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Mitochondria-associated endoplasmic reticulum membranes in the heart

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1. Introduction

Historically, subcellular organelles have been viewed as separate entities with defined compositions and organizations that furnish them with specialized functions. Today, mounting evidence indicates that intercellular organelles in close proximity can form membrane contact sites through protein-protein or protein-lipid tether complexes. At these contact sites, organelle membranes are closely apposed and tethered but do not fuse. Membrane contact sites exist in almost every type of subcellular organelle, including mitochondria-associated endoplasmic reticulum (ER) membranes (MAMs), mitochondria-nucleus contacts, mitochondria-lysosome contacts, and peroxisome-mitochondria contacts. Directly or indirectly through these contact sites, inter-subcellular organelles can alter each other's shape or function by exchanging their contents [1]. This inter-organelle communication appears essential for maintaining fundamental cellular functions ranging from lipid metabolism to cell death [1].

One of the most studied subcellular organelle contacts is MAM [2]. Purification of MAMs demonstrates that ER and mitochondria are physically linked [3] with gap distances of 6–15 nm [4]. Approximately 100 mitochondria-ER contacts are estimated to exist in a yeast cell [5], and 5–20% of the mitochondrial surface is in close apposition to ER in HeLa cells [6]. Here, we briefly review recent findings on the composition of ER-mitochondria contacts and physiological roles of MAMs. The focus of this review is on the newly identified MAM protein FUN14 domain-containing protein 1 (FUNDC1) in hearts, as several excellent

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Disclosures

None.

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reviews on other MAM proteins and functions [7–9] exist for readers to acquire more information on other topics.

2. Functions of MAMs

ER-mitochondria contact sites have received much attention due to their key regulation of several fundamental cellular processes (Table 1), including lipid and calcium (Ca^{2+}) homeostasis, initiation of autophagy and mitochondrial division, and metabolic shifts.

2.1. Ca^{2+} transfer

Ca^{2+} is required by mitochondria to generate adenosine triphosphate (ATP) via the tricarboxylic acid cycle, as several mitochondrial enzymes involved in ATP synthesis are regulated by Ca^{2+} [7, 10, 11]. However, excessive uptake of Ca^{2+} by mitochondria leads to cell apoptosis via the opening of mitochondrial permeability transition pores (MPTPs) [7].

Ca^{2+} transfer from ER to mitochondria is a well-characterized function of ER-mitochondria tethering. Localized Ca^{2+} spikes released from ER via inositol 1,4,5-trisphosphate receptor (IP_3R) channels stimulate mitochondrial Ca^{2+} uptake. Ca^{2+} passes through voltage-dependent anion channel 1 (VDAC1) on outer mitochondrial membranes and mitochondrial Ca^{2+} uniporters on inner mitochondrial membranes [12, 13]. A 75-kDa glucose-regulated protein (GRP75) is required for coupling VDAC1 to IP_3Rs to form VDAC1/GRP75/ IP_3R channel complexes [14]. As overexpression of GRP75 does not increase ER-mitochondria contact, it is likely that GRP75 functions at established contacts to regulate mitochondrial Ca^{2+} uptake [14].

Recent studies identify numerous regulators of IP_3R channels at ER-mitochondria contacts. Promyelocytic leukemia protein (PML) was recently found in MAM fractions in a complex with protein kinase B (PKB/AKT) and protein phosphatase 2A (PP2A) [15]. Active, phosphorylated AKT phosphorylates IP_3R and inhibits IP_3R Ca^{2+} release, protecting mitochondria from mounting a Ca^{2+} -mediated apoptotic response, whereas PP2A phosphatase activity deactivates AKT through dephosphorylation [16, 17]. PML may recruit PP2A, which inactivates AKT and facilitates IP_3R -mediated Ca^{2+} release in response to apoptotic stimuli. Ablation or silencing of *mitofusin 2* (*MFN2*) in mouse embryonic fibroblasts (MEFs) and HeLa cells inhibits ER-mitochondria interactions, which suppresses mitochondrial Ca^{2+} uptake in [9] response to stimuli that generate IP_3 [18]. A mammalian ortholog of yeast Mmm1, SMP domain-containing protein (PDZD8), is also present at ER-mitochondria contact sites [19]. PDZD8 is required for the formation of ER-mitochondria contacts and ER-mitochondria Ca^{2+} transfer in mammalian cells [19].

MAMs are generally considered as signaling platforms from which several modulators regulate VDAC1/GRP75/ IP_3R channel activity under physiological and pathological conditions [9]. Future work is needed to uncover regulatory proteins in the Ca^{2+} microdomain and to clarify the mechanisms by which Ca^{2+} is transferred from the ER into mitochondria.

2.2. Lipid synthesis and transfer

The enzymes involved in some lipid biosynthesis processes are present in both ER and mitochondria, with lipid exchange being required for lipid production [7, 10, 11]. ER-mitochondria contacts may be responsible for phospholipid exchange between the two organelles. For example, ER-synthesized phosphatidylserine requires mitochondria-localized phospholipid synthase to generate phosphatidylethanolamine, which can then be converted to phosphatidylcholine in ER. In addition, phosphatidic acid is synthesized in the ER and must be transferred to mitochondria for modification to generate mitochondrial-specific cardiolipin [20, 21]. Directional transfer of these biosynthetic precursors between ER and mitochondria would be facilitated by a specialized lipid transfer domain [22]. This lipid transfer domain could also sense levels of precursors at each organelle to balance the synthesis and transfer process, thereby regulating the proper composition for each organelle. The MAM-enriched molecular chaperone sigma 1 receptor (Sig1R) binds cholesterol and sphingolipids [23–25], suggesting that Sig1R regulates free cholesterol accumulation at MAMs.

Mitochondria are considered partially autonomous organelles that depend on the import of certain lipids and proteins to form membranes and maintain organelle activity. Although cardiolipin, phosphatidylethanolamine, and phosphatidylglycerol are synthesized by mitochondrial enzymes, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, and sterols are imported from other organelles. Most lipids imported into mitochondria are from the ER, underlining the importance of interactions between these two subcellular compartments. Flis and Daum [26] review the components involved in lipid translocation between the ER and mitochondria.

