Mitochondria-endoplasmic reticulum contact site nexus: Molecular integration, disease pathogenesis and therapeutic opportunities (Review)

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Abstract. Mitochondria-endoplasmic reticulum contact sites (MERCs) are dynamic, nanoscale membrane domains that serve as crucial signaling hubs for inter-organellar communication. These specialized interfaces are maintained by a complex network composed of tethering, promoter, and disruptor proteins and coordinate a wide range of cellular processes, such as calcium and zinc ion homeostasis, lipid biosynthesis and transfer, redox signaling, mitochondrial dynamics (fission, fusion and mitophagy), autophagy, apoptosis, inflammation and cellular senescence. Accordingly, the structural and functional integrity of MERCs is vital for cellular adaptation and survival. Nevertheless, MERC plasticity is often impaired in various human pathologies. Alterations in MERC composition, abundance, or function are regarded as pathogenic mechanisms in neurodegenerative diseases, metabolic disorders, cardiovascular conditions, cancer and orthopedic diseases. Common manifestations of MERC dysfunction include disrupted ion signaling, bioenergetic failure, excessive oxidative stress, and impaired organelle quality control. Therefore, targeted modulation of MERCs represents a promising therapeutic avenue. However, translating this potential into clinical practice faces considerable challenges. This is because MERC function is dynamic, context-dependent and dualistic; both excessive and deficient coupling can drive pathology. Future progress hinges on deciphering the precise regulatory codes that govern MERC assembly, developing tools for real-time, high-resolution *in vivo* analysis, and designing innovative, cell-type-specific interventions that normalize rather than simply inhibit or enhance MERC function. A multidisciplinary approach integrating spatial proteomics, super-resolution imaging, and advanced disease modeling is warranted for unlocking the full diagnostic and therapeutic potential of these organelle contact sites.

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Contents

1. Introduction

In recent years, the role of organelles in the pathogenesis of human diseases has emerged as a central focus of scientific research. Cell survival and function depend on the maintenance of organelle homeostasis. However, organelles do not function in isolation; instead, they establish a highly interconnected and collaborative intracellular network. Inter-organellar communication primarily occurs via two mechanisms: Vesicular transport and the formation of specialized membrane contact sites. These contact sites enable direct material exchange and signal transduction between organelles, thereby facilitating the synergistic or antagonistic regulation of cellular processes (1-4). Advances in imaging, particularly electron microscopy and subcellular fluorescence techniques, have been crucial for identifying and characterizing these membrane contact sites. Membrane contact sites are defined as stable yet dynamic regions where the membranes of two distinct organelles, or an organelle and the plasma membrane (PM), come into close contact without fusing. At these junctions, the membranes are consistently separated by a well-defined gap of ~10-30 nanometers (5). Among the diverse inter-organellar connections, the physical and functional interface between mitochondria and the endoplasmic reticulum (ER), known as mitochondria-ER contact sites (MERCs), is the most extensively studied and characterized.

MERCs are ubiquitous in eukaryotic cells. Rather than acting as static bridges, they are dynamic assemblies that are remodeled in response to cellular signals and can cover ~10-15% of the mitochondrial membrane surface area (6-9). These specialized domains function as concentrated macromolecular hubs accommodating hundreds of proteins, including tethering molecules, structural scaffolds, ion channels and transporters, lipid-modifying enzymes and numerous signaling proteins. The molecular composition of MERCs varies across different tissue types and species, reflecting the adaptation to specialized cellular functions. Over the past decade, extensive research has substantially enhanced our understanding of the interaction between MERCs and core cellular activities. It is now clear that MERCs are crucial for numerous fundamental physiological processes, including the regulation of calcium (Ca^{2+}) (10,11) and zinc (Zn^{2+}) signaling (12-14), the control of mitochondrial dynamics (fission, fusion and motility) (15,16), the modulation of inflammatory responses (17), the maintenance of redox balance (18), the synthesis and transfer of lipids (19), the management of ER stress (20,21), the execution of autophagy and mitophagy (22-24), the initiation of apoptosis (25,26) and the regulation of cellular senescence (27,28). Therefore, the structural integrity and functional plasticity of MERCs are of critical importance for cellular adaptation, survival and overall homeostasis.

Despite substantial progress, a crucial challenge persists in understanding how MERCs hierarchically integrate these diverse functions. Ca^{2+} flux, lipid transfer and redox signaling at MERCs are not isolated activities; instead, they are interdependent processes that are coordinately regulated to determine cellular outcomes. For instance, ER-to-mitochondria Ca^{2+} transfer efficiency is modulated by the local lipid composition of the contact-site membrane, which in turn is influenced by MERC-resident lipid transfer proteins (19,29). Concurrently, the redox environment at MERCs, governed by oxidoreductases such as Ero1α and TMX1, directly regulates the activity of Ca^{2+} channels and pumps. As a result, it calibrates the Ca^{2+} signal that drives mitochondrial metabolism and, under pathological conditions, apoptosis (30-33). The present review aims to move beyond listing individual MERC functions by synthesizing how these activities are integrated into a coherent signaling network.

Given the central role of MERCs in cellular physiology, it is unsurprising that dysregulation of MERC architecture or function is increasingly linked with the pathogenesis of a wide range of human diseases. Abnormalities in MERC composition, abundance, or activity have been implicated in neurodegenerative disorders (for example, Alzheimer's, Parkinson's and amyotrophic lateral sclerosis), metabolic diseases [for example, diabetes and non-alcoholic fatty liver disease (NAFLD)], cardiovascular diseases, cancer and various orthopedic conditions (34-39). These dysfunctions often manifest as disrupted ion homeostasis, bioenergetic failure, excessive oxidative stress, and impaired organelle quality control, ultimately resulting in cellular dysfunction and tissue pathology.

A comprehensive and in-depth understanding of MERCs is therefore essential for the advancement of fundamental cell biology and for the development of precise therapeutic strategies aimed at modulating MERC homeostasis in disease scenarios. The present review provides a comprehensive and integrated overview of the current knowledge about MERCs. The organizational principles and molecular composition that define these contact sites were systematically summarized, the regulatory mechanisms that govern their dynamics were investigated, elaborating on their diverse roles in mediating essential cellular functions, and MERC alterations in disease were described. Finally, the emerging perspectives and future directions for the targeted therapeutic modulation of MERCs were discussed, highlighting the considerable potential and significant challenges involved in translating this knowledge into clinical applications.

To provide a unifying framework for the diverse functions and pathological roles discussed in the present review, a three-tier model of MERC-mediated signal integration were proposed. In the first tier (Sensing), MERCs detect and transduce rapid ionic signals, mainly Ca^{2+} and Zn^{2+}, together with local redox changes that convey information regarding cellular metabolic status and stress. In the second tier (Processing), these signals are decoded by MERC-resident protein complexes that coordinate mitochondrial dynamics (fission, fusion and mitophagy), lipid biosynthesis and transfer and autophagic flux. In the third tier (Execution), the integrated output is translated into cell fate decisions, such as metabolic adaptation, apoptosis, senescence and inflammatory responses, thereby influencing tissue homeostasis or pathology. This hierarchical model, used throughout the review, provides a conceptual framework for comprehending how dysfunction at any level of MERC signaling can propagate and contribute to disease.

A recurring pattern in the literature, and a unifying theme for the present review, is the dualistic nature of MERC function in disease. Evidence from multiple systems demonstrates that the relationship between MERC abundance or

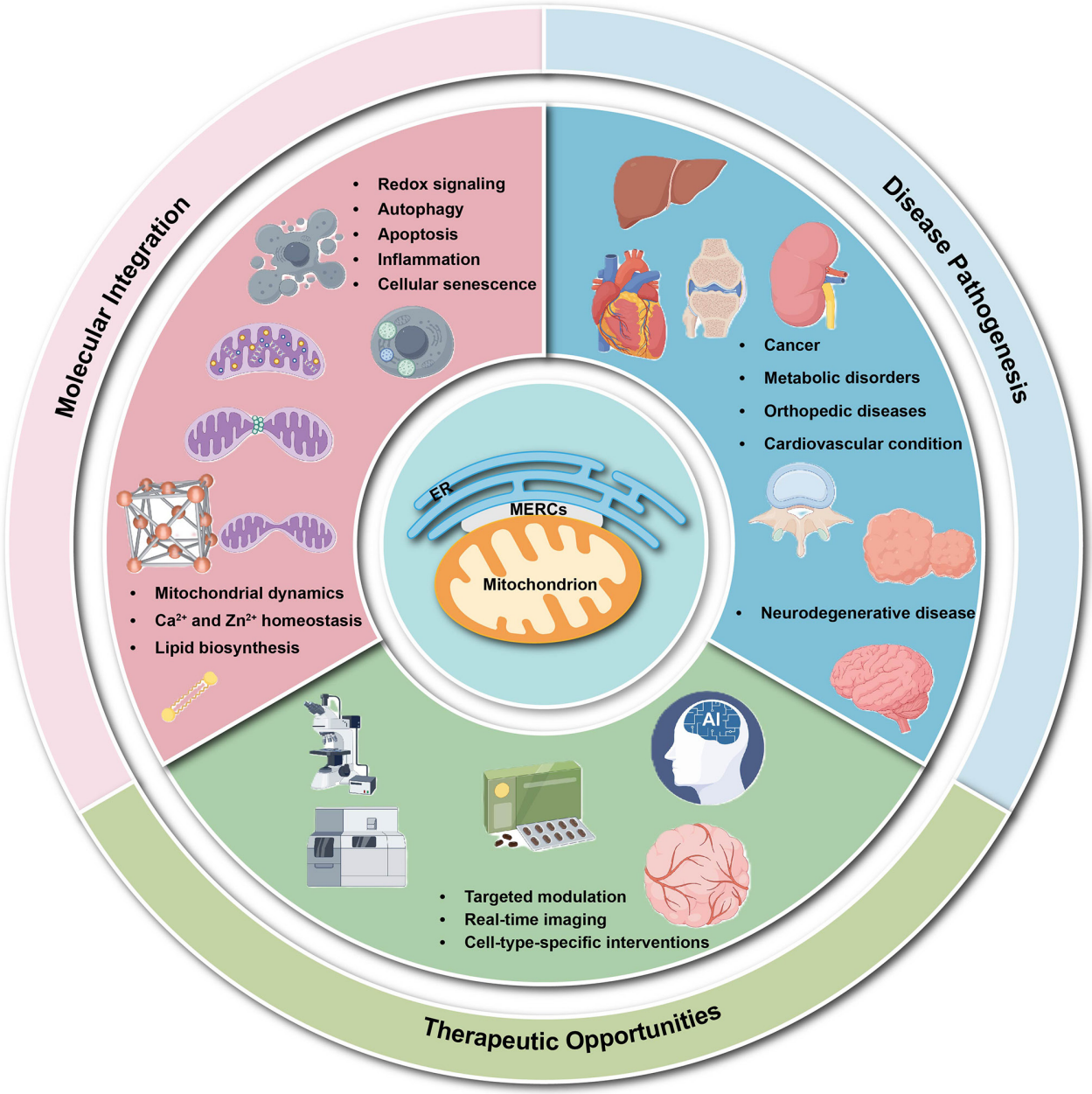

Figure 1. Overview of MERCs molecular integration, disease pathogenesis and therapeutic opportunities. MERCs, mitochondria-endoplasmic reticulum contact sites.

activity and cellular health is non-linear. In pathological contexts, both decreased and increased ER-mitochondria coupling have been observed, with the direction of the change depending on the specific molecular insult, cell type and disease stage. Insufficient coupling impairs the essential inter-organellar transfer of Ca^{2+} and lipids, leading to bioenergetic failure and defective organelle quality control (39-42). Conversely, excessive coupling can result in mitochondrial Ca^{2+} overload, elevated oxidative stress and apoptotic cell death (27,43-45). This context-dependent duality provides a framework for understanding why MERC phenotypes differ across diseases. For example, contact sites are diminished in Charcot-Marie-Tooth disease type 2A and amyotrophic lateral sclerosis, whereas they are increased in certain cancers and diabetic cardiomyopathy (DCM) (42,45,46). Throughout the current review, this dualistic pattern was highlighted and its implications for therapeutic strategies aimed at restoring MERC homeostasis were discussed (Fig. 1).

2. Organization and evaluation of MERCs

MERCs were first detected by electron microscopy in the 1950s (47,48) and were later biochemically identified in the 1990s (49). In 2006, electron tomography revealed that subdomains of the outer mitochondrial membrane (OMM) and the ER are connected by tethers that vary in size and shape (50). Because electron microscopy provides resolution that matches the scale of these nanoscale junctions, it remains the only method capable of direct visualization. Three-dimensional (3D) reconstructions generated through electron tomography provide the most detailed and comprehensive views of ER-mitochondria interactions (50,51). Whole-cell, three-dimensional maps of

ER-mitochondria contacts have since been generated using focused ion beam scanning electron microscopy (FIB-SEM) in neurons at 4 nm resolution (52), serial tilt-angle tomography in yeast cells (51), and, more recently, soft X-ray tomography in lymphoblastoid cells with a 50 nm resolution (53). However, data acquisition and reconstruction processes associated with these 3D techniques remain highly time-consuming, limiting their widespread use for generating statistically robust organelle geometry datasets.

Several groups have analyzed ER-mitochondria interaction parameters using transmission electron microscopy (TEM) to enable statistical comparisons, yet variability in measurement methodologies has made cross-study comparisons difficult. The main quantitative parameters include the length of the contact interface and the width of the gap between the ER and OMM. Some studies have measured contact lengths normalized to mitochondrial perimeter (54), whereas others have quantified the number or frequency of ER-mitochondria contacts (55), typically normalized per mitochondrion. However, TEM is labor-intensive and captures only static snapshots of cellular ultrastructure. More importantly, TEM detects physical proximity rather than functional MERCs; an ER tubule may surround a mitochondrion without forming active MERCs, as close apposition alone does not confirm molecular tethering or biological activity. Immuno-gold labeling in EM has been used to detect specific proteins localized at ER-mitochondria contact sites.

Among various microscopy-based methods, scanning electron microscopy (SEM) has been proposed as a tool for examining ER-mitochondria interactions across large specimen volumes. Conventional SEM uses low-energy electrons to generate surface-specific images (52,56), whereas backscattered electrons can capture signals from the first few nanometers beneath the surface, offering high Z-resolution. Over the past two decades, advanced techniques have markedly improved the quality, efficiency and resolution of full 3D image reconstruction. These approaches use an automated ultramicrotome within the SEM chamber for serial block-face imaging (57) or a focused ion or plasma beam (FIB-SEM) (58,59) to sequentially remove thin layers from a hardened sample. Both methods can be automated to acquire hundreds of serial images. Increasing evidence indicates that immunogold labeling can be combined with SEM to detect resident proteins at the ER-mitochondria interface (60). Despite these technological advances, generating comprehensive 3D reconstructions remains challenging due to the substantial computational power and time required to process large datasets.

Confocal microscopy is another approach for assessing the juxtaposition of mitochondria and the ER, using organelle-specific tracker probes (61) or fluorescent proteins (FPs) (62) targeted to these compartments. The degree of colocalization between the ER and mitochondria is typically quantified using Manders' colocalization coefficient in ImageJ (62). However, mitochondrial membrane potential (MMP) influences the accumulation of MitoTracker dyes thereby affecting organelle labeling efficiency. Therefore, mitochondria-targeted genetically encoded FPs are strongly recommended, as they remain stable under varying mitochondrial conditions, including fluctuations in membrane potential and redox state (63). Morphological changes in organelles can also be misleading and complicate the interpretation of fluorescence imaging data (64). Most critically, the physical thickness of MERCs is far below the resolution limit of conventional confocal microscopes (~150-300 nm). Consequently, this method measures organelle proximity rather than the formation of bona fide MERCs.

To address these limitations, confocal imaging has been used to monitor dynamic changes in MERCs under normal and stress conditions using a split-GFP-based reporter system (65). The split-GFP system consists of two non-fluorescent fragments of a superfolder GFP variant: one containing β-strands 1-10 and the other comprising β-strand 11, which can spontaneously reassemble into a functional β-barrel structure capable of emitting fluorescence (66,67). Fluorescence is restored only when the two fragments are brought into close proximity, allowing each moiety to be targeted to one of the adjacent membranes of interest. Reconstituted split-GFP signals appear as distinct puncta localized between various organelle pairs, including the ER and mitochondria, vacuoles, peroxisomes and lipid droplets. These findings suggest that individual organelles simultaneously establish discrete contact sites with specific membrane domains of their partners (68,69). The split-GFP system therefore holds promise as a tool for identifying regulatory proteins and previously unknown tethering factors at inter-organellar interfaces. Advanced optical microscopy techniques with improved resolution are also available. Super-resolution fluorescence microscopy (SRM) offers enhanced spatial and temporal resolution, enabling more detailed investigation of ER-mitochondria contact site dynamics and fine structural architecture (70,71). Structured illumination microscopy (SIM), a type of SRM suitable for rapid live-cell imaging, has been used to visualize the kinetics and subcellular organization of ER-mitochondria interactions (72,73). Nevertheless, SIM remains costly and technically demanding, limiting its widespread application in characterizing MERCs. It also requires specialized instrumentation and specialized technical expertise.

Several methods detect sites of organelle contact without providing information on their structural architecture. Fluorescence resonance energy transfer (FRET) represents another promising approach for studying contact site dynamics, as it is highly sensitive to intermembrane distance. This technique detects energy transfer between two fluorophores targeted to specific organelles. These fluorophores are engineered with rapamycin-inducible dimerization domains (74); upon addition of rapamycin, they dimerize and generate a maximal FRET signal. Although initially developed to visualize ER-mitochondria juxtaposition (75), this method could theoretically be adapted to assess any potential contact site by attaching appropriate targeting sequences to each fluorescent protein independently. A major limitation of FRET, however, is the requirement for equimolar expression of the two probe components, which can hinder reproducibility and sensitivity.

The proximity ligation assay (PLA) can be tailored to investigate proteins localized at two-membrane interfaces. It enables highly specific detection of proximal protein-protein interactions in cells or tissues (76,77). The method uses proximity probes consisting of oligonucleotide-conjugated antibodies directed against two target proteins. When these proteins are in close proximity, the bound antibodies facilitate

the formation of a circular DNA strand. This circular DNA, covalently linked to the antibody-antigen complex, serves as a template for rolling-circle amplification. The amplified product is then detected via hybridization with fluorescently labeled complementary oligonucleotides. Notably, PLA can function as a molecular ruler for estimating distances between epitopes by adjusting the length of the oligonucleotides on the proximity probes and the size of the protein-binding agents. Söderberg *et al* (78) estimated that the maximum detectable distance, accounting for the sizes of both antibodies and the connecting oligonucleotides, is ~30 nm. Longer oligonucleotides can extend this range when needed, whereas shorter DNA linkers combined with more compact binding agents can reduce the detection distance to just over 10 nm, thereby enhancing spatial resolution (78). A key limitation of PLA, however, is that signal intensity depends on the expression levels of the target proteins, which may vary across samples. Additionally, background signals can arise because the PLA partners are not always exclusively localized at organelle contact sites. Finally, because sample fixation is required prior to analysis, PLA provides only a static snapshot of cellular conditions, raising the possibility of artifacts and limiting insights into dynamic processes.

Subcellular fractionation using sucrose gradient centrifugation is a widely used method for studying the biochemical properties of ER-mitochondria interface sites. When ER-mitochondria interactions were first observed in liver mitochondrial preparations isolated via sucrose-based fractionation in the late 1950s, they were initially attributed to contamination (79). Since then, extensive efforts have been devoted to refining protocols for enriching mitochondrial-associated membranes (MAMs) during cell or tissue fractionation. Throughout the present review, two related but non-interchangeable terms were clearly distinguished. 'MERCs' denote the physical, nanoscale membrane domains where the two organelles closely appose each other and engage in functional crosstalk. 'MAMs' specifically refer to the biochemical membrane fraction that copurifies with mitochondria during subcellular fractionation and is enriched in MERC-resident proteins. MAMs are therefore an experimental preparation that captures MERC components. However, not all MAM proteins are exclusively localized to MERCs, and not all MERC functions can be studied using MAM preparations alone. MAMs represent a distinct membrane fraction that remains biochemically associated with mitochondria and have played a key role in establishing the functional and physical link between the ER and mitochondria (80-82).

