Key genes expressed in mitochondria-endoplasmic reticulum contact sites in cancer (Review)

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Abstract. Cell fate is critically affected by mitochondrial activity, from ATP production to metabolism, Ca^{2+} homeostasis and signaling. These actions are regulated by proteins expressed in mitochondria (Mt)-endoplasmic reticulum contact sites (MERCSs). The literature supports the fact that disruption to the physiology of the Mt and/or MERCSs can be due to alterations in the Ca^{2+} influx/efflux, which further regulates autophagy and apoptosis activity. The current review presents the findings of numerous studies with regard to the involvement of proteins positioned in MERCSs and how they express anti- and pro-apoptotic properties by adjusting Ca^{2+} across membranes. The review also explores the involvement of mitochondrial proteins as hot spots in cancer development, cell death and/or survival, and the method via which they can potentially be targeted as a therapeutic option.

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1. Introduction

In 1990, mitochondria (Mt)-associated membranes (MAMs) were first discovered (1) as the communication network between Mt and endoplasmic reticulum (ER), acting via proteins expressed on the lipid membranes of these organelles (2). To date, >1,000 proteins rest in Mt-ER contact sites (MERCSs) each associated with one or even a variety of cellular biochemical functions, including calcium (Ca^{2+}) homeostasis, lipid metabolism, apoptosis, autophagy and tumour growth (3,4). The integration and coordination between these two organelles in a well-orchestrated network crucial for maintaining homeostasis and serves as a regulatory, signalling and external protein interaction point. Disturbance to the configuration of MERCSs results in miscommunication among the Mt-ER linkage, which leads to a plethora of pathological conditions, such as Alzheimer's disease (5), Parkinson's disease (6,7), lysosomal storage diseases (8), inflammation (9) and cancer (10,11).

The length of MERCSs, depending on cell type, varies from 10-100 nm; 10-15 nm in the smooth ER and 20-30 nm in the rough ER, with 15-20% of their total surface being juxtaposed to the ER (12). The proteins found in the MERCSs are divided into two types: Connective proteins (CPs) that participate in the physical connection between ER and Mt, and interfering proteins that can alter the distance between the two organelles and decrease the contact sites (13). Through MERCSs, the ER and Mt exchange signals and stress stimuli, as well as chemical responses and cell death/survival events, a fact that has numerous times been investigated and the 'mitochondrial-associated membrane structure' considered as an independent sub-organelle (13,14). Evidence supports the fact that the loss of optimal Mt-ER organelle communication affects MERCS activities involving cellular processes such as apoptosis (Bcl2), regulation of cell growth [serine/threonine-specific protein kinase (Akt)], senescence and metabolism (Ras), but also tumour suppression [breast cancer type 1 susceptibility protein, and phosphatase and tensin homolog (PTEN)] (Fig. 1). Dysregulation in the function of these proteins results in multiple pathologies, including cancer (12).

Autophagosome formation by autophagy-related proteins, e.g., Vps34 and Beclin 1, is a cellular activity affected by Mt-ER

interaction, along with anti- and pro-apoptotic proteins (12,15). Should the two organelles not maintain Ca^{2+} homeostasis and result in an Mt Ca^{2+} overload, the permeability transition pore (PTP) will be unlocked, paving the way for the activation of caspases and the release of pro-apoptotic factors (16). This activity also releases cytochrome *c* resulting in apoptotic cell death. MERCSs also promote apoptosis or ferroptosis via reactive oxygen species (ROS) and lipid peroxidation products in cells (10). Targeting MERCSs can aid in cancer therapeutics, such as activating signal transducer and activator of transcription 3 (STAT3), located in this region, resulting in apoptotic resistance due to Ca^{2+} balancing of inositol 1,4,5-trisphosphate receptor type 3 (IP3R3) degradation mediated by IP3R3/STAT3 interaction (17).

In addition, the transfer of Ca^{2+} from the ER to the Mt is crucial for the regulation of several oncogenes and tumour suppressors (18). One of the key activators of IP3R is Akt, which activates IP3R isoform 3 in MERCSs and inhibits apoptosis (19). Akt activity is regulated by its inhibitors, PTEN, the tumor suppressor promyelocytic leukemia protein (PML) and the activator mechanistic target of rampamycin kinase complex 2 (mTORC2), all of which are found to be enriched in MERCSs (20). One of the major tumour suppressors is p53. ER-MAM localization allows tumour suppressor p53 to facilitate Ca^{2+}-dependent apoptosis, via the sarco/ER Ca^{2+}-ATPase (SERCA), which is expressed on the ER membrane (21). Overall, Mt-ER linkage tightening increases Ca^{2+} uptake and induces apoptosis, while loosening of ER-Mt connections/interactions promotes cell survival and Mt respiration (22). The following report summarizes the role proteins expressed in MERCSs play in pharmacological inhibition/activation in cancer environments. Findings have been collaborated using PRISMA guidelines.

2. Key features of MERCSs

Lipid synthesis. Enzymes within the ER are responsible for the synthesis of cellular lipids; however, the activity of these enzymes is affected by alterations to the status quo of both the organelle and cytosol (23). The Mt also act as a factory for lipid membrane components such as cardiolipin, synthesised solely within the Mt, and phosphatidylethanolamine (PE), synthesised within Mt but requiring intervention from the cytosol and ER (24). Phosphatidylserine (PS) is a lipid metabolising protein synthesised by PS synthases 1 and 2 (PSS1 and PSS2) in the ER; it is then stored in MERCSs until it is signalled to enter the Mt for its conversion to PE via carboxylases. Phosphatidylcholine (PC) is another lipid metabolism protein recruited within MERCSs. In cancer cells, lipid membrane integrity and function are affected as the Kennedy pathway (synthesis of PC and PE) is disrupted (25), thus explaining why PE is utilised as a diagnostic marker, as its concentration increases as excess cell proliferation occurs in cancer environments. Interrupting the alignment between the Mt and ER dysregulates lipid synthesis and the transfer of lipids to target organelles, and hence results in damage/loss of lipid membranes (9).

MERCSs are cholesterol-rich membranes carrying a sterol interacting protein, known as caveolin, which is involved in sterol metabolism (26). Especially during acute stress, cholesterol is transported to the Mt for steroidogenesis. An additional protein found in MERCSs that is involved in steroidogenesis is ATPase family AAA domain-containing protein 3B; this protein is involved in transporting cholesterol from the ER to the Mt during MERCS formation, an action that expresses chemoresistance and anti-proliferative abilities through mechanisms that remain unclear (27). Finally, MERCSs play a central role in the formation of ceramide, a sphingolipid product that is also involved in apoptotic cell death, inflammation, cell growth and differentiation (9,19,28,29).