2.3. Mitochondrial fission

Mitochondrial dynamics, which consist of constant fission and fusion processes, are crucial for maintaining cellular homeostasis. The central player in mitochondrial fission is dynamin-related protein 1 (DRP1; called DNM1 in yeast) [27–29]. DRP1 (Dnm1) oligomerizes into spirals that circumscribe mitochondria and mediate their fission [30, 31]. In both yeast and mammalian cells, ER-mitochondria contacts mark fission sites where DRP1 (Dnm1)-dependent fission machinery assembles and consequently divides mitochondria [32, 33]. Moreover, ER-mitochondria contacts coordinate mtDNA replication through downstream mitochondrial division events that distribute newly replicated mtDNA to daughter mitochondria [33, 34]. Recent study demonstrates that mitochondria-lysosome contacts mark the sites of DRP1- and ER-positive mitochondrial fission events via RAB7 GTP hydrolysis [35]. Tagaya and Arasaki [36] review recent progress in understanding the molecular mechanisms of mitochondrial dynamics and mitochondria-related processes in the context of ER interactions.

2.4. Autophagy

ER-mitochondria associations participate in autophagy, through which aggregated proteins and damaged organelles are removed from the cytoplasm. During the process of autophagy, double-membrane autophagosomes engulf damaged proteins and organelles and fuse with lysosomes to execute degradation of their contents. A recent study shows that the source of

autophagosomal membranes forms at ER-mitochondria contact sites [37]. A growing body of evidence indicates that both disturbed ER-mitochondria signaling and dysregulation of autophagy occur in neurodegenerative disorders such as Alzheimer's disease, Parkinson's diseases, and frontotemporal dementia with related amyotrophic lateral sclerosis. Recent findings regarding the contribution of MAMs to autophagosome formation will thus accelerate investigations into the role of autophagy in neurodegenerative diseases [36, 38].

2.5. Intracellular trafficking

In neurons, kinesin 1 drives anterograde (i.e., toward the synapse) transport of mitochondria on tracks of microtubules through axons (i.e., axonal transport) [39]. The outer mitochondrial membrane protein Miro attaches mitochondria to kinesin 1 [39]. Miro localizes at ER-mitochondria contact sites, and some ER are co-transported with mitochondria [40, 41]. Interestingly, Miro also acts as a Ca^{2+} sensor during mitochondria transport, and elevated Ca^{2+} levels stop this transport [42–44]. Thus, it is likely that Miro participates in ER and mitochondria co-transportation through axons, and Miro may sense Ca^{2+} exchange between the two organelles to regulate this transport in response to physiological stimuli. Tang [45] extensively reviews how the small GTPase Miro regulates mitochondrial movement by cooperating with the microtubule-based motor proteins kinesin and dynein.

2.6. Unfolded protein response (UPR)

The accumulation of unfolded proteins activates intracellular signaling pathways, which is referred to as the UPR. The UPR stimulates transcriptional responses that modulate the protein folding capacity of ER [46]. Two known MAM tethering proteins, MFN2 and vesicle-associated membrane protein-associated protein B (VAPB), play roles in the UPR [47–49]. Also, a variety of ER chaperones involved in protein folding, such as BiP, calnexin, calreticulin, endoplasmic reticulum resident protein 44/57 (ERp44/57), and Sig1R, localize in MAMs [50]. Importantly, disruption of ER and mitochondria tethering leads to ER stress and the UPR [51]. Thus, ER-mitochondria contacts may have a role in facilitating stress responses and the UPR.

The ER is crucial for coordinating cellular Ca^{2+} signaling as well as protein synthesis and folding. Mitochondria are central organelles that initiate apoptotic signaling. Not surprisingly, MAMs are a hot spot for the transfer of stress signals from the ER to mitochondria, most notably due to the loss of ER proteostasis. A review by van Vliet and Agostinis [52] summarizes emerging roles of MAMs in the context of ER stress.

2.7. Inflammasome formation

The innate immune system senses microbial invasion and tissue damage and cell stress through pattern recognition receptors. One class of these receptors is the nucleotide oligomerization domain-like receptor (NLR), which senses abnormal cytosolic changes [53]. Upon activation, select NLRs form multiprotein complexes, referred to as the 'inflammasome.' The most well-studied is the pyrin domain-containing 3 protein (NLRP3) inflammasome, which initiates proteolytic maturation of the pro-inflammatory cytokine interleukin 1β [53]. In resting conditions, NLRP3 localizes in the cytosol and ER. Recently,

one study showed that mitochondrial reactive oxygen species (ROS) activate the NLRP3 inflammasome by recruiting both NLRP3 and its adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) to MAM sites. The ER-mitochondria localization of NLRP3 upon activation indicates that NLRP3 strategically accumulates at mitochondria and senses mitochondrial damage [54]. In resting cells, thioredoxin-interacting protein (TXNIP) interacts with thioredoxins and is kept inactivated in the cytosol. Specific inflammasome activators induce TXNIP disassociation from thioredoxins in a ROS-sensitive manner. This free TXNIP binds to NLRP3, which together translocate to MAMs, suggesting a role of TXNIP in interleukin 1 β production [55]. MAMs have emerged as inflammatory platforms from which inflammasome components and their regulators promote the release of pro-inflammatory cytokines that sustain pathological conditions. A review by Missiroli et al. [56] summarizes the critical role of MAMs in initiating inflammation in the cellular defense against pathogenic infections and the association of MAMs with inflammation-mediated diseases.

ER-mitochondria contact sites are dynamic structures with a transient and highly variable MAM composition that is sensitive to changes in intracellular physiological conditions. The unique lipid and protein compositions of MAMs determine their functional roles. Considering that MAMs are composed of many crucial proteins, it is not surprising that structural disturbances to MAMs are involved in the pathogenesis of various diseases. However, the localization and enrichment of some proteins in MAM structures remains under debate. Several elegant reviews provide detailed information on how MAM disturbances are involved in human diseases [56–70].

3. ER-mitochondria tethers

Crosstalk between ER and mitochondria plays fundamental roles in cell metabolism. However, the mechanisms mediating the recruitment of ER membranes to mitochondria are not fully understood. Electron microscopy revealed the presence of structures that appear to tether ER with mitochondria [7], but the biochemical composition of MAMs is not clear. In yeast, proteins of the ER-mitochondria encounter structure (ERMES) serve as molecular tethers between ER and mitochondria [71]. Here, we briefly summarize the proposed ER-mitochondria tethers in mammalian cells (Fig. 1).

3.1. Tethers in non-cardiomyocytes

Homo- and heterotypic interactions between mitochondrial MFN1/2 and ER-located MFN2 have been proposed as a tethering complex [18]. Ablation or silencing of *MFN2* in MEFs and HeLa cells loosens ER-mitochondria interactions [18]. However, later studies from independent laboratories show that loss of MFN2 increases or does not change ER-mitochondria contacts, generating controversy over this finding [72–74].

