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REVIEW

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Mitochondria-associated endoplasmic reticulum membranes and myocardial ischemia: from molecular mechanisms to therapeutic strategies

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Abstract

Myocardial ischemia has the highest disease burden among all cardiovascular diseases making it a significant challenge to the global public health. It can result in myocardial cell damage and death due to impaired mitochondrial and endoplasmic reticulum (ER) functions. These two organelles are important regulators of cell death. In recent years, research has shifted from isolated studies of individual organelles to a more integrative approach, with a particular focus on their membrane contact sites—Mitochondria-Associated Endoplasmic Reticulum Membranes (MAMs). These dynamic microdomains play a crucial role in regulating material exchange and signal transduction between the endoplasmic reticulum and mitochondria. This review comprehensively describes the intricate structure of MAMs and their multifaceted roles in cellular pathophysiological processes. Particular focus was directed at the far-reaching effects of MAMs in regulating key pathological events including calcium homeostasis, mitochondrial dysfunction, ER stress, oxidative stress, and autophagy in ischemic heart disease (IHD). The potential treatment targets and regulatory mechanisms of MAMs were discussed and summarized, providing novel research directions and treatment approaches for improving myocardial ischemia-related diseases.

Keywords Mitochondria-associated endoplasmic reticulum membranes, Myocardial ischemia, Calcium homeostasis, Cellular stress

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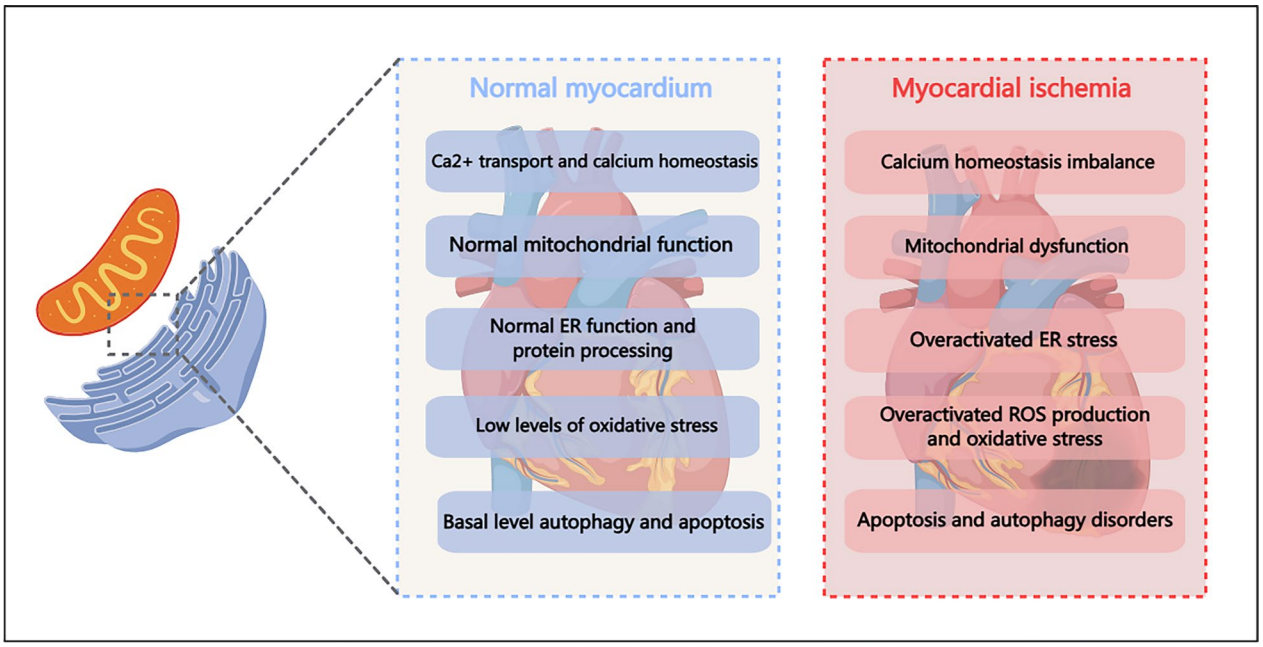
Graphical Abstract



Introduction

Ischemic Heart Disease (IHD) is a clinical syndrome caused by reduced coronary blood perfusion, resulting in an imbalance between myocardial oxygen supply and demand, dysregulated metabolism in cardiomyocytes, as well as impaired cardiac function. Myocardial ischemia causes irreversible damage to cardiomyocytes and may potentially lead to cell death. Several forms of regulated cell death influence the production of reactive oxygen species (ROS), calcium stress, and inflammatory cascade responses, thereby causing cardiomyocyte loss and exacerbating myocardial ischemia/reperfusion (I/R) injury. These processes trigger adverse cardiac remodeling and functional impairment [1]. IHD has the highest disease burden among all cardiovascular diseases, including acute myocardial infarction, chronic stable angina, chronic IHD, and heart failure related to these conditions [2]. The World Health Organization places IHD as the leading cause of death, out of the total 55.4 million deaths recorded in the past two decade. In this context, it is important to comprehensively elucidate specific mechanisms of myocardial ischemia to explore potential novel treatment strategies.

Mitochondria is a primary site for cellular energy production and play a pivotal role in adjustment to environmental stress as well as regulating metabolism, inflammation, calcium homeostasis, and cell death processes [3, 4]. Their functional integrity is vital for the survival of high-energy-demanding cardiomyocytes under ischemia and hypoxia. As the largest multifunctional organelle within the cell, the endoplasmic reticulum (ER) regulates various physiological processes, such as protein synthesis, folding, and transport, lipid metabolism, and calcium signaling [5, 6]. Impaired ER function can induce ER stress, causing the Unfolded protein response (UPR) and cell apoptosis. This process is closely accompanied with pathological changes including ischemia, hypoxia, oxidative stress, and the depletion of Ca^{2+} stores in the ER [7]. As research advances, the mitochondria and ER are no longer considered isolated structures with independent roles, but as interdependent components within a dynamic, integrated functional region called Mitochondria-associated Endoplasmic Reticulum Membranes (MAMs).

MAMs participate in diverse cellular pathophysiological processes, including Ca^{2+} transport, lipid metabolism, mitochondrial dynamics, cellular stress responses (including ER stress and oxidative stress), and regulation of cell death pathways (including autophagy and apoptosis) [8, 9]. MAMs have gained increasing research

attention because of their important and multifaceted roles. Studies have shown that the MAM microdomain significantly affects key pathophysiological mechanisms during myocardial ischemia and reperfusion, including calcium homeostasis, mitochondrial dysfunction, ER stress, oxidative stress, as well as protein–protein crosstalk control [9–11]. Alterations in these processes are recognized as common triggers for various forms of cell death, including apoptosis, autophagy, necrosis, pyroptosis, and ferroptosis [12]. These findings suggest that MAMs can regulate survival and death of cardiomyocytes under pathological conditions including I/R.

Multiple molecules regulate the structural integrity and function of MAMs. Among them is the Phosphofurin Acid Cluster Sorting Protein 2 (PACS-2), which was found to induce severe mitochondrial fragmentation, resulting in mitochondrial fission and separation from the ER, and significantly disrupting the structural integrity of MAMs [13, 14]. Another protein, FUN14 domain-containing protein 1 (FUNDC1) was reported to promote the formation of MAMs by tethering MAMs-specific proteins [15]. Correspondingly, regulatory molecules of MAMs function, including Glycogen Synthase Kinase-3β (GSK3β) and Sigma-1 Receptor (Sig-1R) also play critical roles in myocardial I/R. They can relieve myocardial injury through mechanisms including reducing MAMs-mediated calcium overload and ER stress, thereby exerting cardioprotective effects [16, 17]. With comprehensive research on the role of MAMs in IHD, additional regulatory proteins have been identified, among them CLIC4, Nox4, and ATAD3A, etc. [18–20].

In this review, we systematically describe the complex structure and multifunctional roles of MAMs, focusing on their significant implications in myocardial ischemia. The key regulatory molecules and mechanisms involved in MAMs are discussed, with the aim of identifying potential treatments strategies for myocardial ischemia.

Structure and function of MAMs

The intracellular membrane is the hallmark characteristic of eukaryotic cells, with several organelles or membrane systems interacting through closely connected Multiple Membrane Contact Sites (MCS) to promote material transfer and signal exchange [21, 22]. In 1959, highly ordered tubular structures of the ER were first observed around mitochondria in rat liver cells using transmission electron microscopy although being initially thought to be artifacts of in vitro fixation [23]. With the continuous development of transmission electron microscopy, subcellular fractionation, and mass spectrometry, numerous studies have confirmed the physical relationship between the ER and mitochondria by observing co-sedimentation of ER vesicles with mitochondria and the close link between them and ER vesicles [24, 25]. This physical communication site between the ER and mitochondria is called MAMs; it is a dynamic microdomain maintained by specific tether proteins and spacer proteins [21]. This discovery laid a reference for further research on organelle interactions and their roles in pathophysiological processes.

Morphological structure of MAMs

Mitochondria and the ER, as highly dynamic organelles, the structure of MAMs at their contact sites is not constant. Morphological studies have shown that the contact range between the mitochondria and the ER is approximately 10 to 60 nm, with the two organelles being close but not overlapping. MAMs are considered to be effectively present when the distance between them is less than 30 nm [26]. Chromatographic analysis of isolated mitochondria has also revealed that the distance between the outer mitochondrial membrane (OMM) rough ER (RER) and smooth ER (SER) is different due to the effect of the ribosome [27]. MAMs are also morphologically diverse. Live-cell microscopy and electron tomography investigations have revealed that the tubular structure of ER surrounds the mitochondria in most cells, covering 2% to 5% of the mitochondrial surface area, significantly influencing mitochondrial dynamics [21]. In addition, the ER may partially surround the mitochondria (about 50% of the mitochondrial circumference) or fully envelop the mitochondria.