Ca2+ signalling. SERCA pumps regularly supply the ER with Ca^{2+} from the cytosol (30). A SERCA pump has three isoforms, SERCA1, SERCA2 and SERCA3, of which SERCA2 is expressed in MERCSs. An array of different mechanisms and pathways are triggered by Ca^{2+} signalling in the Mt (31). For example, the presence of Ca^{2+} increases the activity of the tricarboxylic acid cycle enzymes, and stimulates the electron transport chain and oxidative phosphorylation (32). Overall, MERCSs are responsible for the regulation of cellular metabolism through Ca^{2+} signalling driving ATP production, metabolism, gene activation and cell survival pathways (31). While the ER is the main Ca^{2+} storage organelle, the presence of MERCSs is responsible for the transport and accumulation of Ca^{2+} in Mt, which further affects crucial cellular activities, as mentioned throughout this review.

At the same time, the accumulation/overload of Ca^{2+} to the Mt can cause swelling and cell death (33). Apart from SERCA pumps, multiple ER-Ca^{2+} proteins, each with a different role, are found in MERCSs, including, but not limited to, IP3R and SERCA. IP3R regulates Ca^{2+} transmission via mitochondrial voltage-dependent anion channel 1 (VDAC1), which is found at the outer mitochondrial membrane and is connected to the MERCSs. The molecular chaperone glucose-regulated protein 75 succeeds the connection that regulates the IP3R-VDAC1 interaction. When the Ca^{2+} flux from the ER to the Mt declines, cells become more resistant to apoptosis, whereas overexpression of Ca^{2+} results in apoptosis, as seen in vascular smooth muscle cells and epithelial cancer cells, due to the ion's relationship with the Mt-ER associated membrane fusion mediator, Mitofusin 2 (Mfn2) (34). Transfer of this ion across MAMs is also regulated through a supplementary pathway involving PML, Akt and IP3R3, where Ca^{2+} flux from the ER to Mt is increased, thus increasing apoptosis and acting against cancer.

3. Methodology

Evidence regarding Mt-associated ER membranes and cancer was reviewed for the present study. A literature search was performed in PubMed (https://pubmed.ncbi.nlm.nih.gov/), Science Direct (https://www.sciencedirect.com/) and Scopus (https://scopus.com) to identify relevant studies that were published up to and including March 4, 2022, or within the last 10 years of this search date.. The search was based on the following key words and terms in these databases: i) PubMed: (((((((mitochondria-associated ER membranes[Title/Abstract]) OR (MERCSs[Title/Abstract])) AND (cancer[Title/Abstract])) OR (tumor[Title/Abstract])) OR (tumour[Title/Abstract])) OR (carcinoma[Title/Abstract])) OR (malignancy[Title/Abstract])) OR (proliferation[Title/Abstract])) OR (onco*[Title/Abstract]);
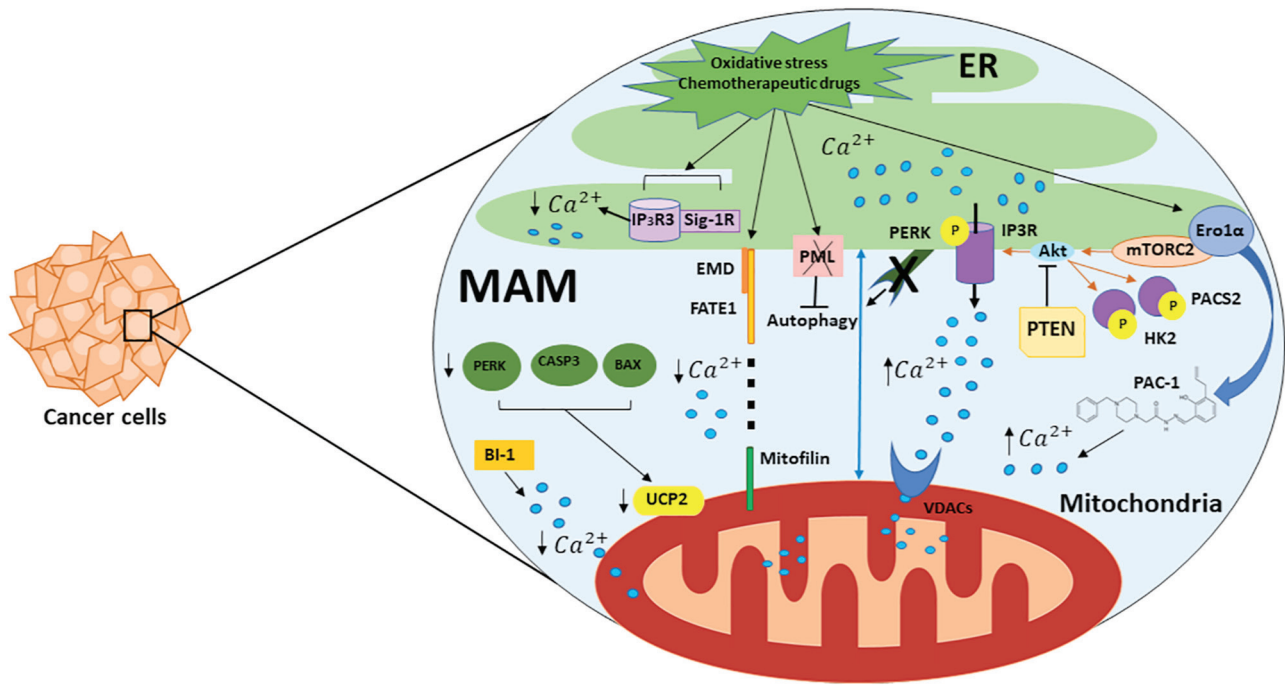
Figure 1. Role of Mt-associated ER membranes in calcium regulation and cancer. Important proteins present in MERCSs used as Ca^{2+} transfer systems at the ER and Mt and contributing to cell death and survival are shown. Regulators of ER Ca^{2+} release machinery are IP3Rs that act as ligand-gated channels and facilitate Ca^{2+} release from the ER to the Mt through VDACs. A fraction of PTEN proteins, localized to the ER and MERCSs, regulate Ca^{2+} release by preventing the activating phosphorylation of Akt, which reduces Ca^{2+} release via IP3Rs. Mt, mitochondria; ER, endoplasmic reticulum; MERCS, Mt-ER contact site; IP3R, inositol 1,4,5-trisphosphate receptor type 3; VDAC, mitochondrial voltage-dependent anion channel; PTEN, phosphatase and tensin homolog; Akt, serine/threonine-specific protein kinase; MAM, Mt-associated membrane; p, phosphate.