VDAC1 is physically linked to IP₃R through the molecular chaperone GRP75 [14], which has been proposed as another tether [14, 75]. However, complete loss of IP₃R does not affect ER-mitochondria contacts, causing debate as to the physical tethering role of the VDAC1/GRP75/IP₃R complex [4].

During the apoptotic process, mitochondrial fission 1 protein (FIS1) forms a complex with the ER-located B-cell receptor-associated protein 31 (BAP31) to link ER and mitochondria. However, the role of the FIS1-BAP31 tether in non-apoptotic processes remains unknown [76]. Moreover, the multifunctional sorting protein phosphofurin acidic cluster sorting protein 2 (PACS-2) controls the tether between mitochondria and ER in a BAP31-dependent manner [51].

Recently, another protein tether was described involving the ER protein VAPB and the outer mitochondrial membrane protein tyrosine phosphatase interacting protein 51 (PTPIP51) [62, 77]. Binding between VAPB and PTPIP51 has been confirmed in several different biochemical assays. Manipulation of VAPB or PTPIP51 expression changes ER-mitochondria contacts and Ca^{2+} exchange between the two organelles [62, 77]. The biochemical interaction between VAPB and PTPIP51 has also been independently verified [78].

In yeast, the ER and mitochondria are contacted by the ERMES complex [71]. Functional and structural orthologs of Mmm1 in the ERMES complex in mammalian cells have been identified as PDZD8 [19]. PDZD8-dependent membrane contacts are required for ER-mitochondria Ca^{2+} transfer [19]. In neuronal dendrites, PDZD8 regulates synaptically evoked Ca^{2+} dynamics, highlighting the significance of inter-organelle membrane contacts in neurobiology [19].

3.2. Tethers in cardiomyocytes

The sarcoplasmic reticulum (SR) is a membrane-bound structure found within muscle cells (cardiac muscle and skeletal muscle) that is similar to the ER in other cells. The main function of the SR is to store Ca^{2+} . MFN2 is a MAM-located protein that was first reported to tether ER and mitochondria and regulate Ca^{2+} transfer from the ER to mitochondria via IP_3R channels in mammalian cells [18]. A remaining question, however, is how *MFN2* deletion disrupts the integrity of IP_3R channels, as there is no evidence of interaction between MFN2 and IP_3R channels. Although this work was challenged by several later studies [71–73], it led to research on the role of MFN2-related MAMs in the heart. In *MFN2* but not *MFN1* knock-out (KO) hearts, transmission electron microscopy (TEM) shows that mitochondria are abnormally large and elongated and that contacts between the SR and mitochondria are destroyed [79]. Furthermore, compared with control myocytes, MFN2-deficient myocytes but not MFN1-deficient myocytes show reduced mitochondrial Ca^{2+} uptake [79].

The existence of SR-mitochondria tethers has been demonstrated in cardiomyocytes over many years [80]. However, the molecular entities constituting the tethers remain elusive. FUNDC1, an outer mitochondrial membrane protein, is highly expressed in cardiac muscle, suggesting important roles in cardiac function [81]. Immunofluorescence analysis of endogenous FUNDC1 combined with live-cell analysis of GFP-tagged exogenous FUNDC1 reveals that FUNDC1 is not evenly anchored at the mitochondrial surface in cardiomyocytes [81]. Instead, the localization of FUNDC1 displays a punctuated pattern in discrete areas of the outer mitochondrial membrane, overlapping with sites of SR-mitochondria juxtaposition (Fig. 2) [81]. Subcellular fractions further substantiate this pattern of FUNDC1 MAM

distribution [81]. This finding is supported by other reports that FUNDC1 localizes to MAMs in both MEFs and HeLa cells [82]. The enrichment of FUNDC1 in cardiac MAM fractions prompted the investigation on its possible role in SR-mitochondria tethering. It is demonstrated that FUNDC1 participates in MAM formation in cardiomyocytes by binding to IP₃R2 [81]. According to our transmission electron microscopy results, *FUNDC1* deletion causes an 80% reduction in SR and mitochondria contacts [81], whereas *MFN2* deletion causes only a 30% reduction [79]. The co-immunoprecipitation of FUNDC1 with IP₃R2 in hearts implies that FUNDC1 forms protein bridges with IP₃R2 [81]. A mutant of FUNDC1 that is unable to interact with IP₃R2 and found that disruption of FUNDC1 and IP₃R2 binding markedly reduces contact between SR and mitochondria, similar to that following *FUNDC1* ablation [81]. Further study shows that FUNDC1 inhibits IP₃R2 ubiquitination and degradation by direct interaction [81]. *FUNDC1* deletion promotes IP₃R2 ubiquitination and degradation, whereas *FUNDC1* overexpression increases IP₃R2 stability [81]. These findings on FUNDC1-related MAMs reveal the molecular underpinnings of the long-sought link between SR and mitochondria in cardiomyocytes.

4. Regulation of MAMs in the heart

The close association of mitochondria to SR in cardiomyocytes may explain efficient mitochondrial Ca²⁺ uptake despite the low Ca²⁺ affinity of mitochondrial Ca²⁺ uniporters [80, 83]. However, in contrast to non-cardiac cells, in which the concept of mitochondrial Ca²⁺ microdomains at the ER-mitochondria interface is well established [84], the existence of Ca²⁺ microdomains and their functional role in cardiomyocytes are poorly understood.

4.1. Cyclophilin D (CypD)-related MAMs

CypD is a mitochondrial matrix chaperone that interacts with MPTP [85]. An increase in mitochondrial matrix Ca²⁺ level activates CypD, which triggers the opening of MPTP [86]. The opening of MPTP is widely recognized as the final step of reperfusion injury and is responsible for mitochondrial and cardiomyocyte death [87, 88]. In mammalian cells, the VDAC1/GRP75/IP₃R complex is the main structure involved in Ca²⁺ transfer from the ER to mitochondria under physiological conditions [75]. CypD interacts with the VDAC1/GRP75/IP₃R1 complex in both adult mouse cardiomyocytes and H9c2 cardiomyoblasts [89]. Restricting the interaction between CypD and the VDAC1/GRP75/IP₃R1 complex by pharmacological inhibition or genetic deletion of CypD inhibits Ca²⁺ transfer from the SR to mitochondria in normoxic conditions [89]. By contrast, in hypoxia-reoxygenation conditions, interactions between CypD and the VDAC1/GRP75/IP₃R1 complex are increased concomitantly with mitochondrial Ca²⁺ overload. Furthermore, inhibition of CypD suppresses Ca²⁺ overload and protects against hypoxia-reoxygenation injury [89]. Therefore, this study reports a new role of CypD at the SR-mitochondrial surface, suggesting that suppression of SR-mitochondria contacts could be a therapeutic strategy for alleviating lethal reperfusion injury [89]. As CypD deficiency reduces susceptibility to cell death by suppressing MPTP opening [90], it seems that CypD deletion in hypoxia-reoxygenation injury serves at least two protective roles: suppressing MPTP pore opening and inhibiting Ca²⁺ transfer. In addition, it is uncertain whether disruption of the interaction between CypD and the VDAC1/GRP75/IP₃R1 complex affects the SR-mitochondria contact structure.