Proteomic analysis of MAMs has unveiled thousands of proteins with different functions [28, 29]. These proteins modulate various cellular physiological processes (Table 1). Based on their localization within MAMs, these proteins can be divided into three categories: (1) MAMs-resident proteins, which are exclusively localized to MAMs; (2) MAMs-enriched proteins, localized in MAMs but also in other cellular compartments; (3) MAMs-associated proteins, which transiently reside in MAMs during biochemical reactions [29]. The stable and dynamic communication between the ER subregion and the OMM is maintained through a series of protein chains and chains. The link between the ER and mitochondria in yeasts is mediated by the ER-mitochondria encounter structure (ERMES). This complex comprises the ER-resident membrane protein Mmm1, soluble protein Mdm12, and two outer mitochondrial membrane proteins, i.e., Mdm10 and Mdm34 [30]. The associations of ER-mitochondria and their structural organization are more intricate in mammals. Multiple protein complexes

Table 1 Major related proteins and functions of MAMs

Role	Protein	Functions and mechanisms	References
MAMs tethers	MFN1/2	Regulating MAMs formation by functioning as ER-mitochondrial ligation complex	[50]
	VAPB-PTPIP51	Modulating MAMs formation by acting as ER-mitochondrial ligation complex	[88]
	ERMES	Composed of Mmm1, Mdm10, Mdm12, and Mdm34, mediates Ca^{2+} transport and phospholipid homeostasis	[30]
Ca^{2+} transport	IP_3R	Regulates the Ca^{2+} release from ER	[37]
	VDAC1	Facilitates mitochondrial uptake of Ca^{2+}	[38]
	Grp75	Links IP_3R and VDAC1 to form a complex that regulates Ca^{2+} transport	[38]
	SERCA	Function as an ER Ca^{2+} uptake pump that interacts with calnexin to maintain intracellular calcium homeostasis	[98]
	Sig-1R	Dissociates from Bip under stress, to influence Ca^{2+} transport between ER-mitochondria by stabilizing IP_3R	[17]
Lipid metabolism	ORP 5/8	Constitutes the ORP5/8-PTPIP51 complex that mediates PS and sterol transport	[43]
	ACAT	Modulates the catalytic transformation of intracellular free cholesterol into cholesterol esters	[46]
Mitochondrial dynamics	MFN1/2	Form a homodimer/heterodimer complex that connects the two organelles and regulates OMM fusion	[32]
	OPA1	Regulates the IMM fusion	[48]
	DRP1	Interacts with receptors MFF, MiD49/51 and Fis1 to drive mitochondria division in a GTPase-dependent manner	[53]
ER stress	PERK	Enriched in MAMs and mediates ER stress and URP response	[64]
	ATF6		
	IRE1		
	MFN2	Binds to the PERK and inhibits its activity, MFN2 depletion activates PERK and ER stress via the CHOP pathway	[71]
Oxidative stress	Ero1	Regulates the ROS production in the ER through the protein oxidative folding mechanism	[73]
	p66Shc	Phosphorylates the Ser36 residue of p66Shc and translocates to the mitochondria/MAMs portion to induce ROS production	[76]
Autophagy	ATG14	Acts as an autophagy marker and regulates autophagosome formation	[87]
	ATG5		
	PINK1/Parkin	Facilitates ER-mitochondrial crosstalk and induces autophagy	[89]
	Beclin 1	Mediates the ER-mitochondrial crosstalk and autophagosome formation	[90]
	VAPB-PTPIP51	Loss of VAPB or PTPIP51 induces autophagy by inhibiting mitochondrial Ca^{2+} uptake	[88]
Apoptosis	Bap31- Fis1	The combination of the Bap31and Fis1 promotes procaspase 8 activation in response to apoptosis signal and induces apoptosis	[31]
	CHOP	Mediates ER stress-dependent apoptosis by promoting ROS production	[70]
	DRP1	Enhances MOMP via stimulating BAX oligomerization on mitochondrial membranes to promote apoptosis	[83]

play critical roles in mediating communication between two organelles, each regulating different physiological functions (Fig. 1). These protein complexes include the inositol 1,4,5-trisphosphate receptors(IP_3Rs)- glucose-regulated protein 75 (Grp75)- voltage-dependent anion channel (VDAC) complex, vesicle-associated membrane protein-associated protein B(VAPB)-protein tyrosine phosphatase interacting protein 51 (PTPIP51) complex, homodimers mitofusin-2 (MFN2)-MFN2 or heterodimers MFN2-MFN1 and B cell receptor-associated protein 31 (Bap31) -mitochondrial fission 1 protein (Fis1) complex, etc. [31, 32]. Based on the complexity of the biological functions mediated by MAMs, we further discussed the composition and role of protein complexes in MAMs in their related different biological functions.

Functions of MAMs

MAMs in Ca^{2+} transfer

As a key second messenger in signal transduction, mitochondrial Ca^{2+} levels influence the various mitochondrial functions, such as signal transduction, ROS generation, oxidative phosphorylation, and apoptosis. Among these, Ca^{2+} transport between mitochondria and ER-mediated by MAMs is a central mechanism in regulating mitochondrial Ca^{2+} levels. Csordás and colleagues discovered that

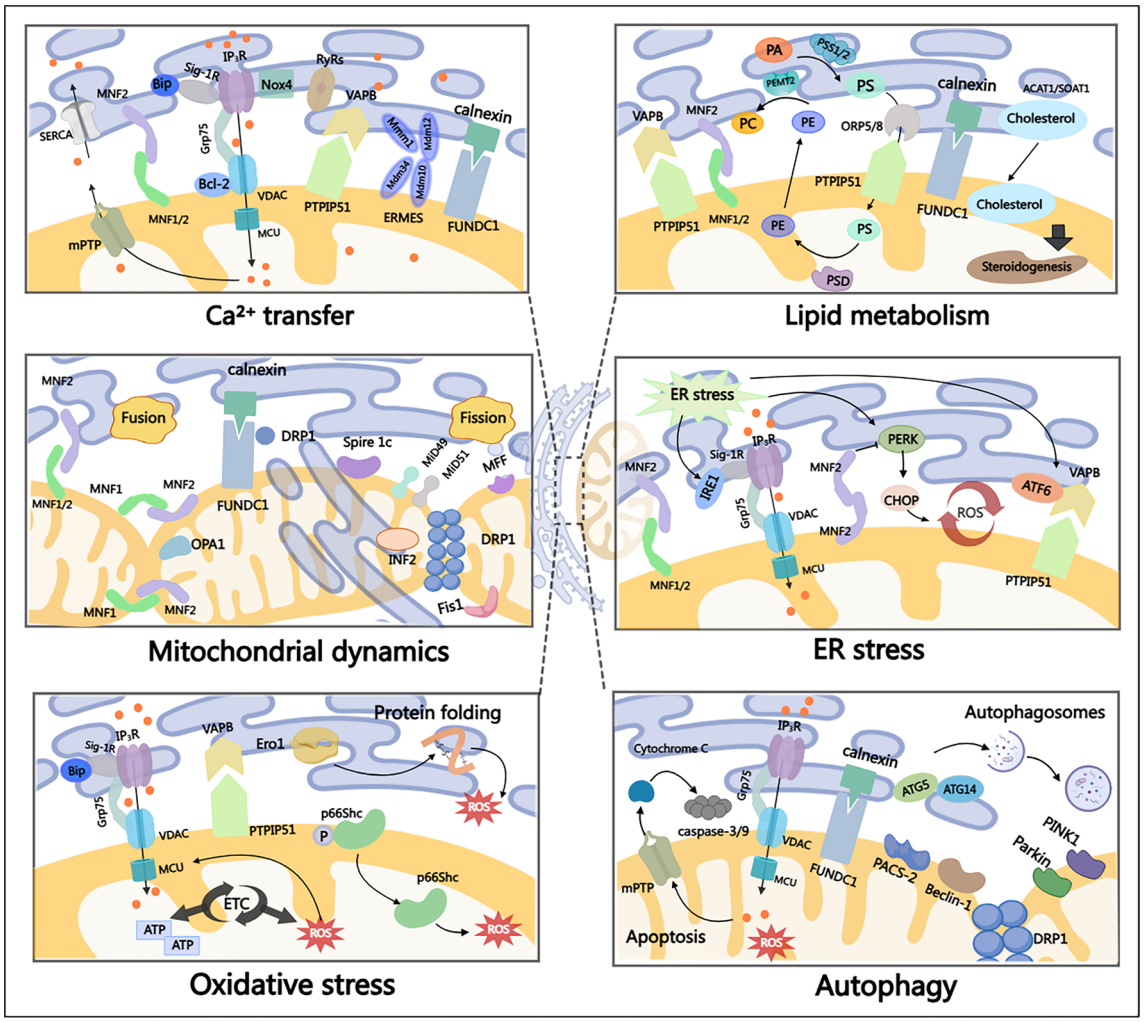


Fig. 1 MAMs play a critical role in regulating various cellular physiological functions, including: Calcium homeostasis, by controlling the transfer of Ca^{2+} between the ER and mitochondria; Lipid biosynthesis and metabolism; Mitochondrial dynamics, including the regulation of fission, fusion, and movement; Cellular stress responses, such as ER stress and oxidative stress; Cell death processes, including autophagy and apoptosis

the intermembrane distance of MAMs and the local Ca^{2+} concentration significantly influence the efficiency of mitochondrial Ca^{2+} absorption. The Ca^{2+} transfer efficiency significantly improves when the intermembrane distance of MAMs increases to 15 nm; conversely, the transfer efficiency markedly reduces when the distance decreases to 5 nm [33, 34]. MAMs also provide a high-concentration Ca^{2+} microenvironment for mitochondria, which is a necessary condition for mitochondrial Ca^{2+} uptake [35]. Therefore, the dynamic regulation of MAMs mediates Ca^{2+} transport.

In MAMs, protein bridge allows direct transportation of Ca^{2+} from the ER to the mitochondria, hence promoting the progression of the Krebs cycle in the mitochondrial matrix and ATP synthesis [36]. The major Ca^{2+} release channels in this process include two receptor families: (1) IP_3Rs enriched in the ER, and (2) ryanodine receptors (RyRs) enriched in the sarcoplasmic reticulum (SR), a specialized form of ER in cardiac cells. These receptors are located on the ER/SR membrane and mediate Ca^{2+} release, producing local Ca^{2+} concentrations higher than the cytoplasmic level in the MAMs region [37]. On the OMM, VDACs, with high conductance, act as key channels for mitochondrial Ca^{2+} uptake. Grp75, located in the cytoplasm, connects IP_3Rs and VDACs via its cytosolic portion, forming the IP_3Rs-Grp75-VDAC complex to promote Ca^{2+} transmembrane transport [38]. Furthermore, this complex acts as a multifunctional molecular scaffold, mediating the dynamic regulation of Ca^{2+} transport in MAMs through various regulatory proteins, including Sig-1R, BiP, Bcl-2, and IRBIT [39, 40]. Similarly, VAPB anchored to the ER can be coupled to PTPIP51 on the OMM to regulate Ca^{2+} transfer by influencing the role of IP_3R-VDAC1 to regulate

autophagy [41]. Once Ca^{2+} crosses the OMM, it enters the mitochondrial matrix through the MCU on the IMM, a crucial process for maintaining mitochondrial function.

