*Review Article*

# Mitochondria-Endoplasmic Reticulum Contacts: The Promising Regulators in Diabetic Cardiomyopathy

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Diabetic cardiomyopathy (DCM), as a serious complication of diabetes, causes structural and functional abnormalities of the heart and eventually progresses to heart failure. Currently, there is no specific treatment for DCM. Studies have proved that mitochondrial dysfunction and endoplasmic reticulum (ER) stress are key factors for the development and progression of DCM. The mitochondria-associated ER membranes (MAMs) are a unique domain formed by physical contacts between mitochondria and ER and mediate organelle communication. Under high glucose conditions, changes in the distance and composition of MAMs lead to abnormal intracellular signal transduction, which will affect the physiological function of MAMs, such as alter the $Ca^{2+}$ homeostasis in cardiomyocytes, and lead to mitochondrial dysfunction and abnormal apoptosis. Therefore, the dysfunction of MAMs is closely related to the pathogenesis of DCM. In this review, we summarized the evidence for the role of MAMs in DCM and described that MAMs participated directly or indirectly in the regulation of the pathophysiological process of DCM via the regulation of $Ca^{2+}$ signaling, mitochondrial dynamics, ER stress, autophagy, and inflammation. Finally, we discussed the clinical transformation prospects and technical limitations of MAMs-associated proteins (such as MFN2, FUNDC1, and GSK3$\beta$) as potential therapeutic targets for DCM.

## 1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by insulin resistance, hyperglycemia, and hyperlipidemia. DM-related complications affect multiple organs. DM causes cardiomyopathy and increases the risk of heart failure, independent of the traditional risk factors, such as hypertension, coronary artery disease, and valvular heart disease, which is clinically known as diabetic cardiomyopathy (DCM) [1]. DCM is a common complication of DM with a poor outcome [2]. The development of DCM is a gradual process. Early-stage DCM is characterized by structural and functional abnormalities, including cardiac hypertrophy, increased myocardial stiffness, myocardial fibrosis, and diastolic dysfunction. As DCM progresses, these abnormalities may lead to heart failure [3]. Effective therapeutic measures for DCM, especially specific therapeutic drugs, are lacking.

The pathophysiology of DCM is multifactorial. Several pathophysiological mechanisms, including hyperglycemia, energy metabolism disturbances, and inflammation, have been proposed. In addition, mitochondrial dysfunction and ER stress are known to be crucial factors in the development and progression of DCM [3] [4]. Both mitochondria and the ER are vital organs in cells, with the functions of mitochondria and the ER highly interconnected. Mitochondria are highly dynamic structures, with their morphologies and compositions constantly changing to meet cellular requirements [5]. They are connected to membranous organelles, including the ER. The contact sites between mitochondria and the ER are known as mitochondria-associated ER membranes (MAMs). MAMs are unique domains that mediate the tight connection between mitochondria and the ER [6]. MAMs serve as the essential hub for intraorganelle communication in cells.

Dysregulation of MAMs contributes to the etiology of many diseases. An increasing amount of data suggests that MAMs play significant roles in pathological conditions, including neurodegenerative disorders [7], metabolic diseases [8], and cardiovascular diseases [9], by regulating intraorganelle lipid exchange, calcium ($Ca^{2+}$) transfer, mitochondrial dynamics, ER stress, inflammation, autophagy, and apoptosis [10] and that these processes are involved in DCM. As such, MAMs are closely related to the pathophysiological mechanism of DCM.

In DM, destruction of the mitochondria-ER contacts in cardiomyocytes leads to dysfunction of several molecular pathways, thereby inducing cell death and cardiac dysfunction [11]. For example, knockout of MAM components such as Mitofusin 2 (MFN2) [12], inositol 1,4,5-triphosphate receptors (IP3R1), or cyclophilin D (CypD) [13] will interrupt MAM integrity and induce insulin resistance. The latter is closely related to mitochondrial dysfunction, ER stress, and altered $Ca^{2+}$ homeostasis in DCM [11, 14]. Excessive mitochondria-ER coupling also has adverse effects, with recent research showing that enhancement of the MAM formation is involved in metabolic pathologies, such as insulin resistance and diabetes [15]. Therefore, a moderate but not excessive level of MAM formation is necessary for cell function.

We hypothesized that MAMs may be the promising regulator in the physiopathology of DCM. In the present review, we first introduce the structure and components of MAMs. We then we discuss the role of MAMs in DCM and summarize their emerging clinical use as potential biomarkers and therapeutic targets for DCM.

## 2. Structure of Mitochondria-ER Contacts

In 1990, Vance et al. described the components of membrane coupling between the ER and mitochondria in rat liver [16]. Since then, researchers have used a combination of electron microscopy and cell fluorescence microscopy to reveal the microstructure of MAMs. Using electronic tomography, researchers showed that the ER and mitochondria are connected by tethers and that the distance between the ER and mitochondria is approximately 25 nm for rough ER and approximately 10 nm for smooth ER [17]. Observations using a wide-field digital 3D deconvolution microscope showed that about 20% of the ER is in direct contact with the mitochondrial surface in MAMs [18].

It is important to consider the frequency and spacing between mitochondria and the ER in MAMs because they are dynamic structures, and the contacts and distance between mitochondria and the ER can vary widely under different cellular physiological conditions. The dynamic and flexible characteristics of MAMs results in a highly variable MAM composition. A proteomic study of MAM components showed that as many as 1212 proteins are localized in MAMs [19]. After excluding contaminated samples, approximately 75 proteins, including sorting proteins, chaperone proteins, and protein kinases, were shown to be associated with MAMs [20, 21].

MAMs have a unique structure, with a large number of proteins with various functions (Table 1). Figure 1 shows the key tethering protein complexes between mitochondria and the ER in mammalian cells (Figure 1). (I) The inositol 1,4,5-triphosphate receptor-glucose-regulated protein 75-voltage-dependent anion-selective channel 1 (IP3R-GRP75-VDAC1) complex is composed of IP3Rs in the ER and VDAC1 at the outer mitochondrial membrane (OMM). GRP75 is a chaperone. It bridges the IP3R and VDAC1 to maintain conformational stability and forms an ER-mitochondrial $Ca^{2+}$ tunnel [22]. (II) MFN2 ethers the ER to mitochondria by forming heterotypic or homotypic complexes with MFN1 or MFN2 on the mitochondrial surface. These complexes mediate mitochondrial dynamics and mitochondrial $Ca^{2+}$ uptake [23]. (III) The vesicle-associated membrane protein-associated protein B-protein tyrosine phosphatase interacting protein 51 (VAPB-PTPIP51) complex consists of the OMM protein PTPIP51 and the ER resident protein VAPB, which maintains ER-mitochondrial $Ca^{2+}$ homeostasis [24]. (IV) The B-cell receptor-associated protein 31-mitochondrial fission 1 (BAP31-FIS1) complex is formed via the interaction of ER-localized BAP31 and mitochondrial FIS1, which participates in mitochondrial dynamics and cell apoptosis [25]. (V) Recently, another important protein bridge was reported, with the bridge formed through the interaction of the OMM human protein FUN14 domain-containing 1 (FUNDC1) with the IP3R2, which modulates the $Ca^{2+}$ transport in the ER into mitochondria and maintains mitochondrial dynamics [26]. Various regulators modulate these tether complexes. For example, the IP3Rs-GRP75-VDAC1 complex is regulated by CypD, Sigma-1 receptor (Sig-1R), glycogen synthase kinase-3$\beta$ (GSK-3$\beta$) and pyruvate dehydrogenase kinase 4 [27, 28].

Abnormality in MAMs resident proteins and regulators may be associated with the pathology of various diseases. For example, the insulin signaling protein AKT is located in MAMs, interacting with promyelocytic leukemia (PML)-protein phosphatase 2A (PP2A)-IP3R to form a large molecular complex in MAMs and controlled ER $Ca^{2+}$ release [29]. Recently, a few of studies support that the presence of ATK regulate MAMs integrity [29–31]. Under DM condition or insulin resistance, an increased phosphorylation of AKT was found in the mice, which induces disruption of MAMs and may be the critical cause of $Ca^{2+}$ disorder and mitochondrial dysfunction in DCM. Additionally, the key MAM proteins, VDAC1, CypD, and PACS2, decreased significantly in insulin-resistant mice [30], which suggest that MAMs are overtly altered under DCM condition; targeted regulation of these MAMs is expected to alleviate the disease to a certain extent.

In the future, more tethers and regulatory proteins will be identified between the mitochondria and the ER, and studies are required to investigate the alteration of MAMs resident proteins contributing to the pathogenesis of DCM.