4.2. MFN2-related MAMs

Downregulation of MFN2 in H9c2 cardiomyoblasts suppresses the interaction between CypD and the VDAC1/GRP75/IP₃R1 complex and prevents mitochondrial Ca²⁺ overload, which protects against cell death after hypoxia-reoxygenation [89]. This finding extends the roles of MFN2-related MAMs in regulating mitochondrial Ca²⁺ hemostasis in cardiomyocytes.

Under basal conditions, cardiac-specific *MFN2* KO mice show cardiac hypertrophy and moderate diastolic dysfunction but no systolic dysfunction [91]. By contrast, under a β -adrenergic stress condition, cardiac-specific *MFN2* KO mice show significant systolic dysfunction [91]. In some areas of adult *MFN2* KO hearts, mitochondria in cardiomyocytes become large, elongated, and disordered, and mitochondrial membrane potential is reduced [91]. In neonatal *MFN2* KO cardiomyocytes, however, mitochondria become fragmented and spherical [91]. *MFN2* KO hearts also show resistance to ischemia and reperfusion (I/R) injury as evidenced by smaller ischemic areas, with isolated mitochondria showing no evidence of respiration dysfunction [91].

In *Drosophila*, *dMFN/MARF*RNAi hearts exhibit larger Ca²⁺ transient amplitudes under both resting and caffeine-stimulated conditions as well as increased diastolic and systolic dimensions and decreased fractional shortening time [79]. Although cardiac function is normal in 6-week-old cardiac-specific *MFN1* or *MFN2* KO mice under both basal and β 1-adrenergic stimulation conditions, Ca²⁺ transient amplitude is increased in *MFN2* KO hearts but not *MFN1* KO hearts under both basal and caffeine stimulation conditions [79]. Interestingly, *MFN1* KO hearts show increased expression of MFN2, whereas *MFN2* KO hearts show normal expression of MFN1 [79]. Both *MFN1* and *MFN2* KO significantly reduce RyR expression [79]. In *MFN2* but not *MFN1* KO hearts, mitochondria are abnormally large and elongated, and contacts between SR and mitochondria are destroyed. *MFN1*-deficient myocytes exhibit normal mitochondrial Ca²⁺ uptake, but *MFN2*-deficient myocytes exhibit reduced mitochondrial Ca²⁺ uptake [79]. Mitochondrial Ca²⁺ accumulation in response to β 1-adrenergic stimulation is blunted in *MFN2* KO myocytes compared with control myocytes. As reduced NADPH is required for elimination of ROS from the matrix, mitochondrial $\cdot\text{O}_2$ production is temporarily increased by isoproterenol challenge of *MFN2* KO cardiomyocytes [79]. These results suggest that Mfn2 plays a role in the regulation of SR-mitochondrial Ca²⁺ crosstalk.

4.3. Glycogen synthase kinase-3 beta (GSK3 β)-related MAMs

Inhibition of GSK3 β , a multifunctional kinase, alleviates myocardial I/R injury [92]. GSK3 β localized at MAMs in the heart specifically interacts with components of the IP₃R Ca²⁺ channeling complex (GRP75, VDAC1, and CypD) [93]. Pharmacological inhibition of GSK3 β suppresses the interaction of IP₃R with the Ca²⁺ channeling complex in adult cardiomyocytes. Inhibition of GSK3 β reduces histamine-stimulated mitochondrial Ca²⁺ uptake in adult cardiomyocytes [93]. In H9c2 myoblasts, inhibition of GSK3 β impairs SR Ca²⁺ release and suppresses mitochondrial Ca²⁺ uptake during the reoxygenation stage. In adult cardiomyocytes, Hypoxia-reoxygenation-induced cell death is associated with increased GSK3 β activity and IP₃R phosphorylation, which results in enhanced transfer of

Ca²⁺ from SR to mitochondria. Suppression of GSK3 β in I/R reduces both IP₃R-dependent SR Ca²⁺ release and cell apoptosis in the heart [93].

4.4. FUNDC1-related MAMs in the heart

FUNDC1 KO cardiomyocytes, in which SR and mitochondria lie distant as a consequence of *FUNDC1* and IP₃R2 disassociation, are a perfect system for investigating the role of MAMs in intracellular Ca²⁺ hemostasis in the heart. Both the mitochondrial Ca²⁺ and cytosolic Ca²⁺ levels are markedly lower in *FUNDC1* KO cardiomyocytes than in WT cells [81]. By contrast, *FUNDC1* KO cells show increased SR Ca²⁺ levels [81]. These results indicate that disruption of *FUNDC1*-related MAMs decreases both mitochondrial and cytosolic Ca²⁺ levels. Conversely, *FUNDC1* overexpression increases mitochondrial and cytosolic Ca²⁺ levels in cardiomyocytes [81]. SR Ca²⁺ levels are markedly lower in *FUNDC1*-overexpressing cardiomyocytes than in control cardiomyocytes [81]. Moreover, silencing of *Itpr*s prevents the increase of mitochondrial and cytosolic Ca²⁺ levels and the reduction of SR Ca²⁺ levels in *FUNDC1*-overexpressing cardiomyocytes [81]. These results indicate that *FUNDC1* promotes Ca²⁺ release from the SR to both mitochondria and cytosol [81].

The results from *FUNDC1* KO cells differ from those obtained from *MFN2* KO cells. Ablation of *FUNDC1* decreases both mitochondrial and cytosolic Ca²⁺ levels [81], whereas, in *MFN2* KO MEFs and HeLa cells, mitochondrial Ca²⁺ uptake is reduced and cytosolic Ca²⁺ levels is increased [18]. In *MFN2* KO cells, the inhibition of mitochondrial Ca²⁺ uptake results from disassociation of ER and mitochondria, and the lack of *MFN2* appears to have minimal impact on ER Ca²⁺ release capacity. Thus, it is likely that *MFN2* deletion, unlike *FUNDC1* deletion, does not affect IP₃R and their related ER Ca²⁺ release capacity. *FUNDC1* deletion promotes IP₃R2 ubiquitination and degradation [81]. Thus, the possibility that *FUNDC1* but not *MFN2* changes IP₃R protein levels might explain the different effects of *MFN2* versus *FUNDC1* on Ca²⁺ levels in the cytosol.