To separate MAMs from pure mitochondria, Wieckowski *et al* (83) established a comprehensive protocol starting with HeLa cells or rat liver tissue. Crude mitochondria containing both MAMs and intact mitochondria are first isolated. Subsequently, differential high-speed centrifugations in solutions of varying mannitol and Percoll concentrations enable the separation of MAM and pure mitochondrial fractions (83). Rigorous controls are required to validate successful MAM isolation. Specifically, western blot analysis of well-characterized marker proteins should be performed to confirm effective fractionation. Voltage-dependent anion channel (VDAC), fatty acid-CoA ligase 4 (FACL4) and inositol 1,4,5-trisphosphate receptor 3 (ITPR3) are commonly recognized as enriched MAM-resident proteins. To assess potential cross-contamination, markers specific to pure mitochondria or ER should also be examined: Cytochrome c and NADH dehydrogenase 1 alpha subcomplex subunit 9 indicate mitochondrial purity, whereas calnexin and calreticulin serve as ER markers and help rule out ER contamination in non-MAM fractions. Finally, contamination from other organelles, such as lysosomes, Golgi apparatus, peroxisomes, nucleus and PM, must be excluded. Given that proteomic analysis can identify novel MAM-localized proteins following purification, subcellular fractionation remains one of the most widely employed approaches for investigating ER-mitochondria tethering and may uncover new molecular components at these contact sites (84). It is important to note, however, that the prolonged purification process may alter protein composition through post-translational modifications (for example, phosphorylation) or disruption of protein complexes (for example, dimerization). Additionally, residual contamination from non-target membranes may compromise the specificity of isolated MAM proteins. Findings derived from this method should therefore be validated using complementary techniques.

Several approaches have been used to identify novel proteins localized at MERCs or involved in their formation and function. A genetic screen in yeast for mutations suppressible by expression of a synthetic tether revealed the ER-mitochondrial encounter structure (85), whereas a functional screen based on the role of ER-mitochondria contacts in lipid transfer identified the ER membrane protein complex (EMC) (86). The VDAC1-GRP75-ITPR and VAPB-PTPIP51 interactions were initially discovered through yeast two-hybrid screening of putative tethering components. Numerous studies have reported mass spectrometry (MS) analysis of ER-mitochondria interactions using MAM fractions isolated via gradient centrifugation from mouse brain and liver (87), as well as from virus-infected cells (88,89). Importantly, resident proteins at ER-mitochondria interfaces have been successfully identified using an engineered ascorbate peroxidase (APEX) (90,91). Unlike horseradish peroxidase, which is commonly used but inactive under reducing conditions, APEX remains functional when expressed in the cytoplasm, mitochondria and other reducing environments. Upon treatment of live cells with H_2O_2 and biotin-phenol for 1 min, APEX catalyzes the one-electron oxidation of biotin-phenol, generating a highly reactive, short-lived biotin-phenoxyl radical that covalently labels proximal endogenous proteins. Biotinylated proteins can then be efficiently enriched using streptavidin-based pull-down assays. Lam *et al* (90) demonstrated that introducing a single A134P mutation enhances the stability and sensitivity of APEX. Cho *et al* (91) showed that this technique can be coupled with MS to identify previously unknown proteins residing at the ER-mitochondria interface. By targeting the APEX probe to the outer membranes of distinct organelles, researchers have mapped the proteomic landscape associated with specific inter-organellar contact sites (92). Furthermore, APEX2 has been split into two inactive fragments that reassemble into an active peroxidase when brought into close proximity. These fragments are fused to rapamycin-inducible dimerization domains, requiring rapamycin addition to induce reconstitution of enzymatic activity (93). Similar to FRET-based systems, the applicability

of this split-APEX system is limited by its dependence on rapamycin.

In summary, research on MERCs has advanced from the initial ultrastructural observations to a well-developed, multimethodological domain. The techniques can be broadly classified into two categories: Those that reveal structural morphology (for example, various EM modalities and super-resolution microscopy) and those that detect functional interaction or molecular composition (for example, biochemical fractionation, split-GFP, PLA, FRET and APEX-based proximity labeling). Each method has distinct advantages and inherent limitations. These limitations range from the high spatial resolution but static and labor-intensive nature of EM to the dynamic and functional readouts of fluorescent probes, which frequently sacrifice direct structural visualization for physiological relevance. Looking ahead, several key challenges and opportunities shape the future direction of MERC research. First, there is an urgent need to integrate complementary techniques, by correlating high-resolution 3D structural data from advanced EM with dynamic, functional readouts from live-cell imaging and molecular mapping, to establish a comprehensive understanding of MERCs. Second, developing more accessible, high-throughput and automated methods for 3D EM analysis will be critical for generating statistically reliable datasets on organelle connectivity and MERC architecture across various cellular states. Third, future tools should minimize perturbation, as numerous current probes rely on overexpression or chemical induction, which may modify native MERC physiology. Fourth, improving the spatial and temporal resolution of live-cell imaging to the nanometer scale, perhaps through next-generation super-resolution techniques or novel biosensors, will be essential for capturing the rapid dynamics of tether formation, disassembly and functional regulation. Finally, expanding the molecular toolbox to systematically discover and validate new tethering components and regulatory mechanisms, perhaps through *in-situ* cryo-EM or refined proximity proteomics in native environments, will enhance our understanding of how MERCs integrate cellular signaling, lipid metabolism and organelle homeostasis in health and disease.

A crucial consideration when interpreting the MERC literature is that no single method can unambiguously define a 'true' functional contact site. Each technique captures a distinct aspect of the ER-mitochondria association, and findings from different approaches are not always concordant. Electron microscopy, whether TEM or FIB-SEM, remains the gold standard for resolving nanoscale architecture, yet it detects physical proximity rather than functional coupling; an ER tubule passing within 30 nm of a mitochondrion may or may not represent a genuine signaling-competent MERC (50,52). Proximity-based assays such as PLA and split-GFP report on protein-protein interactions or membrane apposition, but they depend on the expression levels of the targeted proteins and may detect transient or nonfunctional encounters (65,76). Functional readouts, such as measurements of ER-to-mitochondria Ca^{2+} transfer or lipid exchange, offer evidence of active communication. By themselves, however, they cannot determine whether this communication occurs through stable contact sites or via diffusible intermediates. Biochemical MAM isolation provides proteomic information but is prone to post-lysis artifacts and contamination from other membrane compartments (82). These methodological disparities are a key source of apparent discrepancies in the literature. For instance, a study using TEM-based contact length quantification might report a decrease in MERCs under a specific condition, whereas a FRET-based assay could detect unchanged or even increased functional coupling. Future studies should, therefore, adopt orthogonal and complementary approaches. Ideally, these approaches should integrate high-resolution structural imaging with dynamic functional readouts to draw reliable conclusions about MERC status in both health and disease.

3. Composition of MERCs

A core set of proteins mediates the tethering of mitochondria to the ER in mammalian cells. These proteins either form direct physical linkages or regulate the assembly and stability of tethering complexes at MERCs (Fig. 2). Similar to other PM-anchoring proteins such as junctophilins, STIM1 and extended synaptotagmins, an intermembrane tether can consist of a single protein containing two distinct membrane-binding domains. ATPase family AAA domain-containing protein 3 (ATAD3) is currently the only known natural candidate that functions as a single-protein linker between the ER and mitochondria (94). While certain proteins (for example, ITPR and FATE1/EMD) are associated with relatively wide intermembrane gaps, numerous tethers serve to narrow the distance between the ER and OMM. Notably, FATE1/EMD has been shown to inhibit functions requiring close membrane apposition (95).

Well-characterized MERC-resident proteins include the following: (i) Tethering factors: ATAD3 (94,96), MFN2-MFN1/2 (mitofusin 1/2) (62), MFN2-sarco-ER calcium Ca^{2+} ATPase 2 (SERCA2) (39), GRP75-ITPRs(1, 2, and 3)-VDACs (82), mitochondrial fission 1 protein (FIS1)/translocase of OMM 40 (TOM40)-B-cell receptor-associated protein 31 (BAP31) (81,97), VAPB-PTPIP51 (46,98), PDZ domain containing 8 (PDZD8) (99), inverted formin 2 (INF2)-spire type actin nucleation factor 1 (SPIRE1C) (16,100), oxysterol-binding protein related-protein 5 and 8 (ORP5/ORP8)-PTPIP51 (29), receptor accessory protein 1 (REEP1) (101), synaptojanin 2 binding protein (SYNJ2BP)-ribosome binding protein 1 (RRBP1) (92), motile sperm domain-containing protein 2 (MOSPD2)-PTPIP51 (102), the FUN14 domain containing 1 (FUNDC1)-ITPRs (103), fetal and adult testis expressed 1 (FATE1)-Emerin (EMD) (95), EMC2-solute carrier family 25 member 46 (SLC25A46) (40); (ii) Promoters: phosphofurin acidic cluster sorting protein 2 (PACS2) (25), cyclophilin D (CYPD) (104), Sigma-1 receptor (Sig-1R) (105), thymocyte-expressed, positive selection-associated gene 1 (TESPA1) (106), autocrine motility factor receptor (AMFR) (107-109), Caveolin 1 (CAV1) (110), dynamin-related protein 1 (DRP1) (111,112), IRBIT/adenosylhomocysteinase like 1 (AHCYL1) (113), membrane associated ring-ch-type finger 5 (MARCH5)/mitochondrial ubiquitin ligase (MITOL) (114), mitochondrial Rho GTPase 1 (MIRO1) (115,116), mechanistic target of rapamycin kinase 2 (mTORC2) (117), reticulon 1C (RTN-1C) (118), translocase of the outer membrane 70 (TOM70) (119),

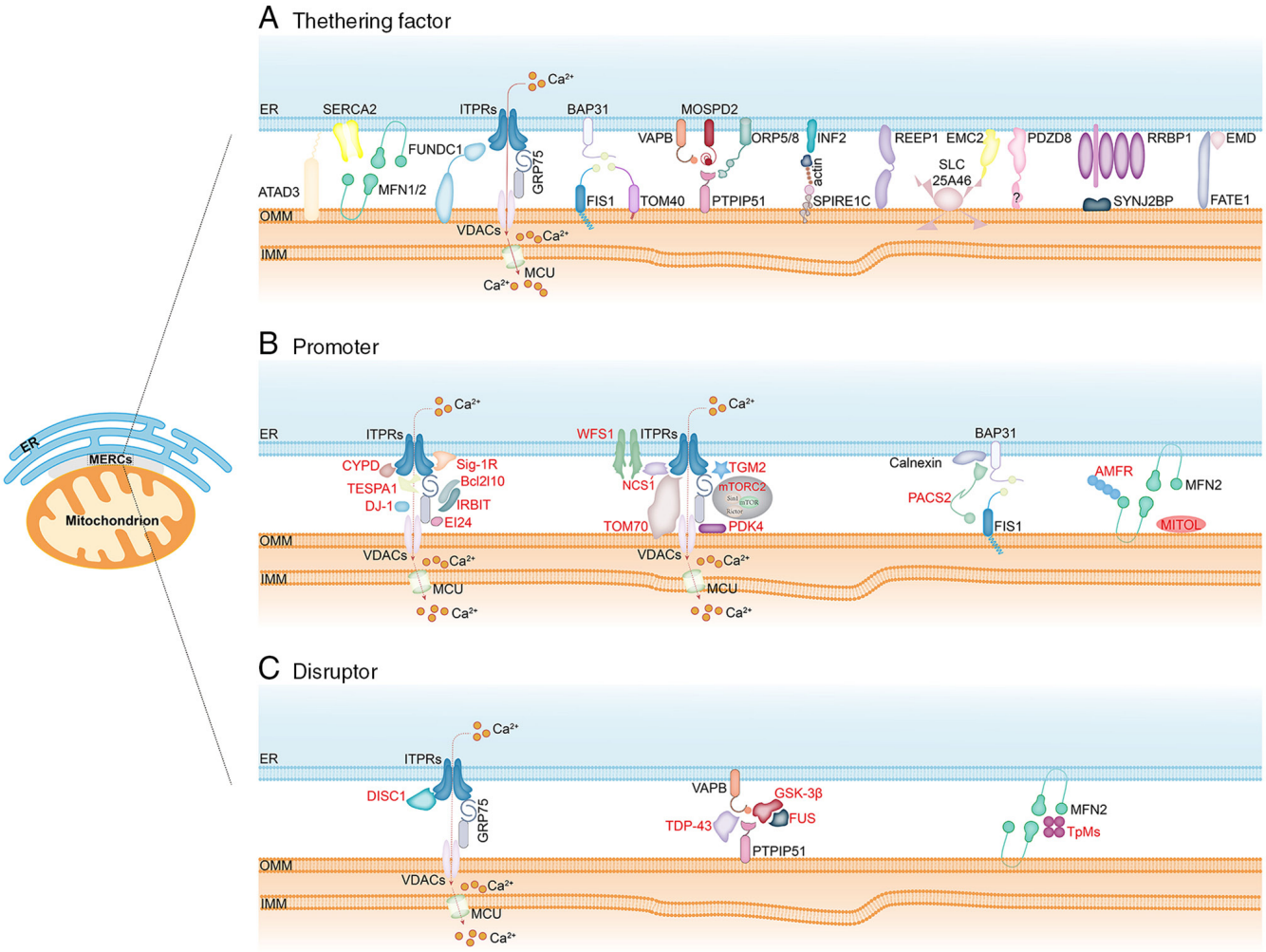

Figure 2. Composition of MERCs. MERCs are specialized membrane domains formed by the close apposition of the endoplasmic reticulum membrane and the OMM, serving as physical bridges between the two organelles. These structures are organized through: (A) Tethering factors, including ATAD3, MFN2-MFN1/2, MFN2-SERCA2, ITPRs-GRP75-VDACs, FUNDC1-ITPRs, BAP31-FIS1, BAP31-TOM40, VAPB-PTPIP51, MOSPD2-PTPIP51, ORP5/8-PTPIP51, INF2-actin-SPIRE1C, REEP1, EMC2-SLC25A46, PDZD8, RRBP1-SYNJ2BP and FATE1-EMD; (B) promoters, such as CYPD, TESPA1, DJ-1, Sig-1R, IRBIT/AHCYL1, EI24, WFS1-NCS1, TOM70, TGM2, mTORC2, PDK4, PACS2, AMFR and MITOL/MARCH5; and (C) disruptors, including DISC1, TDP-43, FUS, GSK3β and Trichoplein/TpMs. MERCs, mitochondria-endoplasmic reticulum contact sites; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; ATAD3, ATPase family AAA Domain-containing protein 3; MFN1/2, mitofusin 1/2; SERCA2, sarco-endoplasmic reticulum calcium Ca^{2+} ATPase 2; ITPRs, inositol 1,4,5-trisphosphate receptors; GRP75, glucose regulate protein 75; VDACs, voltage-dependent anion channels; FUNDC1, the FUN14 domain containing 1; FIS1, mitochondrial fission 1 protein; TOM40, translocase of OMM 40; BAP31, B-cell receptor-associated protein 31; VAPB, vesicle-associated membrane-protein-associated protein B; PTPIP51, protein tyrosine phosphatase-interacting protein 51; MOSPD2, motile sperm domain-containing protein 2; ORP5/ORP8, oxysterol-binding protein related-protein 5 and 8; INF2, inverted formin 2; SPIRE1C, spire type actin nucleation factor 1; REEP1, receptor accessory protein 1; PDZD8, PDZ domain containing 8; SLC25A46, solute carrier family 25 member 46; SYNJ2BP, synaptojanin 2 binding protein; RRBP1, ribosome binding protein 1; FATE1, fetal and adult testis expressed 1; EMD, Emerin; CYPD, cyclophilin D; TESPA1, thymocyte-expressed, positive selection-associated gene 1; Sig-1R, Sigma-1 receptor; EI24, etoposide-induced protein 2.4; WFS1, Wolfram syndrome 1; NCS1, neuronal calcium sensor 1; TOM70, translocase of the outer membrane 70; TGM2, transglutaminase type 2; mTORC2, mechanistic target of rapamycin kinase 2; PDK4, pyruvate dehydrogenase kinases 4; PACS2, phosphofurin acidic cluster sorting protein 2; MITOL, mitochondrial ubiquitin ligase; AMFR, autocrine motility factor receptor; DISC1, disrupted-in-schizophrenia 1; TDP-43, TAR DNA-Binding Protein-43; GSK3β, glycogen synthase kinase-3β.

transglutaminase type 2 (TGM2) (120), Wolfram syndrome 1 (WFS1) (121), pyruvate dehydrogenase kinases 4 (PDK4) (122), etoposide-induced protein 2.4 (EI24) (123,124), DJ-1 (125), DsbA-L (35), Lonp1 (41); (iii) Disruptors: disrupted-in-schizophrenia 1 (DISC1) (126,127), FUS (128), glycogen synthase kinase-3β (GSK3β) (129,130), TAR DNA-Binding Protein-43 (TDP-43) (98), Trichoplein/TpMs (131).

Notably, the precise role of MFN2 in MERCs remains a topic of active research and debate. Although early studies recognized MFN2 as a crucial tether that physically connects the two organelles (62), later work suggested that MFN2 might instead regulate the inter-organellar distance. Paradoxically, its ablation can increase ER-mitochondria coupling in specific contexts (54). These inconsistent findings likely arise from cell-type-specific compensatory mechanisms and the pleiotropic functions of MFN2 in mitochondrial fusion, underscoring the need for careful interpretation of MERC phenotypes in genetic models. The functional roles of the MERC-resident proteins discussed in the present review are summarized in Table I.

In summary, the composition of MERCs is highly complex and dynamic, involving a sophisticated network of proteins that can be functionally classified as tethers, promoters, or disruptors. This comprehensive list emphasizes that MERCs

Table I. Summary of the functional roles of MERCs-resident proteins.

Authors, year	Protein	Mitochondria-ER contact role	Relevant interactions	Relevant functions	(Refs.)
Issop *et al*, 2015	ATAD3	Tethering factor	Interacts with DRP1	Facilitates optimal cholesterol transfer from the endoplasmic reticulum to mitochondria for steroidogenesis.	(96)
Yang *et al*, 2023; de Brito *et al*, 2008	MFN2	Tethering factor	Interacts with MFN1/2, SERCA2	Mediates ER-mitochondria tethering, a prerequisite for efficient mitochondrial Ca^{2+} uptake. Enhances SERCA2-mediated Ca^{2+} reuptake into the ER, thereby preventing excessive mitochondrial Ca^{2+} accumulation and cell death.	(39,62)
Yang *et al*, 2023	SERCA2	Tethering factor	Interacts with MFN2	Enhances SERCA2-mediated Ca^{2+} reuptake into the ER, thereby preventing excessive mitochondrial Ca^{2+} accumulation and cell death.	(39)
Rizzuto *et al*, 1998	ITPRs	Tethering factor	Form complex with GRP75 and VDACs; Interacts with FUNDC1	Participates in the regulation of ER Ca^{2+} release.	(10)
	GRP75	Tethering factor	Form complex with ITPRs and VDACs	Participates in the regulation of ER Ca^{2+} release.	
	VDACs	Tethering factor	Form complex with ITPRs and GRP75	Participates in the regulation of ER Ca^{2+} release.	
Wu *et al*, 2017	FUNDC1	Tethering factor	Interacts with ITPRs	Binds ITPR2 to modulate Ca^{2+} release from the ER into the cytosol and mitochondria.	(103)
Iwasawa *et al*, 2011; Namba *et al*, 2019	BAP31	Tethering factor	Interacts with FIS1, and TOM40	The FIS1-BAP31 complex serves as a platform for apoptosis signaling. The BAP31-TOM40 complex regulates oxygen consumption and mitochondrial complex I activity.	(81,97)
Iwasawa *et al*, 2011	FIS1	Tethering factor	Interacts with BAP31	The FIS1-BAP31 complex serves as a platform for apoptosis signaling.	(81)
Namba *et al*, 2019	TOM40	Tethering factor	Interacts with BAP31	The BAP31-TOM40 complex regulates oxygen consumption and mitochondrial complex I activity.	(97)
De Vos *et al*, 2012	VAPB	Tethering factor	Interacts with PTPIP51	VAPBP56S enhances Ca^{2+} uptake and modifies PTPIP51 binding.	(80)
De Vos *et al*, 2012; Di Mattia *et al*, 2018; Galmes *et al*, 2016	PTPIP51	Tethering factor	Interacts with VAPB, MOSPD2, and OPR5/8	VAPB-P56S enhances mitochondrial Ca^{2+} uptake and alters PTPIP51 binding affinity. Tethers the ER and other organelles. ORP5 and ORP8 require a functional lipid-binding/transfer ORD domain to interact with mitochondrial PTPIP51.	(29,80,102)
Di Mattia *et al*, 2018	MOSPD2	Tethering factor	Interacts with PTPIP51	Tethers the ER and other organelles.	(102)

Table I. Continued.