ii) Science direct: (mitochondria associated ER membranes, MERCSs, cancer, tumor, tumour, carcinoma, malignancy, proliferation); iii) Scopus: TITLE-ABS-KEY ('mitochondria associated ER membrane' AND 'cancer' OR 'tumor' OR 'carcinoma' OR 'malignancy' OR 'proliferation') AND [LIMIT-TO (PUBSTAGE, 'final')] AND [LIMIT-TO (DOCTYPE, 'ar')] AND [LIMIT-TO (LANGUAGE, 'English')]. Abstracts were studied to identify papers on MERCSs in cancer following the PRISMA guidelines (Fig. 2). Publications describing the role of MERCS on cellular integrity and effects on cancer were accessed and reviewed meticulously, and those that did not refer to activity in MERCS space were excluded from the review. The references of the relevant articles were also accessed and reviewed. Finally, expression/alteration data were collected from cBioPortal (https://www.cbioportal.org/) and the University of Alabama at Birmingham Cancer data analysis portal (UALCAN; http://ualcan.path.uab.edu/).

4. Association of key genes/proteins expressed in MERCSs with cell fate

As aforementioned, MAMs within MERCS include an assortment of proteins (Fig. 1) that compose the MERCSs and regulate cellular activity. Silencing the expression of some of these proteins will subsequently affect Mt-ER Ca^{2+} flow, instigate/stifle ER stress and/or threaten cell integrity. Altering Ca^{2+} flow into the Mt affects mitochondrial performance, such as ATP production and autophagy activity. Autophagy is known to provide alternative sources of carbon, e.g., through fatty acid

oxidation, so the cell can meet the higher metabolic demands of tumour microenvironments and permit the cell to elude cell toxicity. For instance, silencing PML within MERCSs results in reduced Ca^{2+} transfer between the ER and Mt via IP3R, and subsequently, ATP production decreases while AMPK activity increases, setting off the AMPK/mTOR/UIK1 pathway that prolongs autophagy. Similarly these effects are recorded in p53-/- cells where delocalisation of PML occurs (34). AMPK interacts with the vesicle-trafficking mediator Beclin-1 following reduced Ca^{2+} transfer to the Mt across MAM, which actuates autophagosome formation, whereas silencing the Beclin-1 gene, BECN1, has the opposite effect (35).

Proteins that complement one another's functionality may compartmentalise across membranes, forming raft-like lipid microdomains ensuring protein interactions take place effortlessly, as is the case with autophagy and Beclin 1 regulator 1 (AMBRA1), ER lipid raft associated 1 (ERLIN1) and mitofusin 2 (MFN2) proteins and ganglioside GD3 (gGD3). AMBRA1 and ERLIN1 interact with support from MFN2 and gGD3 within MERCSs to stimulate the formation of autophagosomes (36). One study showed that knockdown of ST8SIA1 (gGD3) or MFN2 halted autophagosome development, as did the silencing of ERLIN1 (36). This suggests that all proteins are required in MERCSs for autophagy to occur. Thapsigargin (Tg) is an ER stressor drug affecting Ca^{2+} homeostasis. In another study, in both HeLa and Du145 cells, unphosphorylated ER stress sensor IRE was dominant, while following treatment of cells with Tg, readings of phosphorylated IRE (pIRE) increased (37). Knockdown of sigma non-opioid intracellular receptor 1 dephosphorylates IRE, returning cells

Identification of studies via databases and registers

Identification

Records identified from
  PubMed (n=121)
  Science direct (n=167)
  Scopus (n=23)

Screening

Records screened and assessed
for eligibility
(n=311)

Reports excluded:

  Title (n=87)
  Abstract (n=11)
  Full text (n=2)
  Language (n=1)
  Books, Conferences, reviews etc (n=187)
  Duplications (n=3)
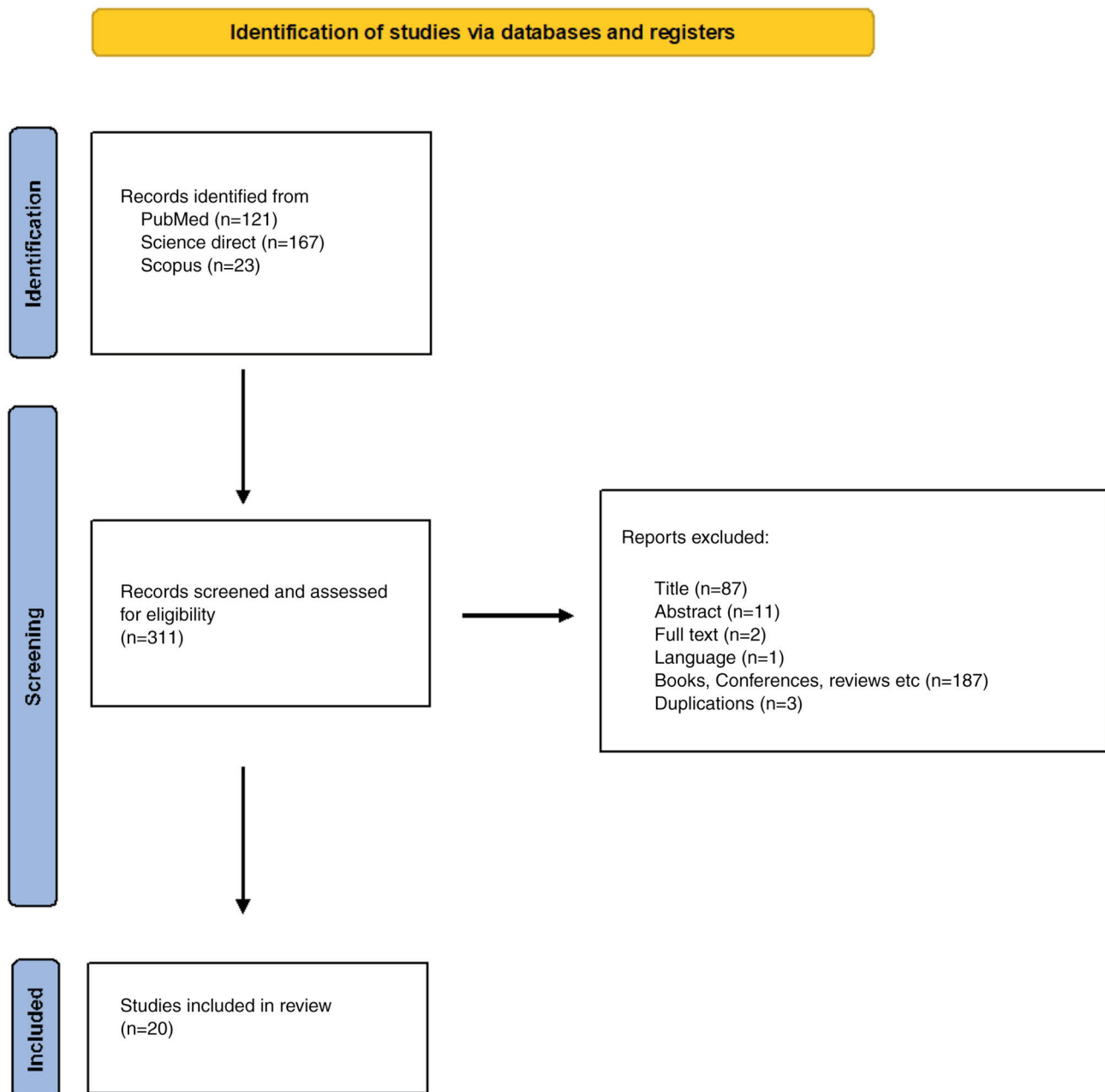
Included