The role of mitochondrial dynamics in cardiac homeostasis is currently unresolved because the highly ordered subcellular architecture of cardiac myocytes physically enforces inter-mitochondrial connectivity, which may obviate the need for molecular tethering [94]. An increasing number of studies demonstrate that mitochondrial fusion and fission occur in the heart [95]. Indeed, growing evidence suggests disordered mitochondrial dynamics plays a causative role in diverse cardiovascular diseases [96]. For example, cardiac-specific *DNM1L* (the gene encoding DRP1) KO mice exhibit mitochondrial elongation, mitochondrial dysfunction, and left ventricular dysfunction [97]. Additionally, cardiac-specific *MFN* deletion suppresses mitochondrial fusion and yields cardiac dysfunction [98]. One of the unveiled roles of MAMs in mitochondrial dynamics is to mark fission sites [32]. However, how specific MAM proteins regulate the mitochondrial fission process remains unclear for both non-cardiomyocytes and cardiomyocytes.

Compared with the ovoid structure of mitochondria in WT hearts, mitochondria in *FUNDC1* KO hearts are larger and more elongated [81]. This result was further evidenced by measuring the size of mitochondria isolated from hearts, which showed that *FUNDC1*-null mitochondria were ~2.5-fold larger than control mitochondria [81]. As mitochondrial

dynamics have a profound impact on mitochondrial function, *FUNDC1* KO neonatal cardiomyocytes and isolated adult mitochondria display significantly lower respiratory capacity than their WT counterparts [81]. Mitochondrial membrane potential (Ψ_m) is also lower in mitochondria isolated from *FUNDC1* KO hearts compared with that from control hearts. As elongated mitochondria likely result from inhibitive fission or enhanced fusion. Using time-lapse confocal imaging. Interestingly, it has been observed that mitochondrial fission occurs at a lower frequency in *FUNDC1* KO cardiomyocytes than in control WT cardiomyocytes [81], suggesting that disruption of FUNDC1-related MAMs inhibits mitochondrial fission. Another line of investigation shows that *MFN2* KO cardiomyocytes have larger mitochondria than WT cells [91]. However, as MFN2 is also a mitochondrial fusion machinery protein, it is difficult to conclude if the impact of MFN2 on mitochondrial morphology is ascribed to impaired ER-mitochondrial coupling or suppressed mitochondrial fusion activity.

An important finding comes from the study of FUNDC1-related MAMs in mitochondrial dynamics in the establishment of a Ca^{2+} /cAMP response element binding protein (CREB)/FIS1 pathway that executes FUNDC1-promoted mitochondrial fission. Specifically, FUNDC1-related MAMs activate Ca^{2+} -sensitive CREB transcription factor and directly bind the promoter of *FIS1*, resulting in its gene transcription [81]. In this context, Ca^{2+} serves as a mediator that facilitates FUNDC1 to induce mitochondrial fission, suggesting the existence of a remarkable pathway in Ca^{2+} -sensitized organs, particularly in cardiac muscle. First, FUNDC1 positively regulates *FIS1* expression without any effect on other mitochondrial dynamic machinery proteins. Second, genetic studies indicate that *FIS1* silencing prevents *FUNDC1* overexpression-induced mitochondrial fission, whereas *FIS1* overexpression prevents *FUNDC1* silencing-induced mitochondrial fusion. Finally, *CREB* silencing blocks *FUNDC1* overexpression-induced *FIS1* expression. These findings extend our knowledge of the role of MAMs in mitochondrial dynamics. That is, MAMs not only mark mitochondrial fission sites [32, 99] but also regulate dynamics machinery gene expression. Another important finding is that FUNDC1-upregulated FIS1 facilitates mitophagy. Indeed, overexpression of *FUNDC1* increases mitochondrial fragmentation followed by increased mitophagy. Inhibition of mitochondrial fission by silencing *FIS1* abolishes mitophagy in cells overexpressing *FUNDC1*. These findings suggest that FUNDC1 induces mitophagy not only by acting as a mitophagy receptor but also by enhancing mitochondrial fragmentation.

A recent study in HeLa cells shows that Ca^{2+} controls mitochondrial dynamics via the calcium/calmodulin-dependent kinase II α /DRP1 pathway [100]. However, there is no any physical interaction between FUNDC1 and DRP1 in cardiomyocytes. Second, overexpression of *FUNDC1* does not affect DRP1 protein expression or phosphorylation, and increased levels of FIS1 induced by *FUNDC1* overexpression do not recruit DRP1 to mitochondria. These results suggest that FIS1 regulates mitochondrial dynamics independently of DRP1 in cardiomyocytes. Consistently, there is no detectable interaction between purified DRP1 and FIS1 [101, 102]. Manipulation of FIS1 levels does not alter mitochondrial translocation of DRP1 in HeLa cells [103]. FIS1 is not a GTPase and is unable to directly drive mitochondrial fission. Thus, additional protein(s) that act as assistants to FIS1 must exist to accomplish the fission process. Two report show that FIS1 recruits TBC1 domain family member 15 (TBC1D15) and regulates mitochondrial

morphology independently of DRP1 [35, 104]. However, there is no interaction between FIS1 and TBC1D15 in cardiomyocytes. Dynamin II was recently found to recruit to mitochondrial fission sites to facilitate the fission process [105], and it is possible that FIS1 functions as the receptor of dynamin II. The molecular function of FIS1 in mammalian mitochondrial morphology regulation warrants further investigation.

Although the physiological role of ER-mitochondria interaction is beginning to be understood, MAM involvement in cardiac pathologies remains unclear. The role of FUNDC1-related MAMs in cardiac function using echocardiography were determined. The ratio of early-to-late ventricular filling velocities in *FUNDC1* KO mice is markedly reduced compared with that in WT mice, and left ventricular isovolumic relaxation time are increased [81]. Moreover, *FUNDC1* KO mice show large decreases in ejection fraction, fractional shortening, and cardiac output compared with WT mice [81]. These results collectively indicate that *FUNDC1* KO mice exhibit both diastolic and systolic dysfunction. Furthermore, additional pathologic evidence that supports an impairment of cardiac function by *FUNDC1* KO, including interstitial fibrosis, positive terminal deoxynucleotidyl transferase dUTP nick-end labeling, and increased cardiac stress gene expression in *FUNDC1* KO hearts compared with WT hearts. These data collectively demonstrate that cardiac-specific deletion of *FUNDC1* leads to heart failure (Fig. 3) [106].