Authors, year	Protein	Mitochondria-ER contact role	Relevant interactions	Relevant functions	(Refs.)
Galmes *et al*, 2016	OPR5/8	Tethering factor	Interacts with PTPIP51	ORP5 and ORP8 require a functional lipid-binding/transfer ORD domain to interact with mitochondrial PTPIP51.	(29)
Manor *et al*, 2015	INF2	Tethering factor	Interacts with SPIRE1C	Spire1C promotes actin assembly on mitochondrial surfaces through interaction with INF2.	(100)
	SPIRE1C	Tethering factor	Interacts with INF2	Spire1C promotes actin assembly on mitochondrial surfaces through interaction with INF2.	
Lim *et al*, 2015	REEP1	Tethering factor		REEP1 facilitates ER-mitochondria interactions.	(101)
Hirabayashi *et al*, 2017	PDZD8	Tethering factor		PDZD8 tethers mitochondria to the ER, regulating dendritic Ca^{2+} dynamics.	(99)
Hung *et al*, 2017	RRBP1	Tethering factor	Interacts with SYNJ2BP	A companion of SYNJ2BP for ERM binding was discovered as RRBP1 byimmunoprecipitation-mass spectrometry.	(92)
	SYNJ2BP	Tethering factor	Interacts with INF2	RRBP1 was identified as a binding partner of SYNJ2BP in ER-mitochondria membrane (ERM) contact sites via immunoprecipitation-mass spectrometry.	
Dong *et al*, 2024	EMC2	Tethering factor	Interacts with SLC25A46	The EMC2-SLC25A46-Mic19 axis represents a pathway regulating ER-mitochondria interactions.	(40)
	SLC25A46	Tethering factor	Interacts with EMC2	One route controlling ER-mitochondria interactions is the EMC2-SLC25A46-Mic19 axis.	
Doghman-Bouguerra *et al*, 2016	FATE1	Tethering factor	Interacts with EMD	FATE1-mediated uncoupling of ER-mitochondria may protect against apoptosis.	(95)
	EMD	Tethering factor	Interacts with FATE1	FATE1-mediated uncoupling of ER-mitochondria may protect against apoptosis.	
Paillard *et al*, 2013	CYPD	Promoter	Interacts with VDAC1/GRP75/ITPR1 complex	Modulates mitochondrial Ca^{2+} overload through the CYPD/VDAC1/GRP75/ITPR1 complex.	(104)
Matsuzaki *et al*, 2013	TESPA1	Promoter	Interacts with VDAC1/GRP75/ITPR3 complex	TESPA1 regulates ITPR3-mediated Ca^{2+} release and mitochondrial Ca^{2+} uptake by directly interacting with GRP75, but not VDAC1.	(106)
Liu *et al*, 2019	DJ-1	Promoter	Interacts with VDAC1/GRP75/ITPR3 complex	Contributes to the regulation of Ca^{2+} crosstalk and structural integrity between the ER and mitochondria.	(125)
Hayashi *et al*, 2007	Sig-1R	Promoter	Interacts with VDAC/GRP75/ITPR3 complex	Plays a role in controlling cell viability and inter-organellar ER-mitochondrial Ca^{2+} signaling.	(105)

Table I. Continued.

Authors, year	Protein	Mitochondria-ER contact role	Relevant interactions	Relevant functions	(Refs.)
Bonneaue *et al*, 2016	IRBIT	Promoter	Interacts with VDAC/GRP75/ITPR complex	Regulates Bcl2l10 activity and ER-mitochondria coupling.	(113)
Yuan *et al*, 2020	EI24	Promoter	Interacts with VDACs/GRP75/ITPRs complex	Promotes ER-to-mitochondria Ca^{2+} flux and enhances DNA damage-induced apoptosis by interacting with VDAC2 to facilitate ER-mitochondria contact formation.	(123,124)
Angebault *et al*, 2018	WFS1	Promoter	Interacts with VDAC1/GRP75/ITPR1 complex	Forms a complex with NCS1 and ITPR1 to activate ER-mitochondria Ca^{2+} crosstalk, thereby stimulating mitochondrial respiratory chain activity and tricarboxylic acid (TCA) cycle function.	(121)
Filadi *et al*, 2018	TOM70	Promoter	Interacts with VDAC1/GRP75/ITPR3 complex	Interacts with ITPR3 to regulate mitochondrial respiration, influencing autophagy, proliferation, and cellular bioenergetics.	(119)
D'Eletto *et al*, 2018	TGM2	Promoter	Interacts with VDAC1/GRP75/ITPR3 complex	Interacts with GRP75 to modulate Ca^{2+} exchange between the ER and mitochondria.	(120)
Betz *et al*, 2013	mTORC2	Promoter	Interacts with VDAC1/GRP75/ITPR2 complex	Regulates mitochondrial function and MERC integrity by Akt-mediated phosphorylation of MERC-associated ITPR2.	(117)
Thoudam *et al*, 2019	PDK4	Promoter	Interacts with VDAC1/GRP75/ITPR1 complex	Stabilizes the ITPR1-GRP75-VDAC1 complex, which plays a role in regulating ER stress, mitochondrial dysfunction, and mitochondrial Ca^{2+} accumulation.	(122)
Simmen *et al*, 2005	PACS2	Promoter	Interacts with BAP31/FIS1 complex	Contributes to the control of apoptosis, ER homeostasis, and Ca^{2+} exchange between the ER and mitochondria.	(25)
Wang *et al*, 2015	AMFR	Promoter	Interacts with MFN2	Maintains MERC integrity.	(107)
Sugiura *et al*, 2013	MITOL	Promoter	Interacts with MFN2	MITOL is required for GTP-dependent MFN2 oligomerization.	(114)
Sala-Vila *et al*, 2016	CAV1	Promoter		CAV1 is an essential component of hepatic MERCs.	(110)
Prudent *et al*, 2015	DRP1	Promoter		SUMOylated DRP1 functionally stabilizes ER-mitochondrial interactions.	(111)
Macaskill *et al*, 2009	MIRO1	Promoter		Links KIF5 motor proteins to mitochondria.	(116)
Reali *et al*, 2015	RTN-1C	Promoter		Regulates lipid exchange between the ER and mitochondria and maintains intracellular Ca^{2+} homeostasis by modulating mitochondrial morphology and function.	(118)

Table I. Continued.

Authors, year	Protein	Mitochondria-ER contact role	Relevant interactions	Relevant functions	(Refs.)
Yang *et al*, 2019	DsbA-L	Promoter		Preserves MERC integrity to exert antiapoptotic effects.	(35)
Li *et al*, 2023	Lonp1	Promoter		Controls the unfolded protein response in the ER, mitochondrial fission, and MERC stability.	(41)
Park *et al*, 2017	DISC1	Disruptor	Interacts with ITPR1	Participates in the regulation of Ca^{2+} dynamics between mitochondria and the ER.	(127)
Stoica *et al*, 2014	TDP-43	Disruptor	Interacts with VAPB-PTPIP51 complex	TDP-43, pathologically linked to frontotemporal dementia and amyotrophic lateral sclerosis, disrupts ER-mitochondria contacts, impairs the VAPB-PTPIP51 interaction, and perturbs cellular Ca^{2+} homeostasis.	(98)
Stoica *et al*, 2016	FUS	Disruptor	Interacts with VAPB-PTPIP51 complex	FUS disrupts ER-mitochondria connections and the VAPB-PTPIP51 interaction, leading to altered Ca^{2+} uptake.	(128)
Stoica *et al*, 2014	GSK3β	Disruptor	Interacts with VAPB-PTPIP51 complex	Regulates the VAPB-PTPIP51 interaction.	(98)
Cerqua *et al*, 2010	TpMs	Disruptor	Interacts with MFN2	Induces apoptosis inhibition, mitochondrial fragmentation, and loss of ER anchoring.	(131)

are not formed by a single, universal mechanism but are organized through a modular and likely cell-type-specific array of molecular complexes. These components jointly regulate the physical distance, stability and functional output of the contact sites. Beyond simple structural bridging, MERC-resident proteins integrate core cellular processes such as calcium signaling, lipid transfer, apoptosis regulation, mitochondrial dynamics and metabolic adaptation, establishing MERCs as central signaling hubs.

4. Important physiological roles of MERCs

MERCs participate in multiple key physiological processes, including Ca^{2+} homeostasis, Zn^{2+} homeostasis, ER stress responses, redox signaling, autophagy, mitochondrial dynamics, apoptosis, inflammation, lipid metabolism and cellular senescence (Figs. 3-5). These diverse functions can be categorized into three interconnected tiers. The first tier consists of ion signaling, specifically Ca^{2+} and Zn^{2+} flux, which functions as the most rapid and direct form of inter-organellar communication at MERCs. The second tier includes organelle quality control mechanisms, such as mitochondrial dynamics (fission, fusion and mitophagy) and autophagy, which are regulated by MERC-localized ion signals and lipid intermediates. The third tier involves cell fate decisions, namely apoptosis, inflammation and cellular senescence, which represent long-term outcomes triggered when MERC-regulated stress signals surpass homeostatic thresholds. These three tiers are underpinned by the fundamental activities of lipid biosynthesis/transfer and redox signaling, which both modulate and are modulated by the ion flux and quality control mechanisms.

5. MERCs and Ca^{2+} homeostasis

As a key intracellular messenger, Ca^{2+} regulates cellular functions such as gene expression, muscle contraction and cell proliferation (132). Numerous proteins localized at MERCs modulate Ca^{2+} dynamics. Among these, the inositol 1,4,5-trisphosphate receptor (ITPR) is a major ER-resident Ca^{2+} release channel. It mediates the release of concentrated Ca^{2+} from the ER lumen into the cytosol. Upon binding of IP_3 to ITPR, the channel is activated, triggering pore opening and enabling Ca^{2+} efflux from the ER into the cytoplasm (133). Additionally, VDAC1, located on the OMM, has been shown to regulate mitochondrial Ca^{2+} uptake (134,135). Through GRP75, ITPRs interact with VDAC1 to form the ITPR-GRP75-VDAC1 complex, which plays a central role in mediating Ca^{2+} exchange between the ER and mitochondria and reflects the coordinated molecular mechanisms operating within MERCs (136). After passage through VDAC1 in the OMM, Ca^{2+} enters the mitochondrial matrix via the mitochondrial calcium uniporter (MCU) at the inner mitochondrial membrane (IMM), a process regulated by additional auxiliary factors (137). Through activation of ITPR-VDAC1 channels at specialized MERCs, the ER

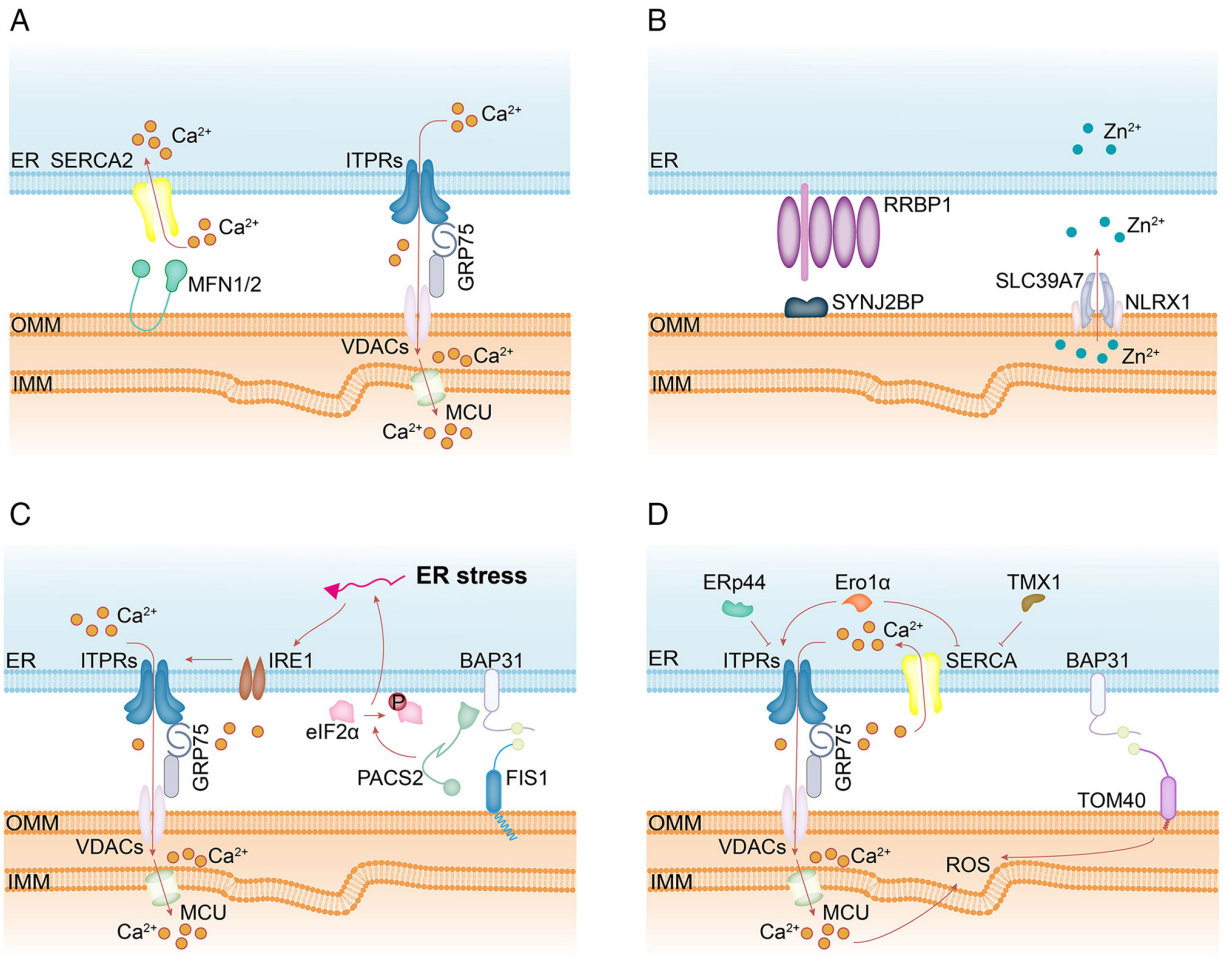

Figure 3. Schematic summary of MERC-associated proteins involved in: (A) Ca^{2+} homeostasis-calcium is transferred from the ER to the outer mitochondrial membrane via the ITPR-GRP75-VDAC complex, promoting mitochondrial activity; MFN2 facilitates ER Ca^{2+} reuptake through SERCA2 at MERCs. (B) Zn^{2+} homeostasis-SYNJ2BP interacts with RRBP1 to support MERC integrity and promotes the interaction between NLRX1 and SLC39A7, thereby regulating mitochondrial Zn^{2+} dynamics. (C) ER stress-the ER stress sensor IRE1, localized at MERCs, enhances mitochondrial Ca^{2+} uptake by activating the ITPR-VDAC axis; depletion of PACS2 leads to a transient increase in phosphorylated eIF2α, indicating induction of ER stress. (D) redox signaling control-MERC-localized ROS are predicted to trigger ITPR activation and SERCA inactivation, amplifying Ca^{2+} transfer; ERp44 and Ero1α modulate ER Ca^{2+} release through regulation of the ITPR-VDAC axis; TMX1 and Ero1α inhibit SERCA activity, reducing cytosolic Ca^{2+} clearance; loss of BAP31 suppresses mitochondrial oxidative phosphorylation, although whether this depends on its interaction with TOM40 remains unclear due to incomplete understanding of BAP31's targeting mechanism to MERCs. ER, endoplasmic reticulum; MERCs, mitochondria-ER contact sites; MFN2, mitofusin 2; SERCA2, sarco-endoplasmic reticulum calcium Ca^{2+} ATPase 2; ITPRs, inositol 1,4,5-trisphosphate receptors; GRP75, glucose regulate protein 75; VDACs, voltage-dependent anion channels; TOM40, translocase of outer mitochondrial membrane 40; BAP31, B-cell receptor-associated protein 31; SYNJ2BP, synaptojanin 2 binding protein; RRBP1, ribosome binding protein 1; PACS2, phosphofurin acidic cluster sorting protein 2; IRE1, inositol-requiring enzyme 1; ERp44, ER protein 44; Ero1α, ER oxidoreductase 1 alpha; TMX1, thioredoxin related transmembrane protein 1; SLC39A7, solute carrier family 39 member 7; NLRX1, NLR family member X1; ROS, reactive oxygen species.

can directly stimulate mitochondrial enzymatic activity (138). Alternatively, the ER indirectly influences mitochondrial function by modulating cytoplasmic Ca^{2+} levels, thereby affecting MMP via SERCA pumps on the ER membrane (139). According to Yang *et al* (39), the MFN2-SERCA2 interaction exerts dual regulatory effects on inter-organellar Ca^{2+} transfer. On the one hand, the physical proximity facilitated by this interaction enhances ER-mitochondria coupling and promotes mitochondrial Ca^{2+} influx. On the other hand, MFN2 supports SERCA2-mediated Ca^{2+} reuptake into the ER at contact sites, potentially preventing excessive mitochondrial Ca^{2+} accumulation and thereby preserving both mitochondrial Ca^{2+} homeostasis and cell survival (39).