Persistent high levels of Ca^{2+} accumulation can however cause calcium overload, causing apoptosis via the mitochondrial permeability transition pore (mPTP) [42]. This highlights the important role of calcium homeostasis in preserving cellular viability. Recent studies have identified several regulatory factors, including FUNDC1, GSK3β, CLIC4, Nox4, and ATAD3A, that regulate calcium homeostasis mediated by MAMs. The subsequent sections in this work will explore specific roles and regulatory mechanisms of these factors.

MAMs in lipid metabolism

The ER and mitochondria are crucial organelles for lipid biosynthesis, with various intermediate molecules requiring multiple movements between them to complete the entire physiological process of lipid synthesis. However, material transport between the ER and mitochondria does not occur via vesicles despite vesicular transport being the primary mechanism for lipid molecule transfer. Studies have shown that the transport of these lipid molecules between the two organelles primarily depends on the MAM pathway [43]. Various tethering proteins, including oxysterol-binding protein-related protein 5/8 (ORP5/8) and PTPIP51, play key roles in MAMs, participating in the aforementioned transmembrane transport [44]. In addition, several enzymes on the membranes of the ER and mitochondria including phosphatidylserine synthases 1/2 (PSS1/PSS2), phosphatidylserine decarboxylase (PSD), and acyl-coenzyme A, cholesterol acyltransferase/sterol O-acyltransferase (ACAT1/SOAT1) play important catalytic roles in lipid biosynthesis [45].

The "phosphatidylserine (PS) decarboxylation pathway" is a key step in the synthesis of phosphatidylethanolamine (PE). In this process, phosphatidic acid (PA) is converted into PS under the catalysis of PSS1/PSS2, before being transferred to mitochondria via the MAMs pathway. The PS subsequently undergoes decarboxylation to form PE, catalyzed by PSD, via a Ca^{2+}-dependent mechanism [45]. Moreover, MAMs has been shown to modulate cholesterol metabolism. ACAT1, which is located on MAMs is a key enzyme that catalyzes the transformation of free cholesterol into cholesterol esters within the cell, playing an important role in maintaining the balance of cholesterol metabolism [46]. Further investigations are still necessary on the mechanisms by which lipid transfer proteins (LTPs) function at membrane contact sites.

Oxysterol-binding protein-related proteins (ORPs), particularly ORP5/8 from the ORP family are localized at MAMs and act as key channels for transporting PS and sterols between the ER and mitochondria [47]. They rely on a functional lipid transfer domain, enabling their interaction with PTPIP51 located on the OMM. Together, they form the ORP5/8-PTPIP51 complex, promoting lipid transport [44]. These MAMs-mediated enzymatic reactions and transmembrane transport mechanisms ensure programmed regulation of lipid biosynthesis, providing important support for the integrity and functional metabolism of the mitochondrial membrane.

MAMs in mitochondrial dynamics

Mitochondria, as highly dynamic organelles, continually reorganize their network through a precise balance of fission and fusion, ensuring the maintenance of genomic integrity and metabolic stability. This complex process is tightly regulated by conserved molecular mechanisms and involves the enrichment of multiple associated proteins in the ER-mitochondria interface. Mitochondrial fusion involves a coordinated merging of the OMM and IMM, with the MFN1/2 complex and optic atrophy 1 (OPA1) acting as important components of this mechanism [48, 49], mediating OMM and IMM fusion, respectively. MFN2 is enriched in the OMM, ER membrane, and MAMs, whereas MFN1 localizes exclusively to the mitochondrial membrane. Together, they form homo- or heterodimeric complexes to connect the two organelles and regulate OMM fusion [32]. Additionally, the efficiency of fusion mediated by MFN1 homodimers, MFN2 homodimers, and MFN1/2 heterodimers varies due to the distinct GTPase activities of MFN1 and MFN2. The MFN1/2 complex also plays a role in the tethering process between the ER and mitochondria [50]. Studies have revealed that the silencing or loss of MFN2 can disrupt the distance between the ER and mitochondria. However, whether the absence of MFN2 loosens the structural connection or increases the contact density between the two organelles remains debatable [51, 52].

MAMs participate in the mitochondrial fission process, specifically in defining the mitochondrial division site [53]. During this process, ER tubules first wrap around the mitochondria, indicating a specific division site, before initiating contraction and fission mechanisms for division. This regulatory pathway involves the sequential action of multiple associated proteins. Mitochondrial contraction begins with the aggregation of actin-myosin assemblies in the MAMs region, a process mediated by inverted formin 2 (INF2) and the mitochondrial actin nucleator, Spire 1c, in the ER [54, 55]. Thereafter, dynamin-related protein 1 (DRP1) is recruited to the OMM, where it interacts with receptors including mitochondrial fission factor (MFF), mitochondrial dynamics 49/51 (MiD49/51), and Fis1. This process mediates mitochondrial constriction and division via a

GTPase-dependent mechanism. Furthermore, studies have shown that ER-associated IMM constriction precedes OMM constriction [56]. Active replication of mitochondrial DNA (mtDNA) has also been observed at the mitochondrial constriction and division sites [57]. These results suggest that signals for mitochondrial fission may originate from mtDNA.

Surprisingly, despite undergoing multiple rounds of reorganization, the ER and mitochondria can maintain a consistent overall morphology and remain connected during movement [58]. The bidirectional transport mechanism of these two organelles along microtubules is linked to the motor proteins kinesin-1 and dynein 2 [59, 60]. Nonetheless, research findings on how they maintain their link without being destroyed during movement remain unclear.

MAMs in stress-ER stress and oxidative stress

A critical function of MAMs is to facilitate cellular adaptation to various stress responses, primarily by promoting the coordinated integration of stress signals between the ER and mitochondria, which are interconnected through MAMs. Considering ER stress as an example, the contact between the ER and mitochondria increases, further improving the Ca^{2+} signaling mediated by MAMs, in turn stimulating an increase in mitochondrial oxidative phosphorylation function. However, abnormal regulation of Ca^{2+} signaling ensues if the ER stress response persists for too long, hence causing apoptosis [61]. During oxidative stress, MAMs regulate the production of ROS and the resulting oxidative damage, thus regulating Ca^{2+} signaling and mitochondrial function to finely tune cellular functions [62]. In certain pathological stress conditions, including myocardial ischemia, ER stress, and oxidative stress simultaneously occur and interact with each other.

The ER serves as the primary site for protein folding and secretion in cells. However, when exposed to various pathological stimuli, its capacity for protein processing may be overwhelmed, leading to the accumulation of unfolded or misfolded proteins within the ER and triggering ER stress. This stress response further activates the UPR, relieving the protein load on the ER and restoring cellular homeostasis by expanding the ER membrane and reducing the input of newly synthesized proteins [63]. During this process, three transmembrane proteins in the ER function as stress sensors and play a central role. These proteins include protein kinase RNA-like kinase(PERK), activating transcription factor 6(ATF6) and inositol requiring enzyme 1(IRE1) [64, 65]. Interestingly, these three UPR-related proteins are enriched in MAMs, which are closely involved in the signaling between ER stress and UPR, hence mediating communication between the ER and mitochondria. In these pathological processes, several proteins regulate mitochondrial Ca^{2+} signaling by modulating the activity of Ca^{2+} channels in the MAMs junctions or by influencing ER Ca^{2+} levels, and ultimately cell apoptosis. For instance, IRE1 in MAMs regulates the availability of IP_3R, promoting Ca^{2+} transfer to mitochondria and further ATP production [66]. Meanwhile, IRE1α accumulation in MAMs can induce mitochondrial Ca^{2+} overload, resulting in cell death [67]. Additionally, tethering proteins in MAMs, including Grp75 and MFN2regulate the UPR process by targeting Ca^{2+} signaling to participate in the adaptive response to ER stress [68]. In response to ER stress, chaperone proteins within the ER, such as calreticulin, calnexin, and BiP (heat shock protein 70 family protein 5), along with redox-regulatory proteins, can influence oxidative phosphorylation and cell apoptosis by enhancing Ca^{2+} flux within the MAMs region [61, 69]. Another mechanism of the UPR response involves the activation of p38 MAPK and C/EBP homologous protein (CHOP), which mediates ER stress-induced cell apoptosis by promoting ROS production. Verfaillie et al. [70] found that PERK in MAMs participates in the formation of MAMs and regulates the transfer of ROS to mitochondria, thereby regulating intrinsic apoptosis caused by ER stress. Additional investigations revealed that the activity of PERK can be suppressed by interacting with the MAMs tethering protein MFN2 [71]. In addition, MAMs tethering protein VAPB can interact with ATF6, directly suppressing the UPR response [67].

Regulating ROS production and mediating oxidative stress damage are primary pathways by which MAMs regulate cellular function during oxidative stress responses. Booth et al. [62] utilized drug-induced ER-mitochondrial contacts and discovered that hydrogen peroxide nanodomains exist at the MAMs interface, originating from mitochondrial cristae. As part of ER-mitochondria communication, this domain can modulate Ca^{2+} signaling and mitochondrial activity. Surprisingly, oxidative stress can influence the integrity of MAMs and alter Ca^{2+} homeostasis [72]. The production of mitochondrial ROS mainly stems from the activity of the mitochondrial electron transport chain (ETC) during oxidative metabolism and ATP synthesis. MAMs promote ROS production by mediating the excessive transfer of Ca^{2+} to mitochondrial cristae. Meanwhile, ROS can also oxidize the MCU, further promoting the transfer of Ca^{2+} into the mitochondrial matrix [15, 62]. Other structural proteins of MAMs directly participate in the redox cross-talk between the mitochondria and the ER, with significant examples including ER oxidoreductase 1 (Ero1) and the 66 kDa subtype of growth factor adapter

Shc (p66Shc). Ero1 promotes the formation of disulfide bonds during protein folding via the oxidation of molecular oxygen, acting as an auxiliary error correction mechanism for protein folding in the ER. However, this oxidative folding process produces numerous ROS, resulting in redox imbalance [73, 74]. Under oxidative conditions, the activation of protein kinase C beta also causes Ser36 phosphorylation on p66Shc [75]. This process promotes the partial translocation of p66Shc to the mitochondria and/or MAMs, where it produces ROS [76]. Interestingly, MAMs regulate cellular function by regulating mitochondrial function during oxidative stress and participate in the genetic mechanisms of damaged mitochondria to regulate cellular functions. Many redox-active proteins, including oxidoreductases TMX1, Ero1α, and Sarco/ER Ca^{2+}-ATPase (SERCA) are enriched in MAMs and regulated by ROS [22]. At the MAMs interface, increased ROS activates the mitogen-activated protein kinase (MAPK) pathway and reduces mitochondrial motility, which is crucial for normal mitochondrial inheritance and quality control [77].