## 3. The Role of MAMs in DCM

In this section, we consider MAM proteins and their regulators that play central roles in ER-mitochondrial crosstalk

TABLE 1: Summary of the functional MAMs proteins.

| Functions | MAMs proteins | Relevant functions in MAMs | Reference |
|---|---|---|---|
| Ca$^{2+}$ transfer | IP3R1/2/3 | Interacts with VDAC via GRP75, a major actor in ER Ca$^{2+}$ release to mitochondria | [22] |
| | VDAC1 | Acts as a Ca$^{2+}$ uptake channel in the OMM | [22] |
| | GRP75 | Chaperone protein connects IP3R and VDAC to form VDAC1/GRP75/IP3R1 channel complex | [22] |
| | PTPIP51 | Interacts with VAPB at MAMs and regulates Ca$^{2+}$ homeostasis | [24] |
| | VAPB | Interacts with PTPIP51 at MAMs and regulates Ca$^{2+}$ homeostasis | [24] |
| | SERCA | Acts as an important pump involved in Ca$^{2+}$ transport into ER | [33] |
| | Sig-1R | Generates a chaperone complex with BiP/GRP78 and prolongs Ca$^{2+}$ signaling stabilizing subunit 3 of IP3R | [105] |
| | P53 | Regulates SERCA activity and modulates ER-mitochondrial transfer | [106] |
| | PML | Regulates Ca$^{2+}$ transfer and control apoptosis | [29] |
| | Calnexin | Interacts with SERCA, regulating Ca$^{2+}$ transfer between contact sites | [107] |
| | Cytc | Interacts with IP3Rs and regulate Ca$^{2+}$ | [108] |
| | Bcl-2 | Inhibit the opening of IP3Rs and downregulate IP3R-mediated Ca$^{2+}$ flux | [109] |
| | CYPD | A partner of the IP3R1-GRP75-VDAC1 complex and changes the MAM spatial structure | [30] |
| | mTORC2 | Regulates Ca$^{2+}$ signaling by Akt regulation | [110] |
| | PP2A | Recruited by PML and inactivates AKT, facilitates IP3R-mediated Ca$^{2+}$ release | [29] |
| | PTEN | PTEN regulates ER Ca2+ release through type 3 IP3R in a protein phosphatase-dependent manner | [111] |
| | Akt | Akt phosphorylates all IP3R isoforms and inhibits Ca$^{2+}$ release from the ER | [38] |
| | GSK3$\beta$ | Regulates organelle Ca$^{2+}$ exchange | [37] |
| | FUNDC1 | Binding of FUNDC1 to IP3R2 at the MAMs increases the Ca$^{2+}$ concentration in both cytosol and mitochondrial matrix | [26] |
| Mitochondrial dynamics | MFN2 | Forms dimers with either MFN1 or MFN2 located on the mitochondria, controls the mitochondrial fusion | [23] |
| | Bax | Interacts with MFN2 to promote mitochondrial fusion | [112] |
| | FUNDC1 | Interacts with OPA1 to promote mitochondrial fusion; promote mitochondrial fission under hypoxic condition | [46] |
| | DRP1 | Regulates mitochondrial fission | [18] |
| | INF2 | Drives initial mitochondrial constriction | [50] |
| | MFF | Recruits DRP1 and regulates mitochondrial fission | [113] |
| | FIS1 | Recruits DRP1 and regulates mitochondrial fission | [114] |
| | MiD49/51 | Recruits DRP1 and regulates mitochondrial fission | [55] |
| Autophagy | ATG14L | Acts as preautophagosome marker, induces autophagosome formation | [59] |
| | ATG5 | Acts as autophagosome marker | [59] |
| | PACS2 | Knocking down PACS2 decreases the number of autophagosomes | [115] |
| | MFN2 | Knocking down MFN2 decreases the number of autophagosomes | [115] |
| | VAPB | Regulates autophagy | [24] |
| | PTPIP51 | Forms a complex with VAPB to regulate autophagy | [24] |
| | BECLIN1 | Enhances the formation of MAMs and autophagosomes | [60] |
| | PINK1 | Promote ER-mitochondrial tethering and autophagosome formation | [60] |
| Inflammation | NLRP3 | NLRP3 inflammasome can be recruited to the MAM sites to sense mitochondrial damage | [70] |
| | ASC | The adaptor of NLRP3 | [70] |
| | TXNIP | TXNIP activates NRLP3 inflammasome activation under mitochondrial oxidative stress conditions | [116] |
| ER stress | PERK | Induces apoptosis after ROS-based ER stress | [117] |
| | IRE1$\alpha$ | Responses to UPR stimulation; IRE1$\alpha$ ubiquitylation at MAM hinder ER-stress-induced apoptosis | [118] |
| | MFN2 | Interacts with PERK and repress its activity | [78] |
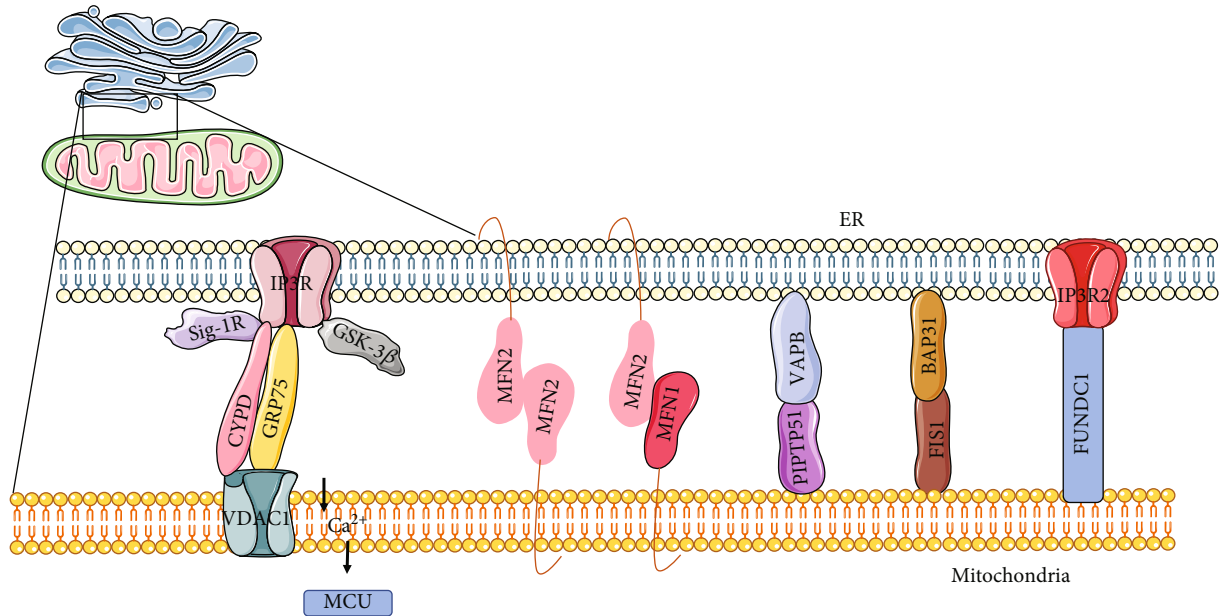
FIGURE 1: A core subset of mitochondria-ER tethering complexes in mammalian cells. IP3R, an ER protein, interacts OMM-localized protein VDAC1 via GRP75. The Sig-1R and GSK3$\beta$ interact with IP3Rs-GRP75-VDAC1 complex. MFN2 on the ER tethers the ER to mitochondria by forming complexes with MFN1 or MFN2 on the mitochondrial surface. An ER resident protein, VAPB interacts with the OMM protein PTPIP51.BAP31, an ER protein, interacts with the mitochondria FIS1. The ER resident IP3R2 interacts the mitochondrial protein FUNDC1. IP3R, inositol 1,4,5-triphosphate receptor; VDAC1, voltage-dependent anion-selective channel; GRP75, glucose-regulated protein 75; MFN2, Mitofusin 2; MFN1, Mitofusin 2; VAPB, vesicle-associated membrane protein-associated protein B; PTPIP51, protein tyrosine phosphatase interacting protein 51; BAP31, B-cell receptor-associated protein 31; FIS1, mitochondrial fission 1; FUNDC1, FUN14 domain containing 1.

(Table 1). In DM condition, destruction of MAMs integrity in cardiomyocytes directly or indirectly causes imbalances in several biological processes, which may explain the metabolic abnormalities in DCM.

3.1. MAMs Regulate $Ca^{2+}$ Transfer in DCM. Mitochondria and the ER (sarcoplasmic reticulum in muscle cells) are the main organelles that regulate $Ca^{2+}$ homeostasis in cells. Mitochondrial $Ca^{2+}$ uptake is dependent on close binding of MAM-mediated ER to mitochondria [32]. The sarcoplasmic reticulum (SR)/ER $Ca^{2+}$ ATPase (SERCA) is greatly enriched in MAMs, which is an ER membrane influx transporter through which $Ca^{2+}$ from the cytoplasm is transported to the ER [33]. $Ca^{2+}$ from the ER is transferred to mitochondria through MAMs [22] and then enters the mitochondrial matrix through the mitochondrial calcium uniporters (MCUs) [34]. In this process, the distance of the ER and mitochondria is a key parameter for $Ca^{2+}$ transport. At shorter distances, $Ca^{2+}$ transport is more efficient. Conversely, $Ca^{2+}$ transport becomes less efficient as the distance increases [35]. Among MAM tethering protein complexes, IP3R-GRP75-VDAC1 is the most important tethering complex mediating $Ca^{2+}$ efficient transfer in MAMs. The releases of $Ca^{2+}$ from the ER to the OMM through the IP3R-GRP75-VDAC1 channel leads to local inflow of $Ca^{2+}$ in the mitochondrial intermembrane space and the formation of a microdomain of high $Ca^{2+}$ close to MCUs, which facilitates the mitochondrial $Ca^{2+}$ uptake by MCU [36]. As shown in recent studies, the IP3R-GRP75-VDAC1 complex also acts

as a molecular scaffold for several other proteins, such as glycogen synthase kinase-3$\beta$ (GSK-3$\beta$) [37], protein kinase B (PKB)/Akt [38], and promyelocytic leukemia (PML) [29]. These proteins are crucial for the fine tuning of $Ca^{2+}$ signaling via IP3R-GRP75-VDAC1 axis (Table 1).