Studies included in review
(n=20)

Figure 2. PRISMA flow chart indicating the inclusion and exclusion criteria for the manuscripts selected.

to an unstressed stage, an action significant for counteracting autophagy, indicating the need of the chaperone to protect IRE from ER-associated degradation. Following Sig-1R knockdown, XBP1 protein splicing is also reduced, as XBP1 cannot bind to fluorescent protein sites due to the absence of the pIRE responsible for its activation (37). Tg was also used to treat AR42J and Neuro2A cells producing excessive phosphorylation of IRE1 (38). Overall, these results present the crucial role of Sig-1Rs in the IRE1-XBP1 signalling pathway involved in cell survival. In the research analysed so far, the IRE1-XBP1 pathway does not induce apoptosis or autophagy as such, but it promotes cell survival by acting against ER stress through Sig-1R chaperones and IRE in MAMs.

The flow of Ca^{2+} into the Mt can also be affected by the distance between the Mt and ER or altering the positioning of the organelles, consequently affecting cell proliferation, integrity and apoptosis. Adrenocortical carcinoma (ACC), for example, is deemed resistant to mitotane drug treatment, as upregulation of fetal and adult testis-expressed 1 (FATE1) disturbs the flow of Ca^{2+} into the Mt as it uncouples the Mt and ER, consequently increasing the resistance of tumour cells to oxidative stress and chemotherapeutic drugs (39). Knockdown of FATE1 reverses these effects and allows ACC to respond to treatment and undergo apoptosis during treatment. Similarly, high FATE1 expression has also been associated with reduced survival time in patients with breast cancer (Fig. S1).

Furthermore, knockdown of the ER stress sensor PERK (PERK$^{-/-}$) in mouse embryonic fibroblasts (MEFs) distorted ER morphology, increasing the distance between the Mt and ER, shielding the Mt from ROS-mediated effector build-up, and subsequently avoiding ER-stress and apoptosis. Cells expressing PERK regulated Mt-ER defense responses against

ROS, but also expressed high levels of pro-apoptotic C/EBP homologous protein (CHOP) triggering apoptosis more effectively than Tg. The effects of PERK$^{-/-}$ were also evaluated in CT26 and MDA-MB468 cell lines that were resistant to cell death due to increased expression of GRP78 chaperone, therefore initiating Ca^{2+} overflow outside the ER, low caspase activation and depletion of CHOP (40). Alterations in ER morphology, as well as a weaker Mt-ER association, were also perceived in MEFs deficient in MFN2. CHOP and Ero1α are upregulated by procaspase-activating compound-1 (PAC-1), a direct caspase-activator, triggering ER stress. To evaluate these effects, both proteins were silenced in HeLa D1ER cells, reducing Ca^{2+} release from the ER and decreasing apoptosis. Furthermore, PAC-1 expression induced GRP78 and GRP94 chaperone activity causing Ca^{2+} leakage subsequent to Ero1a-dependent ER luminal hyper-oxidation in MCF7 and MCF7C3 cells. As a result, ER stress and Mt-mediated apoptosis were recorded (41).

As seen so far, MERCSs may be affected directly or indirectly. Uncoupling protein 2 (UCP2), for example, enhances protein arginine N-methyltransferase 1 activity, which in turn methylates mitochondrial calcium uptake 1, a Ca^{2+} protein pump located in the MAM. Madreiter-Sokolowski *et al* (2021) (42) reported that there was an inverse correlation between UCP2 and the proteins responsible for stabilizing Mt-ER association, in prostate adenocarcinoma tissues, breast invasive cancer, cervical squamous cell carcinoma and multiple other cancer types, affecting cell viability. For example, an IP correlation was observed in HeLa cells where upregulation of Rab32, an A-anchoring protein fostering Mt-ER tethering, resulted in a downregulation of UCP2 and consequently permitted the cancer cells to escape mitochondrial Ca^{2+} overload-induced cell death. Mondet *et al* (2021) (43) reasoned that Mt in acute myeloid leukemia (AML) can be targeted to regulate the proliferation and chemosensitivity within these cells. Assembly of the Mt ultrastructure and its influence on cellular integrity were evaluated in multiple leukaemia cell lines (HEL, HL60, K562, KG1 and OCI-AML3). Alterations were unearthed on a molecular level between the different cell lines. In the case of K562 cells, an ASXL1 gene mutation was recorded, affecting the Mt shape within MERCSs and creating a distance between the Mt and ER, allowing the cells to resist chemotherapy drugs and continue proliferating. This mutation also affected genes expressing MAM complexes, such as VAMP-associated protein B and C (VAPB) and oxysterol binding protein like 5 (OSBPL5), affecting the physiology and integrity of the organelle (43). HL60 cells, where the mutation is lacking, displayed sensitivity to drugs as the distance between the Mt and ER was unchanged.