MAMs in cell death—apoptosis and autophagy

Studies have extensively reported the link between MAMs and cell death, with various physiological events modulated by MAMs being considered common triggers of cell death mechanisms, including apoptosis, autophagy, necrosis, pyroptosis, and ferroptosis [9, 10, 12]. Wang et al. also showcased the role of MAMs in cell death, specifically in integrating apoptotic signaling [78]. Recent studies have shown that death signals involve bidirectional communication between the ER and mitochondria, a process that comprises various cell death mechanisms, with MAMs playing a critical role in the complex crosstalk between these two organelles.

The high-conductance mPTP, comprising VDAC, ATP synthase oligomycin-sensitivity conferring protein (OSCP), adenine nucleotide translocase (ANT), and cyclophilin D (CypD) drives the process of apoptsis [79]. Studies have shown that MAMs mediates Ca^{2+} transfer from the ER to mitochondria. Excessive Ca^{2+} accumulation causes calcium overload, and subsequently, excessive mPTP opening, which promotes cytochrome C release, activates the caspase cascade, ultimately inducing apoptosis [80]. Alterations in MAM tethering are closely associated with cell apoptosis. A key example is the interaction between the mitochondrial protein Fis1 and the ER protein Bap31, which plays a critical role in apoptosis. This interaction facilitates the cleavage of Bap31, transforming it into its pro-apoptotic form. This, in turn, activates procaspase 8, triggering an apoptotic cascade that culminates in cell death [31, 81]. Apoptosis also involves changes in mitochondrial dynamics [82]. During this process, DRP1 mediates mitochondrial fission. However, the role of DRP1 in apoptosis is not limited to this; it may also have functions independent of mitochondrial division. Specifically, DRP1 improves mitochondrial outer membrane permeabilization (MOMP) by promoting BAX oligomerization on the mitochondrial membrane. MOMP is essential for cytochrome c release during apoptosis [83, 84]. In this pathological process, DRP1 and BAX co-localize in MAMs, coordinating MOMP activation.

Autophagy is another important biological role mediated by MAMs. Physiological autophagy is crucial in protein quality control mechanisms and cellular stress response [85]. However, excessive autophagy can overdegrade cellular components, potentially inducing cell death. Although the precise location of autophagosome formation and the sources of proteins and lipids required for its expansion are still controversial, increasing evidence suggests that MAMs may act as important sites for autophagosome biogenesis [86]. In a starvation-induced pathological autophagy model, it was found that the autophagosome marker proteins, ATG14 and ATG5 were found to be recruited to MAMs, where they participates in the formation of autophagosomes [87]. Autophagosome formation is blocked when MAMs are disrupted by knockdown of PACS-2 or MFN2. Gomez-Suaga et al. further reported a correlation between the MAM tether protein VAPB-PTPIP51 complex and autophagy. They discovered that the deletion of VAPB or PTPIP51 caused autophagy by suppressing mitochondrial Ca^{2+} uptake, whereas their overexpression promoted Ca^{2+} flux by tightening the ER-mitochondria connection, hence suppressing autophagosome formation [88]. Moreover, critical autophagy proteins like PINK1/Parkin and Beclin-1 are concentrated at MAMs, where they mediate communication between the ER and mitochondria. These proteins also drive the formation of autophagosomes during mitochondrial autophagy, simultaneously identifying and removing damaged mitochondria through the autophagic process [89, 90]. Research reports have shown that FUNDC1, located at MAMs, mediates mitochondrial fission and mitophagy under hypoxic conditions, and recruits Drp1 to MAMs, further promoting mitochondrial fission during hypoxia [91]. These findings highlight the close link between MAMs and autophagy.

In summary, MAMs mediate the regulation of various cellular processes. In addition to the above-mentioned functions, MAMs modulate other physiological processes, such as inflammation and antiviral responses.

The role of MAMs in myocardial ischemia

The cell biology of the cardiovascular system is tightly regulated, with growing interest being directed towards the role of mitochondria-ER interactions. The importance of these interactions in maintaining cardiovascular health has been emphasized in recent studies. MAMs are broadly present in cardiomyocytes, where they regulate many pathophysiologically relevant signaling processes, including intracellular Ca^{2+} transport, lipid metabolism, mitochondrial dynamics, cellular stress responses, and cell death. Dysfunctions in MAMs are responsible for the onset and progression of cardiovascular diseases including atherosclerosis(AS), myocardial I/R injury, heart failure, and diabetic cardiomyopathy [27]. As a common cardiovascular condition, the incidence of IHD has been on the rise due to the increase in global population aging, hence the first cause of death in human diseases. Currently, there are few interventions for suppressing mortality rates and improving long-term outcomes. Modern medicine can effectively treat ischemic myocardium with timely thrombolysis and interventional procedures; however, these treatments are limited by narrow time windows and the risk of inducing bleeding. Consequently, it is important to explore the potential treatment targets and strategies for IHD.

Under myocardial ischemic conditions, cardiomyocytes shift to anaerobic metabolism, resulting in rapid ATP depletion, ion imbalance (particularly calcium homeostasis disruption), and intracellular acidosis [92]. ATP depletion, calcium dysregulation, and changes in environmental pH disrupt various intracellular signaling pathways, causing a cascade of pathological events including mitochondrial dysfunction, oxidative stress, ER stress, inflammatory responses, autophagy, and apoptosis. Although rapid reoxygenation after restoring myocardial blood flow can alleviate ATP depletion and pH imbalance, it also causes excessive ROS production and calcium overload. These factors ultimately trigger cardiomyocyte apoptosis and necrosis, contributing to myocardial contractile dysfunction [92, 93]. Several studies have highlighted the signifcance of MAMs tether proteins in the regulation of calcium homeostasis, mitochondrial function, cellular stress, and cell death during myocardial ischemia.

Calcium homeostasis

Calcium signaling regulates post-ischemic cardiac function, encompassing both its physiological role in excitation–contraction coupling (ECC) and its pathological impact during calcium overload. During ECC in cardiomyocytes, a transient increase in cytosolic Ca^{2+} concentration causes the formation of cross-bridges, driving myocardial contraction [94]. Simultaneously, mitochondria absorb Ca^{2+} during contraction to stimulate mitochondrial oxidative phosphorylation and the ETC, enabling ATP synthesis, which is critical to meeting the high energy demands of cardiomyocytes [95]. However, in the post-ischemic state, IP_3Rs and RyRs on the ER membrane become overly activated, inducing a massive Ca^{2+} release into the cytoplasm [96, 97]. Although SERCA alleviates cytoplasmic Ca^{2+} overload using ATP to pump Ca^{2+} back into the ER [98], its expression and activity are significantly reduced during ischemia. This suppression contributes to the further increase in intracellular Ca^{2+} levels, excessive mitochondrial ROS production, and activation of mitochondrial fission pathways [99]. These changes exacerbate calcium overload in the cytoplasm and mitochondria, creating a vicious cycle. As discussed in Sect. 2.2.1, MAMs play a critical role in Ca^{2+} transport. Being a bridge between the ER and mitochondria, MAMs tether proteins provide the necessary channels for Ca^{2+} transfer and significantly influence the efficiency of this process (Fig. 2).

Maintaining Ca^{2+} homeostasis is crucial for protecting cardiomyocytes during myocardial ischemia and reperfusion. mPTP-dependent cell death induced by Ca^{2+} overload is one of the primary mechanisms of cardiomyocyte death [42]. Under ischemic conditions, severe disruption of ionic balance and energy supply in cardiomyocytes triggers the opening of voltage-gated Ca^{2+} channels. Subsequently, massive Ca^{2+} transfer from the ER to mitochondria occurs via the IP_3R-Grp75-VDAC complex on MAMs, causing mitochondrial calcium overload [38]. Excessive Ca^{2+} accumulation in mitochondria triggers numerous pathological changes, including mitochondrial membrane potential collapse, mitochondrial swelling, and the release of pro-apoptotic factors including cytochrome C. These changes further activate CypD in the mitochondrial matrix, a key regulator of mPTP activity [100, 101]. Studies have shown that CypD also can interact with the IP_3R-Grp75-VDAC complex, directly regulating Ca^{2+} transfer from the SR to mitochondria [102]. These disruptions cause excessive mPTP opening, resulting in increased mitochondrial membrane permeability. The subsequent activation of the mitochondrial apoptotic signaling pathway, through caspase-9 and caspase-3 activation, ultimately induces cardiomyocyte apoptosis. Excessive contraction induced by MAMs-mediated calcium overload represents another critical factor leading to cell death during myocardial I/R. In mitochondria, ETC complexes I, III, IV, and V exhibit pronounced Ca^{2+}-dependent phosphorylation regulation [93]. Increased intracellular Ca^{2+} levels improve the conductance of these complexes, significantly boosting ATP production. Although the restored energy supply helps alleviate energy deficit during ischemia, excessive