Due to its role in excitation-contraction coupling in muscle tissue, $Ca^{2+}$ signaling is crucial for heart function, and $Ca^{2+}$ disturbance mediated by destruction of the integrity of MAMs is closely associated with cardiac dysfunction in DCM. Interaction between several cellular $Ca^{2+}$ transporter complexes controls the diastolic and systolic functions of the myocardium. During diastole, myocardial relaxation occurs due to reuptake of $Ca^{2+}$ into the SR via SERCA2a [14]. In diabetes, reduced activity and expression of SERCA2a in cardiomyocytes result in altered $Ca^{2+}$ handling, which leads to impairment of left ventricular diastolic function and the development of DCM [39]. Disordered $Ca^{2+}$ transfer through MAMs is the crucial cause for DCM. In the early stage of DCM, a reduced formation of the IP3R-GRP75-VDAC1 $Ca^{2+}$ channeling complex and decreased IP3R-stimulated $Ca^{2+}$ transfer to mitochondria trigger mitochondrial dysfunction [40]. A decrease in the ER-mitochondrial $Ca^{2+}$ transfer leads to insufficient mitochondrial bioenergetics to match the energy demand for normal heart contraction [40]. However, inconsistent finding on the role of $Ca^{2+}$ transport in DCM has been reported. Hu et al. reported that a high glucose level enhances the connections between the SR and mitochondria and increases the efficiency of $Ca^{2+}$ transfer, which

causes $Ca^{2+}$ overload in mitochondria [41]. Wu's study showed that FUNDC1 is important in mediating MAMs formation in diabetes, with high glucose increasing FUNDC1 levels, IP3R2 levels, and MAMs formation, resulting in increased $Ca^{2+}$ levels, mitochondrial dysfunction, and deterioration of cardiac function [11]. High glucose increases MAMs-associated FUNDC1 levels by downregulating AMP-activated protein kinase (AMPK). And binding of FUNDC1 to the IP3R2 inhibits IP3R2 ubiquitination and proteasome-mediated degradation, which promotes contacts between the ER and mitochondria, resulting in increased $Ca^{2+}$ transport and a decline in the mitochondrial membrane potential [11]. These events trigger long-term mitochondrial permeability transition pore (mPTP) opening and cell apoptosis by promoting mitochondrial $Ca^{2+}$ uptake [42] (Figure 2(a)). We hypothesize that this inconsistent result of $Ca^{2+}$ homeostasis in DCM is due to different severities and stages of the disease.

According to the literature, MFN2 regulates $Ca^{2+}$ transfer in high glucose conditions, with high glucose upregulating the expression of MFN2, decreasing the distance between the ER and mitochondria, and increasing mitochondrial $Ca^{2+}$ uptake in atrial cardiomyocytes [43]. And knocking down MFN2 significantly disrupted ER-mitochondrial tethering and decreased the $Ca^{2+}$ transportation, thereby preventing mitochondrial dysfunction and cell death [43]. However, there is still controversy regarding the role of MFN2. Filadi et al. demonstrated that MFN2 ablation or silencing increased the ER-mitochondrial contacts, enhancing $Ca^{2+}$ transfer between the two organelles, with the function of MFN2 similar to that of a tethered antagonist [44]. The use of different physiological conditions, cell types, and experimental approaches may explain the discord in the literature on the role of MFN2 in DCM. However, the detailed mechanism of how MFN2 participates in the $Ca^{2+}$ transport in DCM is still unclear. In addition to mediating the spatial distance from the ER to mitochondria and affecting the transport efficiency of $Ca^{2+}$, it is interesting to explore whether it forms a complex with $Ca^{2+}$ channel proteins. The exact mechanism of MFN2 in DCM deserves to be further investigated.

### 3.2. MAMs Modulate Mitochondrial Dynamics in DCM.

Mitochondria are highly dynamic organelles, and balancing mitochondrial dynamics is essential to maintain heart function in response to metabolic or environmental stresses [5]. Various GTPases are involved in the regulation of mitochondrial dynamics, including dynamin-like GTPases optic atrophy 1 (OPA1), which plays a role in inner mitochondrial membrane (IMM) fusion, together with MFN1 [45]. FUNDC1 interacts with OPA1 to coordinate mitochondrial fusion [46]. Under normal physiological conditions, FUNDC1 anchor OPA1 to the inner surface of OMM through its charged lysine residue. Mitochondrial stresses disrupt the connection of FUNDC1 and OPA1 and induce OPA1 cleavage or even degradation, which promotes mitochondrial fission [46]. Extracellular hyperglycemia and metabolic dysregulation create an energy stress in DCM, which reduce the interaction of OPA1 and FUNDC1, increasing mitochondrial fragmentation. MFN1 and MFN2 are crucial for the fusion of

OMM [23], with deletion of MFN1 or MFN2 reduces the mitochondrial fusion rate [47]. In DCM, downregulation of MFN2 contributes to unbalanced mitochondrial dynamics and mitochondrial dysfunction [48]. The downregulation of MFN2 is partly attributed to the decreased expression of the peroxisome proliferator-activated receptor alpha (PPARα) caused by the lipid metabolism disorder in DCM [49] (Figure 2(b)). Reconstitution of MFN2 improves mitochondrial function by promoting mitochondrial fusion [48]. Accordingly, modulation of mitochondrial dynamics by regulating MFN2 might be a potentially effective target for DCM treatment.

Dynamin-related protein 1 (DRP1) plays a central role in mitochondrial fission [18]. DRP1 is a cytoplasmic protein that can be recruited from the cytosol to the OMM, which is a critical step in the fission process. Friedman et al. showed that MAM is an important platform for mitochondrial fission. In their study, they demonstrated oligomerization and translocation of DRP1 to MAMs, where it induced fission events [18]. Subsequent studies revealed the mechanism that MAMs participate in initial mitochondria contraction. This mechanism involves ER-localized inverted formin 2 (INF2) inducing actin polymerization, which promotes MAMs formation, facilitating $Ca^{2+}$ transfer from ER to mitochondria, followed by IMM contraction and initial mitochondrial constriction. This is followed by DRP1-driven secondary constriction, which completes the fission process [50, 51].

In patients diagnosed with diabetes, myocardial contractile dysfunction is closely associated with mitochondria fission. In diabetes, increased expression of DRP1 initiates mitochondrial fission. Conversely, decreased expression of DRP1 decrease alleviates mitochondrial dysfunction and cardiac dysfunction [52]. Mechanically, lipid overload decreased $NAD^+$ levels and increased the acetylation of DRP1 at a specific lysine residue (K642). A DRP1 point mutation, K642E, appears to reverse the impact of lipid toxicity. Excessively activation of DRP1 results in DRP1 translocated to mitochondria, induces mPTP and apoptosis, and compromises cardiomyocyte contractile function via VDAC1 [53].

Several proteins have been shown to regulate DRP1 activity at MAMs. Under normal physiological condition, mitochondrial FIS1, mitochondrial fission factor, and mitochondrial dynamics proteins of 49/51 kDa have been reported to recruit DRP1 during mitochondrial fission [54, 55]. Regulating DRP1 activity by handing these molecules at contact site may be an appropriate strategy to prevent the abnormal mitochondrial fission and mitochondrial dysfunction-related DCM.

Despite the high abundance of fusion and fission regulatory proteins in the heart, mitochondria in adult cardiomyocytes exhibit static morphology and infrequent dynamic changes. These fusion and fission proteins may have functions beyond morphology regulation, or they may regulate cardiac function in DCM by regulating the mitochondrial dynamics of other cells in the heart, such as cardiac fibroblasts. As shown by Zhang et al. [56], they pointed to a novel noncanonical function of DRP1, in which DRP1 maintained or positively stimulated mitochondria respiration, biogenetics, and reactive
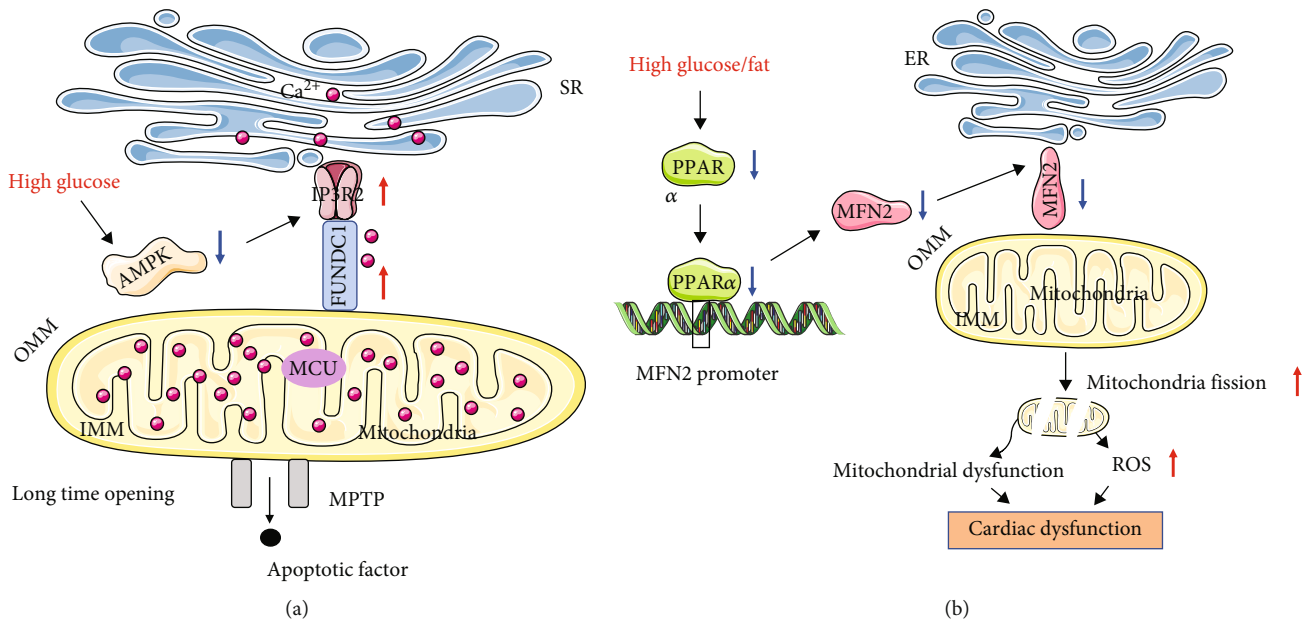
FIGURE 2: (a). The role of FUNDC1 and MFN2 in DCM. Under high glucose condition, FUNDC1 is upregulated by AMPK inactivation. The upregulated FUNDC1 increases MAMs formation by interacts with IP3R2. The increased MAMs contribute to the increased mitochondrial $Ca^{2+}$ uptake, which induce the long-time opening of mPTP and trigger cell apoptosis. (b). High glucose or high fat reduces the PPAR$\alpha$ expression. PPAR$\alpha$ directly binding to MFN2 promoter and decreases the expression of MFN2. The downregulation of MFN2 induces mitochondrial fission and leads to the ROS production, which cause mitochondrial dysfunction and cardiac dysfunction. MFN2, Mitofusin 2; FUNDC1, FUN14 domain containing 1; AMPK, AMP-activated protein kinase; IP3R2, inositol 1,4,5-triphosphate receptor 2; PPAR$\alpha$, peroxisome proliferator-activated receptor alpha.