By facilitating the formation of specialized microdomains characterized by high Ca^{2+} flux efficiency, MERCs enable the rapid and precise transfer of Ca^{2+} from the ER lumen directly to the mitochondrial matrix via coordinated protein complexes such as ITPR-GRP75-VDAC and the downstream MCU. This direct coupling supports key mitochondrial metabolic processes but is also precisely regulated by interactions such as MFN2-SERCA2, which can adjust the transfer efficiency to avoid cytotoxic Ca^{2+} overload. The structural and functional integrity of MERCs is therefore essential for maintaining calcium homeostasis, directly connecting organelle communication to fundamental cellular processes and survival. The highly organized spatial arrangement of these Ca^{2+} handling complexes defines MERCs as privileged signaling microdomains. Importantly, this Ca^{2+} flux does not function in isolation; it serves as the central mediator of MERC communication, interpreted in a context-specific manner. The

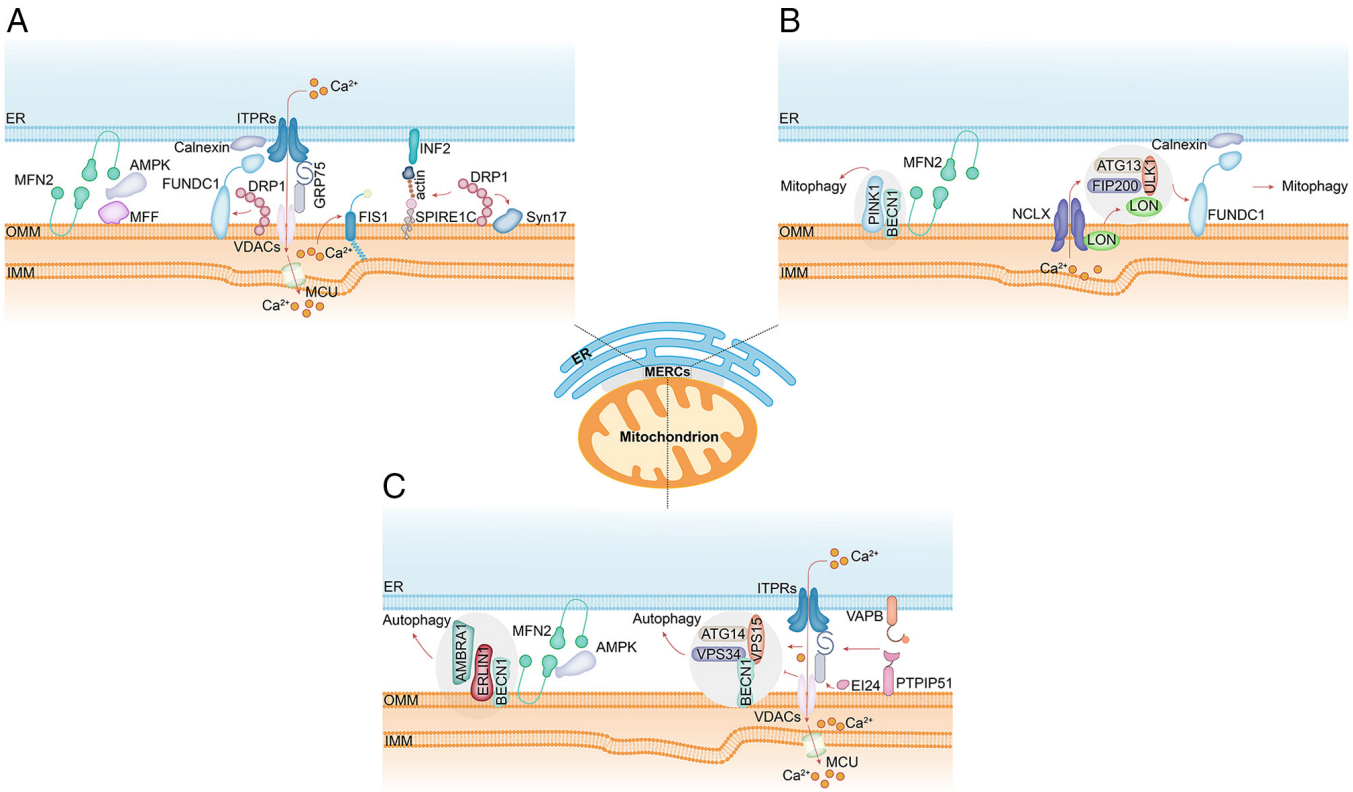

Figure 4. Schematic summary of MERC-associated proteins involved in: (A) Mitochondrial fission-under energy stress, the interaction between MFN2 and AMPK is reduced; this may release active AMPK to phosphorylate MFF, thereby regulating mitochondrial division. FUNDC1 accumulates at MERCs through interaction with the ER-resident protein calnexin and acts as a novel mitochondrial receptor for DRP1 to initiate fission under hypoxic conditions. Spire1C promotes actin assembly on mitochondrial surfaces by binding INF2; disruption of its actin- or formin-binding activities impairs mitochondrial constriction and division. MERCs contain raft-like microdomains enriched with syntaxin 17 (Syn17), which regulates DRP1 localization and activity to promote mitochondrial fission. (B) Mitophagy-during autophagy, PINK1 and Beclin 1 (BECN1) relocalize to MERCs, facilitating autophagosome initiation site formation and enhancing ER-mitochondrial interface functionality. Under hypoxic conditions, stress-induced mitochondrial protease Lon accumulates at MERCs and functions as a molecular chaperone by stabilizing the FUNDC1-ULK1 complex in an NCLX-dependent manner, thereby triggering mitophagy. (C) Autophagy-upon energy stress, substantial levels of AMPK translocate from the cytosol to mitochondria and MERCs, where it directly interacts with MFN2 to promote mitochondrial fission. At MERC raft-like microdomains, ERLIN1 interacts with AMBRA1 and recruits it to the BECN1 complex, a critical step for autophagosome formation. Core components of the autophagy initiation complex-including BECN1, ATG14, VPS15 and VPS34-localize to MERCs. Overexpression of VAPB or PTPIP51 inhibits autophagosome biogenesis. The C-terminal domain of EI24 is essential for both MERC integrity and autophagic flux, and it interacts with the ITPR-VDAC axis. ER, endoplasmic reticulum; MERCs, mitochondria-ER contact sites; MFN2, mitofusin 2; MFF, mitochondrial fission factor; ITPRs, inositol 1,4,5-trisphosphate receptors; VDACs, voltage-dependent anion channels; FUNDC1, the FUN14 domain containing 1; VAPB, vesicle-associated membrane-protein-associated protein B; PTPIP51, protein tyrosine phosphatase-interacting protein 51; INF2, inverted formin 2; SPIRE1C, spire type actin nucleation factor 1; EI24, etoposide-induced protein 2.4; DRP1, dynamin-related protein 1; PINK1, PTEN-induced putative kinase 1; BECN1, Beclin 1; ULK1, unc-51 like autophagy activating kinase 1; ATG14, autophagy related 14; VPS34, vacuolar protein sorting 34; VPS15, vacuolar protein sorting 15; ERLIN1, ER lipid raft associated 1; AMBRA1, autophagy and beclin 1 regulator 1.

amplitude, frequency and spatial localization of mitochondrial Ca^{2+} transients are decoded by Ca^{2+}-sensitive dehydrogenases in the TCA cycle to align ATP production with cellular demand. Meanwhile, sustained increases in matrix Ca^{2+} reduce the threshold for permeability transition pore opening, directly connecting metabolic control to cell fate decisions (140,141).

6. MERCs and Zn^{2+} homeostasis

Like Ca^{2+}, Zn^{2+} acts as a second messenger, and its precise spatiotemporal control is crucial for cellular function. Intriguingly, numerous of the same MERC platforms that coordinate Ca^{2+} transfer also participate in Zn^{2+} regulation. Zinc, an essential trace element with diverse physiological functions, plays a key role in regulating various cellular signaling pathways, including Zn^{2+} signaling (142). Previous studies have shown that intracellular free Zn^{2+}, similar to elevated cytosolic Ca^{2+}, acts as a signaling ion by modulating second messenger metabolism, protein activation and phosphorylation, and signal transduction processes (143,144). Cellular Zn^{2+} levels must be tightly regulated, as both deficiency and excess can impair cell function.

Two families of Zn^{2+} transporters mediate Zn^{2+} flux across cellular and organellar membranes: Zrt- and Irt-like proteins (ZIPs/SLC39A) facilitate Zn^{2+} efflux from intracellular stores into the cytosol (145), whereas Zn^{2+} transporters (ZnTs/SLC30A) promote Zn^{2+} sequestration into organelles or extrusion from the cell. ZIP7 (solute carrier family 39 member 7, SLC39A7), one of the best-characterized ZIP transporters, was initially identified in the early secretory pathway, particularly within the ER and Golgi apparatus, where it regulates Zn^{2+} mobilization between the cytosol and intracellular vesicles. Recent evidence suggests that SLC39A7 may also redistribute to or localize within mitochondria (13). Pathological dysregulation of SLC39A7 leads to mitochondrial Zn^{2+} accumulation and impaired mitophagy (146), thereby

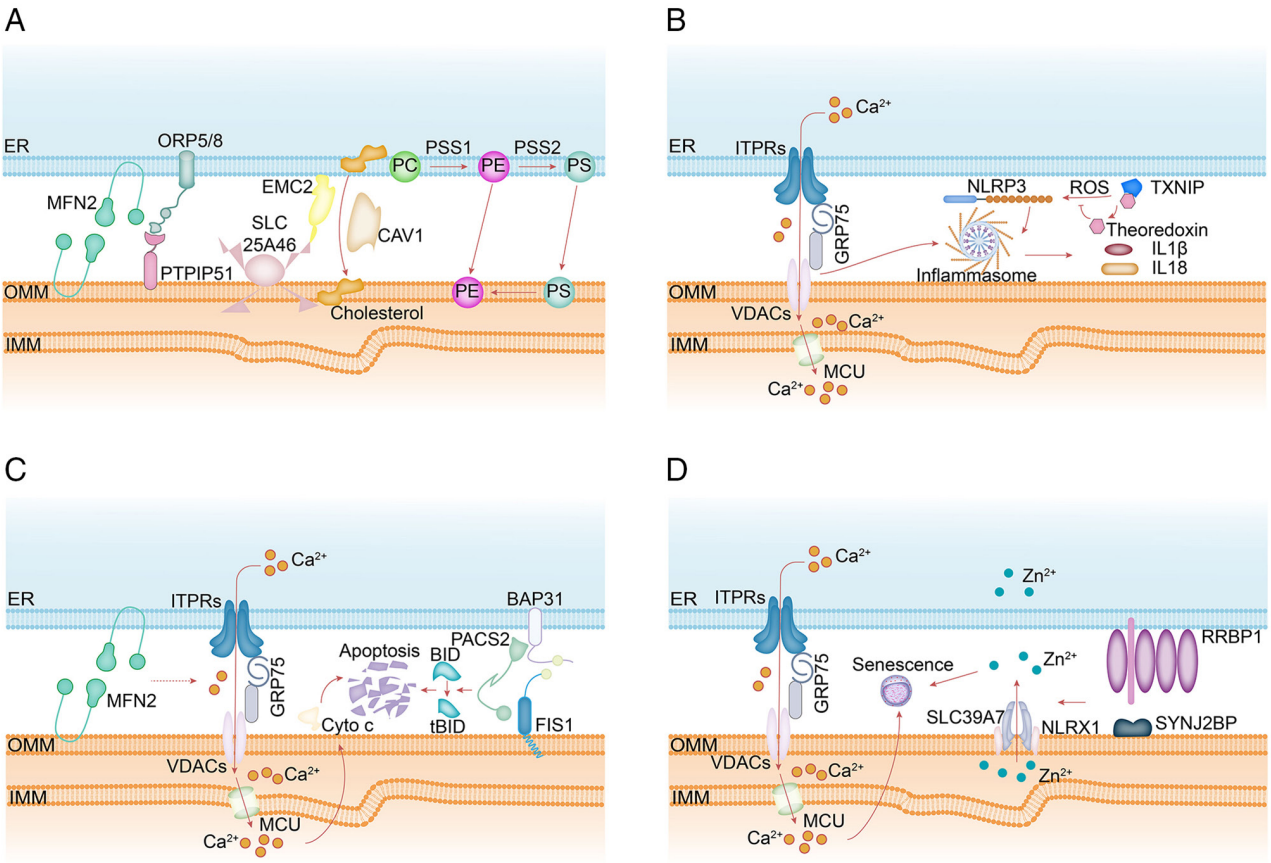

Figure 5. Schematic summary of MERC-associated proteins involved in: (A) Lipid metabolism-ORP5 and ORP8, ER membrane proteins, together with PTPIP51 localized on the mitochondrial membrane, mediate PS transport to mitochondria for PE synthesis. CAV1 regulates cholesterol transfer from the ER to mitochondria to support lipid biosynthesis. PSS1 catalyzes the conversion of PC to PS, while PSS2 facilitates the reverse conversion of PE to PS. The SLC25A46-EMC2 axis may regulate disruptions in mitochondrial phospholipid metabolism, including reduced cardiolipin synthesis. MFN2 specifically binds PS and mediates its transport into mitochondria. (B) Inflammation-NLRP3 localizes to the ER membrane in its inactive state; upon activation, both NLRP3 and its adaptor protein ASC redistribute to the ER-mitochondria interface. Inhibition of VDAC disrupts mitochondrial function and suppresses inflammasome activation. ROS-induced dissociation of TXNIP from thioredoxin promotes formation of TXNIP-NLRP3 complexes, which translocate to MERCs and facilitate inflammasome assembly and activation. (C) Apoptosis-PACS2 facilitates the translocation of Bid to mitochondria, leading to cytochrome c release and generation of tBid, thereby initiating the intrinsic apoptotic cascade and resulting in cell death. Following hypoxic injury, elevated levels of ITPR2-enriched at MERCs-cause mitochondrial Ca^{2+} overload, which triggers photoreceptor apoptosis. (D) Cellular senescence-calcium transfer to mitochondria via the ITPR-VDAC complex induces premature senescence. ER, endoplasmic reticulum; MERCs, mitochondria-ER contact sites; PS, phosphatidylserine; PE, phosphatidylethanolamine; CAV-1, caveolin-1; PC, phosphatidylcholine; tBid, truncated Bid; SYNJ2BP, a senescence-promoting factor, interacts with RRBP1 to maintain mitochondrial Zn^{2+} homeostasis and enhance the NLRX1-SLC39A7 interaction. MFN2, mitofusin 2; SLC25A46, solute carrier family 25 member 46; ITPRs, inositol 1,4,5-trisphosphate receptors; GRP75, glucose regulate protein 75; VDACs, voltage-dependent anion channels; PTPIP51, protein tyrosine phosphatase-interacting protein 51; ORP5/ORP8, oxysterol-binding protein related-protein 5 and 8; SYNJ2BP, synaptojanin 2 binding protein; RRBP1, ribosome binding protein 1; PACS2, phosphofurin acidic cluster sorting protein 2; PSS1, phosphatidylserine synthase1; NLRP3, NOD-like receptor family, pyrin domain-containing protein 3, TXNIP, tioredoxin-interacting protein; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane.

exacerbating mitochondrial oxidative stress. Song *et al* (147) showed that NLR family member X1 (NLRX1) is required to maintain SLC39A7 localization in mitochondria. Upon activation of NLRX1 using the agonist NX-13, they observed reduced association between SLC39A7 and NLRX1, along with diminished mitochondrial targeting of SLC39A7. NLRX1 transitions into an active conformation to promote mitophagy. Thus, NLRX1-mediated retention of SLC39A7 in mitochondria is critical for preserving local MMP, and its loss at sites of mitochondrial damage suggests a role in localized depolarization without affecting global membrane potential (147). This finding highlights how fine-tuned mitochondrial adaptations, such as transient fluctuations or 'flickering' of membrane potential, are physiologically regulated (148,149).

Another group of Zn^{2+} transporters, including SLC30A9, SLC25A25 and SLC30A5, also contributes to MERC-dependent regulation of mitochondrial Zn^{2+} distribution. Their colocalization at MERCs facilitates efficient Zn^{2+} transfer from the ER to mitochondria. Moreover, certain Zn^{2+} transporters exhibit dynamic relocalization among organelles, enabling spatiotemporal control of Zn^{2+} concentrations. In aged cardiomyocytes, altered expression of Zn^{2+} transporters in mitochondria (increased SLC30A7 and SLC30A8, no change in SLC39A7 and SLC39A8) and ER (increased SLC39A7, decreased SLC30A7, no change in SLC39A8 and SLC30A8) correlates with oxidative stress-induced cardiac dysfunction (13). Recent findings indicate that SYNJ2BP enhances the interaction between NLRX1 and SLC39A7, thereby supporting mitochondrial Zn^{2+} homeostasis. As a structural component of MERCs through its binding to RRBP1, SYNJ2BP plays a key role in maintaining contact site integrity. Loss of SYNJ2BP expression or disruption of MERC architecture not only

reduces formation of the SLC39A7-NLRX1 complex but also prevents mitochondrial localization of SLC39A7 (14). Similar to calcium, Zn^{2+} functions as a crucial signaling ion, and its accurate compartmentalization is essential for cellular well-being. These findings indicate that MERCs act as strategic platforms that promote this regulation by localizing key Zn^{2+} transporters, such as SLC39A7/ZIP7, and their regulatory partners, including NLRX1 and SYNJ2BP. The formation of dynamic protein complexes at the contact interface, as exemplified by the SYNJ2BP-RRBP1 tether that enables the SLC39A7-NLRX1 interaction, supports efficient Zn^{2+} transfer and buffering between the ER and mitochondria. This localized control is important for maintaining MMP, alleviating oxidative stress, and supporting processes such as mitophagy. Dysregulation of these MERC-centered Zn^{2+} regulatory mechanisms, as observed in aging cardiomyocytes, is directly associated with mitochondrial dysfunction and tissue pathology. Collectively, the evidence indicates that MERCs are not merely passive bridges but active, integrative hubs that coordinate Zn^{2+} signaling to preserve mitochondrial quality and cellular resilience. Notably, disruption of MERC integrity, which impairs Zn^{2+} homeostasis, can simultaneously trigger ER stress, since the protein-folding environment of the ER is highly sensitive to alterations in both ion flux and MERC architecture. This bidirectional relationship between MERC status and ER stress is discussed in the following section.

7. MERCs and ER stress

When ER homeostasis is disrupted, misfolded or unfolded proteins accumulate, leading to ER stress and activation of the unfolded protein response (UPR) (150). The adaptive phase of the UPR aims to restore cellular homeostasis by promoting ER-associated degradation of misfolded proteins and protecting cells from damage; however, prolonged ER stress can ultimately trigger cellular dysfunction and apoptosis (151). Although the protective effect of enhanced MERC formation on ER stress depends on timing, a previous study showed that tunicamycin-induced ER stress increases MERC formation in liver and muscle tissues (20). Specifically, during the early stages of ER stress, transient MERC expansion enhances Ca^{2+} transfer from the ER to the mitochondrial matrix, thereby boosting mitochondrial bioenergetics and ATP production. This increased energy supply supports efficient ER protein folding and facilitates the clearance of misfolded proteins, contributing to cellular resilience against ER stress. By contrast, in obese hepatocytes, chronic enrichment of MERCs results in sustained elevation of mitochondrial matrix Ca^{2+} levels, eventually leading to Ca^{2+} overload, mitochondrial dysfunction, and impaired oxidative phosphorylation (OXPHOS) (20).

The ER stress sensor inositol-requiring enzyme 1 (IRE1), enriched at MERCs, plays a key role in regulating mitochondrial Ca^{2+} uptake by mediating the splicing of X-box binding protein 1 (XBP1) (152). XBP1 is generally considered cytoprotective and, through transcriptional regulation, modulates various cellular processes in a context- and cell-type-specific manner (153). Notably, MERCs are not merely downstream effectors of ER stress; conversely, MERC dysfunction can itself induce ER stress by impairing inter-organellar Ca^{2+} signaling (25). For instance, depletion of phosphofurin acidic cluster sorting protein 2 (PACS2) leads to a transient increase in phosphorylated eIF2α, followed by restoration of ER homeostasis and normal protein synthesis rates after two days (25). MERCs are dynamically regulated by the UPR and, in turn, actively help determine cellular fate during ER stress. In the initial, adaptive stage, a transient increase in MERC formation promotes beneficial Ca^{2+} transfer to mitochondria, enhancing ATP production to support protein folding and restore ER homeostasis. In chronic pathological conditions, however, sustained MERC expansion disrupts this delicate equilibrium, resulting in mitochondrial Ca^{2+} overload, dysfunction and worsening of stress. Key molecular factors, such as the MERC-localized sensor IRE1 and its downstream effector XBP1, are crucial for this crosstalk. Importantly, the relationship is cyclic: MERC dysfunction can itself trigger ER stress, as demonstrated by PACS2 depletion, creating a harmful feedback loop. MERCs thus serve as critical decision points, where their functional state can either resolve or propagate ER stress, highlighting the therapeutic potential of targeting MERC dynamics to interrupt this cycle in diseases characterized by chronic ER stress.

8. MERCs and redox signaling control

A key downstream consequence of ER stress is increased generation of reactive oxygen species (ROS), especially at the interface between the ER and mitochondria. As described below, MERCs function as crucial platforms for redox communication between these two organelles. Moreover, the ROS generated during ER stress can further regulate MERC function, establishing bidirectional regulatory loops. ROS are normally generated at physiological levels necessary for maintaining cellular homeostasis. Excessive ROS production, however, can lead to oxidative damage to DNA, lipids, and proteins (154). Under basal conditions, mitochondria, peroxisomes, and the ER maintain low ROS levels due to intrinsic antioxidant defense mechanisms. However, perturbations in redox balance, particularly within the mitochondrial respiratory chain-can significantly enhance ROS generation and trigger mitochondrial-derived oxidative stress (155,156). The amount of ROS produced by organelles varies depending on oxygen tension, tissue type and cell type. Among intracellular compartments, the ER is estimated to be the largest contributor of ROS to the cytosol, accounting for ~60% of total cellular ROS, with mitochondria and peroxisomes each contributing ~20%. This estimation is based on both the relative permeability to ROS and the absolute rates of ROS production across these three organelles (157).