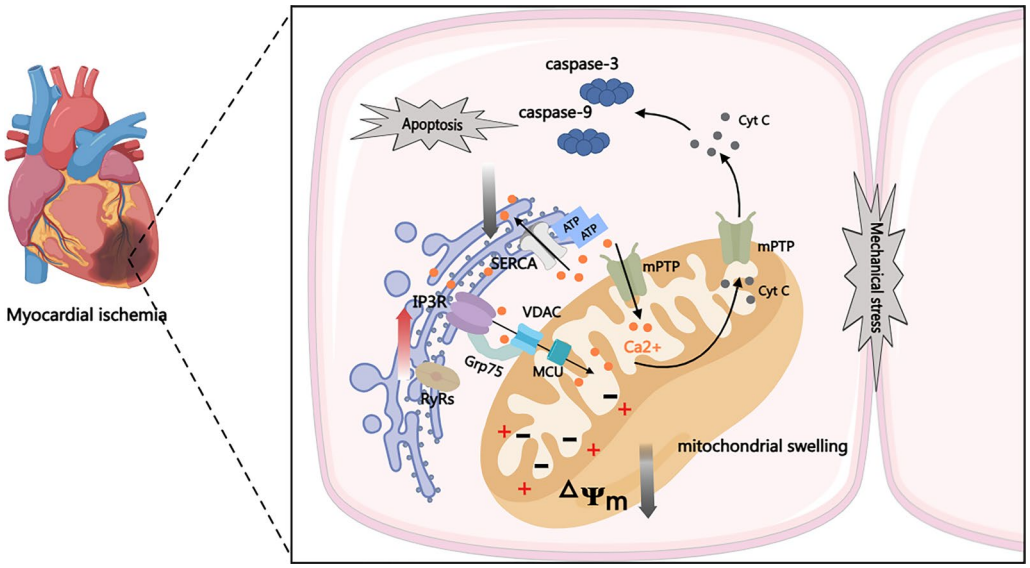


Fig. 2 MAMs regulates calcium homeostasis during myocardial ischemia and reperfusion. Myocardial ischemia induces the overactivation of IP3Rs and RyRs on the endoplasmic reticulum membrane, leading to massive Ca^{2+} efflux, while the activity of SERCA, responsible for Ca^{2+} reuptake into the endoplasmic reticulum, is inhibited. These pathological changes exacerbate calcium overload in the cytoplasm and mitochondria, inducing mPTP-dependent apoptosis. In addition, calcium overload and restored energy production during reperfusion trigger excessive myocardial contraction, leading to mechanical strain-induced mutual damage of adjacent cardiomyocytes

ATP generation paradoxically drives abnormally intense cellular contractions. This hypercontractile activity imposes mechanical strain on myocardial tissue, causing structural disruption and necrosis of adjacent cells [103].

However, MAMs-mediated Ca^{2+} transfer is not entirely harmful in pathological processes like tissue I/R. Göbel et al. demonstrated that after acute brain injury and blood–brain barrier disruption, accumulation of MAMs around blood vessels could promote vascular remodeling. Upon MFN2 deletion, impairing MAMs function, mitochondrial Ca^{2+} uptake was significantly reduced, and the ability to remodel blood vessels was weakened accordingly [104]. This suggests that in acute injuries like myocardial ischemia, appropriately improving the role of MAMs might help maintain ATP production under ischemic conditions by promoting Ca^{2+} transfer, supporting cardiovascular remodeling, and ultimately benefiting myocardial tissue. In summary, a moderate increase of mitochondrial Ca^{2+} induced by MAMs microdomains improves cellular energy metabolism. However, under myocardial I/R pathological conditions, uncontrolled mitochondrial Ca^{2+} accumulation induced by MAMs microdomains can exacerbate myocardial injury. Therefore, interventions targeting molecules involved in Ca^{2+} transfer within MAMs microdomains and maintaining mitochondrial Ca^{2+} homeostasis is an important therapeutic intervention for preventing and alleviating myocardial I/R injury.

Mitochondrial dysfunction

After myocardial ischemia, mitochondria, as sensitive oxygen-sensing organelles, experience significant inhibition of their ETC activity and related ATP production in the hypoxic environment, causing the cell metabolism to shift towards anaerobic glycolysis. Hypoxia weakens mitochondrial oxidative phosphorylation and disrupts mitochondrial ion homeostasis. In this condition, cells attempt to compensate by mechanisms including Na^+ and Ca^{2+} overload mediated by the Na^+/H^+ exchanger and Na^+/Ca^{2+} antiporter to relieve excess H^+ [105, 106]. Meanwhile, MAMs play an important role in this process by mediating the transfer of Ca^{2+} from the ER to the mitochondria, further causing mitochondrial Ca^{2+} overload. This Ca^{2+} overload induces excessive mitoROS production and exacerbates mitochondrial damage. Especially during the reperfusion phase, the accumulation of mitoROS triggers the opening of the mPTP, hence mediating myocardial cell death [106–108]. Damage-associated molecular patterns (DAMPs) released from mitochondrial damage mediate the activation of NLRP3 inflammasome and related inflammatory cytokines within MAMs, thereby promoting inflammatory injury of cells and generating harmful effects [109]. Notably, excessive ROS production and mitochondrial dysfunction key factors driving endothelial cell injury toward necrosis or apoptosis. This pathological progression further exacerbates myocardial ischemia/I/R injury [110].

Another alteration in mitochondria following myocardial ischemia is the pronounced increase in fission activity. This process is essential for producing additional mitochondria to support the increased energy demands of myocardial cells during both ischemia and reperfusion. However, studies have discovered that excessive mitochondrial fission is also one of the key pathogenic factors in cardiac I/R injury [111]. MAMs play an important role in maintaining the balance between mitochondrial fission and fusion. Korobova et al. [54] found that mitochondrial fission is significantly reduced when communication at the ER-mitochondria contact sites is interfered with. MAMs tether proteins, specifically the MFN1-MFN2 heterodimer, not only play an important role in stabilizing the ER-mitochondria microdomain but are also vital molecules regulating mitochondrial fission [52]. Research has shown that although combined ablation of MFN1 and MFN2 can cause mitochondrial dysfunction, it can also reduce myocardial cell damage related to mitochondria/SR tethering during I/R [112]. The ablation of MFN1-MFN2 reduces MAMs, alleviating I/R-induced Ca^{2+} overload and ROS production at the same time lowering the sensitivity of mitochondrial mPTP opening. This suggests that disrupting mitochondria/SR tethering may protect against ischemia. As a key regulator of mitochondrial fission, DRP1 is recruited in response to hypoxic stress after myocardial ischemia. However, Ong et al. [111] revealed that DRP1 inactivation suppressed mitochondrial fission and reduced the sensitivity of HL-1 cells to mPTP opening, exerting cardioprotective effects. Under ischemic conditions, the level of OPA1, a key protein in mitochondrial fusion, is reduced. However, studies in the cardiac myogenic cell line H9c2 have shown that both reduced and excessive OPA1 expression can trigger cytochrome c release. This indicates that although OPA1 overexpression improves mitochondrial fusion, it does not offer protection against ischemia-induced apoptosis in myocardial cells [113] (Fig. 3).

Cellular stress: ER stress

Under pathological conditions including ischemia and reperfusion, stress responses experienced by myocardial tissue cause damage and necrosis, with ER stress and oxidative stress being prominent features. In this process, MAMs, as critical subcellular structures linking mitochondria and the ER, play a vital role in the transduction of stress response signals and the crosstalk in redox regulation (Fig. 4).

ER stress is caused by the imbalance between the protein folding capacity of the ER and the demand for protein processing. Its normal protein folding function relies on ER calcium homeostasis and energy metabolism [114]. After myocardial ischemia, the massive efflux of Ca^{2+} from the ER-mediated by MAMs, is considered a major factor that contributes to ER stress and decreased protein folding capacity [115]. Excess accumulation of

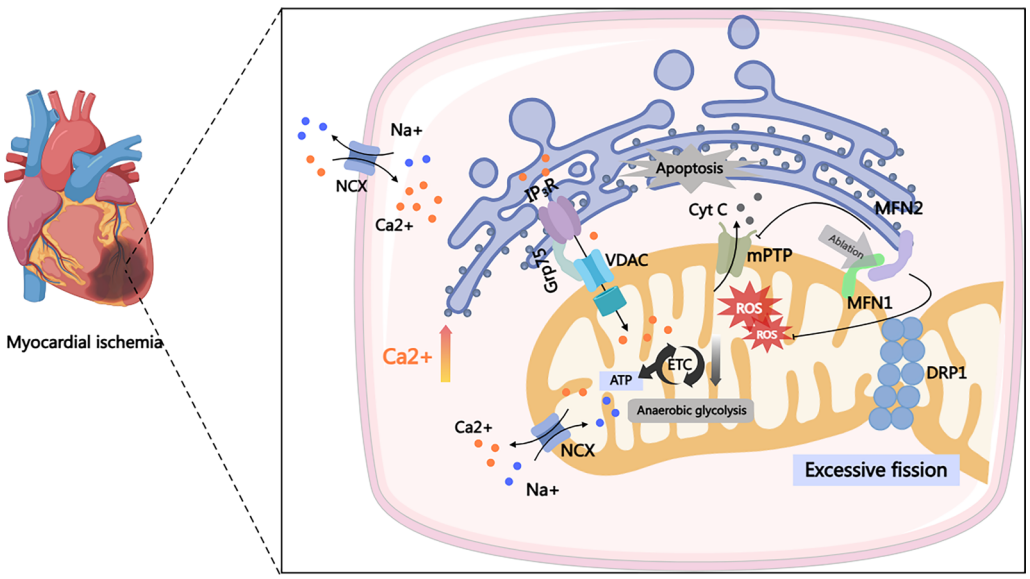


Fig. 3 MAMs regulate mitochondrial dysfunction during myocardial ischemia and reperfusion. Under ischemic and hypoxic conditions, the activity of the ETC and associated ATP production are suppressed, triggering a metabolic shift toward anaerobic glycolysis. This metabolic alteration disrupts ion homeostasis, activating various compensatory mechanisms such as the Na^+/H^+ exchanger and Na^+/Ca^{2+} antiporter. Furthermore, mitochondria undergo excessive fission to meet the energy demands of cardiomyocytes. MAMs contributes to the delicate balance between mitochondrial fission and fusion, a process tightly regulated by MAM-associated tether proteins MFN1-MFN2 heterodimer, DRP1, and OPA1