Deletion of *MFN2*, another MAM protein, is reported to induce modest cardiac hypertrophy [91]. Similarly, another line of investigation shows that depletion of *MFN2* decreases mitochondrial Ca^{2+} accumulation but does not affect normal cardiac function [79]. These findings are inconsistent with our demonstration that *FUNDC1* KO induces obvious cardiac dysfunction. One possible explanation stems from the degree of MAM levels that are affected by *FUNDC1* versus *MFN2* deletion. That is, *FUNDC1* deletion results in an 80% reduction in SR and mitochondria contacts [81], which is higher than the 30% reduction resulting from *MFN2* deletion [79].

FUNDC1-dependent mitophagy plays an important role in cardiac I/R injury [106, 107], which mainly results from cardiomyocyte death. Mitophagy sustains mitochondrial function and exerts a pro-survival effect on reperfused heart tissue [108], with suppression of mitophagy during reperfusion presumed to cause cardiac cell death. Reperfusion inhibits FUNDC1-related mitophagy by upregulating macrophage-stimulating 1 (MST1) via the extracellular signal-regulated kinase (ERK)-CREB pathway [109]. Another study reports that I/R decreases FUNDC1-related mitophagy by upregulating the nuclear receptor subfamily 4 group A member 1-nuclear protein kinase CK2 α subunit pathway [110].

The role of FUNDC1-related MAMs in ischemic heart failure has also been investigated. MAM disruption by *FUNDC1* deletion leads to cardiac dysfunction via disturbed intracellular Ca^{2+} homeostasis [81]. The experimental model of myocardial infarction (MI) after coronary artery ligation in mice is a widely used model of ischemic heart failure [111]. Under our experimental conditions, echocardiography revealed that after MI, mice show a significant increase in left ventricular internal diameter at end-diastole and end-systole compared with mice that received sham treatment [81]. Ejection fraction and fractional shortening are significantly lower in mice treated with MI than in sham mice [81].

Moreover, the fibrosis area is significantly higher in MI-treated mice than in sham-treated mice, indicative of aggravation of myocardial scarring by MI [81]. Overall, these results indicate the successful establishment of a heart failure phenotype by MI challenge.

As expected, MI reduced FUNDC1 protein expression, concurrent with a reduction in MAM-related proteins (IP₃R2 and PACS-2), phosphorylated CREB, and FIS1 [81]. These results indicate that the MI-induced heart failure phenotype is associated with downregulation of FUNDC1-related MAMs. To establish the clinical relevance of FUNDC1-related MAMs, heart specimens from heart failure patients diagnosed with ischemic cardiomyopathy and control donors were examined. Together with a reduction in MAM-related proteins, including IP₃R1 and IP₃R3, levels of FUNDC1, phosphorylated CREB, and FIS1 were significantly reduced in heart failure patients compared with control donors [81]. There is a clear reduction in the association between SR and mitochondria in failed hearts [81]. Similar to mitochondrial morphology in *FUNDC1* KO hearts, mitochondria in failed hearts were more elongated compared with the ovoid structure of mitochondria in donor's hearts [81]. These findings indicate that the downregulation of FUNDC1-related MAMs is correlated with the pathogenesis of ischemic heart failure.

A large majority of diabetes patients die from diabetic cardiomyopathy. Hyperglycemia has been recognized as a crucial pathologic factor in type 1 diabetes, whereas, the exact mechanisms involved in the pathological processes of diabetic cardiomyopathy are poorly characterized. In particular, numerous evidence suggests that mitochondrial dysfunction, which triggers mitochondrial oxidative stress and thereby induces cardiomyocyte death, is a central event in diabetic cardiomyopathy [80, 81]. However, the molecular mechanisms of mitochondrial dysfunction in diabetic cardiomyopathy remain elusive [82–85]. The mitochondria where ATP is mainly produced, and the SR/ER where proteins are synthesized, are two key players that regulate cellular functions, and their structural and functional interactions are essential for cellular homeostasis [86]. As illustrated by TEM images and the following quantitative analysis, the association of the SR with mitochondria was dramatically increased in the hearts of diabetic Akita mice. Consistently, confocal imaging and Pearson's correlation coefficient analysis revealed an increased association between the SR and mitochondria in high glucose (HG)-treated mouse neonatal cardiomyocytes. This result indicates that hyperglycemia, a key pathological factor in type 1 diabetes, is highly likely the reason to induce MAM formation in the heart. This is the first-time examination of MAMs in the animal model of type 1 diabetes. The studies of MAMs in obesity-induced type 2 diabetes are controversial. It has been shown that ER-mitochondria contacts in anorexigenic pro-opiomelanocortin (POMC) neurons in the hypothalamus are decreased in diet-induced obesity [112]. A later study shows that in the liver, obesity markedly increases MAMs, which results in mitochondrial Ca²⁺ overload, compromised mitochondrial oxidative capacity, and augmented oxidative stress [113].

A new protein bridge has been reported in cardiomyocytes, which is formed via the interaction between outer mitochondrial membrane protein FUNDC1 and the SR protein IP₃R2 [81]. Interestingly, both FUNDC1 and IP₃R2 expression were increased in cardiac MAM fractions isolated from both streptozotocin (STZ)-treated mice and Akita diabetic mice [114]. Consistently, HG significantly increased the expression of FUNDC1 and IP₃R2

in mouse neonatal cardiomyocytes [114]. To be clinically relevant, FUNDC1 levels were significantly elevated in cardiac tissues from diabetic patients compared to those in non-diabetic donors [114]. Furthermore, Akita hearts show increased co-immunoprecipitation of FUNDC1 and IP₃R2, which suggests that diabetes may increase MAM formation by promoting FUNDC1 and IP₃R2 interaction [114]. Earlier study shows that FUNDC1 is an upstream regulator of IP₃R2 [81]. The increased IP₃R2 in the diabetic heart was prevented by *FUNDC1* deletion [114]. Therefore, it can be speculated that FUNDC1 is the key regulator of cardiac MAM formation. As illustrated by TEM images and the following quantitative analysis, *FUNDC1* deletion dramatically reduced the association between the SR and mitochondria in Akita hearts [114]. These results demonstrate that FUNDC1 is required for diabetes-induced cardiac MAM formation [114]. This result was validated our hypothesis in mouse neonatal cardiomyocytes [114]. TEM analysis indicated that *FUNDC1* ablation reduced the proportion of SR adjacent to mitochondria in HG-treated cells [114]. Similarly, confocal microscopic images showed that deletion of *FUNDC1* abolished increased SR-mitochondria contacts in mouse neonatal cardiomyocytes under HG treatment [114].

