarly, VAPB also has a growth-stimulatory activity of tumor tissue that might be tied to increased activity of Akt when VAPB is highly expressed (174). The oncoproteins TDP-43 (175) and fused in sarcoma (FUS) inhibit the PTPIP51-VAPB complex (176), again suggesting that the proteins of this complex generally act to accelerate tumor growth, albeit not necessarily through their roles at the MAM.

Together, it appears that the currently known multimeric protein complexes of the MAM have unclear roles for tumorigenesis that appear not always linked to their functions as MAM tethers. But given their rather recent identification as such tethers, and the many open questions about this biological role, such statements should not be considered as final.

CONCLUSION

Research from the past decade has identified the MAM as a potentially central regulator of tumor cell metabolism, as exemplified by the presence of critical tumor suppressors and oncoproteins on this structure. Moreover, findings from our lab and others have shown that MAM proteins such as the oxidoreductase TMX1 indeed can determine the balance between tumor cell glycolysis and oxidative phosphorylation (89, 177). From these findings and early insights (78), we could postulate that in particular solid, glycolytic tumor tissue is frequently characterized by a loss of normal MAM architecture and formation. Further research will have to determine whether this is indeed the case for a majority of cancer types.

There is no doubt that proteins forming connections between the ER and mitochondria are differentially expressed in tumor

tissue, as shown by numerous examples mentioned in this review. Additionally, many of these proteins are multifunctional, leading to complex significance for tumor growth that is not limited to the maintenance of the MAM. Therefore, with the exception of mitofusin-2 and PACS-2, most MAM tethering regulators show no clear association with the progression of cancer or no logical connection of their expression pattern to their role as MAM tethers. One reason for this lack of a clear link could be the often multifunctional properties of MAM regulatory proteins. Another reason is that the bigger picture of changes at the MAM may impact the survival and proliferation of cancer cells in more ways than one.

Given the field is rapidly developing, and the exact roles of MAM regulators are still evolving, such connections may solidify in the coming years. Additionally, since the entire set of MAM tethers in mammalian cells is almost certainly incomplete, new tethers may emerge that show better or cleaner association with tumorigenesis than the ones we currently know. Therefore, researchers studying the role of ER-mitochondria contacts in tumor cell metabolism and tumorigenesis are expected to read

about further exciting findings in the near future that will identify more oncoproteins and tumor suppressors on this suborganellar domain of the ER.

AUTHOR CONTRIBUTIONS

TS wrote the manuscript. MH-C contributed to text sections, edited the text, and produced the figure and table for the manuscript.

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