The physiology of the Mt and ER is greatly dependent on the integrity and expression of proteins within MERCSs. Transient receptor potential melastatine 8 (TRPM8) channel isoforms are expressed on the ER lipid membrane, whose knockdown initiates apoptosis in cancer cells (44). It is understood that the 4TM-TRPM8 isoform aids the survival of prostate cancer epithelial cells by actively regulating Ca^{2+} trafficking from the ER into the cytosol, and subsequently Mt uptake, which as a result protects the cells from ER stress. Alternatively, survival of tumor cells is affected by the expression of NLRX1, coding for a NOD-like receptor immune system regulator, where

expression of this gene regulates TNF-α-induced metabolism and cell death (45). Overexpression of Bax inhibitor-1 (BI-1) in HT1080 cells (HT1080/BI-1) permitted them to avert apoptosis by leaking Ca^{2+} out of the ER, so no ER stress resulted, and reducing mitochondrial Ca^{2+} intake, therefore reducing cytochrome *c* release (involved in apoptosis) and PTP opening. To maintain mitochondrial homeostasis in HT1080/BI-1, mitoK$_{ca}$ channels open, permitting an influx of K$^+$, further protecting the cell against ER stress and its consequences (46). Silencing BI-1 decreased Ca^{2+} in the ER and simultaneously increased mitochondrial Ca^{2+}, permitting the cell to respond to drugs and undergo apoptosis (46). Additionally, the crucial role of FUNDC1 in angiogenesis has been investigated both *in vitro* and *in vivo*. Disruption of FUNDC1-related MAM formation contributed to intracellular Ca^{2+} dys-homeostasis, resulting in decreased levels of pSRF and VEGFR2, and subsequent reduction of VEGF-induced angiogenesis (12). A recent study supported that chronic increases in MAM formation resulted in mitochondrial Ca^{2+} overload, impairing mitochondrial bioenergetics function and increasing ROS production *in vivo,* also leading to aging-associated pathologies, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (47). The expression levels of IP3R, FUNDC1 and MFN2 were significantly elevated in MAM fractions isolated from VEGF-treated endothelial cells. The interaction between FUNDC1 and IP3R1 in MAMs mediated the changes in Ca^{2+} levels. IP3R1 knockdown significantly inhibited vascular angiogenesis *in vivo* (48).

ER stress, Ca^{2+} regulation and gene knockdown all influence MAM behavior, and these in turn are dependent on the length of MERCSs to ensure optimum interplay to succeed in affecting the vulnerability of cancer cells (40). Separation of the two organelles can result in resistance to chemotherapeutic drugs, ER stress and/or apoptosis. This is supported by the study by Doghman-Bouguerra *et al* (2016) (39), where increased FATE1 expression in ACC cells caused the Mt and ER to detach and apoptosis to be eliminated. FATE1 protein localised near calreticulin and HSP60 (ER and Mt markers, respectively) in MERCSs. In the same study, FATE1 knockdown caused a significant increase in angiotensin II-stimulated aldosterone levels, which resulted in the increase of blood pressure, dehydroepiandrosterone and cortisol, as well as aldosterone, with multiple biological pathways affected.

The mTORC2 protein facilitates the phosphorylation of IP3R, hexokinase 3 and phosphofurin acidic cluster sorting protein 2 (PACS2) via Akt in non-small cell lung carcinomas (NSCLC). Triggering PACS2 with a compound such as oxyphyllanene B, increases its activity that, as a result, distorts Mt-ER communication and allows cancer cells, such as glioblastoma, to overcome chemotherapy resistance (49). At the same time, pharmacologically inhibiting mTOR decreases cell proliferation and increases apoptosis (50). Alternatively, tyrosine kinase inhibitors (TKIs) relocate the expression of mTORC2 from MERCSs to the cytoplasm, assisting cancer cells such as H1299 and H1975 to overcome CD20 mono-antibody EGFR-TKI resistance (39). To overcome the resistance of NSCLC to EGFR-TKIs, the drug rituximab was administered in synergy with erlotinib to move the expression of the mTORC2 protein from MERCS region to the cytoplasm. Consequently, both H1299 and H1975 cells overcame

EGFR-TKI resistance (51). PTEN expresses multiple actions and is localised in the ER and MAM region, affecting Ca^{2+} transport and apoptosis induction. Stimulation of the silenced PTEN cells with ATP led to IP3 expression, meaning greater interaction with IP3Rs, as aforementioned, resulting in the ensuing cell apoptotic activity. High localization of PTEN in the ER during ArA-mediated apoptosis supports the involvement of PTEN in Ca^{2+} mediated apoptosis via IP3Rs (52).

Finally, the key MAM proteins reported by the literature that affect cancer outcomes are summarized in the present review, and using bioinformatics analysis, their levels of expression in different cancer types, including their role in cell outcome, are reviewed. Gene expression analysis using The Cancer Genome Atlas data from UALCAN indicated that MERCS genes (Table I) are differentially expressed in respect to cancer type. Consequently, genes regularly expressing proteins in MERCSs affect patient survival, including FATE1, EIF2AK3 and TRPM8 in breast cancer, AMBRA1 and ERLIN1 in pancreatic cancer, mTORC2 in ovarian cancer, PML and PRKAA2 in kidney renal clear cell carcinoma, MFN2 in thyroid cancer, UCP2 in sarcoma, BECN1 in liver hepatocellular carcinoma and ASXL1 in adenoid cystic carcinoma.

5. Adjusting the MT-ER microenvironment determining cell fate

Cancer cells require excessive amounts of energy to grow, proliferate and migrate (53). This energy demand is attained when adequate Ca^{2+} uptake in Mt occurs and is processed by the Krebs cycle and oxidative phosphorylation. The current review presents evidence emphasizing that the association between Mt and ER, across MERCSs, influences cancer cell proliferation and migration, and induces cancer cell death (Table I) activities that are determined by which proteins are expressed (37).

Further analysis of FATE1 behaviour in additional cancer cell lines would be appropriate to determine its potential as a cancer treatment target, given its ability to decrease caspase-3/7 activity and increase H_2O_2, both elements amplified in cellular stress environments (39,40). The aforementioned evidence supports the fact that regulating Ca^{2+} homeostasis in cancer cells aids in the perseverance of cellular stress and the prevention of autophagy, as indicated by increasing expression of 4TM-TRPM8 isoforms in prostate epithelial cells. Therefore, 4TM-TRPM8 channels, partially localized in MERCSs, are 'new gatekeepers' for the regulation of Ca^{2+} and any complementary outcomes expressed (44). Alternatively, Mt-ER coupling could be increased by prescribing tunicamycin in HeLa cells, which upregulated the concentration of three proteins (Rab32, PEMT1 and GPR75), responsible for stabilizing MAM, and decreased UCP2, thus effectively decreasing cancer cell viability (37).