oxygen species (ROS) signaling in adult cardiomyocytes, independent of morphological changes. In DCM, whether the fusion and fission proteins function through other mechanisms independent of mitochondrial dynamics remains to be elucidated.

3.3. MAMs Regulate Autophagy in DCM. Autophagy is an evolutionarily conserved lysosome-mediated degradation process that has fundamental roles in cellular homeostasis. Autophagosome and autolysosome formation are key processes in autophagy, which are mediated by autophagy-related genes (ATGs) [57, 58]. Autophagosome formation at MAMs in mammalian cells has been reported, with preautophagosome marker, autophagy-related 14-like, and the omegasome marker double FYV1 domain-containing protein1, localized in MAMs initiating autophagosome formation [59]. Another autophagosome formation marker, autophagy-related 2/5 (ATG2/5), also localizes at contact sites until the autophagy process is completed [59]. In addition, Beclin-1, a pro-autophagic protein localized in MAMs, plays a role in autophagosome formation and the autophagy process [60]. Inducing MAM dysfunction by knockout of MFN2 or PACS2 decreases the number of autophagosomes, and MFN2 deficiency impairs autophagosome-lysosome fusion [61]. Hu et al. have also shown that AMPK interacts directly with MFN2 to increase MAM numbers and induce autophagy [62]. Thus, these facts indicated that MAMs play key roles in the induction and execution of autophagy.

Autophagy is a double-edge sword, and basal autophagy is beneficial to the heart, whereas insufficient autophagy or

excessive autophagy may promote pathological cardiomyopathy [63]. Previous studies reported that cardiac autophagy was suppressed in diabetes, accompanied by decrease in ATG5 and Beclin-1 expression levels [64]. However, the role of autophagy in DCM is controversial, as another study pointed to an increase in cardiac autophagy in type 2 diabetes through a Beclin-1-mediated pathway [65]. This discord in the findings is likely related to the unresolved question of whether an accumulation of autophagosomes in cells is the result of upregulation of autophagy or blockade of autophagic flux. As the autophagy process is highly dynamic, quantification of autophagy becomes a challenge [66]. It is important to consider not only the number of intracellular autophagosomes but also the autophagic degradative activity and autophagic flux. As reported previously, increased autophagic flux alleviates diabetes-induced cardiac injury [67]. Thus, autophagic flux insufficiency, resulting in maladaptive cardiac remodeling, should be considered a pivotal pathology in in DCM [68].

3.4. MAMs Regulate Inflammasome in DCM. The crucial role of inflammation in the pathogenesis of DCM is widely recognized. Inflammasomes comprise innate immune system receptors and sensors, which are activated in response to cellular stress and trigger the maturation of proinflammatory cytokines and the immune response [69]. To date, NOD-like receptor pyrin domain-containing 3 (NLRP3) is the sole inflammasome reported to be associated with MAMs, and it comprises NLRP3 protein, the adapter apoptosis-associated speck-like protein containing a C-

terminal caspase recruitment domain (ASC) and pro-caspase-1. Under normal physiological conditions, NLRP3 is localized in the cytosol. When inflammasome is activated, the NLRP3 protein and its adaptor ASC are recruited to MAMs and are activated by MAM-derived effectors [70]. The NLRP3 inflammasome is activated by saturated fatty acids, ceramides, modified low density lipoprotein, and hyperglycemia in obesity and type 2 diabetes [71]. Continuous activation of inflammasomes ultimately leads to cardiac dysfunction [72]. In DCM, an early inflammatory response occurs as a protective mechanism against hyperglycemia. If hyperglycemia continues, a chronic inflammatory response will eventually lead to cardiomyocyte hypertrophy, apoptosis, and myocardial fibrosis [73].

The NLRP3 inflammasome recognizes signs of cellular stress, such as mitochondrial ROS production and $Ca^{2+}$ signaling from damaged cells. Sustained influx of $Ca^{2+}$ into the mitochondria via MAMs triggers mPTP opening, releasing risk-associated molecular patterns, finally leading to the activation of the NLRP3 inflammasom [70]. Yin et al. suggested that ROS could promote NLRP3 inflammasome activation [74]. Zhou et al. showed that mitophagy/autophagy blockade led to the accumulation of ROS, which activated the NLRP3 inflammasome [70]. Therefore, mitochondrial ROS and $Ca^{2+}$ signaling pathways in MAMs are closely bound up with inflammasome activity. Based on the literature, $Ca^{2+}$ communication between mitochondria and the ER may link MAMs to NLRP3 inflammasome activation in DCM. More research is needed to investigate the link of MAMs and inflammasome in DCM.

*3.5. MAMs Regulate ER Stress in DCM.* The ER plays a vital role in proteins folding. Disruption of ER homeostasis leads to an accumulation of misfolded proteins in the lumen, which triggers unfolded protein response (UPR) and ER stress. The stimulation of the UPR is sensed predominantly by three transmembrane proteins, protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring protein 1 (IRE1$\alpha$), and activating transcription factor 6 (ATF6), which regulate the protein folding ability of ER [75]. Substantial evidence suggests that ER stress is a key mechanism in the development and progression of DCM. Continuous activation of the UPR mediates upregulation of apoptosis-related gene expression by affecting mitochondrial function, which eventually leads to apoptosis of cardiomyocytes and deterioration of cardiomyopathy.

IRE1$\alpha$ is expressed mainly in the MAMs, where it binds to the Sigma-1 receptor (Sig1R). Our previous study reported that the IRE1 pathway mediated the stimulatory effect of Sig1R on cardiac fibroblast activation [76]. The PERK signaling pathway plays a key role in ROS-mediated ER stress in DCM [77]. Previous research showed that MFN2 physically interacts with PERK and negatively regulates its activity [78]. Gao et al. indicated that with the aggravation of oxidative stress injury in DM, the cardiac MFN2 mRNA level decreased [79]. A recent study revealed that in high glucose (HG)-induced podocytes, HG activated the PERK pathway by downregulating MFN2 expression and reducing MFN2-PERK interaction [80]. Therefore, we spec-

ulate that the downregulation of MFN2 in cardiomyocytes under high glucose environment may lead to dissociation and continuous activation of PERK, which subsequently initiates UPR and ER stress.

# 4. MAMs Targeting as a Potential Therapeutic Strategy

*4.1. Current Drugs in Treatment of DCM.* Metformin is widely used to treat type 2 diabetes. A large-scale prospective study of 1,519 type 2 diabetes mellitus (T2DM) patients with heart failure indicated that treatment with metformin reduces cardiovascular mortality in this patient population [81]. In a preclinical study that examined the effect of metformin treatment for 4 months in type 1 diabetes mice, the authors observed clear improvements in the systolic and diastolic function of the heart [82].

The major mechanism of action of metformin is activation of AMPK [83]. In this review, we focus mainly on metformin's therapeutic effect on DCM through the MAMs proteins-related pathways. The NLRP3 inflammasome is activated in DCM, and AMPK inhibits NLRP3 expression by initiating autophagy. In DCM, metformin exerts cardioprotective and anti-inflammatory effects by activating AMPK autophagy pathway and inhibiting the NLRP3 inflammasome [53]. AKT and GSK3$\beta$ are essential MAM components. Yang et al. showed that metformin ameliorates high glucose-induced cardiac damage through the AKT-GSK3$\beta$ pathway in high glucose-exposed cardiomyocytes and animal models of diabetes [84]. Combined treatment with metformin and atorvastatin attenuated DCM by inhibiting oxidative stress and the impression of inflammation-related proteins, such as NLRP3, caspase-1, and 1L-1$\beta$. Combination therapy also restrained the apoptosis of cardiomyocytes by decreasing the expression of pro-apoptotic related proteins, including caspase-3 and BAX [85].