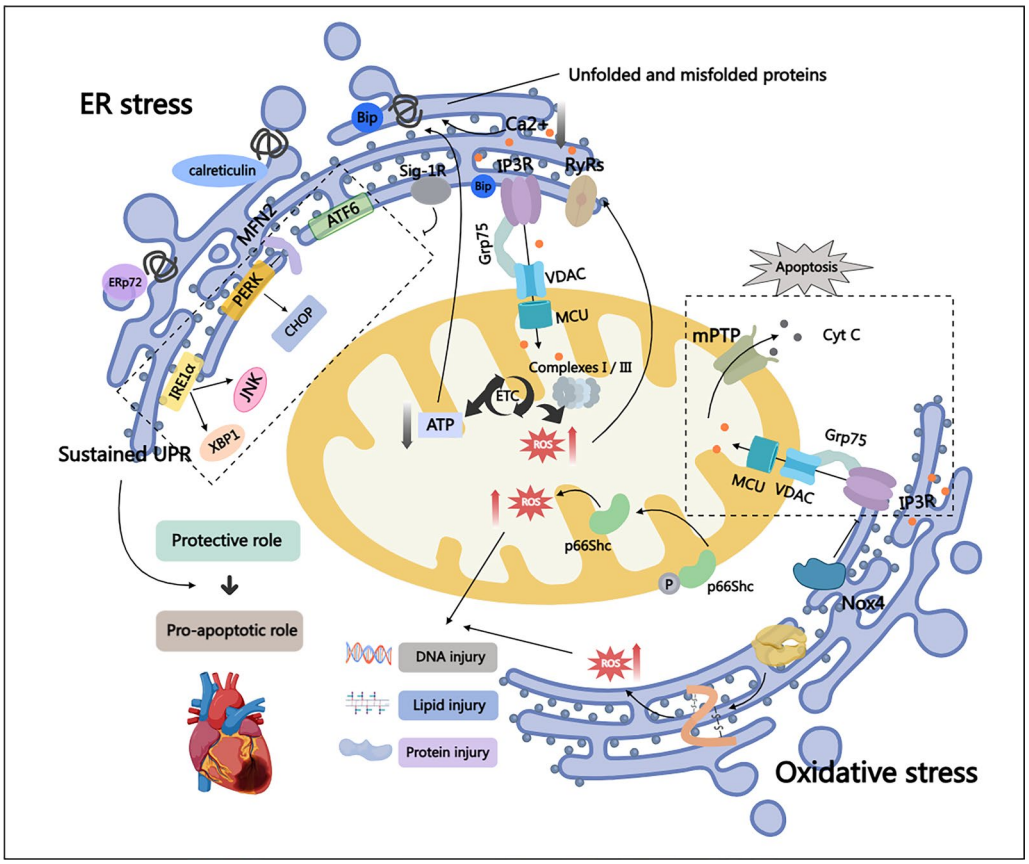


Fig. 4 MAMs mediate the regulation of ER stress and oxidative stress during the pathological process of myocardial ischemia and reperfusion. Following myocardial ischemia, MAM-mediated Ca^{2+} efflux from the ER triggers ER stress and protein misfolding, which activates the UPR to restore cellular homeostasis. However, prolonged stress can shift UPR signaling from a cardioprotective role to a pro-apoptotic response. Furthermore, excessive Ca^{2+} transfer from the ER to mitochondria induces calcium overload, resulting in elevated mitoROS production. Structural proteins within MAMs, such as Ero1 and p66Shc, play a direct role in redox crosstalk between the mitochondria and ER, further exacerbating ROS generation

unfolded polypeptides or improperly folded proteins within the ER, exceeding the capacity of ER chaperones stimulates the UPR pathway to restore cell homeostasis and prevent tissue damage [63, 64]. The major function of the UPR is to induce protective ER-targeted proteins to cope with ER stress, including ER chaperones (including GRP78, and calreticulin) and protein disulfide isomerases (PDIs) (including ERp72). These proteins are enriched in MAMs and can re-establish ER calcium as well as redox homeostasis, thereby restoring protein folding functions in the ER and protecting myocardial cells from excessive ER stress under ischemic conditions. Studies suggest that this protective effect is achieved partly by binding UPR-activated ATF6 and X-box binding protein-1 (XBP1) to regulatory elements of ER stress response genes [116]. Nonetheless, ER stress-dependent apoptotic pathways are activated when prolonged stress causes the protein load in the ER to exceed the regulatory capacity of the UPR, resulting in cell death. In this process, UPR signaling switches from a protective role to a pro-apoptotic role via the transcriptional induction of CHOP, activation of c-Jun N-terminal kinase (JNK), and caspase-12-dependent pathways, ultimately causing myocardial cell damage during I/R [117–119].

Sig-1R, a ubiquitously expressed MAMs-resident protein in the heart, normally binds to GRP78 (also known as Bip) to form an inactive complex. Under ER stress or related factors stimulation, the uncoupling of the Sig-1R-GRP78 complex occurs, and Sig-1R shifts to the ER, where it mitigates ER stress-induced myocardial damage by suppressing the UPR response. Studies have shown that overexpression of Sig-1R can attenuate the intensity of the ER stress response by activating PERK and ATF6, whereas Sig-1R knockdown interferes with MAMs integrity and causes mitochondrial dysfunction, ultimately causing apoptosis [17]. According to Alam et al., Sigmar1-siRNA knockdown in myocardial cells (NRCs) significantly upregulated CHOP expression and caused myocardial cytotoxicity via sustained activation of ER stress pathways. Conversely, Sigmar1 overexpression

enhanced IRE1α phosphorylation, promoted XBP1s expression and nuclear translocation, and ameliorated these effects [120]. These findings suggest that Sig-1R, being an important component of the adaptive ER stress response is a potentially effective treatment target for myocardial ischemic injury.

Cellular stress: oxidative stress

Oxidative stress is closely linked to the pathophysiological processes of myocardial I/R injury. After myocardial ischemia, MAMs-mediated mitochondrial calcium overload induces the production of mitoROS, subsequently causing mitochondrial damage. Especially during reperfusion, reoxygenation of ischemically injured myocardial tissue further causes massive ROS generation [106, 107]. The accumulated ROS can overwhelm cellular antioxidant defenses, resulting in oxidative damage to DNA, lipids, and proteins, thereby exacerbating myocardial I/R injury.

During the pathophysiology of myocardial I/R, mitochondrial calcium overload causes Ca^{2+} accumulation within mitochondria, resulting in mitochondrial depolarization and disruptions in oxidative phosphorylation. These abnormalities decouple the mitochondrial ETC from respiratory complexes I and III, hence promoting ROS production [15]. More importantly, excessive superoxide radicals further oxidize RyRs, exacerbating mitochondrial calcium overload and ROS production [121]. Additionally, structural proteins within MAMs, including Ero1 and p66Shc, contribute to redox crosstalk between the mitochondria and ER, playing a significant role in ROS production (Sect. "MAMs in stress-ER stress and oxidative stress"). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Noxs) are also a major source of ROS in myocardial I/R injury; Nox1, Nox2, and Nox4 are the important isoforms involved in this pathological process [122]. Notably, Beretta et al. discovered that Nox4 is enriched in the MAMs regions of cardiomyocytes, where it regulates redox signaling within MAMs under stress conditions. By improving AKT-dependent phosphorylation of IP_3R, Nox4 suppresses excessive Ca^{2+} flux and reduces mPTP-dependent cell necrosis [20]. In the I/R-injured heart, Nox4 limits infarct size via this mechanism, suggesting that Nox4 upregulation during I/R stress is a protective mechanism for cell survival.

Autophagy

Autophagy, a highly conserved intracellular degradation mechanism, has been implicated in the regulation of cardiac homeostasis and function. During myocardial ischemia, autophagy activation is generally considered a protective mechanism that promotes cell survival. It generates substrates needed for ATP regeneration, decreasing the accumulation of misfolded proteins and oxidative stress, and clearing of damaged mitochondria, thereby mitigating ischemic damage and improving the adaptation of the heart to pressure overload (PO) [123, 124]. Under starvation conditions, the autophagosome marker protein ATG14 was found to relocate to the MAMs [87], indicating that ATG14, an indispensable factor in mitophagy activation, may be regulated by MAMs microdomains. However, under certain pathological conditions, such as I/R or the acute phase of PO, excessive autophagy activation may lead to myocardial damage. Evidence shows that autophagy-related protein Beclin 1 can play a detrimental role in I/R injury development. Matsui et al. [125] reported that the Beclin 1 expression was significantly upregulated during cardiac reperfusion through a ROS-dependent mechanism, whereas mice with systemic heterozygous deletion of Beclin 1 showed significant reductions in autophagy and ischemic injury. One plausible mechanism is that autophagy during I/R may induce autosis in cardiomyocytes [126]. This form of cell death is characterized by the excessive formation of autophagosomes, and these autophagosomes are formed at the expense of the cellular membrane rather than relying on lysosomal degradation. This process may cause severe damage to the cellular structure and function, resulting in significant cellular dysfunction and death. As discussed earlier, key autophagy-related proteins such as ATG14, PINK1/Parkin, and Beclin 1 are enriched in MAMs and regulated by ER-mitochondria microdomains [87, 89, 90]. Numerous studies have confirmed that under pathological conditions such as myocardial I/R, these factors mediate pro-survival or pro-apoptotic signals in myocardial injury by modulating mitophagy activation.

Different tether proteins within MAMs are involved in the formation of autophagosomes and clearance of target structures via fine-tuning the contact between the ER and mitochondria. Some MAMs proteins' specific roles in ischemic injury have gradually been revealed. For example, PTPIP51, a mitochondrial resident protein, was reported to form a complex with VAPB on the ER and is an important component of MAMs. This complex regulates Ca^{2+} transport by modulating ER-mitochondrial contact via the IP_3R/VDAC1 pathway [41]. In a mouse model of I/R injury, PTPIP51 was found to be significantly upregulated, and its knockout suppressed myocardial damage and the size of infarct size induced by I/R [127]. Further studies by Sciarretta and colleagues revealed that both downregulation of PTPIP51 or its interacting protein VAPB induced autophagy by lowering mitochondrial Ca^{2+} levels, thus exerting partial protection against

I/R injury. However, excessive autophagy during reperfusion may potentially aggravate myocardial damage [128]. In addition, FUNDC1, a mitochondrial autophagy receptor, in located in MAMs and binds to IP_3R2 to regulate Ca^{2+} homeostasis, mitochondrial fission, and mitophagy [15]. In a cardiac I/R model, genetic knockout of FUNDC1 compromised the ER-mitochondria microdomain, causing significant decreases in IP_3R2 and Ca^{2+} levels in mitochondria. Mice with specific FUNDC1 knockout experienced more severe heart dysfunction. Therefore, autophagy-related proteins located in MAMs can improve ischemic myocardial injury by regulating autophagy, and thus promising treatments for myocardial ischemia.