Given that both the ER and mitochondria function as major intracellular redox hubs, redox-mediated communication between them is highly plausible. It has been proposed that MERC-localized ROS can activate inositol 1,4,5-trisphosphate receptors (ITPRs) (30) and inhibit sarco/ER Ca^{2+}-ATPase (SERCA) (31), thereby establishing a feed-forward loop that amplifies Ca^{2+} transfer between the ER and mitochondria. In reducing environments, ER protein 44 (ERp44) binds to ITPR1 to prevent ER Ca^{2+} release, serving a protective role (32). By contrast, certain oxidoreductases promote ER-mitochondrial Ca^{2+} crosstalk. Thioredoxin-related transmembrane protein 1 (TMX1) and ER oxidoreductase 1 alpha (Ero1α) are two

well-characterized examples. Ero1α enhances ER Ca^{2+} release by modifying ITPRs and potentially competing with ERp44 (33), while simultaneously reducing mitochondrial Ca^{2+} uptake (158). Conversely, TMX1 decreases cytosolic Ca^{2+} clearance by inactivating SERCA (159). Glutathione peroxidase 8 (GPx8) has a more complex role: Although it inactivates SERCA, it also reduces ER Ca^{2+} stores, resulting in diminished ER-mitochondria Ca^{2+} flux (160). BAP31, a key calnexin-binding partner localized at MERCs, interacts with the mitochondrial outer membrane translocase TOM40. Additionally, BAP31 associates with NDUFS4, a core subunit of mitochondrial complex I (ubiquinone oxidoreductase), which is part of the MERC-associated proteome (97). Loss of BAP31 suppresses mitochondrial OXPHOS; however, whether this effect depends on its interaction with TOM40 remains unclear, as the mechanism underlying BAP31 targeting to MERCs has not yet been fully elucidated. These oxidoreductases, once considered solely components of the ER protein folding machinery, are now recognized to cooperate in forming a redoxosome at MERCs, where they are predominantly localized (159,160). The redoxosome is a multimeric, multi-organellar protein complex hypothesized to facilitate or regulate the formation and function of MERCs in a redox-sensitive manner. As the two primary intracellular sources and targets of ROS, the ER and mitochondria employ MERCs to promote essential redox communication. This inter-organellar crosstalk has two key features: First, ROS localized at MERCs can enhance inter-organellar calcium signaling through the modulation of ITPRs and sarco/ER calcium-ATPase (SERCA), establishing a potential positive feedback loop. Second, MERCs contain a set of specialized oxidoreductases, such as ERp44, TMX1, Ero1α and GPx8, which jointly regulate calcium flux in response to the local redox environment, fulfilling protective or regulatory functions. The emerging concept of a 'redoxosome', a multimeric protein complex at MERCs, encompasses this coordinated activity, defining these contact sites as highly sophisticated redox-sensitive centers. Moreover, the interaction between MERC proteins, such as BAP31, and mitochondrial complex I subunits directly links contact site integrity to the regulation of OXPHOS. Therefore, MERCs are not merely passive bystanders but active integrators that convert redox fluctuations into precisely calibrated functional outputs, safeguarding cellular homeostasis and highlighting how their malfunction could propagate oxidative stress in diseases.

9. MERCs and mitochondrial dynamics

The redox environment at MERCs not only modulates ion flux but also directly affects mitochondrial morphology. As described below, the sites of mitochondrial fission are physically determined by contacts between the ER and mitochondria, and local redox conditions can regulate the recruitment and activity of the fission machinery. Mitochondria are double-membrane-bound organelles that regulate cellular metabolism and cell function. Mitochondrial dynamics include mitophagy, motility, fusion and fission (161). Mitochondrial fission is a multistep process by which one mitochondrion divides into two daughter mitochondria (162). This division predominantly occurs at sites where the OMM undergoes constriction, driven by actin polymerization or physical contact with the ER. During this process, several OMM adaptor proteins-including mitochondrial fission 1 protein (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamics proteins MiD49 and MiD51-recruit the cytosolic GTPase dynamin-related protein 1 (Drp1) to the mitochondrial surface (163,164). Subsequently, the mitochondrial-specific phospholipid cardiolipin (CL) activates highly oligomerized DRP1, promoting the formation of large helical structures and enhancing GTPase activity at fission sites. Nucleotide-induced allosteric regulation enables DRP1 to self-assemble, undergo conformational changes, and disassemble, thereby wrapping around the mitochondrion to mediate membrane scission.

Notably, recent studies have shown that MERCs form prior to Drp1 recruitment and mark the initial sites of mitochondrial constriction. This finding indicates that MERCs actively participate in the early stages of mitochondrial fission by defining division sites. Drp1 is then recruited and assembled at these pre-constricted locations (15). Further research has demonstrated that the initial mitochondrial constriction and division can be initiated by actin polymerization at MERCs, which is triggered by ER-localized inverted formin 2 (INF2) (16,165). On the one hand, actin polymerization induces OMM constriction by narrowing the mitochondrial tubule to a diameter compatible with DRP1 oligomerization. On the other hand, spire type actin nucleation factor 1C (Spire1C) localizes to mitochondria and establishes direct links between the ER and the actin cytoskeleton via mitochondrial anchoring. Spire1C promotes actin assembly on mitochondrial surfaces through interaction with INF2. Disruption of Spire1C's actin- or formin-binding activities reduces mitochondrial division and constriction (100). In addition to actin, several other proteins regulate DRP1 activity at MERCs. Syntaxin 17 (Syn17), a SNARE protein localized to raft-like microdomains within MERCs, controls the localization and function of DRP1 in nutrient-replete conditions, thereby stimulating mitochondrial fission. This regulatory mechanism depends on the C-terminal hydrophobic hairpin domain of Syn17 (166). Another MERC-associated protein, FUNDC1, regulates hypoxia-induced mitochondrial dynamics. By interacting with the ER-resident chaperone calnexin, FUNDC1 accumulates at MERCs and acts as a novel mitochondrial receptor for DRP1, initiating fission under hypoxic conditions (167). Additional studies revealed that ablation of FUNDC1 reduces intracellular Ca^{2+} levels, leading to decreased binding of the cAMP response element-binding protein (CREB) to the FIS1 promoter, which in turn suppresses mitochondrial fission and FIS1 expression (103). Hu *et al* (168) found that while a subset of AMP-activated protein kinase (AMPK) binds to MFN2 under basal conditions, this interaction decreases during energy stress. Intriguingly, the released active AMPK may phosphorylate MFF, thereby regulating mitochondrial fission (168). Collectively, MERCs play a crucial role in both the initiation and completion of mitochondrial fission by determining division sites and facilitating fission through multiple coordinated molecular pathways.

Mitochondrial fusion is a multistep process involving several key proteins. First, dynamin-related GTPases such as mitofusin 1 and 2 (MFN1/2) on the OMM, FAM73a/FAM73b, and optic atrophy protein 1 (OPA1) on the IMM are activated.

GTP hydrolysis then drives OMM fusion, followed by IMM fusion, and finally the mixing of intramitochondrial contents (169-171). Mitochondrial fusion contributes to the maintenance of mitochondrial homogeneity and functional stability by diluting mutant mtDNA and damaged proteins through mixing with healthy matrix components. Genetic knockout of MFN1 and/or MFN2 disrupts mitochondrial architecture, leading to severe cellular defects including fragmented and smaller mitochondria, reduced MMP, impaired respiratory activity and diminished ATP production. These abnormalities ultimately inhibit cell proliferation (172). The human ubiquitin ligase MITOL regulates MERC formation and selectively ubiquitinates the mitochondrial pool of MFN2, but not the ER-localized MFN2, thereby enhancing its fusogenic activity. Sugiura *et al* (114) identified lysine 192 (K192) in the GTPase domain of MFN2 as the primary ubiquitination site targeted by MITOL. While both MFN1 and MFN2 mediate OMM fusion, only MFN1 is required for OPA1-dependent IMM fusion (173). This finding suggests intermembrane communication during fusion events and supports the possibility of direct interaction between MFN1 and OPA1, a hypothesis that has become increasingly plausible given recent insights into the topology and conformational dynamics of MFN proteins (174). Notably, MERCs appear to mark sites of mitochondrial fusion, providing compelling evidence for their involvement in facilitating fusion, although the underlying molecular mechanisms remain largely undefined.

Mitophagy is essential for maintaining both mitochondrial quantity and quality. It is an evolutionarily conserved process that selectively eliminates damaged or excess mitochondria through autophagy (175). Mitophagy involves two key stages: First, double-membrane autophagosomes recognize and selectively engulf impaired mitochondrial segments; second, the fusion of autophagosomes with lysosomes forms autolysosomes, where hydrolytic enzymes degrade the engulfed mitochondria. Two critical regulators of mitophagy, PTEN-induced putative kinase 1 (PINK1) and Beclin 1 (BECN1), relocalize to MERCs during autophagy, promoting the formation of autophagosome initiation sites and enhancing ER-mitochondrial interface functionality. Following mitophagy induction, Parkin RBR E3 ubiquitin protein ligase (PARK2) is also upregulated at MERCs. However, PINK1 silencing reduces BECN1 enrichment at MERCs regardless of PARK2 status, indicating a PARK2-independent role for PINK1 in regulating mitophagy (22). The adaptor protein FUNDC1 has been shown to play a crucial role in hypoxia-induced mitophagy by enabling the recruitment of LC3 to the OMM. FUNDC1 accumulates at MERCs through interaction with calnexin and subsequently binds DRP1 to promote mitochondrial fission. The fragmented mitochondria then recruit the unc-51-like autophagy-activating kinase 1 (ULK1) complex to initiate mitophagy (167,176). Recent findings using colon and oral cancer cells have demonstrated that under hypoxic conditions, the stress-induced mitochondrial protease Lon accumulates at MERCs and acts as a molecular chaperone by stabilizing the FUNDC1-ULK1 complex in a manner dependent on the mitochondrial Na^+/Ca^{2+} exchanger (NCLX), thereby triggering mitophagy (177). Rather than acting as passive bystanders, MERCs actively organize these events by acting as privileged platforms for the spatiotemporal assembly of molecular machinery. They determine the initial sites for mitochondrial division by recruiting and coordinating proteins such as INF2, Spire1C and actin polymers to induce constriction before DRP1-mediated scission. Simultaneously, through proteins such as MFN2 and its regulator MITOL, MERCs promote and demarcate sites of mitochondrial fusion, ensuring content mixing and functional homogenization. Moreover, MERCs are crucial hubs for initiating quality control through mitophagy, where proteins such as PINK1, FUNDC1 and Lon protease converge to connect mitochondrial stress sensing, fission and autophagosome formation. These processes are highly interconnected; for example, fission promoted at MERCs often provides substrates for subsequent mitophagy. MERCs thus emerge as central decision-making nodes that physically and functionally integrate the biogenesis, remodeling and turnover of mitochondria, directly linking organelle dynamics to cellular energy status, stress adaptation, and overall health.

10. MERCs and autophagy

Autophagy is a fundamental, evolutionarily conserved cellular degradation process that eliminates various macromolecules and subcellular components, including proteins, lipids, nucleic acids and organelles. This lysosome-dependent mechanism has attracted considerable attention for its critical function in maintaining cellular homeostasis under both physiological and pathological conditions (178). Autophagy contributes to numerous cellular responses and functions, extending well beyond basic clearance to include roles in metabolism, immunity and stress adaptation. Previous studies have revealed that MERCs play a key role in autophagosome biogenesis. Notably, core components of the autophagy initiation complex, including Beclin 1 (BECN1), autophagy-related protein 14 (ATG14) and vacuolar protein sorting 34 (VPS34), localize to MERCs (179,180). The two main modes of autophagy induction are starvation-induced autophagy and rapamycin-inducible autophagy, which occurs through inhibition of the mammalian target of rapamycin (mTOR). Intriguingly, overexpression of VAPB or PTPIP51 impairs rapamycin-inducible autophagy, whereas starvation-induced autophagy remains unaffected (180). Subsequent research has linked VAPB and PTPIP51 to ER-mitochondrial Ca^{2+} exchange, clarifying their regulatory roles in autophagy. Specifically, blockade of ITPR-mediated Ca^{2+} transfer from the ER to mitochondria abolishes the inhibitory effect of VAPB and PTPIP51 overexpression on autophagy (180). By contrast, autophagy is suppressed in primary pancreatic β cells lacking EI24. It has been shown that EI24 interacts with VDAC1, ITPR, and the chaperone GRP75 on the OMM. Further mechanistic studies reveal that EI24 is enriched at the ER-mitochondria interface and that both autophagic flux and MERC integrity depend on the C-terminal domain of EI24 (123). Consistent with these findings, energy stress induces mitochondrial fission and autophagy, but more notably increases MERC formation, a process mediated by AMP-activated protein kinase (AMPK). Under energy stress, substantial levels of AMPK translocate from the cytosol to mitochondria and MERCs, promoting mitochondrial fission. Notably, AMPK directly interacts with MFN2 (168). More recently, Manganelli *et al* (181)

demonstrated that knocking down ERLIN1 disrupts its interaction with autophagy and beclin 1 regulator 1 (AMBRA1), preventing recruitment of AMBRA1 to the BECN1 complex at raft-like microdomains within MERCs. This interaction is essential for autophagosome formation during nutrient starvation. Moreover, the structural integrity of key proteins such as MFN2 is required for this association to occur (181). The localization of core autophagy machinery components, such as the BECN1-ATG14-VPS34 complex, to MERCs emphasizes these contact sites as preferential locations for autophagosome formation. The regulatory mechanism is multi-faceted, incorporating signals from nutrient status, energy stress and inter-organellar communication. For example, the VAPB-PTPIP51 tether regulates autophagy by influencing ER-to-mitochondria Ca^{2+} signaling. Meanwhile, proteins such as EI24 and AMPK directly associate MERC integrity and mitochondrial dynamics with autophagic flux. Notably, the data indicate that specific protein complexes at specialized MERC subdomains, such as the ERLIN1-AMBRA1 interaction at raft-like microdomains, are crucial for recruiting and activating the autophagy machinery. Collectively, this evidence demonstrates that MERCs are not simply passive structural bridges but rather dynamic signaling hubs that physically and functionally integrate cellular stress perception, organelle remodeling, and the autophagic degradation pathway, thereby playing a key role in cellular homeostasis and adaptation.

11. MERCs and lipid metabolism

A growing body of evidence indicates that the regulation of phospholipid biosynthesis and intermembrane lipid trafficking is one of the primary functions of MERCs (19). MERCs establish an aqueous microenvironment between the ER and mitochondria that facilitates the bidirectional, non-vesicular transport of lipids (182). Although most mitochondrial phospholipids and their precursors are initially synthesized in the ER, certain phospholipids, such as CL and phosphatidylethanolamine (PE), are essential for mitochondrial respiratory function and can be further processed or generated within mitochondria (183). Phosphatidylserine synthase (PSS), localized at MERCs (184), catalyzes the synthesis of phosphatidylserine (PS), a key precursor for PE production. It has been previously shown that downregulation of PSS1 and PSS2 leads to reduced PS levels, which alters the steady-state distribution of PS in the ER and redirects lipid metabolism toward triacylglycerol and diacylglycerol synthesis, ultimately promoting intracellular lipid accumulation (185). MFN2 specifically binds PS and mediates its transfer from the ER to mitochondria. Knockout of MFN2 results in decreased PSS1 expression and impaired PS translocation, disrupting phospholipid synthesis and inducing ER stress. Moreover, while PSS1 overexpression enhances PS synthesis, it fails to restore PE production (36). Similarly, oxysterol-binding protein-related proteins ORP5 and ORP8 localize to MERCs and interact with OMM protein PTPIP51, the mitochondrial contact site and cristae organizing system complex, and the mitochondrial intermembrane bridging complex, thereby mediating PS transfer from the ER to mitochondria. Depletion of ORP5 or ORP8 leads to defects in mitochondrial respiratory function and abnormal mitochondrial morphology (29,186). Cholesterol is likely transported into mitochondria via specific MERC subdomains. Caveolin-1 (CAV1), a MERC-associated protein, has been shown to regulate cholesterol trafficking from the ER to mitochondria (110). Inhibition of CAV1 is associated with reduced physical extension and structural integrity of MERCs, as well as abnormal intracellular accumulation of free cholesterol (187). Recent findings highlight the role of the Mic19-SLC25A46-EMC2 axis in regulating ER-mitochondria interactions. Deficiency in Mic19 disrupts mitochondrial phospholipid metabolism, including diminished CL biosynthesis, which compromises mitochondrial membrane architecture and ultimately impairs fatty acid metabolism (40). MERCs establish a specialized microenvironment that enables the efficient transfer of lipid precursors, such as PS, from their synthesis site in the ER to mitochondria for subsequent processing into essential phospholipids, including PE and CL. This coordinated transfer is mediated by specific tethering and lipid-transfer proteins located at MERCs, including MFN2, ORP5/ORP8 (which interact with PTPIP51), and the enzyme PSS. Disruption of these components undermines lipid homeostasis, resulting in defective mitochondrial respiration, abnormal morphology and ER stress. Moreover, proteins such as CAV1 and the Mic19-SLC25A46-EMC2 axis highlight the role of MERCs in cholesterol trafficking and the broader regulation of membrane architecture and lipid metabolism. Collectively, the evidence indicates that MERCs are not merely structural bridges but active metabolic platforms; their integrity is crucial for maintaining lipid balance, and their dysfunction directly contributes to lipotoxicity and associated pathologies.

12. MERCs and inflammation

Upon infection or cellular stress, inflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-18 (IL-18) are processed to their mature forms and activate the immune system. This maturation process is mediated by inflammasomes, cytosolic molecular platforms that assemble in response to danger signals. Inflammasomes are classified into four main subfamilies based on their sensor proteins: NLR family CARD domain-containing 4 (NLRC4), NOD-like receptor family pyrin domain-containing 3 (NLRP3), NOD-like receptor family pyrin domain-containing 1 (NLRP1), and absent in melanoma 2 (AIM2) (188). NLRP3 is known to reside at the ER membrane in its inactive state; upon activation, both NLRP3 and its adaptor protein ASC relocalize to the ER-mitochondria interface. To date, the NLRP3 inflammasome is the only inflammasome complex shown to associate with MERCs (17). Notably, unlike other NLR family members, NLRP3 can recognize not only pathogen-associated molecular patterns but also danger-associated molecular patterns released by damaged cells, including mitochondrial DNA (mtDNA), perturbed Ca^{2+} signaling, and mitochondrial ROS (mtROS) (189).

Suppression of the VDAC)disrupts mitochondrial function, leading to reduced ROS production and impaired inflammasome activation (17). In response to oxidative stress, the NLRP3 inflammasome translocates to MERCs via the ROS sensor thioredoxin-interacting protein (TXNIP). ROS inducers such as uric acid crystals trigger the dissociation of TXNIP from

thioredoxin and promote the formation of TXNIP-NLRP3 complexes, which then translocate to MERCs and facilitate inflammasome assembly and activation (190,191). MERCs function as a strategic platform at which danger signals, such as mtROS, released mtDNA and disrupted calcium signaling, converge to initiate the assembly and activation of the inflammasome complex. The recruitment of NLRP3 and its adaptor ASC to the ER-mitochondria interface, facilitated by the ROS-sensitive protein TXNIP, underscores MERCs as a specialized domain for integrating metabolic and oxidative stress signals into immune activation. Moreover, the reliance on functional mitochondrial channels such as VDAC demonstrates how MERC integrity and mitochondrial health directly govern inflammatory output. Collectively, this indicates that MERCs are not only structural connections but also dynamic signaling hubs that enhance innate immune responses; their dysregulation may contribute to pathological inflammation. The identified gap in understanding how TXNIP or inflammasome activation mutually modifies MERC composition points to an important area for future research on the bidirectional communication between organelle contact sites and inflammatory pathways.

13. MERCs and apoptosis

Apoptosis is a form of programmed cell death executed by caspases, leading to the ordered disassembly of cellular structures (192). In earlier studies, Yang *et al* (35) demonstrated that overexpression of disulfide bond A oxidoreductase-like protein (DsbA-L) in HK-2 cells preserved MERC integrity and reduced apoptosis induced by high glucose. The upregulation of FATE-1, a MERC-uncoupling protein, partially reversed these protective effects, suggesting that maintenance of MERC integrity exerts an antiapoptotic role (35). PACS2, a multifunctional sorting protein, also contributes to MERC formation and inter-organellar communication. PACS2 specifically links ER-mitochondria crosstalk to mitochondria-dependent apoptosis. In response to apoptotic stimuli, PACS2 facilitates the translocation of Bid to mitochondria, thereby initiating the intrinsic apoptotic cascade through cytochrome c release and generation of truncated Bid (tBid) (25).