Sodium-glucose co-transporter 2 (SGLT2) inhibitors, such as empagliflozin, dapagliflozin, and canagliflozin, also serves as potential antidiabetic agents. In a cardiac magnetic resonance imaging study of changes in cardiac structure and function in 25 patients with T2DM, 6-month treatment with empagliflozin lowered left ventricle end diastolic volume compared to that in a control group [86]. Empagliflozin also suppressed oxidative stress and fibrosis by activating Nrf2/ARE signaling, as well as inactivating the TGF-$\beta$/SMAD pathway [87]. In 37 patients (25 males and 12 females) with T2DM, 32% of whom had pre-existing cardiovascular diseases, a 3-month treatment with canagliflozin significantly improved the left ventricular diastolic function [88]. Treatment with dapagliflozin for 6 months in 58 T2DM patients with stable heart failure appeared to have beneficial effects on left ventricle diastolic function [89]. Dapagliflozin reduced NLRP3/ASC inflammasome activation and activated AMPK both *in vivo* and *in vitro*. Attenuation of the NLRP3 inflammasome activation depends on AMPK activation [90]. In addition to activating AMPK, dapagliflozin has been identified activating mTOR [91]. Large-scale clinical trials aiming at investigating the impact of SGLT2 inhibitors on DCM are needed.

The glucagon-like peptide-1 receptor (GLP-1R) agonist is used to treat advanced stage T2DM. Preclinical studies have indicated that GLP-1R agonists, including exendin-4 and liraglutide, induce robust cardio protection [92, 93]. Exendin-4 relieves mitochondrial oxidative stress in diabetic heart. In models of T2DM models, the MFN1/MFN2 ratio was reduced in exendin-4-treated group [94]. Exendin-4 and liraglutide also protected cardiomyocytes under high glucose condition. High glucose incubation of cardiomyocytes significantly upregulated the expression of pro-apoptotic factor, but exendin and liraglutide abrogated this effect. These two drugs activate autophagy through the classical mTOR/ULK1-dependent pathway [95]. Younce et al. reported that exendin-4 attenuated high glucose-induced cardiomyocyte apoptosis in association with decreased ER stress and enhanced the activity of SERCA2a [96]. In addition, lipid regulation of GLP-1R agonists may improve diastolic function and attenuate diabetic cardiomyopathy. Wu et al. showed that exendin-4 improved the structure and function of diabetic hearts by inhibiting PPAR$\alpha$-mediated lipid accumulation and toxicity regulated by the PKA/ROCK pathway [97].

Melatonin is widely used to treat insomnia and sleep disorders. Notably, the cardioprotective effects of melatonin have been described [98, 99]. Recent research confirms that melatonin acts as a regulator of MAMs. Application of melatonin inhibits IP3R, stabilizing the physical contacts between mitochondria and ER, and thus improving mitochondrial function and reducing cardiomyocytes damage. Moreover, some other MAM markers, Fis1, BAP31, and MFN2, were also inhibited by melatonin [100]. These data suggest that melatonin-induced cardioprotective effect is mediated via normalization of mitochondria-ER interaction.

*4.2. Potential Targets.* MFN2 was originally thought to be a mitochondrial protein that mediated OMM fusion. Recent research suggests that MFN2 appears to have a variety of roles in a nonfusion way. For example, MFN2 tether the ER to the mitochondria for $Ca^{2+}$ signaling from the ER to mitochondria. MFN2 ablation or reduction increases the physical distance between mitochondria and the ER and disturb mitochondrial $Ca^{2+}$ uptake [23, 44]. MFN2 is also involved in the cardiac autophagic process. Deficiency of MFN2 impairs autophagosome-lysosome fusion and leads to cardiac vulnerability and dysfunction [61]. Hu demonstrated that MFN2 expression is reduced in diabetic hearts, resulting in excessive mitochondrial fission, leading to mitochondrial dysfunction in DCM [48]. Reconstitution of MFN2 promotes mitochondrial fusion and alleviated mitochondrial dysfunction, consequently inhibiting the development of DCM [48]. Taken together, these findings suggest that regulation of MFN2 might be a potentially effective strategy for DCM treatment.

FUNDC1, an OMM protein, is highly conserved across species from drosophila to humans and highly expressed in cardiac muscle [26]. Diabetes increases FUNDC1 expression and aberrant MAM formation in cardiomyocytes, resulting in an increase in mitochondrial $Ca^{2+}$ levels, mitochondrial dysfunction, and cardiac dysfunction. Cardiac-specific dele-

tion of FUNDC1 improves mitochondrial function and attenuates cardiomyopathy in diabetic mice, confirming the causative role of FUNDC1 in this disorder [11]. However, there is some controversy regarding the role FUNDC1. According to Ren et al., FUNDC1 deficiency accentuated high fat diet-induced cardiac anomalies, including cardiac remodeling and intracellular $Ca^{2+}$ mishandling [101]. The discord in the findings may be due to differences in the expression of FUNDC1 in different stages of DCM disease progression. As mentioned above, the development of DCM is a gradual process. During this process, it is likely that FUNDC1 expression may first increased and then decreased or vice versa. In conditions like DCM, where the disease progresses over time, the findings of these two studies may not necessarily be contradictory. Despite the discord in the findings, both studies prove that FUNDC1 is an extremely important regulator in DCM. FUNDC1 also interacts with OPA1 to coordinate mitochondrial fusion under normal conditions [46]. We speculate that extracellular hyperglycemia and metabolic dysregulation in DCM might reduce the interaction of OPA1 and FUNDC1, thereby increasing mitochondrial fragmentation. These findings collectively support the unique role of FUNDC1 as a powerful therapeutic target in DCM.

GSK3$\beta$ is a multifunctional kinase. A fraction of GSK3$\beta$ is localized to the MAM in the heart, where it specifically interacts with the IP3R $Ca^{2+}$ channeling complex in MAMs. Apoptosis of cardiomyocytes in DCM involves overexpression of GSK3$\beta$ [102]. Pharmacological or genetic inhibition of GSK3$\beta$ may decrease the cardiomyocytes apoptosis of T2DM patients.

## 5. Conclusions

MAMs connect two important organelles (the ER and mitochondria), which have important roles in cellular functions. The importance of the contacts between the ER and mitochondria in the pathogenesis of DCM has been recognized. Several MAM-related proteins participate directly or indirectly in the regulation of the pathophysiological process of DCM via the regulation of lipids synthesis, insulin signaling, $Ca^{2+}$ signaling, mitochondrial dynamics, ER stress, autophagy, and inflammation. Targeting MAMs could lead to the development of more efficient pharmacological approaches and potential biomarkers for the treatment of DCM.

Currently, various existing antidiabetic drugs, such as metformin, SGLT2 inhibitors, and GLP-1 agonists, provide significant cardiovascular protection in both animal models and patients with DCM, and these drugs represent the primary treatment options for patients with DCM. Several MAM proteins, such as MFN2, FUNDC1, and GSK3$\beta$, have been identified that play key roles in DCM. These proteins may have clinical applications as therapeutic targets and intervention strategies in DCM. In addition, several herbal compounds are known to regulate MAM and thus might have potential in DCM treatment. For example, Shengmai injection, a tradition Chinese herbal medicine extracted from Panax ginseng C.A. Mey., *Ophiopogon japonicus* (Thunb.) Ker Gawl., and *Schisandra chinensis* (Turcz.) Baill.,

can activate the AMPK signaling pathway [103]. As AMPK regulates FUNDC1 expression, targeting AMPK may provide a cardioprotective effect. Moreover, obacunone, a natural bioactive compound isolated from the Rutaceae family, downregulates the activity of GSK-3$\beta$, which may target on MAMs to stabilize the mitochondrial membrane potential [104]. Taken together, these findings provide strong evidence that MAMs may be the crucial target of DCM.

There are a number of unanswered questions surrounding the potential of MAMs as therapeutic targets in DCM. First, the pathways or mechanisms of some MAM proteins in DCM have not been clearly explained, even some contradictory views appeared in different researches. Second, the cell types and intervention methods in addition to the carriers, dosages, and targeted treatment sites differ from different researches. Third, current research is limited to *in vitro* cell experiments and animal experiments, and clinical studies are some ways off yet. Uniform research standards and intervention methods are necessary to further explore the functional mechanisms, clinical efficacy, and long-term effects of MAMs in DCM. In the future, more tether proteins will likely be identified between the ER and mitochondria. Further studies are required to shed light on how changes in activity of MAMs resident proteins contribute to the pathogenesis of DCM and how these proteins are expected to be clinically attractive therapeutic strategies.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Yan Chen drafted and proofread the manuscript. Yanguo Xin edited the manuscript. Xiaojing Liu determined the theme and innovation. Yue Cheng revised the manuscript. All authors have agreed upon the submission and publication of this work. Yan Chen and Yanguo Xin contributed equally to this work.

## Acknowledgments

## References

[1] T. J. Regan, M. M. Lyons, S. S. Ahmed et al., "Evidence for cardiomyopathy in familial diabetes mellitus," *The Journal of Clinical Investigation*, vol. 60, no. 4, pp. 884–899, 1977.

[2] B. Bozkurt, D. Aguilar, A. Deswal et al., "Contributory risk and management of comorbidities of hypertension, obesity, diabetes mellitus, hyperlipidemia, and metabolic syndrome in chronic heart failure: a scientific statement from the American Heart Association," *Circulation*, vol. 134, no. 23, pp. e535–e578, 2016.

[3] G. Jia, M. A. Hill, and J. R. Sowers, "Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity," *Circulation Research*, vol. 122, no. 4, pp. 624–638, 2018.

[4] R. H. Eckel, K. E. Bornfeldt, and I. J. Goldberg, "Cardiovascular disease in diabetes, beyond glucose," *Cell Metabolism*, vol. 33, no. 8, pp. 1519–1545, 2021.