Potential regulatory molecules targeting MAMs

In summary, this review discusses the critical role of MAMs in myocardial ischemia and reperfusion injury, highlighting their various pathological mechanisms such as calcium homeostasis regulation, mitochondrial dysfunction, ER stress, oxidative stress, and autophagy. As a pivotal hub that coordinates energy metabolism, protein homeostasis, and cell fate regulation, the integrity and functional maintenance of MAMs are important regulators of myocardial protection. Further investigation into the regulatory mechanisms and molecular components associated with MAMs are warranted to not only enhance our understanding of the pathogenesis of myocardial ischemia but also provide valuable insights for uncovering novel therapeutic targets for preventing or mitigating such diseases (Table 2).

PACS-2

The PACS-2, a sorting protein localized on the surface of the ER, is the first MAMs regulatory protein that integrates the ER-mitochondria communication, maintaining ER homeostasis, and regulating apoptotic signaling. Simmen et al. [13] first reported that PACS-2 is involved in Bid-mediated apoptosis by preserving the structural and functional integrity of MAMs through the regulation of mitochondrial fission. Specifically, PACS-2 deficiency can trigger Bap31 cleavage, producing the pro-apoptotic fragment p20, which further triggers early ER Ca^{2+} release into mitochondria and recruits Drp1 to the mitochondrial surface. This process stimulates mitochondrial fission, causes extensive mitochondrial fragmentation and causes the decoupling of the fragmented mitochondria from the ER [13, 129]. This suggests that PACS-2 may suppress Bap31 cleavage through a specific mechanism, thereby effectively preventing excessive mitochondrial fission and its associated dysfunction during myocardial I/R.

AS is the primary pathological process underlying myocardial ischemia. It is marked by the accumulation of LDL in the arterial intima and the phenotypic transformation of vascular smooth muscle cells (VSMCs), both of which play pivotal roles in the progression of AS [130]. The PACS-2 is localized at ER-mitochondria contact sites in human VSMCs (hVSMCs) and responds to

Table 2 The major regulatory molecules of MAMs morphology and function

Regulatory molecule	Regulation mechanism	References
PACS-2	Regulates the structural and functional integrity of MAMs by affecting mitochondrial fission, thereby participating in Bid-mediated apoptosis; Accumulates in the MAMs of VSMCs and responds to LDL damage, thereby mediating AS; Interferes with MAMs function, playing a key role in calcium homeostasis, formation of ER lipid synthesis centers, and autophagosome formation	[13, 87, 131, 132]
GSK3β	Interacts with ANT on the IMM, competitively inhibiting the binding of CypD and thereby preventing mPTP opening; Interacts with the CypD-IP_3R- Grp75-VDAC complex, thereby modulating IP_3R-dependent Ca^{2+} transport	[16, 136]
FUNDC1	Enriched in the MAMs by binding to the ER protein CANX, acting as a central regulatory molecule for mitochondrial fission and autophagy, and mediating the activation of mitochondrial autophagy; Genetic ablation of FUNDC1 disrupts ER-mitochondria coupling by inhibiting ubiquitin-dependent degradation of IP_3R2, thereby interfering with mitochondrial Ca^{2+} uptake	[91, 143, 144]
CLIC4	Maintaining mitochondrial calcium homeostasis as a regulator of ER/SR-mitochondrial Ca^{2+} transfer; Inhibits apoptosis and mitochondrial membrane depolarization by suppressing the ROS production in HR injury	[18]
ATAD3A	Regulates the IP_3R1- Grp75-VDAC1 complex to influence the morphology and functional integrity of MAMs Its overexpression can prevent mitochondrial Ca^{2+} accumulation and ROS generation, thereby reducing mitochondrial oxidative stress and calcium overload	[19, 153]
NOX4	Regulates the redox signaling within MAMs under stress conditions to increase AKT-dependent phosphorylation of IP_3R, thereby inhibiting calcium flux and reducing mPTP-dependent cell necrosis	[20]
Sig-1R	Inhibits UPR response by downregulating PERK and ATF6 activation and increasing IRE1α phosphorylation, and ultimately ameliorating ER stress injury	[17, 120]

LDL-induced damage. Moulis et al. reported that exposure to oxidized LDL (OX-LDL) significantly upregulates PACS-2 expression in the MAMs regions of hVSMCs. Silencing PACS-2 disrupts the structural integrity of MAMs, impairs mitochondrial autophagy, and accelerates apoptosis in VSMCs [131]. Moreover, PACS-2 participates in the maintenance of calcium homeostasis, establishing ER lipid synthesis hubs, and facilitating autophagosome formation by regulating the function of MAMs [13, 87, 132].

Overall, the precise mechanism of PACS-2 action remains to be fully understood. At present, there are no drug agonists or inhibitors specifically targeting PACS-2. While RNAi-based therapies hold potential, they still face significant challenges in terms of safety and efficacy [133]. The phosphorylation status of PACS-2, influences the effectiveness of targeting PACS-2 by acting as a molecular switch. Therefore, when developing chemical modulators of PACS-2 function, an indirect strategy targeting the phosphorylation or degradation of the PACS-2 protein need to be considered.

GSK3β

GSK3 is a serine/threonine kinase with two isoforms, GSK-3α and GSK3β, known to participate in the development of cardiac ischemia and reperfusion. GSK3β is constitutively active in cells, and phosphorylation at the Ser9 site inhibits its activity [16]. It has been reported that the phosphorylated Ser9-GSK3β confers cytoprotection by increasing the threshold for mPTP opening [134, 135], though the precise mechanisms remain unclear. Research has indicated that GSK3β interacts with ANT on the IMM, competitively inhibiting the binding of CypD to prevent mPTP opening [16, 136]. Interestingly, Gomez et al. uncovered an additional regulatory mechanism of GSK3β, in which it partially localizes at the MAMs interface in cardiac cells, where it interacts with the CypD-IP_3R- Grp75-VDAC complex, regulating IP_3R-dependent Ca^{2+} transport in cardiomyocytes. During reperfusion, GSK3β inhibition reduces IP_3R-mediated Ca^{2+} leakage at MAMs, limiting mitochondrial calcium overload and subsequent cell death [16]. Furthermore, various strategies to inhibit GSK3β activity have been identified, including the activation of signaling pathways such as AKT, PKC, and protein kinase A [137, 138]. These results suggest that targeting these molecular mechanisms may offer new therapeutic strategies for alleviating cardiac I/R injury. Unexpectedly, Stoica et al. reported a contrasting finding: in neuronal cells, inhibition of GSK3β enhances the VAPB-PTPIP51 interaction and ER-mitochondria association, resulting in elevated mitochondrial Ca^{2+} levels [139]. This may be due to the tissue-specific role of MAMs.

Similarly, Zhai et al. observed that GSK3β has opposing effects in myocardial ischemia and ischemia/I/R injury. Inhibition of GSK3β worsens ischemic injury but helps protect against I/R injury by regulating mTOR and autophagy [140]. In this context, limiting GSK3β inhibition to only reperfusion time is a feasible strategy. GSK3β inhibitors provide a potential therapeutic approach. Jiang et al. [141] reported a list of GSK3β inhibitors currently undergoing clinical studies, which have demonstrated beneficial effects in various disease models, including Alzheimer's disease, multiple sclerosis, and carotid artery diseases, among others. However, their clinical application in myocardial ischemic diseases has yet to be implemented. This may be attributed to the role of GSK3β in multiple pathophysiological systems, and whether its inhibition may lead to adverse effects on other systems remains to be clarified.

FUNDC1

FUNDC1, a novel hypoxia-induced mitochondrial autophagy receptor, was first reported in 2012. It selectively responds to hypoxic/ischemic stimuli but not to starvation conditions [142]. Wu et al. revealed that under hypoxic conditions, FUNDC1 accumulates at MAMs by interacting with the ER-resident protein calnexin (CANX) and serves as a central regulatory molecule for mitochondrial fission and autophagy during hypoxic responses. In the context of mitochondrial autophagy, the interaction between FUNDC1 and CANX is blocked, exposing the cytosolic loop of FUNDC1, which preferentially recruits DNM1L/DRP1, accelerating mitochondrial fission under hypoxic stress [91, 143]. However, downregulation of the FUNDC1 under hypoxic conditions increases the number of elongated mitochondria, reduces colocalization of autophagosomes with mitochondria, and inhibits mitochondrial fission and autophagy. At the molecular level, genetic ablation of FUNDC1 disrupts ER-mitochondria coupling by inhibiting ubiquitin-dependent degradation of IP_3R2, which interferes with mitochondrial Ca^{2+} uptake [144]. Reduced cytosolic Ca^{2+} levels inhibit the binding of cAMP response element-binding protein (CREB) to promoter regions, suppressing Fis1 expression and thus impairing mitochondrial fission [15]. Studies have also demonstrated that FUNDC1 promotes mitochondrial autophagy under ischemic conditions, confering cardioprotection and inhibiting cardiomyocyte apoptosis. Phosphorylation-induced inactivation of FUNDC1 reduces mitochondrial autophagy, which in turn heightens stress responses to unfolded mitochondrial proteins and disrupts mitochondrial homeostasis.

This impairment weakens the mitochondrial quality control system, causing the accumulation of damaged mitochondria and triggering the pan-apoptotic program [145–147].

FUNDC1 regulates mitochondrial autophagy by phosphorylating and dephosphorylating three key residues: Ser13, Ser17, and Tyr18 [148]. These sites present promising therapeutic targets for strategies designed to induce protective mitochondrial autophagy. Early research has already shown promising progress in translating FUNDC1 findings into practical applications. For example, cell-permeable functional peptides composed of the HIV-1 Tat protein transduction domain can promote FUNDC1-mediated mitochondrial autophagy in cell experiments. Moreover, intraperitoneal injection of synthesized cell-penetrating peptides has been shown to effectively modulate FUNDC1-mediated mitochondrial autophagy in vivo [149].