ER stress on the other hand occurs in concentrated environments of ROS, a characteristic common in tumours. Therefore, determining the association between mitochondrial apoptosis and oxidative stress is detrimental. NLR Family Member X1 (NLRX1) protein, expressed in the Mt, activates Caspase-8, which allows TNF-α/cycloheximide to reduce ROS assembly, and overall neutralises the acidity expressed by tumour cells.

This regulation marks NLRX1 as a tumor suppressor (45). A study by Verfaillie *et al* (2012) (40) illustrated the expression of PERK, through unfolded protein response mechanisms, in MAM, and described its essential role in coupling the Mt and ER, therefore regulating ROS-induced cell death. Additional effects included reduced caspase-3 activity, prolonged XBP1 accumulation and consequent IRE1 activation, which together with CHOP facilitated apoptosis. These characteristics were not expressed in wild-type cells. Furthermore, treating PERK$^{-/-}$ cells with Tg led to ER Ca^{2+} store depletion, due to the inhibition of SERCA, thus abolishing the survival of clonogenic cells and stimulating cell death. The decline in Ca^{2+} signalling by PERK$^{-/-}$ MEFs following treatment with Tg can be caused by IP3 (40).

Numerous MAM proteins determine whether the cell will undergo apoptosis or survive. In the case of BI-1, mostly localized in the ER with a smaller portion expressed in the Mt, Ca^{2+} uptake by the Mt is reduced, affecting the regulation of cytochrome *c* and the mitochondrial permeability transition pore. This consequently protects the cell against cell death. Analysing the differences between HT1090/BI-1 and HT1080/Neo cells, HT1090/BI-1 showed a lower calcium ion capacity, allowing these cells to close the permeability transition pore, preventing ion evacuation and avoiding stress-induced apoptosis (46). Leakage of mitochondrial Ca^{2+} can affect the physiology of the organelle and hence its activities. Having said that, alternative channels, such as $mitoK_{Ca}$, can be opened to maintain homeostasis within the organelle by drawing K^+ into the Mt, increasing water uptake and thus preventing cell destruction. Additional information is necessary to determine the relationship of BI-1 with other proteins within MERCSs and to analyse the mechanisms involved.

6. Targeting MERCSs

Chemotherapy drugs are designed to target cancer cells with the sole purpose of inducing death. However, once at its target site, drug activity may be obstructed by multiple mechanisms, including ion imbalances, cellular activity (e.g., triggering autophagy), gene expression and other factors (e.g., drugs) (34,54). The following proteins indicating autophagy activity in MERCSs can be utilized as diagnostic tools for PML levels: Microtubule-associated protein 1A/1B-light chain 3, autophagy-related 14 and syntaxin-17 (34). Administering an autophagy inhibitor in synergy with 5-FU (Fluorouracil), a chemotherapeutic drug, reduces solid tumour size in mice transfected with acute promyelocytic leukemia (34).

Patients with the ASXL1 mutation in AML presented with downregulation of VAPB and PTPIP51, which collaborate to assist delivery of Ca^{2+} via IP3R (55). Two more proteins affected by this mutation include ITPR1, which is also involved in regulating Ca^{2+}, while the effects of OSBL5 on cholesterol expression in the region could explain why leukaemia cells have previously been described as being sensitive to cytostatic agents that inhibit cell growth or induce cell death (56). This sensitivity, however, can be hindered via morphological alterations and the integrity of ER communication.

In MCF7, MCF7C3, SiHa and HeLa cells, PAC-1 arrests the cell cycle at the G_1 phase, ultimately inducing cell death. PAC-1 is a caspase-activating compound released in response to

Table I. Data extracted from the literature search indicating type of study, cancer type and the gene or protein studied and findings.

First author, year	Type of cancer/cells	Aim	Gene	Protein	Findings/results	(Refs.)
Bidaux et al, 2018	Prostate cancer	Explore the molecular properties of the TRPM8 channel subfamily and their function	TRPM8	Transient receptor potential cation channel subfamily M (melastatin) member 9	Ca^{2+} flow into the Mt and ER is also affected by 4TM-TRPM8.	(44)
Mori et al, 2013	Prostate cancer	Assess the role of Sig-1R chaperones at MAM when cells are under oxidative/ER stress	SIGMAR, ERN1	Sigma-1, Serine/threonine-protein kinase/endoribonuclease IRE1	IRE1 is chaperoned by Sig-1Rs in MAM when the cell is under ER stress. The cell avoids apoptosis via this physical support but also through the transmission of stress signals from ROS from the Mt to the nucleus via MAM	(37)
Wang et al, 2021	Lewis lung carcinoma	Determine how to expression of FUNDC1 affects the ER-Mt association and consequently how it affects MAM-related proteins such as VEGFR2	FUNDC1	FUN14 domain-containing protein 1	Deleting FUNDC1 gene results in decreased ER-Mt co-localisation, consequently decreasing VEGFR2 expression. VEGFR2 plays a critical role in angiogenesis; therefore, in its absence, angiogenesis is disrupted thus limiting the progress of cancer	(12)
Lee et al, 2014	Fibrosarcoma	This study considers regulatory effects of the concentration of Ca^{2+} ions in the Mt on cell death. Also how BI-1 reduces $[Ca^{2+}]$ mito	TMBIM6	Bax inhibitor-1	Both Ca^{2+} uniporter and Ca^{2+}-dependent K^+ channels are regulated by BI-1, which causes them to open. As a result, the mitochondrial permeability transition pore is inhibited preventing the inflow of Ca^{2+} and mitochondrial swelling. Through this mechanism the cell can escape cell death	(46)
Manganelli et al, 2020 2020	Fibrosarcoma	Understand the interaction between ERLIN1 and AMBRA1, and how MFN2 and ganglioside GD3 affect autophagy activity in MERCSs	ERLIN1	ER lipid raft associated 1	Upon analysis of 2FTGH cells, a physical relationship is presented between ERLIN1 and AMBRA1. This relationship is severed if MFN2 or ST8SIA1 are knocked down. For autophagy to occur, assembly of AMRA1 with additional molecules is necessary in MERCSs (AMBRA1/BECN1/WIPI1)	(36)
Xu et al, 2017	Non-small cell lung cancer (T790M EGFR mutation)	To evaluate if erlotinib can inhibit cell proliferation in NSCLC cells positive for EGFR T790M mutation or whether it	RICTOR	mTORC2	NSCLC with the T790M mutation is resistant to erlotinib (EGFR-TKI), as EGFR phosphorylation is prevented; hence, cancer cells resist apoptosis. However,	(57)

Table I. Continued.