[5] R. J. Youle and A. M. van der Bliek, "Mitochondrial fission, fusion, and stress," *Science*, vol. 337, no. 6098, pp. 1062–1065, 2012.

[6] R. Filadi, P. Theurey, and P. Pizzo, "The endoplasmic reticulum-mitochondria coupling in health and disease: molecules, functions and significance," *Cell Calcium*, vol. 62, pp. 1–15, 2017.

[7] S. Paillusson, R. Stoica, P. Gomez-Suaga et al., "There's something wrong with my MAM; the ER-mitochondria axis and neurodegenerative diseases," *Trends in Neurosciences*, vol. 39, no. 3, pp. 146–157, 2016.

[8] E. Tubbs and J. Rieusset, "Metabolic signaling functions of ER-mitochondria contact sites: role in metabolic diseases," *Journal of Molecular Endocrinology*, vol. 58, no. 2, pp. R87–r106, 2017.

[9] P. Gao, Z. Yan, and Z. Zhu, "Mitochondria-associated endoplasmic reticulum membranes in cardiovascular diseases," *Frontiers In Cell And Developmental Biology*, vol. 8, article 604240, 2020.

[10] G. Csordás, D. Weaver, and G. Hajnóczky, "Endoplasmic reticulum-mitochondrial contactology: structure and signaling functions," *Trends in Cell Biology*, vol. 28, no. 7, pp. 523–540, 2018.

[11] S. Wu, Q. Lu, Y. Ding et al., "Hyperglycemia-driven inhibition of AMP-activated protein kinase $\alpha$2 induces diabetic cardiomyopathy by promoting mitochondria-associated endoplasmic reticulum membranes in vivo," *Circulation*, vol. 139, no. 16, pp. 1913–1936, 2019.

[12] D. Sebastián, M. I. Hernández-Alvarez, J. Segalés et al., "Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 14, pp. 5523–5528, 2012.

[13] J. Rieusset, J. Fauconnier, M. Paillard et al., "Disruption of calcium transfer from ER to mitochondria links alterations of mitochondria-associated ER membrane integrity to hepatic insulin resistance," *Diabetologia*, vol. 59, no. 3, pp. 614–623, 2016.

[14] L. T. Al Kury, "Calcium homeostasis in ventricular myocytes of diabetic cardiomyopathy," *Journal Diabetes Research*, vol. 2020, article 1942086, 12 pages, 2020.

[15] A. P. Arruda, B. M. Pers, G. Parlakgül, E. Güney, K. Inouye, and G. S. Hotamisligil, "Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity," *Nature Medicine*, vol. 20, no. 12, pp. 1427–1435, 2014.

[16] J. E. Vance, "Phospholipid synthesis in a membrane fraction associated with mitochondria," *The Journal of Biological Chemistry*, vol. 265, no. 13, pp. 7248–7256, 1990.

[17] G. Csordaas, C. Renken, P. Varnai et al., "Structural and functional features and significance of the physical linkage between ER and mitochondria," *The Journal of Cell Biology*, vol. 174, no. 7, pp. 915–921, 2006.

[18] J. R. Friedman, L. L. Lackner, M. West, J. R. DiBenedetto, J. Nunnari, and G. K. Voeltz, "ER tubules mark sites of

mitochondrial division," *Science*, vol. 334, no. 6054, pp. 358–362, 2011.

[19] C. N. Poston, S. C. Krishnan, and C. R. Bazemore-Walker, "In-depth proteomic analysis of mammalian mitochondria-associated membranes (MAM)," *Journal of Proteomics*, vol. 79, pp. 219–230, 2013.

[20] A. Raturi and T. Simmen, "Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM)," *Biochimica et Biophysica Acta*, vol. 1833, no. 1, pp. 213–224, 2013.

[21] C. Li, L. Li, M. Yang, L. Zeng, and L. Sun, "PACS-2: a key regulator of mitochondria-associated membranes (MAMs)," *Pharmacological Research*, vol. 160, article 105080, 2020.

[22] G. Szabadkai, K. Bianchi, P. Várnai et al., "Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels," *The Journal of Cell Biology*, vol. 175, no. 6, pp. 901–911, 2006.

[23] O. M. de Brito and L. Scorrano, "Mitofusin 2 tethers endoplasmic reticulum to mitochondria," *Nature*, vol. 456, no. 7222, pp. 605–610, 2008.

[24] P. Gomez-Suaga, S. Paillusson, R. Stoica, W. Noble, D. P. Hanger, and C. C. J. Miller, "The ER-mitochondria tethering complex VAPB-PTPIP51 regulates autophagy," *Current Biology*, vol. 27, no. 3, pp. 371–385, 2017.

[25] R. Iwasawa, A. L. Mahul-Mellier, C. Datler, E. Pazarentzos, and S. Grimm, "Fis 1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction," *Embo Journal*, vol. 30, no. 3, pp. 556–568, 2011.

[26] S. Wu, Q. Lu, Q. Wang et al., "Binding of FUN14 domain containing 1 with inositol 1,4,5-trisphosphate receptor in mitochondria-associated endoplasmic reticulum membranes maintains mitochondrial dynamics and function in hearts in vivo," *Circulation*, vol. 136, no. 23, pp. 2248–2266, 2017.

[27] T. Thoudam, C. M. Ha, J. Leem et al., "PDK4 augments ER-mitochondria contact to dampen skeletal muscle insulin signaling during obesity," *Diabetes*, vol. 68, no. 3, pp. 571–586, 2019.

[28] P. Gao, W. Yang, and L. Sun, "Mitochondria-associated endoplasmic reticulum membranes (MAMs) and their prospective roles in kidney disease," *Oxidative Medicine and Cellular Longevity*, vol. 2020, Article ID 3120539, 21 pages, 2020.

[29] C. Giorgi, K. Ito, H. K. Lin et al., "PML regulates apoptosis at endoplasmic reticulum by modulating calcium release," *Science*, vol. 330, no. 6008, pp. 1247–1251, 2010.

[30] E. Tubbs, P. Theurey, G. Vial et al., "Mitochondria-associated endoplasmic reticulum membrane (MAM) integrity is required for insulin signaling and is implicated in hepatic insulin resistance," *Diabetes*, vol. 63, no. 10, pp. 3279–3294, 2014.

[31] C. Betz, D. Stracka, C. Prescianotto-Baschong, M. Frieden, N. Demaurex, and M. N. Hall, "Feature article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 31, pp. 12526–12534, 2013.

[32] R. Rizzuto, P. Pinton, W. Carrington et al., "Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses," *Science*, vol. 280, no. 5370, pp. 1763–1766, 1998.

[33] D. M. Bers, "Cardiac excitation-contraction coupling," *Nature*, vol. 415, no. 6868, pp. 198–205, 2002.

[34] J. M. Baughman, F. Perocchi, H. S. Girgis et al., "Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter," *Nature*, vol. 476, no. 7360, pp. 341–345, 2011.

[35] G. Csordás, P. Várnai, T. Golenár et al., "Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface," *Molecular Cell*, vol. 39, no. 1, pp. 121–132, 2010.

[36] A. Bononi, S. Missiroli, F. Poletti et al., "Mitochondria-associated membranes (MAMs) as hotspot Ca2+ signaling units," *Advances in Experimental Medicine and Biology*, vol. 740, pp. 411–437, 2012.

[37] L. Gomez, P. A. Thiebaut, M. Paillard et al., "The SR/ER-mitochondria calcium crosstalk is regulated by GSK3 $\beta$ during reperfusion injury," *Cell Death and Differentiation*, vol. 23, no. 2, pp. 313–322, 2016.

[38] T. Szado, V. Vanderheyden, J. B. Parys et al., "Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca2+ release and apoptosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 7, pp. 2427–2432, 2008.

[39] A. Zarain-Herzberg, G. García-Rivas, and R. Estrada-Avilés, "Regulation of SERCA pumps expression in diabetes," *Cell Calcium*, vol. 56, no. 5, pp. 302–310, 2014.

[40] M. Dia, L. Gomez, H. Thibault et al., "Reduced reticulum-mitochondria Ca2+ transfer is an early and reversible trigger of mitochondrial dysfunctions in diabetic cardiomyopathy," *Basic Research in Cardiology*, vol. 115, no. 6, p. 74, 2020.

[41] X. Hu, T. Bai, Z. Xu, Q. Liu, Y. Zheng, and L. Cai, "Pathophysiological fundamentals of diabetic cardiomyopathy," *Comprehensive Physiology*, vol. 7, no. 2, pp. 693–711, 2017.

[42] S. Marchi and P. Pinton, "The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications," *The Journal of Physiology*, vol. 592, no. 5, pp. 829–839, 2014.

[43] M. Yuan, M. Gong, Z. Zhang et al., "Hyperglycemia induces endoplasmic reticulum stress in atrial cardiomyocytes, and mitofusin-2 downregulation prevents mitochondrial dysfunction and subsequent cell death," *Oxidative Medicine and Cellular Longevity*, vol. 2020, Article ID 6569728, 14 pages, 2020.

[44] R. Filadi, E. Greotti, G. Turacchio, A. Luini, T. Pozzan, and P. Pizzo, "Mitofusin 2 ablation increases endoplasmic reticulum-mitochondria coupling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 17, pp. E2174–E2181, 2015.

[45] S. Cipolat, O. Martins de Brito, B. Dal Zilio, and L. Scorrano, "OPA1 requires mitofusin 1 to promote mitochondrial fusion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 45, pp. 15927–15932, 2004.

[46] M. Chen, Z. Chen, Y. Wang et al., "Mitophagy receptor FUNDC1 regulates mitochondrial dynamics and mitophagy," *Autophagy*, vol. 12, no. 4, pp. 689–702, 2016.