CLIC4

Chloride intracellular channel (CLIC) proteins are among the primary intracellular Cl^- channels, which function to confer cardioprotective effects. Studies show that blocking CLIC proteins increases myocardial infarction size following I/R injury [150] and abolishes the cardioprotective effects of ischemic preconditioning [151]. Ponnalagu and colleagues further revealed that CLIC4 is expressed in cardiac MAMs, where it regulates Ca^{2+} transfer between the ER/SR and mitochondria, maintaining mitochondrial calcium homeostasis. In cardiomyocytes with CLIC4 gene knockout (clic4 -/-), increased Ca^{2+} levels can trigger the premature mPTP opening under stress stimuli such as I/R injury. Furthermore, clic4 -/- cardiomyocytes exposed to hypoxia-reoxygenation (HR) injury exhibit significantly increased ROS production, greater mitochondrial membrane depolarization, and higher rates of apoptosis [18]. These findings suggest that the CLIC4 channel in MAMs confers cardioprotection against hypoxia and I/R injury by regulating mitochondrial calcium homeostasis and oxidative stress. This also demonstrates its significant potential as a promising therapeutic target for cardiac protection.

ATAD3A

ATPase Family AAA-domain Containing Protein 3A (ATAD3A) is a mitochondrial membrane-anchored protein that participates in various functions including mitochondrial dynamics, mtDNA expression, and lipid metabolism. Previous studies have shown that ATAD3A prevents mitochondrial disruption and is essential for mantainance of the integrity of the mitochondrial ultrastructure [152]. Furthermore, several studies have indicated that the loss of ATAD3A impairs the interaction between the ER and mitochondria to modulate the formation of MAMs [153]. Building on this, Li et al. found that ATAD3A is localized to the MAMs region and interacts with the IP_3R1- Grp75-VDAC1 complex to maintain the morphology and functional integrity of MAMs. They found that ATAD3A overexpression decreased the integrity of the IP_3R1- Grp75-VDAC1 complex, inhibiting isoproterenol (ISO)-induced mitochondrial Ca^{2+} accumulation and ROS generation. This mechanism alleviated mitochondrial oxidative stress and calcium overload, protecting cardiomyocytes from hypertrophic stimuli [19]. Although the specific role of ATAD3A in myocardial I/R models has not been investigated, its role in MAMs microdomains sugggests its potential involvement in myocardial ischemia.

Several studies have revealed various important roles of MAMs regulatory targets in cardiovascular protection. However, its translation into clinical settings need to be further explored. Currently, there is a lack of research on certain regulatory targets of MAMs in relevant translational fields, but some of their mechanisms provide directions for our exploration. For example, since the multiple functions of Nox4 may depend on its subcellular localization, position-specific regulation of Nox4 could be considered in the treatment of I/R injury. Steroids may act as endogenous ligands that bind to Sig-1R, inducing its inhibition or activation, among which dehydroepiandrosterone (DHEA) and its sulfate ester DHEAS have been shown to be strong Sig-1R agonists [154]. Exploring endogenous ligands of Sig-1R is a feasible direction. Moreover, the effects of various chemical molecules or drugs on MAMs and their regulatory targets have been investigated in diverse studies. Notably, sulforaphane (SFN) was shown to regulate Ca^{2+} flux through IP3R and MCU, helping to maintain calcium homeostasis within MAMs [155]. Pravastatin, a drug used for the treatment of hypercholesterolemia, has been recently found to reverse hypercholesterolemia-induced changes in bone marrow-derived macrophages (BMDMs), including affecting alterations in MAMs, ox-LDL uptake, and phagocytic contact points, while enhancing the expression of mitochondrial dynamics-related genes, Mfn2 and Fis1 [156]. Furthermore, fluvoxamine, a selective serotonin reuptake inhibitor (SSRI) with high affinity for Sig-1R, can also act as a Sig-1R agonist to protect against transverse aortic constriction (TAC)-induced cardiac dysfunction [157]. Future studies are needed to identify the potential roles and targets of MAMs to promote the development of MAMs-based therapeutic approaches.

Discussion and perspectives

MAMs are important reglators of the cellular processes in the mitochondria and the endoplasmic reticulum. Due to the changes in MAMs and their significant pathophysiological correlation in ischemic heart disease (IHD), further understanding of the upstream and downstream regulatory mechanisms of MAMs is expected to provide new solutions in this field. However, the complexity and dynamic nature of MAMs present significant challenges for further research. Emerging tools and technologies, such as split-GFP-based contact site sensors (SPLICS), offer promising solutions for real-time in vitro monitoring of MAMs, providing valuable support for advancing studies in this field [158, 159]. Similarly, the development of Contact-ID and OrthoID methods is expected to promote proteomic analysis of MAMs in living cells [160, 161]. Furthermore, several functional detection tools for MAMs need to be developed. Notably, Cho et al. developed the MAM-Calflux, a MAM-specific BRET-based Ca^{2+} indicator, which facilitates ratiometric measurement of dynamic intercellular calcium signaling [162]. Further development of these tools to investigate MAMs alterations in pathophysiology plays a crucial role in gaining new insights into the mechanisms of MAMs in related diseases.

Overall, in the pathological responses associated with myocardial ischemia and subsequent reperfusion, the MAMs microdomain serves as a critical signaling platform that coordinating the regulation of calcium homeostasis, mitochondrial dysfunction, oxidative stress, ER stress, autophagy, and apoptosis. These signaling molecules rely on the MAMs microdomain to transmit signals, thereby collectively mediating myocardial injury and protection. Building on this foundation, therapies that modulate the homeostasis of MAM microdomains are emerging as crucial targets for the clinical treatment of myocardial ischemia. In this review, we have systematically examined key regulatory molecules of MAMs, including PACS-2, GSK3β, and FUNDC1, etc., exploring their mechanisms of action and highlighting recent advancements in translational medicine research related to these targets. However, the development of MAMs-targeted therapies for myocardial ischemia-related conditions still faces several significant challenges. First, some MAMs-related regulatory mechanisms are still being debated, such as the effect of MFN2 deficiency on the structure between mitochondria and the endoplasmic reticulum, the bidirectional regulatory mechanism of GSK3β on mitochondrial Ca^{2+} levels, and the tissue-specific roles of MAMs. Second, the communication between organelles is complex and dual-faceted. MAMs-mediated ER Ca^{2+} transfer to mitochondria enhances ATP production, while excessive mitochondrial calcium accumulation can trigger mPTP-dependent cell death. Similarly, autophagy activation during myocardial ischemia acts as a protective survival mechanism; however, excessive autophagy can exacerbate myocardial damage. Consequently, it is essential to investigate how to precisely regulate its formation and function. Such understanding will help navigate the complexity of this process and inform the development of targeted therapeutic strategies.

Abbreviations

IHD	Ischemic heart disease
ROS	Reactive oxygen species
I/R	Ischemia/reperfusion
ER	Endoplasmic reticulum
UPR	Unfolded protein response
MAMs	Mitochondria-associated endoplasmic reticulum membranes
PACS-2	Phosphofurin acid cluster sorting protein 2
FUNDC1	FUN14 domain-containing protein 1
GSK3β	Glycogen synthase kinase-3β
Sig-1R	Sigma-1 Receptor
MCS	Membrane contact sites
OMM	Outer mitochondrial membrane
RER	Rough ER
SER	Smooth ER
ERMES	ER-mitochondria encounter structure
IP_3Rs	Inositol 1,4,5-trisphosphate receptors
Grp75	Glucose-regulated protein 75
VDAC	Voltage-dependent anion channel
VAPB	Vesicle-associated membrane protein-associated protein B
PTPIP51	Protein tyrosine phosphatase interacting protein 51
MFN2	Mitofusin-2
Bap31	B cell receptor-associated protein 31
Fis1	Fission 1 protein
RyRs	Ryanodine receptors
SR	Sarcoplasmic reticulum
MCU	Mitochondrial calcium uniporter
IMM	Inner mitochondrial membrane
mPTP	Mitochondrial permeability transition pore
ORP5/8	Oxysterol-binding protein-related protein 5/8
PSS1/PSS2	Phosphatidylserine synthases 1/2
PSD	Phosphatidylserine decarboxylase
ACAT1/SOAT1	Acyl-coenzyme A, cholesterol acyltransferase/sterol O-acyltransferase
PS	Phosphatidylserine
PE	Phosphatidylethanolamine
PA	Phosphatidic acid
LTPs	Lipid transfer proteins
OPA1	Optic atrophy 1
INF2	Inverted formin 2
DRP1	Dynamin-related protein 1
MFF	Mitochondrial fission factor
MiD49/51	Mitochondrial dynamics 49/51
mtDNA	Mitochondrial DNA
PERK	Protein kinase RNA-like kinase
ATF6	Activating transcription factor 6
IRE1	Inosital requiring enzyme 1
BiP/GRP78	Heat shock protein 70 family protein 5
CHOP	C/EBP homologous protein
ETC	Electron transport chain
Ero1	ER oxidoreductase 1
p66Shc	66 KDa subtype of growth factor adapter Shc
SERCA	Sarco/ER Ca^{2+}-ATPase
MAPK	Mitogen-activated protein kinase
OSCP	ATP synthase oligomycin-sensitivity conferring protein
ANT	Adenine nucleotide translocase
CypD	Cyclophilin D
MOMP	Mitochondrial outer membrane permeabilization

AS	Atherosclerosis
ECC	Excitation–contraction coupling
DAMPs	Damage-associated molecular patterns
PDIs	Protein disulfide isomerases
XBP1	X-box binding protein-1
JNK	C-Jun N-terminal kinase
Noxs	Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases
PO	Pressure overload
VSMCs	Vascular smooth muscle cells
hVSMCs	Human VSMCs
OX-LDL	Oxidized LDL
CANX	Calnexin
CREB	CAMP response element-binding protein
CLIC	Chloride intracellular channel
clic4 -/-	CLIC4 gene knockout
HR	Hypoxia-reoxygenation
ATAD3A	ATPase family AAA-domain containing protein 3A

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Author contributions
CC designed research topics and wrote manuscripts, FMX and WXM conducted literature search and preliminary screening, FMX and NKB revised manuscript language readability, CC and WXM created schematics and reviewed manuscripts. DGH and GWL are the co-corresponding authors responsible for the overall conception, framework design, and revision of the review. All authors read and approved the fnal manuscript.

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