The study performed in two type 1 diabetes mouse models shows that FUNDC1-related MAMs play essential roles in diabetic cardiomyopathy [114]. Intriguingly, cardiac-specific deletion of *FUNDC1* almost completely prevented the cardiac abnormalities in STZ-treated diabetic mice, which preserved LVID, EF, FS, E/A ratio, and IVRT at normal values [114]. *FUNDC1* ablation also reduced cardiac fibrosis in STZ-treated mice [114]. Moreover, *FUNDC1* ablation completely prevented cardiac hypertrophy, which was revealed by the increased ratio of heart weight to tibia length [114]. Supportably, *FUNDC1* ablation reduced cardiomyocyte death in STZ-treated mice indicated by TUNEL staining [114]. Similarly, *FUNDC1* ablation prevents diabetic cardiomyopathy in Akita mice, which are genetically-induced type 1 diabetes model [114].

Tethering of ER to mitochondria is thought to facilitate local Ca²⁺ delivery from the ER to mitochondrial matrix, which promotes mitochondrial Ca²⁺ signaling. Whereas, mitochondrial Ca²⁺ overload, which impairs mitochondrial function, is a key determinant in heart failure [115]. Defects in intracellular Ca²⁺ homeostasis have been implicated in diabetic cardiomyopathy; however, the exact mechanisms for diabetes-induced Ca²⁺ homeostasis disruption remain unclear. It has been demonstrated that FUNDC1-related MAMs control cardiac function by regulating the Ca²⁺ transfer from SR to mitochondria and cytosol [81]. In the meanwhile, FUNDC1-related MAMs increase and contribute to diabetic cardiomyopathy. Therefore, it can be speculated that diabetes through increasing FUNDC1-related MAMs induce mitochondrial Ca²⁺ overload, which yields cardiac dysfunction. Indeed, Akita hearts showed higher resting mitochondrial Ca²⁺ levels than WT hearts [114]. This indicates that diabetes causes an abnormal mitochondrial Ca²⁺ increase. Both *FUNDC1* deletion prevents mitochondrial Ca²⁺ increase in Akita heart. Both aberrant ROS production and reduced Ψ_m were observed in diabetic Akita hearts [114]. Importantly, *FUNDC1* KO Akita hearts show significantly improved Ψ_m and less ROS production when compared with those in Akita mice [114]. Moreover, *FUNDC1* deletion restored mitochondrial respiration capacity in Akita hearts [114]. Akita cells uptake more Ca²⁺ in mitochondria than WT cells [114]. Importantly, *FUNDC1* deletion abolished mitochondrial Ca²⁺ uptake in

Akita cells [114]. After histamine stimulation, Akita cells showed a quicker mitochondrial depolarization than WT cells [114]. Histamine-induced mitochondrial depolarization in Akita cells was blocked by *FUNDC1* deletion [114]. Ru360, a specific inhibitor of mitochondrial Ca^{2+} uptake, prevented mitochondrial depolarization in Akita cells after histamine stimulation [114]. Taken together, these results suggest that *FUNDC1* deletion protects Akita cells against from mitochondrial dysfunction by suppressing mitochondrial Ca^{2+} overload.

One of the body's master guardians of metabolism is AMP-activated protein kinase (AMPK), which senses the cellular energy status by monitoring ATP, ADP, and AMP levels [116, 117]. Liver kinase B1 (LKB1) phosphorylates and activates AMPK in stress conditions when the ratio of AMP/ATP is elevated [118–120]. Activated AMPK regulates metabolism on purpose to compensate for energy depletion by upregulating energy sources and downregulating energy-consuming processes [116, 117]. By contrast, AMPK is dephosphorylated and inactivated when cellular energy levels are depleted when the ratio of AMP/ATP was decreased. AMPK has received a large number of attention as a potential target for treating metabolic diseases, which includes diabetes, obesity, fatty liver diseases, and cancer. There is a growing recognition that AMPK plays particularly important roles in the heart, which continuously demands more energy than most other organs [121, 122]. Metformin, one of the most commonly applied anti-type 2 diabetes drug, is a well-characterized AMPK activator [123–126]. According to the UK Prospective Diabetes Study, metformin activates AMPK, improves cardiac function, and reduces the incidence of MI in obese-related diabetic patients [127]. It is shown that metformin-induced chronic AMPK activation prevents diabetic OVE26 mice against cardiomyopathy by restoring mitochondrial and cardiac ultrastructure [124]. AMPK appears to be a critical regulator of cardiac energy status and may be an attractive therapeutic target for heart disease. AMPK seems to be a major regulator of MAMs, at least in the heart. Inactivated AMPK contributes to diabetic cardiomyopathy by increasing *FUNDC1*-related MAMs [114]. This conclusion can be supported by the following evidence: 1) Enhanced MAM formation along with mitochondrial Ca^{2+} overload is shown in *PRKAA2* (*gene encoding AMPK α 2*)-null hearts compared with WT hearts; 2) Decreased AMPK activity exists together with enhanced MAM formation in diabetes heart; 3) Constitutive activation of AMPK inhibits MAM formation and mitochondrial Ca^{2+} overload in HG-treated cultured mouse neonatal cardiomyocytes. The impact of AMPK on *FUNDC1*-related MAMs offers evidence supporting the concept that metformin might be a useful tool for treating diabetic cardiomyopathy in type 1 diabetes. Larger trials are warranted to evaluate the potential effects of metformin in cardiovascular outcomes in patients with type 1 diabetes.