As described in Section 'MERCs and Ca^{2+} homeostasis', the ITPR-GRP75-VDAC axis mediates tightly regulated Ca^{2+} transfer from the ER to mitochondria at MERCs (141). Under physiological conditions, this Ca^{2+} flux supports oxidative metabolism by activating Ca^{2+}-dependent TCA cycle enzymes (193). However, under pathological stress, this same machinery can drive excessive mitochondrial Ca^{2+} accumulation, which sensitizes the permeability transition pore (mPTP) to opening, resulting in the release of proapoptotic factors including cytochrome c (140). Li *et al* (43) showed that MERCs are significantly enhanced in photoreceptors following hypoxia induction, both *in vivo* and *in vitro* (43). This increase coincided with activation of mitochondrial apoptosis and disruption of mitochondrial homeostasis. Following hypoxic injury, elevated levels of ITPR2, enriched at MERCs, led to mitochondrial Ca^{2+} overload, ultimately causing photoreceptor cell death. Notably, ITPR2 knockdown reduced MERC formation and consequently attenuated mitochondrial Ca^{2+} accumulation during hypoxia, not only restoring mitochondrial morphology but also rescuing mitochondrial function in photoreceptors (43). Another study revealed that sevoflurane-induced MERC dysfunction is associated with MFN2 downregulation, and that enhanced ER-mitochondria coupling promotes mitochondrial Ca^{2+} overload, leading to mitochondrial permeability transition pore (mPTP) opening and neuronal apoptosis (44).

MERCs function as a central hub where pro-survival and pro-death signals converge, ultimately determining cell fate. First, MERC integrity has an antiapoptotic effect, as evidenced by proteins such as DsbA-L. Maintaining contact site stability safeguards cells against apoptotic stimuli. Second, MERCs are essential platforms for initiating and amplifying apoptotic signaling. This proapoptotic function is mainly mediated through precise regulation of mitochondrial Ca^{2+} uptake via MERC-localized channels, for example, the ITPR-GRP75-VDAC complex. Under stress conditions, heightened ER-mitochondria coupling can result in pathogenic mitochondrial Ca^{2+} overload, which triggers mPTP opening and cytochrome c release. Moreover, MERCs facilitate the recruitment and activation of key apoptotic factors, such as PACS2-dependent translocation of Bid to mitochondria. MERCs thus act as a dynamic signaling switch: Their proper formation and function are essential for cellular health, yet their dysregulation, whether excessive coupling or disruption, can actively drive the cell toward apoptosis. Nevertheless, the precise molecular mechanisms by which individual MERC components govern the critical steps of cytochrome c release and mPTP opening remain incompletely understood, highlighting a crucial area for future research to clarify how MERC architecture determines the commitment to cell death.

14. MERCs and cellular senescence

Whereas the aforementioned MERC-mediated Ca^{2+} overload acts as an acute trigger for apoptosis, lower-intensity or chronic MERC dysfunction can, by contrast, promote cellular senescence, a state of permanent proliferative arrest with distinct functional consequences. Cellular senescence can be triggered by various stressors and is characterized by a sustained arrest in cell proliferation accompanied by the development of a senescence-associated secretory phenotype, which includes proinflammatory cytokines, profibrotic factors and matrix metalloproteinases (194). Calcium plays a crucial role in regulating numerous cellular and molecular processes, including secretion, autophagy, migration, proliferation and cell death (195). More recent evidence has shown that calcium signaling regulates cellular senescence and influences its progression (196,197).

Consistent with the central role of MERC-mediated Ca^{2+} signaling aformentioned, evidence demonstrates that calcium transfer from the ER to mitochondria via ITPR channels at MERCs is a key driver of cellular senescence. In normal human cells, this ITPR-dependent mitochondrial calcium accumulation induces senescence (197,198). ITPR2 knockout (KO) mice exhibit delayed aging phenotypes, including extended lifespan, improved metabolic stress resistance, reduced immuno-senescence, and decreased hepatic steatosis and fibrosis. Notably, both *in vivo* and *in vitro* ablation of ITPR2 reduces the number of mitochondria-ER contacts, and artificially enforced

ER-mitochondria tethering accelerates aging (27). Additionally, other studies have shown that cyanidin 3-O-arabinoside suppresses p38-mediated VDAC1 expression, a pathway involved in MERC formation and calcium flux through VDAC1-ITPR1 complexes. Furthermore, dihydrotestosterone-induced MERC formation was found to increase senescence in dermal papilla cells (199). A link between MERC-associated genes and cellular senescence in osteoarthritis (OA) has been also proposed by the authors. Two MERC-related genes, PTPN1 and ITPR1, show positive correlations with senescence-associated genes (MAP2K1, ABL1, PTEN and MAPK14) (200).

In addition to calcium regulation, intracellular free Zn^{2+} has been shown to play a significant role in cellular senescence. Recent research revealed that SYNJ2BP promotes the NLRX1-SLC39A7 interaction and maintains mitochondrial Zn^{2+} homeostasis. SYNJ2BP is essential for MERC structural organization through its interaction with RRBP1. Loss of SYNJ2BP expression or disruption of MERC structure not only reduces formation of the SLC39A7-NLRX1 complex but also prevents mitochondrial localization of SLC39A7 (14). By integrating published MERC proteomic data with transcriptomic profiles from nucleus pulposus cells in a tert-butyl hydroperoxide-induced degenerative model, it was further demonstrated that SYNJ2BP downregulation is a key pathological feature of intervertebral disc degeneration and nucleus pulposus cell senescence, strongly associated with MERC disruption (14). MERCs therefore emerge as crucial signaling nodes. The dysregulation of inter-organellar ion homeostasis, specifically calcium and zinc, directly drives the aging process. Excessive calcium transfer through the ITPR-VDAC axis at MERCs has been established as a key factor in senescence, as evidenced by the significant antiaging phenotypes observed in ITPR2 knockout models. Conversely, the integrity of MERCs is essential for maintaining mitochondrial zinc homeostasis via complexes such as SYNJ2BP-RRBP1 and SLC39A7-NLRX1. Disruption of this structure accelerates senescence-associated pathologies, such as intervertebral disc degeneration. These findings collectively indicate that MERCs are not passive bystanders but active regulators of cellular aging. Their enhanced, diminished, or dysfunctional state can either induce or alleviate senescence. The association between MERC-associated genes and senescence markers in OA further emphasizes their broad significance in age-related diseases. Ultimately, the evidence presents MERCs as dynamic therapeutic targets. Modulating their structure and function could provide a novel approach to intervene in the fundamental processes of aging and senescence-related disorders.

Collectively, the aforementioned physiological functions exemplify the three-tier integration model of MERCs. Ion fluxes and redox changes act as rapid sensing signals (Tier 1), which are processed by organelle quality control and metabolic mechanisms (Tier 2), ultimately determining cell fate outcomes (Tier 3). In the following section, it is examined how disease-associated perturbations at each of these tiers contribute to the development of diseases.

15. Pathophysiology of MERCs

Before discussing individual diseases, it is useful to define the core functional deficits arising from MERC dysfunction and differentiate them from secondary pathological consequences. The primary disruptions due to altered MERC biology can be categorized into three interrelated groups: (i) Dysregulated ion signaling, specifically abnormal ER-to-mitochondria Ca^{2+} and Zn^{2+} transfer; (ii) Bioenergetic failure, caused by impaired mitochondrial Ca^{2+}-dependent metabolism and lipid homeostasis; and (iii) defective organelle quality control, including disrupted mitochondrial dynamics (fission, fusion and mitophagy) and autophagy. These core defects, in turn, trigger secondary pathological processes (such as excessive oxidative stress, chronic inflammation and cellular senescence) that exacerbate tissue injury and contribute to disease progression. In the disease-specific discussions below, it is indicated, whenever possible, which of these core deficits are the primary drivers and which represent the downstream consequences of MERC dysfunction.

The dual nature of MERC dysfunction offers a conceptual framework for interpreting the diverse alterations observed across diseases. As shown in the following subsections, pathologies as distinct as CMT2A and DCM can both be associated with MERC dysfunction. However, one is marked by a loss of contacts, whereas the other entails excessive coupling (42,45). This pattern emphasizes the context-specific nature of MERC biology and warns against therapeutic approaches that aim to uniformly augment or diminish MERC abundance without considering the specific disease context. It is increasingly evident that alterations in MERC plasticity and composition significantly contribute to the pathogenesis of certain diseases, as supported by a growing body of evidence emphasizing the critical role of MERCs in maintaining cellular homeostasis and regulating cell fate (201) (Table II). It is crucial to note that the interpretation of altered MERC parameters in disease contexts is complicated by several factors. First, the structural and functional definition of MERCs lacks uniformity across studies. Electron microscopy-based measurements of inter-organellar distance, biochemical MAM isolation and PLA-based detection of protein proximity capture different aspects of ER-mitochondria association and may lead to non-concordant results. Second, MERC composition and abundance are highly cell-type-specific. This means that pathological changes observed in one tissue may not be applicable to others. Third, the dynamic nature of MERCs means that their abundance and coupling efficiency can vary over the course of disease progression. For example, an early compensatory increase in ER-mitochondria Ca^{2+} transfer may be followed by a maladaptive loss of contact site integrity in later stages. Therefore, the following disease-specific discussions aim to distinguish, whenever possible, between causal mechanistic evidence obtained from genetic or pharmacological rescue experiments and correlative observations from patient samples or descriptive models.

16. Genetic disorders

Charcot-Marie-Tooth disease (CMT) is usually inherited in an autosomal dominant manner, although X-linked and autosomal recessive forms also exist (202). The most common genetic mutations associated with CMT involve gap junction protein beta 1 (GJB1), peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), and MFN2, which have

Table II. Roles of MERCs in diseases.

Authors, year	Diseases	MERCs resident protein	Principal function	(Refs.)
Bernard-Marissal *et al*, 2019	CMT	MFN2↓	Increases ER stress. Alters Ca^{2+} homeostasis	(42)
Cartes-Saavedra *et al*, 2021	ADOA	ITPRs↑	Alters Ca^{2+} homeostasis	(206)
Stoica *et al*, 2014; Stoica *et al*, 2016; Gomez-Suaga *et al*, 2022	ALS/FTD	VAPB-PTPIP51↓	Alters Ca^{2+} homeostasis. Decreases mitochondrial ATP production	(98,128,208)
Paillusson *et al*, 2017	PD	VAPB↓	Alters Ca^{2+} homeostasis. Decreases mitochondrial ATP production	(212)
Lau *et al*, 2020	AD	VAPB-PTPIP51↓	NR	(218)
Paillusson *et al*, 2017		Sig-1R↓	Alters Ca^{2+} homeostasis. Promotes mitochondrial dysfunction	(212)
Cherubini *et al*, 2020	HD	MFN2↓	NR	(224)
		ITPR3↓	NR	
		GRP75↓	NR	
		DRP1↑	Alters Ca^{2+} homeostasis. Increase ROS production	
Yang *et al*, 2019	DKD	DsbA-L↓	Increases ER stress. Increases apoptosis	(35)
Xue *et al*, 2021		PACS2↓	Promotes mitochondrial dysfunction	(229)
Chen *et al*, 2024		MFN2-VDR↓	Inhibits mitophagy	(230)
Li *et al*, 2023		DRP1↑	Promotes mitochondrial fission	(231)
Xie *et al*, 2022		VDAC1-RTN1A↑	Increases apoptosis. Activates inflammasome pathways	(232)
Xu *et al*, 2022	NAFLD	Cds2↓	Reduces lipid metabolism	(237)
Dong *et al*, 2024		EMC2-SLC25A46-Mic19↓	Reduces lipid metabolism. Reduces mitochondrial unfolded protein stress response	(40)
Wu *et al*, 2017	HF	FUNDC1↓	Alters Ca^{2+} homeostasis. Inhibits mitochondrial fission	(103)
LI *et al*, 2023		LonP1↓	Promotes mitochondrial fission. Activates ER unfolded protein stress response	(41)
Wu *et al*, 2019; Salin Raj *et al*, 2023	DCM	FUNDC1↑ PACS2↑ VDAC1-ITPR2↑	Alters Ca^{2+} homeostasis. Promotes mitochondrial fission. Increases apoptosis	(45,245)
Li *et al*, 2024	OA	PTPN1↑	Promotes cellular senescence	(201)
		ITPR1↑	Promotes cellular senescence	
Song *et al*, 2024	IDD	SYNJ2BP-RRBP1↓	Alters Zn^{2+} homeostasis. Promotes cellular senescence	(14)
Hernández-Alvarez *et al*, 2019	Cancer	MFN2↓	Reduces lipid metabolism. Increases ER stress	(36)
Wu *et al*, 2016; Chai *et al*, 2021		FUNDC1↑	Promotes mitochondrial fission	(167,250)
Hu *et al*, 2021		MFF↑	Promotes mitochondrial fission	(168)
Gomez-Suaga *et al*, 2017		VAPB-PTPIP51↑	Inhibits autophagy. Alters Ca^{2+} homeostasis	(180)
Ponneri Babuharisankar *et al*, 2023		FUNDC1-ULK1↑	Promotes autophagy	(177)
Raturi *et al*, 2016		SERCA2b↑	Alters Ca^{2+} homeostasis	(159)
Zheng *et al*, 2018		EI24-VDAC2↑	Alters Ca^{2+} homeostasis. Increases apoptosis	(124)
Li *et al*, 2022		GRP75-VDAC1↑	Alters Ca^{2+} homeostasis	(252)

NR, not reported; ↑, upregulated expression; ↓, downregulated expression; CMT, Charcot-Marie-Tooth disease; ADOA, autosomal dominant optic atrophy; PD, Parkinson's disease; AD, Alzheimer's disease; ALS/FTD, amyotrophic lateral sclerosis/Frontotemporal dementia; HD, Huntington's disease; DKD, diabetic kidney disease; NAFLD, non-alcoholic fatty liver disease; HF, heart failure; DCM, diabetic cardiomyopathy; OA, osteoarthritis; IDD, Intervertebral disc degeneration.

become key targets in research efforts aimed at developing disease-modifying therapies (202). The hallmark of CMT is progressive degeneration of peripheral nerve axons and myelin sheaths, leading to reduced nerve conduction velocity (203). Based on clinical and electrophysiological features, four main subtypes of CMT can be distinguished (203). CMT1 is characterized by demyelination and is inherited in an autosomal dominant pattern. CMT2 is an axonal form that may be inherited in either a dominant or recessive pattern. CMTX, which is predominantly X-linked, is associated with intermediate nerve conduction velocities, although recessive and autosomal dominant intermediate forms have also been reported. CMT4 exhibits a demyelinating phenotype but follows an autosomal recessive inheritance pattern.

Because dominant mutations in the MFN2 gene predominantly impair mitochondrial fusion and motility, CMT2A is the most common subtype among patients with CMT2 (204). It has been recently demonstrated that expression of MFN2R94Q induces distal axonal degeneration even in the absence of overt neuronal death (42). In primary neurons, motor neurons from a CMT2A animal model, and fibroblasts derived from CMT2A patients, the mutant protein reduces the extent of ER-mitochondria contacts. These alterations occur concurrently with ER stress, dysregulation of calcium homeostasis, and abnormalities in mitochondrial morphology and axonal transport. Notably, pharmacological interventions that alleviate ER stress or enhance ER-mitochondria crosstalk restore mitochondrial morphology and prevent axonal degeneration. These findings establish MERC dysfunction as a key pathogenic mechanism through which mutant MFN2 drives CMT2A pathology (42).

Autosomal dominant optic atrophy (ADOA) leads to degeneration of retinal neurons, resulting in optic nerve atrophy and progressive visual impairment (205). Studies have shown that ADOA is associated with mutations in two genes (OPA1 and OPA3) and three established loci (OPA4, OPA5 and OPA8), which encode proteins localized to the IMM. Additionally, other genetic loci (OPA2, OPA6 and OPA7) may be linked to X-linked or recessive forms of optic atrophy (205). Previous research has demonstrated that human fibroblasts derived from patients with ADOA lacking functional OPA1 exhibit enhanced ER-mitochondria coupling and a leftward shift in cytoplasmic Ca^{2+} dependence, indicating more efficient ER-to-mitochondria calcium transfer compared with wild-type cells (206).

The evidence indicates that mutations in genes encoding MERC-associated proteins, such as MFN2 in CMT2A and OPA1 in ADOA, cause distinct but significant alterations in MERC structure and function. In CMT2A, the MFN2R94Q mutation decreases ER-mitochondria contacts, initiating a series of events including ER stress, calcium dyshomeostasis and mitochondrial dysfunction, which ultimately leads to axonal degeneration. In ADOA, OPA1 deficiency results in increased ER-mitochondria coupling and more efficient calcium transfer, which may paradoxically contribute to retinal neuron pathology. These examples demonstrate that MERCs represent a crucial functional interface where genetic lesions converge to disrupt cellular homeostasis, and that the pathological outcome, whether due to reduced or excessive connectivity, depends on the specific molecular lesion and cellular context. Importantly, the discovery that pharmacological restoration of ER-mitochondria communication can reverse phenotypes in CMT2A models underscores the therapeutic potential of targeting MERC dynamics to alter disease progression. Thus, CMT2A and ADOA exemplify the two contrasting poles of MERC dysfunction: In CMT2A, a primary loss of ER-mitochondria contacts leads to axonal degeneration, whereas in ADOA, pathological hyper-coupling and excessive Ca^{2+} transfer result in retinal neuron loss. These opposite deviations from the normal MERC state emphasize how the functional requirements at these contact sites are cell-type-specific.

17. Neurodegenerative diseases

Amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) share overlapping clinical, pathological and genetic features, establishing them as closely linked neurodegenerative disorders. Clinical manifestations in patients with FTD often resemble those observed in ALS, and vice versa (207). A key pathological hallmark shared by both conditions is the abnormal aggregation of TDP-43, which plays a critical role in disease progression and is associated with neuronal dysfunction (207). Stoica *et al* (98) demonstrated that MERCs are significantly reduced in the presence of both wild-type and mutant TDP-43, a protein implicated in familial ALS. Notably, this reduction occurs without changes in the expression levels of MFN2, PTPIP51, or VAPB. Specifically, VAPB levels were markedly decreased in ALS tissues compared with controls, whereas no significant alterations were detected in PTPIP51, ITPR3, or VDAC1. Although TDP-43 does not directly bind to VAPB or PTPIP51 *in vitro* or *in vivo*, it disrupts their interaction (98). The binding between VAPB and PTPIP51 is diminished upon GSK3β activation, but enhanced by GSK3β inhibitors, which promote MERC formation (98,128). Furthermore, overexpression of both wild-type and four distinct mutant forms of TDP-43 was shown to activate GSK3β (98). Similarly, both wild-type and mutant FUS impair VAPB-PTPIP51 tethering and ER-mitochondria connectivity. These disruptions adversely affect mitochondrial ATP synthesis and calcium homeostasis. Additional studies indicate that neither wild-type nor mutant FUS directly interacts with VAPB or PTPIP51; instead, their effect is mediated through GSK3β activation. Treatment with the GSK3β inhibitor AR-A014418 mitigates FUS-induced defects in ER-mitochondrial coupling, restoring Ca^{2+} signaling and mitochondrial ATP production (128).