[47] H. Chen, S. A. Detmer, A. J. Ewald, E. E. Griffin, S. E. Fraser, and D. C. Chan, "Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development," *The Journal of Cell Biology*, vol. 160, no. 2, pp. 189–200, 2003.

[48] L. Hu, M. Ding, D. Tang et al., "Targeting mitochondrial dynamics by regulating Mfn2 for therapeutic intervention in diabetic cardiomyopathy," *Theranostics*, vol. 9, no. 13, pp. 3687–3706, 2019.

[49] T. I. Lee, Y. H. Kao, Y. C. Chen, J. H. Huang, F. C. Hsiao, and Y. J. Chen, "Peroxisome proliferator-activated receptors modulate cardiac dysfunction in diabetic cardiomyopathy," *Diabetes Research and Clinical Practice*, vol. 100, no. 3, pp. 330–339, 2013.

[50] F. Korobova, V. Ramabhadran, and H. N. Higgs, "An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2," *Science*, vol. 339, no. 6118, pp. 464–467, 2013.

[51] R. Chakrabarti, W. K. Ji, R. V. Stan, S. J. de Juan, T. A. Ryan, and H. N. Higgs, "INF2-mediated actin polymerization at the ER stimulates mitochondrial calcium uptake, inner membrane constriction, and division," *The Journal of Cell Biology*, vol. 217, no. 1, pp. 251–268, 2018.

[52] M. Ding, N. Feng, D. Tang et al., "Melatonin prevents Drp1-mediated mitochondrial fission in diabetic hearts through SIRT1-PGC1α pathway," *Journal of Pineal Research*, vol. 65, no. 2, article e12491, 2018.

[53] Q. Hu, H. Zhang, N. Gutiérrez Cortés et al., "Increased Drp1 acetylation by lipid overload induces cardiomyocyte death and heart dysfunction," *Circulation Research*, vol. 126, no. 4, pp. 456–470, 2020.

[54] H. Otera, C. Wang, M. M. Cleland et al., "Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells," *The Journal of Cell Biology*, vol. 191, no. 6, pp. 1141–1158, 2010.

[55] C. S. Palmer, K. D. Elgass, R. G. Parton, L. D. Osellame, D. Stojanovski, and M. T. Ryan, "Adaptor proteins MiD49 and MiD51 can act independently of Mff and Fis1 in Drp1 recruitment and are specific for mitochondrial fission," *The Journal of Biological Chemistry*, vol. 288, no. 38, pp. 27584–27593, 2013.

[56] H. Zhang, P. Wang, S. Bisetto et al., "A novel fission-independent role of dynamin-related protein 1 in cardiac mitochondrial respiration," *Cardiovascular Research*, vol. 113, no. 2, pp. 160–170, 2017.

[57] C. A. Lamb, T. Yoshimori, and S. A. Tooze, "The autophagosome: origins unknown, biogenesis complex," *Nature Reviews. Molecular Cell Biology*, vol. 14, no. 12, pp. 759–774, 2013.

[58] Y. Liao, B. Duan, Y. Zhang, X. Zhang, and B. Xia, "ER-phagy results in ER stress, UPR, and cell death," *The Journal of Biological Chemistry*, vol. 294, no. 52, pp. 20009–20023, 2019.

[59] M. Hamasaki, N. Furuta, A. Matsuda et al., "Autophagosomes form at ER-mitochondria contact sites," *Nature*, vol. 495, no. 7441, pp. 389–393, 2013.

[60] V. Gelmetti, P. De Rosa, L. Torosantucci et al., "PINK1 and BECN1 relocalize at mitochondria-associated membranes during mitophagy and promote ER-mitochondria tethering and autophagosome formation," *Autophagy*, vol. 13, no. 4, pp. 654–669, 2017.

[61] T. Zhao, X. Huang, L. Han et al., "Central role of mitofusin 2 in autophagosome-lysosome fusion in cardiomyocytes," *The Journal of Biological Chemistry*, vol. 287, no. 28, pp. 23615–23625, 2012.

[62] Y. Hu, H. Chen, L. Zhang et al., "The AMPK-MFN2 axis regulates MAM dynamics and autophagy induced by energy stresses," *Autophagy*, vol. 17, no. 5, pp. 1142–1156, 2021.

[63] J. M. Bravo-San Pedro, G. Kroemer, and L. Galluzzi, "Autophagy and mitophagy in cardiovascular disease," *Circulation Research*, vol. 120, no. 11, pp. 1812–1824, 2017.

[64] M. H. Zou and Z. Xie, "Regulation of interplay between autophagy and apoptosis in the diabetic heart: new role of AMPK," *Autophagy*, vol. 9, no. 4, pp. 624–625, 2013.

[65] P. E. Munasinghe, F. Riu, P. Dixit et al., "Type-2 diabetes increases autophagy in the human heart through promotion of Beclin-1 mediated pathway," *International Journal of Cardiology*, vol. 202, pp. 13–20, 2016.

[66] K. C. Yang, P. Sathiyaseelan, C. Ho, and S. M. Gorski, "Evolution of tools and methods for monitoring autophagic flux in mammalian cells," *Biochemical Society Transactions*, vol. 46, no. 1, pp. 97–110, 2018.

[67] B. Wang, Q. Yang, Y. Y. Sun et al., "Resveratrol-enhanced autophagic flux ameliorates myocardial oxidative stress injury in diabetic mice," *Journal of Cellular and Molecular Medicine*, vol. 18, no. 8, pp. 1599–1611, 2014.

[68] J. Xie, K. Cui, H. Hao et al., "Acute hyperglycemia suppresses left ventricular diastolic function and inhibits autophagic flux in mice under prohypertrophic stimulation," *Cardiovascular Diabetology*, vol. 15, no. 1, p. 136, 2016.

[69] K. Schroder and J. Tschopp, "The inflammasomes," *Cell*, vol. 140, no. 6, pp. 821–832, 2010.

[70] R. Zhou, A. S. Yazdi, P. Menu, and J. Tschopp, "A role for mitochondria in NLRP3 inflammasome activation," *Nature*, vol. 469, no. 7329, pp. 221–225, 2011.

[71] H. Wen, D. Gris, Y. Lei et al., "Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling," *Nature Immunology*, vol. 12, no. 5, pp. 408–415, 2011.

[72] R. Zhou, A. Tardivel, B. Thorens, I. Choi, and J. Tschopp, "Thioredoxin-interacting protein links oxidative stress to inflammasome activation," *Nature Immunology*, vol. 11, no. 2, pp. 136–140, 2010.

[73] G. Frati, L. Schirone, I. Chimenti et al., "An overview of the inflammatory signalling mechanisms in the myocardium underlying the development of diabetic cardiomyopathy," *Cardiovascular Research*, vol. 113, no. 4, pp. 378–388, 2017.

[74] Y. Yin, Z. Zhou, W. Liu, Q. Chang, G. Sun, and Y. Dai, "Vascular endothelial cells senescence is associated with NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation via reactive oxygen species (ROS)/thioredoxin-interacting protein (TXNIP) pathway," *The International Journal of Biochemistry & Cell Biology*, vol. 84, pp. 22–34, 2017.

[75] S. Song, J. Tan, Y. Miao, and Q. Zhang, "Crosstalk of ER stress-mediated autophagy and ER-phagy: involvement of UPR and the core autophagy machinery," *Journal of Cellular Physiology*, vol. 233, no. 5, pp. 3867–3874, 2018.

[76] J. Qu, M. Li, D. Li et al., "Stimulation of sigma-1 receptor protects against cardiac fibrosis by alleviating IRE1 pathway and autophagy impairment," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 8836818, 25 pages, 2021.

[77] Z. W. Liu, H. T. Zhu, K. L. Chen et al., "Protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling pathway plays a major role in reactive oxygen species (ROS)-mediated endoplasmic reticulum stress-induced apoptosis in diabetic cardiomyopathy," *Cardiovascular Diabetology*, vol. 12, no. 1, article 158, 2013.

[78] J. P. Muñoz, S. Ivanova, J. Sánchez-Wandelmer et al., "Mfn2 modulates the UPR and mitochondrial function via

repression of PERK," *The EMBO Journal*, vol. 32, no. 17, pp. 2348–2361, 2013.

[79] Q. Gao, X. M. Wang, H. W. Ye et al., "Changes in the expression of cardiac mitofusin-2 in different stages of diabetes in rats," *Molecular Medicine Reports*, vol. 6, no. 4, pp. 811–814, 2012.

[80] Y. Cao, Z. Chen, J. Hu et al., "Mfn2 regulates high glucose-induced MAMs dysfunction and apoptosis in podocytes via PERK pathway," *Frontiers In Cell And Developmental Biology*, vol. 9, article 769213, 2021.

[81] "Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34)," *The Lancet*, vol. 352, no. 9131, pp. 854–865, 1998.

[82] S. P. Romero, J. L. Andrey, A. Garcia-Egido et al., "Metformin therapy and prognosis of patients with heart failure and new-onset diabetes mellitus. A propensity-matched study in the community," *International Journal of Cardiology*, vol. 166, no. 2, pp. 404–412, 2013.

[83] Z. Xie, K. Lau, B. Eby et al., "Improvement of cardiac functions by chronic metformin treatment is associated with enhanced cardiac autophagy in diabetic OVE26 mice," *Diabetes*, vol. 60, no. 6, pp. 1770–1778, 2011.

[84] Z. Yang, M. Wang, Y. Zhang et al., "Metformin ameliorates diabetic cardiomyopathy by activating the PK2/PKR pathway," *Frontiers in Physiology*, vol. 11, p. 425, 2020.