5. Conclusion and perspective

The contractile activity of cardiac muscle requires mitochondria-generated ATP, the synthesis of which depends on the propagation of Ca^{2+} from SR to mitochondria, highlighting the importance of MAMs in cardiomyocytes. So far, a limited number of proteins have been identified in cardiac MAM fractions. Of these, only *FUNDC1* is demonstrated to regulate MAM structure and function. *FUNDC1* is highly expressed in cardiac muscle, supporting its crucial role in cardiac function. Levels of *FUNDC1*-related

MAMs are critically relevant to cardiac function in that they affect Ca^{2+} transfer from SR storage to mitochondria and cytosol. Inhibition of FUNDC1-related MAMs induces cardiac dysfunction, whereas increased FUNDC1-related MAMs contribute to the progression of diabetic cardiomyopathy. These findings reveal the molecular nature of the tethers between SR and mitochondria, provide support for the Ca^{2+} microdomains theory, and suggest a role for SR-mitochondria juxtaposition in the pathogenesis of heart diseases. FUNDC1-related MAMs might be effective therapeutic targets for heart diseases. Future work is needed to investigate the role of FUNDC1-related MAMs in other types of heart disease and non-cardiovascular disease, such as cancer. Furthermore, it is equally important to explore the molecular mechanisms regulating the dynamics of FUNDC1-related MAMs

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Abbreviations used:

ASC	apoptosis-associated speck-like protein containing a caspase recruitment domain
ATP	adenosine triphosphate
BAP31	B-cell receptor-associated protein 31
CL	cardiolipin
CREB	cAMP response element binding protein
CypD	cyclophilin D
DRP1	dynamin-related protein 1
EF	ejection fraction
EM	electron microscopy
ER	endoplasmic reticulum
SR	sarcoplasmic reticulum
ERMES	ER-mitochondria encounter structure
FIS1	mitochondrial fission 1 protein
FS	fractional shortening
FUNDC1	FUN14 domain-containing protein 1
GRP75	75 kDa glucose-regulated protein

GSK3β	glycogen synthase kinase-3 beta
IP₃R	inositol 1,4,5-trisphosphate receptor
I/R	ischemia and reperfusion
KO	knock-out
MAMs	mitochondria-associated endoplasmic reticulum membranes
MEFs	mouse embryonic fibroblasts
MFN2	mitofusin 2
MI	myocardial infarction
MST1	macrophage-stimulating 1
NLRP3	pyrin domain-containing 3 protein
NLRs	nucleotide oligomerization domain-like receptors
PA	phosphatidic acid
PACS-2	phosphofurin acidic cluster sorting protein 2
PC	phosphatidylcholine
PDZD8	SMP domain-containing protein
PE	phosphatidylethanolamine
PKB/AKT	protein kinase B
PML	promyelocytic leukemia protein
POMC	pro-opiomelanocortin
PP2A	protein phosphatase 2A
PS	phosphatidylserine
PTPIP51	protein tyrosine phosphatase interacting protein 51
ROS	reactive oxygen species
Sig1R	sigma 1 receptor
TBC1D15	TBC1 domain family member 15
TEM	transmission electron microscopy
TXNIP	thioredoxin-interacting protein
UPR	unfolded protein response
VAPB	vesicle-associated membrane protein-associated protein B

VDAC1	voltage-dependent anion channel 1
WT	wild-type
Ψ_m	mitochondrial membrane potential

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Figure 1.

Proteins involved in MAM formation. (A) Non-cardiomyocyte: ER-localized IP₃R and outer mitochondrial membrane protein voltage-dependent anion channel 1 (VDAC1) interact via chaperone GRP75; ER-localized MFN2 interacts with mitochondrial MFN1/2; ER-localized B-cell receptor-associated protein 31 (BAP31) binds to mitochondrial fission 1 protein (FIS1); ER resident VAPB binds to mitochondrial protein tyrosine phosphatase interacting protein 51 (PTPIP51). (B) Cardiomyocyte: SR-localized IP₃R2 and outer mitochondrial membrane protein FUNDC1; SR-localized MFN2 interacts with mitochondrial MFN1/2.

Figure 2. Representative confocal microscopy images of subcellular localization of FUNDC1 in neonatal mouse cardiomyocytes. Mitochondria were labeled with MitoTracker Red; SR was labeled with DsRed2-ER; FUNDC1 was labeled with anti-FUNDC1; nuclei were stained by DAPI. Scale bars, 10 μ m. This image is cited from Ref. 81.

Figure 3.

Illustration of the role of FUNDC1-related MAMs under physiological conditions. Persistent downregulation of FUNDC1 inhibits the integrity of MAMs. Reduced MAM formation results in lower mitochondrial and intracellular Ca^{2+} levels, which in turn leads to inhibition of the cAMP response element binding protein (CREB)/FIS1 pathway, elongation of mitochondria, impairment of mitochondrial function, and consequent cardiac dysfunction.

Figure 4.

The scheme shows the role of FUNDC1-related MAMs in diabetic cardiomyopathy. FUNDC1 was upregulated by AMP-activated protein kinase (AMPK) inactivation in diabetic mouse hearts. The increased FUNDC1 was associated with increased MAM formation by binding to IP₃R2. The increased MAMs contribute to the increased mitochondrial Ca^{2+} levels $[Ca^{2+}]_m$, which in turn induces mitochondrial dysfunction, and consequent cardiac dysfunction in diabetic hearts.

Table 1.

Protein composition and functions of mitochondria-associated endoplasmic reticulum membranes (MAMs) in mammalian cells.

Functions of MAMs	Involved proteins
Ca ²⁺ transfer	IP ₃ R, AKT, PP2A, PML, MFN2, FUNDC1, RyR, Sig1R, p53, PDZD8, GSK3β, CypD
Lipid synthesis and transfer	SERAC1, FAC1/4, acyl-CoA desaturase, FATP4, PEMT2, Sig1R
Mitochondrial fission	DRP1, FUNDC1, RAB7, FIS1
Autophagy	FUNDC1
Intracellular trafficking	MIRO
Unfolded protein response (UPR)	GRP75, MFN2, VAPB, BIP, Calnexin, Calreticulin, ERp57/44, Sig1R
Inflammasome formation	NLRP3, ASC, TXNIP

IP₃R: inositol 1,4,5-trisphosphate receptor. PKB/AKT: protein kinase B. PP2A: protein phosphatase 2A. PML: promyelocytic leukemia protein. MFN2: mitofusin 2. FUNDC1: FUN14 domain-containing 1. Sig1R: sigma 1 receptor. DRP1: dynamin-related protein 1. GRP75: 75 kDa glucose-regulated protein. VAPB: resident vesicle-associated membrane protein-associated protein B. NLRP3: pyrin domain-containing 3 protein. ASC: apoptosis-associated speck-like protein containing a caspase recruitment domain. TXNIP: thioredoxin-interacting protein. RyR: ryanodine receptor. PDZD8: SMP domain-containing protein. GSK3β: glycogen synthase kinase-3 beta. CypD: cyclophilin D. SERAC1: serine active site containing 1. FAC1: palmitoyl-CoA ligase. FATP4: fatty acid transport protein 4. PEMT2: phosphatidylethanolamine N-methyltransferase 2. RAB7, Ras-related protein Rab-7. FIS1: mitochondrial fission 1 protein.