Disruption of the VAPB-PTPIP51 tether has also been observed in neurons derived from induced pluripotent stem (iPS) cells of patients carrying the C9orf72 mutation, as well as in transgenic mice expressing the ALS/FTD-associated mutant C9orf72. All three C9orf72 dipeptide repeat proteins (DPRs) significantly reduce VAPB-PTPIP51 and ITPR1-VDAC1 interactions, thereby impairing ITPR-mediated Ca^{2+} transfer from the ER to mitochondria (208), in contrast to control samples. However, the absence of direct binding between DPRs and either VAPB or PTPIP51 suggests an indirect mechanism, supporting further evidence that DPR-induced damage to the VAPB-PTPIP51 complex is facilitated by GSK3β activation (208). It is crucial to note that although the

disruption of VAPB-PTPIP51 tethering by TDP-43, FUS and C9orf72 DPRs has been repeatedly verified across multiple model systems, the causal chain that links this disruption to specific neurodegenerative endpoints *in vivo* remains an area under active investigation. The correlative evidence from postmortem human tissue and iPSC-derived neurons is robust, yet conclusive proof that restoring this single tether is sufficient to halt disease progression in animal models has not been established (46,128,208). In this context, the disruption of Ca^{2+} homeostasis and ATP production caused by defective VAPB-PTPIP51 tethering can be regarded as a core MERC-driven pathogenic mechanism. Meanwhile, the downstream activation of GSK3β and the subsequent protein aggregation are secondary events that amplify the initial insult.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the death or degeneration of dopamine-producing neurons in the substantia nigra (SN), a brain region critical for motor control. Since dopaminergic neurons regulate movement and coordination, patients with PD commonly exhibit symptoms such as bradykinesia, tremors and muscle rigidity (209). Two primary pathophysiological hallmarks of PD are the abnormal accumulation of α-synuclein (α-Syn) protein, leading to the formation of Lewy bodies, and the dysfunction of dopaminergic neurons in the SN. These pathological changes disrupt neural circuitry, ultimately giving rise to the clinical manifestations of PD. As the disease progresses, misfolded α-Syn aggregation, dysregulated dopamine metabolism, oxidative stress and neuronal loss form a self-reinforcing cycle (210). Guardia-Laguarta *et al* (211) demonstrated that in both human and mouse brain tissues, wild-type α-Syn localizes predominantly to MERCs, rather than to mitochondria alone. Notably, pathogenic point mutations in α-Syn reduce MERC formation. In contrast to cells expressing wild-type α-Syn, mutant α-Syn-expressing cells exhibit weakened ER-mitochondria associations, impaired MERC function, and increased mitochondrial fragmentation (211). Another study focused on the interaction between α-Syn and VAPB, showing that overexpression of both wild-type and familial mutant α-Syn disrupts the VAPB-PTPIP51 tether, thereby loosening ER-mitochondria contacts (212). This disruption is also observed in neurons derived from iPSCs of patients with PD with triplication of the α-Syn locus. α-Syn-mediated weakening of ER-mitochondria coupling impairs inter-organellar Ca^{2+} exchange and compromises mitochondrial ATP production (212).

Alzheimer's disease (AD) is the leading cause of cognitive impairment in a significant proportion of older adults. The primary pathogenic features of AD include the degeneration of neuronal connections and cells, along with the abnormal accumulation of misfolded proteins, particularly intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein and extracellular β-amyloid (Aβ) plaques (213). Familial forms of AD are associated with mutations in presenilin-1 (PSEN1), presenilin-2 (PSEN2) and the amyloid precursor protein (APP). APP processing generates Aβ, the pathological peptide that accumulates in AD brains. The γ-secretase complex, which cleaves APP to produce Aβ, contains presenilins as catalytic subunits (214). Both presenilins and APP localize to MERCs, where they participate in APP processing and Aβ generation (215,216). Mutations in these proteins, as well as Aβ accumulation itself, disrupt MERC functions, including mitochondrial biogenesis, ATP production, ER stress responses, Ca^{2+} signaling, lipid metabolism, autophagy, inflammation and synaptic activity (217). Lau *et al* (218) investigated the neuropathological impact of AD across brain regions by analyzing postmortem brain tissues from control subjects and AD patients. Their findings indicate that the cerebellum is relatively spared in AD, whereas the temporal cortex is highly vulnerable. In late-stage AD, VAPB and PTPIP51 protein expression remained unchanged in the cerebellum but was significantly reduced in the cerebral cortex. Further analysis revealed that the VAPB-PTPIP51 tethering complex was disrupted in pyramidal neurons from individuals at Braak stages III-IV, whereas Purkinje cells retained intact ER-mitochondria contacts. To determine whether VAPB loss correlates with reduced PTPIP51 levels in the temporal cortex, neuron-specific enolase-normalized VAPB and PTPIP51 signals were analyzed in individual samples. The results demonstrated a strong positive correlation between the two proteins (218). The chaperone protein sigma-1 receptor (Sig-1R) is predominantly localized at MERCs and performs multiple critical cellular functions. Deletion of Sig-1R leads to diminished ER-mitochondria coupling, altered mitochondrial dynamics, disrupted Ca^{2+} homeostasis, and increased ER stress (219). It has been recently shown that N,N-dimethyltryptamine (DMT) restores neuronal ER-mitochondria crosstalk by activating Sig-1R, a mechanism linked to DMT's potential anti-AD effects. DMT enhances TCA cycle activity, alleviates mitochondrial dysfunction in AD models, and modulates ER-mitochondria contacts and mitochondrial calcium uptake in both *in vitro* and *in vivo* systems (220).

Huntington's disease (HD) is an autosomal dominant, progressive and fatal neurological disorder characterized by motor dysfunction, including limb tremors, and cognitive decline (221). Histopathological examination of HD patient brains has revealed neurodegeneration in multiple brain regions, particularly the striatum, subthalamic nucleus, hypothalamus, caudate nucleus, and putamen. HD is caused by an expanded CAG trinucleotide repeat in exon 1 of the huntingtin (HTT) gene, which encodes a polyglutamine tract and leads to the production of a mutant protein with toxic gain-of-function properties (222). Selective degeneration of the striatum in HD is associated with well-established pathological features, including disruption of Ca^{2+} homeostasis and mitochondrial dysfunction (223). A significant reduction in the levels of the chaperone GRP75 and the ER-anchored ITPR3 in the striatum of both patients with HD and two distinct HD animal models, but not in the cortex or hippocampus, has been previously demonstrated (224). MFN2 expression was found to be decreased specifically in the putamen of patients with HD. The association between reduced MERC-associated protein levels and the onset of behavioral abnormalities in HD animal models suggests that impaired ER-mitochondria connectivity contributes to striatal vulnerability, rather than being merely a secondary consequence. Moreover, reductions in MERC proteins occur during early to mid-stages of the disease, indicating that these changes actively participate in HD progression rather than simply reflecting late-stage neuronal damage. Disruptions in ER-mitochondria interface proteins

have also been implicated in other neurodegenerative diseases, where they can either enhance or diminish ER-mitochondria juxtaposition (224). Excessive mitochondrial fission (overfission) has been observed in HD models, and treatment of R6/1 mutant huntingtin striatal neurons with the Drp1 inhibitor Mdivi-1 restored ER-mitochondria contacts and Ca^{2+} transfer, thereby ameliorating Ca^{2+} homeostasis defects (224).

Despite having distinct etiologies, a common theme emerges: The specific disruption of key tethering complexes, most notably the VAPB-PTPIP51 interaction, impairs inter-organellar communication. This disruption is instigated by disease-specific proteins (for example, TDP-43, FUS, α-synuclein, Aβ, mutant huntingtin), often via indirect pathways involving kinases such as GSK3β. The subsequent loss of MERC integrity results in a characteristic triad of defects: Dysregulated Ca^{2+} signaling, impaired mitochondrial bioenergetics (decreased ATP production), and heightened cellular stress. Significantly, MERC alterations frequently occur at an early stage of disease progression, contributing to neuronal vulnerability rather than merely being a late-stage consequence. The evidence indicates a vicious cycle in which protein aggregates disrupt MERCs, and dysfunctional MERCs in turn promote further proteostasis failure and metabolic deficiency. Collectively, these findings firmly establish MERCs as crucial hubs, the dysfunction of which is integral to neurodegenerative pathogenesis, thus positioning the restoration of ER-mitochondria connectivity as a promising therapeutic strategy for multiple disorders.

18. Metabolic diseases

Diabetic kidney disease (DKD) is characterized by early alterations in glomerular filtration rate and increased urinary excretion of proteins, ions and other solutes. Advanced stages of DKD are marked by extracellular matrix (ECM) protein accumulation, interstitial fibrosis, cellular senescence, and ultimately renal cell loss and organ failure (225). The pathogenesis of DKD involves multiple mechanisms, including hemodynamic and metabolic disturbances that trigger proinflammatory and profibrotic signaling pathways in the kidney, leading to progressive histopathological changes (226). Both human patients and experimental murine models of DKD have been shown to exhibit compromised MERC integrity. In a study by Yang *et al* (227), the structural integrity of MERCs was examined in renal biopsies from individuals at various stages of DKD. Electron microscopy revealed a reduced number of MERCs in DKD samples compared with controls. Additionally, the expression levels of key MERC components, including the tethering protein MFN2, the structural regulator PACS2, and disulfide-bond A oxidoreductase-like protein (DsbA-L), a MERC-localized enzyme, were found to be downregulated in DKD biopsies. Furthermore, co-immunoprecipitation analysis showed decreased interaction between the ER-resident ITPR1 and the mitochondrial VDAC1, two MERC components critical for calcium transfer, in the tubular compartment of DKD biopsies relative to controls (227). MERC integrity was negatively correlated with markers of kidney injury and fibrosis in DKD (227). A more recent study reported diminished expression of MFN2, a core MERC protein, in kidney biopsies from patients with DKD. MFN2 was nearly undetectable in DKD tissues, whereas it was robustly expressed in the glomerular compartment of control samples (228). DsbA-L-deficient mice exhibited elevated markers of ER stress and an exacerbated DKD phenotype, whereas overexpression of DsbA-L in renal tubular cells conferred protection against high glucose-induced apoptosis, ER stress and MERC disruption (35). Similarly, diabetic mice lacking PACS2, a structural component of MERCs, displayed worsened kidney function and increased proteinuria. By contrast, *in vivo* overexpression of PACS2 via intravenous delivery of an adenoviral vector, as well as its overexpression in cultured renal tubular cells (HK-2), preserved MERC integrity and protected against glucose-induced cellular dysfunction (229). Chen *et al* (230) demonstrated a significant downregulation of vitamin D receptor (VDR), PINK1, Parkin, FUNDC1, LC3II, ATG5, MFN2 and MFN1 in renal tubular cells of diabetic rats. In streptozotocin-induced diabetic rats, calcitriol treatment attenuated tubulointerstitial fibrosis and reduced urinary albumin and serum creatinine levels. Moreover, VDR agonists restored MERC integrity, alleviated mitophagy impairment, and suppressed mitochondrial fission and ROS production (230).

Other studies suggest a more nuanced role for MERCs in DKD progression through dynamic changes in their abundance or composition. For instance, upregulation of A-kinase anchoring protein 1 (AKAP1) at MERCs explains the increased MERC enrichment observed in podocytes from biopsies of patients with DKD and in podocytes exposed to high glucose *in vitro*. AKAP1 promotes mitochondrial fission by enhancing DRP1 phosphorylation and translocation. Pharmacological inhibition of DRP1 mitigates podocyte dysfunction and excessive mitochondrial fission induced by AKAP1 overexpression (231). Tubule-specific overexpression of RTN1A exacerbated DKD in diabetic mice, as evidenced by increased tubular damage, tubulointerstitial fibrosis and impaired renal function. However, RTN1A overexpression did not aggravate diabetes-induced albuminuria or glomerular injury. Notably, RTN1A overexpression amplified ER stress and mitochondrial dysfunction in diabetic tubular epithelial cells by disrupting MERCs. ER-localized RTN1A interacted with both VDAC1 and mitochondrial hexokinase-1 (HK1), interfering with their association. Dissociation of VDAC1 from HK1 triggered apoptotic and inflammasome activation pathways, contributing to TEC damage and loss (232). The literature on MERC alterations in DKD emphasizes the importance of distinguishing correlative relationships from causal ones. Although the decreased expression of MERC components, such as MFN2 and PACS2, is associated with the disease severity in patient biopsies, the functional rescue experiments, in which the overexpression of DsbA-L or PACS2 mitigates renal injury in diabetic models, provide more convincing evidence for a causal role of MERC dysfunction in DKD pathogenesis (35,227,229). Taken together, the evidence from DKD models indicates that the loss of MERC integrity represents an early, primary event that initiates a cascade of secondary pathologies, such as ER stress, mitochondrial fission and inflammasome activation. These secondary pathologies collectively lead to tubular injury and fibrosis.

NAFLD is a collective term encompassing a spectrum of liver conditions, including hepatic steatosis and non-alcoholic

steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma. A defining feature of NAFLD232 is steatosis, defined as the accumulation of fat in >5% of hepatocytes (233). Contributing factors to NAFLD include diabetes, insulin resistance, metabolic syndrome and genetic variants in TM6SF2 (transmembrane 6 superfamily member 2) and PNPLA3 (patatin-like phospholipase domain-containing protein 3) (234,235). In individuals with NASH, mitochondrial dysfunction is frequently observed and plays a critical role in the progression from simple steatosis to NASH (236). Liver biopsies from patients with NASH show reduced MFN2 expression. Similarly, mouse models of steatosis or NASH exhibit decreased Mfn2 levels, and re-expression of Mfn2 in a NASH model ameliorates disease pathology. Mice with liver-specific ablation of Mfn2 develop fibrosis, hepatocellular malignancy, lipid accumulation and inflammation. MFN2 has been shown to bind PS and facilitate its transport into specific membrane domains, thereby promoting PS transfer to mitochondria and subsequent mitochondrial PE synthesis. Consequently, loss of hepatic Mfn2 impairs phospholipid biosynthesis and PS trafficking, leading to ER stress, development of a NASH-like phenotype, and increased susceptibility to liver cancer (36). Recently, Xu *et al* (237) demonstrated that Cds2 expression is downregulated in both diet-induced and genetically engineered NAFLD animal models. Hepatic deficiency of Cds2 results in steatosis, inflammation and fibrosis. Loss of Cds2 alters the MERCs proteome, potentially affecting both the structure and function of MERCs. The ER-mitochondria PS transfer, mediated by EMC subunits and MFN2, is significantly impaired in Cds2-deficient livers due to substantial downregulation of these proteins (237). Additionally, MFN2 serves as a physical tether between mitochondria and the ER. Reduced PS transport to mitochondria likely results from diminished MFN2 protein levels and increased distance between the ER and mitochondria in the livers of Cds2f/f; AlbCre mice (237). Mic19, a core component of the mitochondrial contact site and cristae organizing system (MICOS) complex, was shown by Dong and colleagues to regulate ER-mitochondria interactions via the EMC2-SLC25A46-Mic19 axis. Hepatocytes from mice with liver-specific Mic19 knockout (LKO) exhibit impaired mitochondrial fatty acid β-oxidation and disrupted lipid metabolism, which can spontaneously lead to NASH and liver fibrosis. LKO also leads to reduced ER-mitochondrial contacts, disordered mitochondrial lipid metabolism, cristae disorganization, and activation of the mitochondrial UPR. By contrast, re-expression of Mic19 in Mic19 LKO hepatocytes prevents the development of liver disease. Furthermore, overexpression of Mic19 attenuates methionine and choline-deficient diet-induced fatty liver disease (40).

Dysfunction of MERCs represents a central and active pathogenic mechanism in metabolic diseases, particularly DKD and NAFLD. A common characteristic in both conditions is the downregulation or impaired function of core tethering and regulatory proteins, such as MFN2, PACS2 and DsbA-L, which results in a loss of MERC integrity. This structural disruption triggers a series of functional deficits. In DKD, it impairs Ca^{2+} signaling through the ITPR1-VDAC1 axis, exacerbates ER stress, and promotes renal cell apoptosis and fibrosis. In NAFLD, it disrupts the crucial transfer of phospholipids such as PS, leading to hepatic lipid accumulation, ER stress, inflammation, and the progression to steatohepatitis. Significantly, the evidence indicates a vicious cycle in which metabolic insults (for example, hyperglycemia and lipotoxicity) damage MERCs, and dysfunctional MERCs in turn intensify metabolic dysfunction and cellular injury. Interventions that restore MERC structure or function, either by overexpressing tethering components or by using agents such as VDR agonists, exhibit protective effects in disease models, highlighting the therapeutic potential of targeting these organelle contact sites. MERCs are therefore identified as critical integrators of cellular metabolism, and their deterioration is a key factor in the pathogenesis and progression of complex metabolic disorders.

19. Cardiovascular diseases

Heart failure (HF) is a clinical syndrome characterized by the heart's inability to pump sufficient oxygen and nutrients to meet the body's demands, leading to impaired physical function and commonly presenting with fatigue, edema and dyspnea (238). Mitochondrial function and Ca^{2+} homeostasis are well-established contributors to ventricular remodeling and the progression of HF (239,240). Cardiac contraction and rhythmicity require substantial energy, which is primarily generated through mitochondrial OXPHOS (241). Additionally, optimal Ca^{2+} levels necessary for excitation-contraction coupling are regulated by the sarcoplasmic reticulum (SR), a specialized membrane system enriched with Ca^{2+}-ATPases. Disruptions in Ca^{2+} homeostasis can lead to aberrant energy metabolism and ER stress, both of which contribute to the development of HF (242). MERCs have been shown to play critical roles in cellular physiology, including regulation of mitochondrial dynamics and Ca^{2+} signaling (43,231).

Patients with HF exhibit significantly reduced expression of FUNDC1 and fewer SR-mitochondria contacts compared with healthy individuals. The diminished FUNDC1 levels at MERCs result in impaired ubiquitin-dependent inhibition of ITPR2 degradation, thereby compromising SR-to-mitochondria Ca^{2+} transfer. This disruption ultimately affects cardiac function through dysregulation of the CREB/Fis1 pathway. Furthermore, FUNDC1-deficient animals display both diastolic and systolic dysfunction (103). LonP1, a protease localized at MERCs, plays a key regulatory role. Depletion of LonP1 leads to a significant reduction in MERC formation and causes mitochondrial fragmentation. Moreover, loss of LonP1 in mouse cardiomyocytes disrupts mitochondrial fusion and MERC integrity and activates the ER UPR. Consequently, cardiomyocyte-specific deletion of LonP1 induces abnormal metabolic reprogramming and pathological cardiac remodeling (41).

DCM is a distinct form of cardiomyopathy that occurs independently of coronary artery disease or hypertension. In individuals with diabetes, DCM is strongly associated with an increased incidence of HF and elevated mortality rates (243). The pathophysiological hallmarks of DCM include myocardial interstitial fibrosis, cardiomyocyte hypertrophy, necrosis and apoptosis. Its development involves multiple complex mechanisms, including insulin resistance, disturbances in cardiac energy metabolism, oxidative stress, inflammatory responses, Ca^{2+} imbalance, impaired autophagy, and other contributing factors (244). A direct link between MERCs and the onset

of DCM has been recently suggested (45). Compared with cardiac tissues from non-diabetic donors, those from diabetic individuals exhibit significantly higher levels of FUNDC1. Cardiomyocyte-specific deletion of Fundc1 abolished diabetes-induced MERC formation, prevented mitochondrial Ca^{2+} overload, mitochondrial fragmentation and apoptosis, and was associated with improved mitochondrial functional capacity and cardiac performance (45). Based on these findings, several research groups have investigated the effects of high glucose exposure and demonstrated that such conditions reduce mitochondrial biogenesis, fusion and OXPHOS while promoting MERC formation through upregulation of PACS2, ITPR2, FUNDC1 and VDAC1. These observations highlight the critical roles of mitochondria-driven apoptotic pathways, such as the SMAC-HTRA2-ARTS-XIAP cascade, in the molecular pathogenesis of DCM. Additionally, diabetic rats exhibited signs of cardiomyopathy, including elevated levels of troponin, TNNI3K and heart mass index. Both *in vitro* and *in vivo*, ferulic acid effectively suppressed hyperglycemia-induced alterations in MERCs and ameliorated the associated cellular abnormalities (245).

The evidence indicates that MERC dysfunction is a common factor, albeit presenting differently in various disease contexts. In HF, a decline in MERC integrity, mediated by reduced levels of proteins such as FUNDC1 and LonP1, disrupts the accurate calcium signaling between the SR and mitochondria, impedes mitochondrial fusion, and initiates maladaptive stress responses. Collectively, these effects compromise cardiac energetics and contractile function. Conversely, in DCM, a pathological elevation in MERC formation, driven by the upregulation of proteins such as FUNDC1, ITPR2 and PACS2, leads to excessive mitochondrial calcium influx, resulting in Ca^{2+} overload, oxidative stress, and the activation of apoptotic pathways. These contrasting dysregulations, whether it is diminished or excessive ER-mitochondria coupling, both culminate in the same adverse outcomes: Disrupted mitochondrial bioenergetics, impaired calcium homeostasis, and ultimately, cardiomyocyte death and cardiac remodeling. The protective effects observed following genetic or pharmacological modulation of MERC components emphasize that restoring the physiological balance of these contact sites is a feasible therapeutic strategy. Therefore, MERCs emerge as dynamic and crucial regulators of cardiac health, where their precise structural and functional adjustment is essential to prevent the progression from metabolic stress to overt HF. The contrast between HF and DCM clearly illustrates the dualistic nature of MERC dysfunction in cardiac disease. In HF, the reduced levels of FUNDC1 and LonP1 result in diminished MERC formation and impaired Ca^{2+} transfer. By contrast, in DCM, the upregulation of FUNDC1, PACS2 and ITPR2 leads to excessive MERC coupling and mitochondrial Ca^{2+} overload. Both of these extreme situations ultimately compromise cardiac function, which reinforces the concept that MERC integrity must be maintained within a physiological range appropriate to the cell type and metabolic context.