First author, year	Type of cancer/cells	Aim	Gene	Protein	Findings/results	(Refs.)
		requires the addition of rituximab			administration of a CD20 mono-antibody, such as rituximab, inhibits the expression of rictor, an essential mTORC2 protein, in MERCSs, thus allowing greater EGFR kinase activity. As a consequence, EGFR-TKI resistance is reversed, suggesting that synergistic administration of both drugs can act as a targeted therapy for T790M-mutated NSCLC.	
Verfaillie *et al*, 2012	Undifferentiated colon carcinoma and breast cancer	Identify the role of PERK in ROS signalling	*EIF2AK3*	PERK	Silencing of EIF2AK3 results in deformed ER morphology and disrupted Ca²⁺ signalling. This absence of PERK increases the distance between the two organelles, hence shielding Mt from ROS-mediated events triggering apoptosis. BAX-mediated mitochondrial apoptosis is also suspended in PERK silenced cells as cytosolic Ca²⁺ and other components are delayed.	(40)
Shioda *et al*, 2012	Neuroblastoma	Determine whether σ1SR (isoform of chaperone σ1R) overexpression initiates neuro-2a cell differentiation and what affect it has on cell survival	*Sigma-1*	Sigma-1 receptor	Autophagic response by σ1SR when under ER stress and also decrease mitochondrial ATP levels, as it destabilizes IP3R by inhibiting σ1R.	(38)
Çoku *et al*, 2022	Neuroblastoma	Understanding the role of MERCSs in therapy resistance	*BCL2L11*	Bcl-2-like protein 11	Analysis of relapsed neuroblastoma samples after they have undergone treatment, revealing a mitigated response to stress (via MERCSs) compared with the same cells analysed at diagnosis prior to treatment. Samples from relapsed cases are more resistant to stress-induced MOMP, as supported by their shape and size, while resistance to MOMP is expressed in tumour cells where ceramide is naturally reduced.	(59)

Table I. Continued.

First author, year	Type of cancer/cells	Aim	Gene	Protein	Findings/results	(Refs.)
Bononi et al, 2013	Human embryonic kidney cell line [transfected with PTEN (C124S) and PTEN (G129E)]	Investigate the effect of PTEN activity on Ca^{2+} signalling and apoptosis.	PTEN	Phosphatase and tensin homolog deleted on chromosome 10	Cancer cells can avoid cell death through apoptosis-inducing Ca^{2+} signals by driving deletion or mutation of PTEN; hence, the protein cannot be expressed in MERCSs or ER. PTEN shuts down Akt-mediated phosphorylation of IP3R3; therefore, the cell is exposed to Ca^{2+}-mediated apoptosis.	(52)
Doghman-Bouguerra et al, 2016	Adrenocortical carcinoma	Investigate, in cancer cells, the function of FATE1 in regulating Ca^{2+}-dependent apoptosis but also drug-dependent apoptosis	FATE1	Steroidogenic factor 1	In adrenocortical carcinoma, ER-Mt uncoupling occurs due to increased expression of FATE1, by effect of SF-1, permitting the cells to resist chemotherapeutic drugs but also escape apoptosis.	(39)
Ciscato et al, 2020	Chronic lymphocytic leukaemia	Provide a detailed presentation of the molecular mechanisms of HK2 that lead to cell damage and express anti-neoplastic characteristics. All of this supported by a detailed comprehension of HK2 mechanisms	HK2	Hexokinase 2	Silencing of HK2 prevents cells from proliferating and finally induces death in B-chronic lymphatic leukaemia cells via calpain-dependent events.	(60)
Missiroli et al, 2016	Promyelotic leukaemia	To investigate the rold of PML in in certain molecular pathways which affect cell death and proliferation of cancer	Pml	Promyelocytic leukemia protein	Autophagosome formation is suppressed when PML is localised within MAM; hence, autophagy induction does not occur. However, p53 is also involved in ensuring the placement of PML from MERCSs. ER-mitochondrial Ca^{2+} transfer via IP3R is reduced if PML is displaced.	(34)
Mondet et al, 2021	Leukaemia	Analyse five different subtypes of acute myeloid leukaemia to explore the mitochondrial ultrastructure and its function	ASXL1	Polycomb group protein ASXL1	Molecular variations are expressed in the different leukemic cells, which also present with altered mitochondrial functions. HL60 cells express more ER-mitochondrial contact sites. Cells carrying the ASXL1 mutation have significantly depleted expression of MAM genes.	(43)

Table I. Continued.

First author, year	Type of cancer/cells	Aim	Gene	Protein	Findings/results	(Refs.)
Ciscato *et al*, 2020	Neurofibroma	Define the mechanism by which HK2 triggers cell death in cancer cell lines	*CAPN1*	Calpain	With pre-prepared cleaved HK2-peptides, damage can be avoided to secondary targets and still be tailored to multiple malignancies. HK2-inactivation delays mitochondrial polarisation followed by cell death mediated via Ca^{2+}-dependent protease calpains when displaced from MERCSs.	(60)
Ciscato *et al*, 2020	Colorectal cancer	Evaluate the outcome of silencing HK2 in solid tumours	*HK2*	Hexokinase 2	Displacing of HK2 further impedes colorectal cancer cell growth in solid tumours, while injection in allografts of colon cancer significantly decreases proliferation of cells.	(60)
Ciscato *et al*, 2020	Breast cancer	Evaluate the outcome of silencing HK2 in solid tumours	*HK2*	Hexokinase 2	A cleaved peptide that displaces HK2 from MERCSs causing an overload of calcium ions in Mt decreases colony formation and kills the tumour cells.	(60)
Seervi *et al*, 2013	Breast cancer	Recognise the effects PAC-1 has on cancer cells lines	*ERO1A*	ERO1-like protein α	Systematic action of PAC-1 results in G_1 arrest and autophagy of cells as a response to stress. Cytochrome *c* release and cell death in MCF7 cells is reduced by PAC-1 when PUMA is silenced	(41)
Singh *et al*, 2015	Breast cancer	Does NLRX1 regulate ATP production by Mt.	*NRLX1*	NLR family member X1	Downregulation of NLRX1 permits cells to grow in the presence of TNF-α, and ATP is increased. Expression of NLRX1 accompanied by TNF-α/CHX increases mitochondrial ROS levels and consequently induces cell death by activating caspase-8	(45)
Seervi *et al*, 2013	Cervical cancer	Provide a breakdown of the mechanisms involved in PAC-1 cell death	*ERO1A*	ERO1-like protein α	Silencing of Ero1a results in decreased Ca^{2+} release from ER and PAC-1 activates cell death. Therefore, PAC-1 and Ero1a affect ion movement between ER and Mt via MERCSs	(41)
Ciscato *et al*, 2020	Cervical cancer	Define the mechanism by which HK2 triggers cell death in cancer cell lines	*CAPN1*	Calpain	With pre-prepared cleaved HK2-peptides, damage to secondary targets can be avoided and still be tailored to multiple malignancies.	(60)

Table I. Continued.