[85] W. Jia, T. Bai, J. Zeng et al., "Combined administration of metformin and atorvastatin attenuates diabetic cardiomyopathy by inhibiting inflammation, apoptosis, and oxidative stress in type 2 diabetic mice," *Frontiers in Cell and Developmental Biology*, vol. 9, article 634900, 2021.

[86] N. D. Cohen, S. J. Gutman, E. M. Briganti, and A. J. Taylor, "Effects of empagliflozin treatment on cardiac function and structure in patients with type 2 diabetes: a cardiac magnetic resonance study," *Internal Medicine Journal*, vol. 49, no. 8, pp. 1006–1010, 2019.

[87] C. Li, J. Zhang, M. Xue et al., "SGLT2 inhibition with empagliflozin attenuates myocardial oxidative stress and fibrosis in diabetic mice heart," *Cardiovascular Diabetology*, vol. 18, no. 1, p. 15, 2019.

[88] D. Matsutani, M. Sakamoto, Y. Kayama, N. Takeda, R. Horiuchi, and K. Utsunomiya, "Effect of canagliflozin on left ventricular diastolic function in patients with type 2 diabetes," *Cardiovascular Diabetology*, vol. 17, no. 1, p. 73, 2018.

[89] F. Soga, H. Tanaka, K. Tatsumi et al., "Impact of dapagliflozin on left ventricular diastolic function of patients with type 2 diabetic mellitus with chronic heart failure," *Cardiovascular Diabetology*, vol. 17, no. 1, p. 132, 2018.

[90] Y. Ye, M. Bajaj, H. C. Yang, J. R. Perez-Polo, and Y. Birnbaum, "SGLT-2 inhibition with dapagliflozin reduces the activation of the Nlrp3/ASC inflammasome and attenuates the development of diabetic cardiomyopathy in mice with type 2 diabetes. Further augmentation of the effects with saxagliptin, a DPP4 inhibitor," *Cardiovascular Drugs and Therapy*, vol. 31, no. 2, pp. 119–132, 2017.

[91] H. Chen, D. Tran, H. C. Yang, S. Nylander, Y. Birnbaum, and Y. Ye, "Dapagliflozin and ticagrelor have additive effects on the attenuation of the activation of the NLRP3 inflammasome and the progression of diabetic cardiomyopathy: an AMPK-mTOR interplay," *Cardiovascular Drugs and Therapy*, vol. 34, no. 4, pp. 443–461, 2020.

[92] M. Almutairi, K. Gopal, A. A. Greenwell et al., "The GLP-1 receptor agonist liraglutide increases myocardial glucose oxidation rates via indirect mechanisms and mitigates experimental diabetic cardiomyopathy," *The Canadian Journal of Cardiology*, vol. 37, no. 1, pp. 140–150, 2021.

[93] D. J. Drucker, "The cardiovascular biology of glucagon-like peptide-1," *Cell Metabolism*, vol. 24, no. 1, pp. 15–30, 2016.

[94] A. Monji, T. Mitsui, Y. K. Bando, M. Aoyama, T. Shigeta, and T. Murohara, "Glucagon-like peptide-1 receptor activation reverses cardiac remodeling via normalizing cardiac steatosis and oxidative stress in type 2 diabetes," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 305, no. 3, pp. H295–H304, 2013.

[95] W. Yu, W. Zha, and J. Ren, "Exendin-4 and liraglutide attenuate glucose toxicity-induced cardiac injury through mTOR/ULK1-dependent autophagy," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 5396806, 14 pages, 2018.

[96] C. W. Younce, M. A. Burmeister, and J. E. Ayala, "Exendin-4 attenuates high glucose-induced cardiomyocyte apoptosis via inhibition of endoplasmic reticulum stress and activation of SERCA2a," *American Journal of Physiology. Cell Physiology*, vol. 304, no. 6, pp. C508–C518, 2013.

[97] L. Wu, K. Wang, W. Wang et al., "Glucagon-like peptide-1 ameliorates cardiac lipotoxicity in diabetic cardiomyopathy via the PPARα pathway," *Aging Cell*, vol. 17, no. 4, article e12763, 2018.

[98] H. Zhou, Y. Yue, J. Wang, Q. Ma, and Y. Chen, "Melatonin therapy for diabetic cardiomyopathy: a mechanism involving Syk- mitochondrial complex I-SERCA pathway," *Cellular Signalling*, vol. 47, pp. 88–100, 2018.

[99] H. Zhou, Y. Zhang, S. Hu et al., "Melatonin protects cardiac microvasculature against ischemia/reperfusion injury via suppression of mitochondrial fission-VDAC1-HK2-mPTP-mitophagy axis," *Journal Of Pineal Research*, vol. 63, no. 1, p. e12413, 2017.

[100] W. Li, B. Liu, L. Wang, J. Liu, X. Yang, and J. Zheng, "Melatonin attenuates cardiac ischemia-reperfusion injury through modulation of IP3R-mediated mitochondria-ER contact," *Oxidative Medicine And Cellular Longevity*, vol. 2021, Article ID 1370862, 12 pages, 2021.

[101] J. Ren, M. Sun, H. Zhou et al., "FUNDC1 interacts with FBXL2 to govern mitochondrial integrity and cardiac function through an IP3R3-dependent manner in obesity," *Science Advances*, vol. 6, no. 38, 2020.

[102] W. Wu, X. Liu, and L. Han, "Apoptosis of cardiomyocytes in diabetic cardiomyopathy involves overexpression of glycogen synthase kinase-3β," *Bioscience Reports*, vol. 39, no. 1, 2019.

[103] Y. Li, X. Ruan, X. Xu et al., "Shengmai injection suppresses angiotensin II-induced cardiomyocyte hypertrophy and apoptosis via activation of the AMPK signaling pathway through energy-dependent mechanisms," *Frontiers in Pharmacology*, vol. 10, p. 1095, 2019.

[104] J. Zhou, T. Wang, H. Wang, Y. Jiang, and S. Peng, "Obacunone attenuates high glucose-induced oxidative damage in NRK-52E cells by inhibiting the activity of GSK-3β," *Biochemical and Biophysical Research Communications*, vol. 513, no. 1, pp. 226–233, 2019.

[105] T. Hayashi and T. P. Su, "Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate $Ca^{2+}$ signaling and cell survival," *Cell*, vol. 131, no. 3, pp. 596–610, 2007.

[106] C. Giorgi, M. Bonora, G. Sorrentino et al., "p53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner," *Proceedings of the National Academy of Sciences of the United States of America.*, vol. 112, no. 6, pp. 1779–1784, 2015.

[107] E. M. Lynes, M. Bui, M. C. Yap et al., "Palmitoylated TMX and calnexin target to the mitochondria-associated membrane," *The EMBO Journal*, vol. 31, no. 2, pp. 457–470, 2012.

[108] D. Boehning, R. L. Patterson, L. Sedaghat, N. O. Glebova, T. Kurosaki, and S. H. Snyder, "Cytochrome _c_ binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis," *Nature Cell Biology*, vol. 5, no. 12, pp. 1051–1061, 2003.

[109] H. Ivanova, L. E. Wagner 2nd, A. Tanimura et al., "Bcl-2 and IP3 compete for the ligand-binding domain of IP3Rs modulating Ca2+ signaling output," *Cellular and Molecular Life Sciences*, vol. 76, no. 19, pp. 3843–3859, 2019.

[110] M. S. Yoon, "The role of mammalian target of rapamycin (mTOR) in insulin signaling," *Nutrients*, vol. 9, no. 11, p. 1176, 2017.

[111] A. Bononi, M. Bonora, S. Marchi et al., "Identification of PTEN at the ER and MAMs and its regulation of $Ca^{2+}$ signaling and apoptosis in a protein phosphatase-dependent manner," *Cell Death and Differentiation*, vol. 20, no. 12, pp. 1631–1643, 2013.

[112] M. Karbowski, K. L. Norris, M. M. Cleland, S. Y. Jeong, and R. J. Youle, "Role of Bax and Bak in mitochondrial morphogenesis," *Nature*, vol. 443, no. 7112, pp. 658–662, 2006.

[113] S. Gandre-Babbe and A. M. van der Bliek, "The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells," *Molecular Biology of the Cell*, vol. 19, no. 6, pp. 2402–2412, 2008.

[114] D. Stojanovski, O. S. Koutsopoulos, K. Okamoto, and M. T. Ryan, "Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology," *Journal of Cell Science*, vol. 117, no. 7, pp. 1201–1210, 2004.

[115] D. W. Hailey, A. S. Rambold, P. Satpute-Krishnan et al., "Mitochondria supply membranes for autophagosome biogenesis during starvation," *Cell*, vol. 141, no. 4, pp. 656–667, 2010.

[116] X. Zhang, J. H. Zhang, X. Y. Chen et al., "Reactive oxygen species-induced TXNIP drives fructose-mediated hepatic inflammation and lipid accumulation through NLRP3 inflammasome activation," *Antioxidants & Redox Signaling*, vol. 22, no. 10, pp. 848–870, 2015.

[117] T. Verfaillie, N. Rubio, A. D. Garg et al., "PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress," *Cell Death and Differentiation*, vol. 19, no. 11, pp. 1880–1891, 2012.

[118] K. Takeda, S. Nagashima, I. Shiiba et al., "MITOL prevents ER stress-induced apoptosis by IRE1α ubiquitylation at ER-mitochondria contact sites," *The EMBO Journal*, vol. 38, no. 15, article e100999, 2019.