20. Orthopedic diseases

OA is a whole-joint disease characterized by structural alterations in articular cartilage, subchondral bone, ligaments, joint capsule, synovial membrane and periarticular muscles (246). OA has a complex etiology involving mechanical, inflammatory and metabolic factors that ultimately lead to structural failure of the synovial joint. Rather than being a passive degenerative condition or a so-called 'wear-and-tear' disease as it is often described, OA represents an active and dynamic process resulting from an imbalance between tissue repair and degradation in the joint (247). Research on the involvement of MERCs in OA remains limited; however, the authors' most recent work suggests a potential role for MERCs in OA pathogenesis (200). The present findings revealed a strong association between genes linked to cellular senescence in OA and hub genes related to MERCs. Further evidence from *in vitro* experiments demonstrated positive correlations between OA-related senescence genes (PTEN, ABL1, MAPK14 and MAP2K1) and MERCs-associated genes (PTPN1 and ITPR1) (200).

Intervertebral disc degeneration (IDD) and its associated pathological changes are the leading causes of low back pain and neck pain, two of the most prevalent medical conditions contributing to increased years lived with disability and heightened demand for rehabilitation services (248). Accumulating evidence indicates that disrupted mitochondrial function exacerbates key pathological processes during IDD progression, including ECM degradation, inflammatory responses, cellular senescence and cell death (249). Previous studies have demonstrated that MERCs facilitate the transfer of SLC39A7 from the ER to mitochondria, thereby regulating intracellular Zn^{2+} homeostasis (12,13). Furthermore, proteomic analyses have revealed that NLRX1 localizes to both the ER membrane and the OMM, suggesting a potential role in MERC formation and stabilization (92). A recent study found that SYNJ2BP promotes the interaction between NLRX1 and SLC39A7 and is essential for maintaining mitochondrial Zn^{2+} homeostasis. SYNJ2BP contributes to MERC structural organization through its interaction with RRBP1. In addition to reducing the formation of the SLC39A7-NLRX1 complex, loss of SYNJ2BP expression and disruption of MERC architecture impair mitochondrial localization of SLC39A7. Moreover, SYNJ2BP downregulation was identified as a key pathological feature of nucleus pulposus cell senescence and IDD progression, strongly associated with MERC disruption (14).

Although research is still in its nascent stages, preliminary evidence firmly indicates that MERC dysfunction contributes to disease progression via distinct yet converging pathways. In OA, a correlative association has been established between MERC-associated genes (PTPN1 and ITPR1) and key factors driving cellular senescence, suggesting that disrupted inter-organellar communication may expedite the senescent phenotype that is central to joint degradation. In IDD, a more well-defined molecular mechanism is presented. Specifically, the downregulation of the MERC component SYNJ2BP disrupts contact site architecture, impedes formation of the NLRX1-SLC39A7 complex, and consequently leads to dysregulation of mitochondrial zinc homeostasis. This dysregulation promotes senescence of nucleus pulposus cells, degradation of the ECM, and ultimately disc failure. Collectively, these findings establish MERCs as crucial, yet relatively under-investigated, cellular hubs in orthopedic health. The impairment of their structural integrity or functional capacity appears to intensify core degenerative processes, specifically, the impairment of ion

homeostasis (Zn^{2+}) and the acceleration of cellular senescence, which underlie both OA and IDD. This highlights a common theme across various diseases: MERC dysfunction disrupts basic cellular housekeeping, resulting in tissue-specific pathology. In the future, these insights present a promising new direction for research, indicating that therapeutic strategies designed to preserve or restore MERC function may potentially alleviate the progression of degenerative orthopedic conditions by targeting their fundamental cellular origins.

21. Cancer

Increasing evidence indicates that MERCs play essential roles in tumor initiation and progression. Key intracellular mechanisms supported by MERCs, such as lipid exchange, mitochondrial fission, autophagy, Ca^{2+} signaling and ROS regulation, are critically involved in cancer cell survival, susceptibility to cell death, invasion and metastasis (1). Galmes *et al* (29) demonstrated that in HeLa cells, the lipid-binding/transfer domains of ORP5 and ORP8, oxysterol-binding protein-related proteins, target ER-mitochondria contact sites and interact with the mitochondrial outer membrane protein PTPIP51, thereby modulating mitochondrial morphology and function. Additionally, hepatic deficiency of MFN2 has been shown to impair phospholipid biosynthesis and ER-mitochondrial PS transfer, ultimately leading to ER stress, development of a NASH-like phenotype, and hepatocellular carcinogenesis (36). Multiple studies have linked MERC-associated proteins to the regulation of tumor growth through the mitochondrial fission pathway. For example, Wu *et al* (167) reported that under hypoxic conditions in HeLa cells, the ER membrane protein calnexin interacts with FUNDC1, an OMM protein localized at MERCs, promoting its accumulation at ER-mitochondria interfaces. This interaction facilitates the recruitment of Drp1 to MERCs, initiating mitochondrial fission. Thus, FUNDC1 is specifically required for mitochondrial division in cervical cancer cells under hypoxic stress (167). Moreover, ubiquitin-specific protease 19 (USP19), an ER-resident deubiquitinase, enhances Drp1 homopolymerization by binding to and deubiquitinating FUNDC1 at ER-mitochondria contact sites in hypoxic HeLa cells. USP19 also modulates Drp1's hydrolytic enzyme activity and GTP binding capacity, thereby mediating mitochondrial fission (250).

Notably, MERC-mediated regulation of autophagy also contributes to cancer development. In a study by Hu *et al* (168), acute energy stress induces translocation of the cellular energy sensor AMPK from the cytosol to MERCs in human cervical carcinoma HeLa cells. There, AMPK directly interacts with the MERC tethering protein MFN2 and plays a critical role in energy stress-induced autophagy. Remarkably, following prolonged stress, a significant fraction of AMPK switches to bind and activate the MFF, thereby coordinating mitochondrial fission (168). Furthermore, Gomez-Suaga *et al* (180) showed that the ER protein VAPB and the mitochondrial protein PTPIP51 form tethering structures at MERCs, enhancing ER-mitochondria connectivity and facilitating ER-mitochondrial Ca^{2+} exchange, which suppresses autophagosome formation in HeLa cells (180). Ponneri Babuharisankar *et al* (177) further demonstrated that under hypoxia, the stress-induced mitochondrial chaperone Lon accumulates at ER-mitochondria contact sites in colon and oral cancer cells. Lon stabilizes the FUNDC1-ULK1 complex in a manner dependent on the mitochondrial NCLX, thereby promoting mitophagy and supporting cancer cell survival and progression (177). The fate of cancer cells is also influenced by oncogenes and tumor suppressors that disrupt Ca^{2+} homeostasis through complex interactions at ER-mitochondria contact sites, directly altering Ca^{2+} flux from the ER to mitochondria within the context of MERC-mediated Ca^{2+} signaling (251). TMX1 inhibits SERCA2b, a Ca^{2+} pump enriched at ER-mitochondria contact sites, thereby reducing ER-mitochondrial Ca^{2+} transfer in HeLa cervical cancer and A375P melanoma cells (159). Additionally, Zheng *et al* (124) showed that tubular ER undergoes extensive expansion in response to DNA damage, a process dependent on p53-mediated transcriptional activation of ER-shaping proteins REEP1, REEP2 and EI24. Subsequently, EI24 interacts with VDAC2 to promote ER-mitochondria contact formation, increasing Ca^{2+} transfer from the ER to mitochondria and promoting DNA damage-induced apoptosis (124). Li *et al* (252) also found that cisplatin-resistant ovarian cancer (OC) cells exhibit elevated levels of GRP75 and VDAC1, proteins known to stabilize MERCs. These cells display resistance to cisplatin by preventing proapoptotic ROS accumulation and mitigating cisplatin-induced mitochondrial dysfunction (252). The evidence indicates that MERCs are not passive structures; rather, they are actively remodeled within tumors to support cell survival, proliferation and adaptation to stress. Through specific tethering complexes, such as ORP5/ORP8-PTPIP51 for lipid transfer and VAPB-PTPIP51 for calcium exchange, MERCs regulate fundamental processes that contribute to tumorigenesis. They enable cells to survive under hypoxic conditions by promoting fission through the FUNDC1-Drp1 axis and modulate the energy stress response by recruiting AMPK to regulate autophagy. Moreover, MERCs play a crucial role in determining cell fate in response to therapy. They can mediate proapoptotic calcium transfer in response to DNA damage (for example, via the EI24-VDAC2 axis), yet they can also be exploited to promote therapy resistance, as observed in cisplatin-resistant OC, where upregulated GRP75-VDAC1 stabilizes MERCs to inhibit apoptosis. This dual functionality highlights the context-dependent nature of MERC function in cancer, positioning them as dynamic therapeutic targets whose modulation may disrupt the adaptive mechanisms that enable tumors to grow and resist treatment.

22. Strategies for targeting MERCs

Building upon the comprehensive overview of MERC biology and pathology presented in the present review, the development of therapeutic strategies targeting these contact sites represents both a compelling opportunity and a formidable challenge. Current approaches, although in their infancy, can be broadly categorized into three levels of intervention: Direct modulation of the contact-site machinery, regulation of individual MERC-associated proteins, and indirect modulation through upstream metabolic or signaling pathways. Direct strategies are aimed at precisely manipulating the physical tethering or functional output at the interface. The Drp1 inhibitor Mdivi-1 (253), which restores MERC integrity and calcium homeostasis in HD

models (224), serves as an example of this by targeting a key executor of MERC-defined mitochondrial fission. Similarly, the utilization of a cell-penetrating peptide to displace Hexokinase 2 from MERCs, thereby inducing lethal Ca^{2+} overload specifically in cancer cells (254,255), demonstrates the potential of precisely disrupting pro-survival microdomains. These examples highlight the therapeutic potential of molecules that can stabilize beneficial contacts (for example, in neurodegeneration) or destabilize pathological ones (for example, in cancer or diabetic complications). The second level involves modulating the expression or post-translational modification of core tethering components, such as genetically or pharmacologically targeting MFN2, PACS2, or cyclophilin D (104). While effective in specific experimental settings, such as protecting cardiomyocytes from ischemia-reperfusion injury, this approach is severely restricted by the pleiotropic functions of these proteins outside of MERCs. As demonstrated by the systemic metabolic disruptions in liver-specific Mfn2 knockout mice (256) or the hepatic insulin resistance in CypD knockout animals (257), global manipulation often results in unacceptable on-target, off-site effects. This underscores a fundamental challenge: The majority of MERC regulators are not confined solely to the contact site but are essential components of more extensive cellular networks that govern fusion, calcium signaling and metabolism. Consequently, the future of MERC-targeted therapy depends on overcoming this limitation through the attainment of context-specificity, which involves specifically influencing the protein's function at the ER-mitochondria interface, and functional-selectivity, which entails modulating only its tethering-related activity without interfering with its other functions.

The translation of MERC-targeted strategies into clinical applications encounters several fundamental obstacles. First, the majority of MERC-resident proteins are not solely localized at contact sites but also carry out essential functions in other parts of the cell. For instance, MFN2 is essential for mitochondrial fusion in all cell types, and its deficiency in liver-specific knockout mice results in severe metabolic disruption and hepatocarcinoma (36,256). Likewise, cyclophilin D, while regulating MERC stability, serves as a crucial regulator of the mitochondrial permeability transition pore throughout the body (104,257). Pharmacological modulation of these pleiotropic proteins will inevitably result in on-target but off-site effects, which may overshadow the therapeutic benefits. Second, the molecular composition and functional output of MERCs are highly specific to cell types. A MERC-stabilizing intervention aimed at restoring neuronal calcium homeostasis in AD could, in theory, exacerbate cardiomyocyte pathology in HF by promoting excessive mitochondrial calcium loading, as indicated by the pathological MERC expansion observed in DCM (45,245). Third, the dynamic nature of MERCs determines that their abundance, inter-membrane distance, and protein composition continuously adapt to the cellular metabolic status. This plasticity undermines the concept of a simple 'inhibitor' or 'activator' of MERCs. Instead, therapeutic interventions may need to achieve context-dependent normalization of MERC function. Strategies relying on cell-type-specific delivery systems, such as ligand-functionalized nanoparticles or viral vectors with tissue-specific promoters, may ultimately address the specificity challenge. Nevertheless, such approaches are still in the early pre-clinical stage and demand extensive validation in physiologically relevant human models, including patient-derived organoids and organ-on-chip platforms.

To actualize this paradigm shift, future research should pivot towards addressing several fundamental biological questions and developing next-generation technologies. First, a significant knowledge gap pertains to the molecular code for MERC targeting. Specifically, do conserved structural motifs or lipid-binding domains universally direct proteins to this interface, or is targeting accomplished through unique, protein-specific combinatorial signals? Deciphering this 'MERC zip code' via comparative proteomics of contact-site subdomains and structural biology is of utmost importance. Such knowledge would facilitate the rational design of targeted therapeutics, including small molecules that allosterically modulate a tether only when it is engaged at the contact site, or biologics conjugated to MERC-homing peptides. Second, there exists an urgent requirement for advanced tools that enable real-time, high-resolution *in vivo* observation and manipulation of MERCs. The development of more robust, minimally perturbative dynamic reporters beyond split-GFP, perhaps based on novel biosensors for local lipid flux, nanometer-scale distance changes, or specific enzymatic activities at MERCs, will be vital for screening and validating candidate drugs. Third, nanotechnology-based delivery systems present a promising approach to surmount specificity challenges. Designing liposomes or nanoparticles functionalized with ligands for MERC-enriched surface proteins could enable site-specific delivery of therapeutic cargoes. For example, gene-editing tools could be used to correct mutant MFN2 exclusively in affected neurons in CMT2A, or small interfering RNA could be employed to knock down pathological FUNDC1 overexpression specifically in diabetic cardiomyocytes. Finally, considering the dynamic and cell-type-specific characteristics of MERCs identified in diverse diseases, therapeutic strategies need to be highly customized. This requires transitioning from conventional cell lines to conducting tests in more physiologically relevant models, such as patient-derived organoids or organ-on-a-chip systems that maintain tissue-specific MERC architecture. In summary, the future direction involves moving from a simplistic, protein-focused pharmacopeia to a refined, interface-focused toolkit. The ultimate objective is to develop 'MERC stabilizers' and 'MERC normalizers' that can accurately adjust the distance, duration and functional output of these crucial communication hubs, thus restoring cellular homeostasis in a broad spectrum of diseases marked by their dysfunction, ranging from neurodegeneration and cardiomyopathy to cancer and metabolic syndrome. The three-tier model of MERC signaling, namely sensing, processing and execution, further suggests that therapeutic interventions can be strategically targeted at a specific tier according to the disease context. For example, normalizing abnormal ion sensing might be adequate in early-stage diseases, whereas interventions targeting downstream execution pathways may be necessary once organelle damage has been established.

23. Prospects and remarks

Over the past several decades, scientific interest has significantly shifted towards comprehending intracellular

communication through close-range, non-vesicular organelle interactions. Among these, the contact sites between the ER and mitochondria, two organelles essential for cellular homeostasis, have emerged as prominent, multifunctional signaling hubs. As elaborated in the present review, MERCs are not simply static bridges but dynamic, protein-rich platforms that integrate and regulate an astonishing variety of cellular processes, including Ca^{2+} and Zn^{2+} flux, lipid biosynthesis and transfer, redox balance, mitochondrial dynamics (fission, fusion and mitophagy), autophagy, apoptosis, inflammation and senescence. Current evidence clearly indicates that the exact structure and function of these nanoscale domains are often altered during the pathogenesis of a broad range of diseases, spanning neurodegeneration and metabolic disorders to cancer and cardiovascular conditions. MERC dysfunction is therefore now widely recognized as a central pathogenic mechanism, rendering targeted modulation of these interfaces a compelling yet complex therapeutic frontier.

However, the translation of this knowledge into effective clinical strategies is hindered by several fundamental challenges that delineate the future trajectory of the field. First, the intrinsic dynamic and heterogeneous characteristics of MERCs pose a substantial obstacle (258,259). Their composition, abundance and inter-membrane distance are not static but are precisely regulated by cellular metabolic status, stress signals and tissue context (260). This plasticity renders broad, non-specific pharmacological manipulation perilous. A more viable approach involves initially deciphering the endogenous, homeostatic mechanisms that regulate MERC assembly and disassembly. Progress in tool development is crucial in this regard. Emerging techniques, including split-BioID for *in situ* proteomic mapping of contact sites (261,262), next-generation split-fluorescent or bioluminescent reporters for real-time monitoring of MERC dynamics in live cells and organisms, and high-throughput CRISPR screens targeting MERC morphology, will be essential for uncovering these regulatory networks. Second, the functional impact of MERCs is highly tissue- and context-specific, which necessitates the adoption of a precision medicine approach. In neurons, MERCs play a crucial role in supplying Ca^{2+} and ATP to maintain synaptic activity and plasticity. In cardiomyocytes, they regulate the excitation-contraction coupling; however, their over-activation may lead to Ca^{2+} overload and apoptosis. In hepatocytes, they are essential for lipid metabolism, and any disruption to them can result in steatosis. Therefore, a therapeutic intervention aimed at stabilizing MERCs to benefit neurons in AD might inadvertently aggravate the pathology in DCM by facilitating pathological calcium transfer. Future research should prioritize the creation of detailed 'MERC atlases' that define their distinct molecular compositions and functional outputs across various cell types and disease states to guide the development of cell-selective modulators. Third, MERCs demonstrate a crucial dualism, in which their activity needs to be sustained within a narrow physiological range. Optimal coupling improves mitochondrial bioenergetics and promotes survival. By contrast, inadequate or excessive coupling can lead to pathology, either through bioenergetic failure or via apoptotic Ca^{2+} overload, excessive fission and impaired quality control (39-41,229,232). This Yin-Yang characteristic emphasizes that therapeutic strategies cannot simply aim to 'increase' or 'decrease' MERCs across the board. Instead, the objective must be to' 'normalize' or 'rescue' their function, restoring homeostasis regardless of whether the initial defect is hyper-or hypo-connectivity. This necessitates drugs that can detect and rectify the underlying functional deficit, rather than merely addressing the structural parameter. Fourthly, the potential of MERCs to act as sources of novel biomarkers remains largely unexploited, yet it confronts both technical and conceptual obstacles. Although the circulating levels of MERC-associated proteins (such as GRP78 in diabetes) (263) exhibit promising prospects, their lack of specificity represents a significant limitation, given that these proteins play diverse roles within cells (264). More sophisticated strategies are required. These may encompass PLA in circulating immune cells to detect specific tethering interactions, advanced lipidomic profiling of plasma to uncover signatures of disrupted MERC lipid transfer, or the isolation and proteomic analysis of MERC-enriched fractions from accessible tissues. The advancement of non-invasive imaging techniques capable of assessing MERC integrity *in vivo* persists as a distant yet potentially transformative objective.

In conclusion, the research on MERCs has advanced from descriptive ultrastructural analysis to an understanding of their role as key integrators in cellular life-and-death decisions. Their malfunction represents a common pathological node across various diseases. The future research path requires a multidisciplinary approach: (i) Fundamental discovery: Employing cutting-edge tools in spatial proteomics, super-resolution imaging, and organelle-specific gene editing to elucidate the regulatory mechanisms of MERCs. (ii) Contextual precision: Systematically mapping the biological characteristics of MERCs across various tissues and disease stages to enable targeted interventions. (iii) Therapeutic innovation: Transcending conventional targets to develop a novel category of 'interface therapeutics'-small molecules, peptides, or nanotechnologies that can precisely modulate the MERC interactome. (iv) Biomarker translation: Developing specific, MERC-centered diagnostic markers for early disease detection and patient stratification. By adopting this comprehensive framework, future research will not only enhance the understanding of these remarkable organelles but also open up new possibilities for diagnosing and treating some of the most widespread and challenging human diseases. The ultimate objective is to harness the potential of inter-organellar communication, shifting the therapeutic paradigm from targeting individual pathways to restoring the balance of the cellular network.

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Authors' contributions

HML, YL, WW and XC wrote the manuscript, and prepared the figures and tables. WW and CT conceived and revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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