First author, year	Type of cancer/cells	Aim	Gene	Protein	Findings/results	(Refs.)
					HK2-inactivation delays mitochondrial polarisation followed by cell death mediated via Ca^{2+}-dependent protease calpains when displaced from MERCSs.	
Singh et al, 2015	Cervical cancer	Determine the role of NLRX1 expression on migration and growth in response to physiological stress such as cancer	NLRX1	NLR family member X1	Following treatment with TNF-α/CHX, cells transfected with NLRX1, which localizes in the Mt, are sensitized to TNF-α-induced death. Knockdown of NLRX1 results in decreased ROS stimulation and reverses acidification by tumour cells.	(45)
Madreiter-Sokolowski et al, 2021	Cervical cancer	Define the role of UCP2 in cancer cell viability through its involvement in MERCSs	UCP2	Uncoupling Protein 2	In response to histamine-induced ER Ca^{2+}, mitochondrial Ca^{2+} uptake is depleted upon UCP2 knockdown whereas the opposite occurs with UCP2 overexpression. Administration of tunicamycin to HeLa cells upregulates proteins known to be involved in MAM stabilization.	(42)
Ahumada-Castro et al, 2018	Cervical cancer	Identify the mechanism that interrupts mTORC1-dependent autophagy	PRKAA2 and mTOR	AMP-activated protein kinase and mammalian target of rapamycin complex 1	If MAM is disrupted and communication between the ER and MERCSs is lost, AMPK approaches the lysosomal membrane where it directly or indirectly phosphorylates the mTORC1 binding complex consequently inhibiting mTORC1.	(35)
Cui et al, 2022	Glioblastoma	Analyse the SAR of 56 sesquiterpenes and confirm whether OLB is cytotoxic in pa-resistant glioblastoma cells	PACS2	Phosphofurin acidic cluster sorting protein 2	MAM and ER-specific chaperone concentration is increased and ER stress is induced via OLB. OLB further causes morphological alterations in the MAM network and induces glioblastoma cell apoptosis. OLB activity is reduced following PACS2 deletion.	(49)

MAM, MERCS, ER, endoplasmic reticulum; Mt, mitochondria; OLB, oxyphyllanene B; MAM, Mt-associated membrane; SAR, structure-activity relationship analysis; TKI, tyrosine kinase inhibitor; TMZ, temozolomide; ROS, reactive oxygen species.

cellular stress and regulating autophagosome activity, while as aforementioned, Ero1a favors the environment of the cell by hyper-oxidizing the ER lumen, admitting Ca^{2+}, which is followed by PAC-1 signaling and ER stress, causing the MAM to become narrower. Using PAC-1 as the objective treatment in multiple cell lines (breast, cervical, ovarian and colon cancer cells) resulted in upregulation of Ero1α, increasing ER calcium release and cell death (41). ER stress upregulated PUMA in the MAM region, which in turn activated pro-apoptotic Bax/Bak proteins. The product of these events was calcium leakage at the MAM, and ultimately the induction of autophagy and Mt-mediated apoptosis (46).

A crucial mechanism responsible for regulating eukaryotic cellular growth is the serine/threonine protein kinase known as mTOR. One of the complexes formed by mTOR is mTORC1. Blocking ER-Mt Ca^{2+} flow recruits AMPK for the consumption of glucose, as higher ATP levels are required, while simultaneously inhibiting mTORC1, which induces anabolic pathways, to cutback energy consumption. Eventually AMPK activates autophagy via BECN1, a class III pshosphodylinositol 3-kinase member expressed in the main site where autophagosome fragments assemble (35). A subsequent mTOR complex is mTORC2, which phosphorylates Akt and further phosphorylates PACS2, IP3R and HK2 proteins, any of which can act as a drug target for regulating calcium flux, cell metabolism, integrity of the space and overall cell survival (50). The involvement of IP3R in Ca^{2+}-mediated apoptosis was also supported by the study by Bononi *et al* (2013) (52), which presented PTEN as a compound expressed in the MAM and ER and is capable of neutralising Akt activation. Inactivity of Akt signifies that IP3R is not phosphorylated and hence Ca^{2+} apoptosis occurs due to unregulated ER-Ca^{2+} release (43).

Finally, multiple mutations of NSCLC responsible for affecting behaviour and treatment have been identified over the years (57). One genetic alteration that occurs in NSCLC cell lines (H1975 and H1299) is the substitution of a threonine by methionine at amino acid position 790 (T790M) resulting in cancer cell lines expressing resistance to tyrosine kinase inhibitors, such as erlotinib. This is an impediment often encountered in chemotherapy treatments. Possible solutions involve reversing resistance to anticancer drugs or selecting a different treatment target that will require the use of other drugs. Xu *et al* (2017) (57) chose to evaluate the first option, as EGFR TKI-resistant cells are known to be directly linked to mTORC2, and determined that prescribing rituximab, a CD20 monoclonal antibody, adjusted mTORC2 expression in MERCSs and allowed resistance to erlotinib to be reversed (54). Supplementary evidence on mTORC2 analysis is essential as to its behavior in cancer cells and role in treatments (58).

7. Conclusion

Evaluation of the literature supports the fact that disruption of protein expression within MERCSs, affecting Ca^{2+} influx/efflux, can also impact the physiology of the Mt and ER, inducing autophagy or the apoptosis of cells. Given the vast number of proteins expressed in MERCSs, further analysis is necessary to define protein function, as well as for biomarkers and their potential use as targets for treatment. Multiple examples mentioned in the present review show promising results and insinuate broader exploration is necessary. Finally, the genes highlighted in the review are all expressed within MERCSs and are capable of affecting cellular survival, and furthermore, the clinical attitude of the patients with different cancer types.

Availability of data and materials

Not applicable.

Authors' contributions

ST and AY were responsible for the study concept. Data collection and interpretation was performed by ST, PC, TK, AZ and AY. The manuscript was drafted by ST, CF and AY. All authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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