Review

Mitochondrial-Endoplasmic Reticulum Axis (MERA) in Pulmonary Hypertension: A Critical Inter-Organellar Crossroads for Pathogenesis and Therapeutic Targets

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Mitochondrial-Endoplasmic Reticulum Axis (MERA) in Pulmonary Hypertension: A Critical Inter-Organellar Crossroads for Pathogenesis and Therapeutic Targets

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Highlights

1. Mitochondria-associated endoplasmic reticulum (ER) membrane (MAM) governs mitochondrial dynamics, Ca^{2+} homeostasis, ER stress, oxidative stress, autophagy, apoptosis, lipid transport, and inflammation.
2. MAM may become a new and effective therapeutic target for pulmonary hypertension.

Abstract

Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized primarily by sustained pulmonary vasoconstriction, pulmonary vascular remodeling, and elevated pulmonary artery pressure. Recent emerging studies have indicated that mitochondria-endoplasmic reticulum (ER) membrane (MAM) is increasingly implicated in pathogenesis of pulmonary hypertension (PH). Dysfunction of mitochondria or ER can trigger a series of pathological processes, including disrupted intracellular Ca^{2+} homeostasis, oxidative stress, ER stress, and inflammation, which in turn drive dysfunction of pulmonary artery smooth muscle (PASMCs) and endothelial cells (PAECs). This review article aims to systematically evaluates the pathogenic mechanisms of the MAM in PH, covering the latest research advances in mitochondrial dynamic disorders, Ca^{2+} homeostasis, ER stress, oxidative stress, cellular metabolic reprogramming, and inflammatory responses. Special focus is placed on the structural and functional regulatory mechanisms of MAMs and their roles in pulmonary vascular functional and structural dysfunction. Furthermore, the current review also discusses the potential of new and efficacious therapeutic strategies targeting mitochondrial dysfunctions and ER stress for treatments of PH.

Keywords: pulmonary hypertension; mitochondria-associated endoplasmic reticulum membrane; mitochondrial dynamics; endoplasmic reticulum stress; Ca^{2+} homeostasis; oxidative stress

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1. Introduction

Pulmonary hypertension (PH) is a severe cardiovascular disorder. This disorder is mainly characterized by pulmonary vasocontraction and pulmonary vascular remodeling, thereby causing elevated blood flow resistance and right heart failure. PH is divided into five groups: Group I specifically refers to PH, which includes idiopathic, heritable, and medical conditions including congenital heart disease and other diseases; Group II occurs due to left heart disease; Group III results from chronic lung disease and/or hypoxia; Group IV is caused by chronic thromboembolic pulmonary hypertension; Group V is secondary to sarcoidosis, sickle cell anemia, chronic hemolytic anemia, and certain metabolic disorders[1]. PH represents a significant global health challenge, affecting individuals across all age groups. The current prevalence of PH is approximately 1% of the world population with its higher occurrence among people aged over 65 years[2]. The epidemiological characteristics of PH vary among countries. In developing countries, key contributors to PH include coronary heart disease, infectious diseases, HIV, and hypoxia[3]. In the UK, the observed prevalence of PH has doubled over the past decade and now stands at 125 cases per million inhabitants[3].

The histopathological features of PH include intimal and medial thickening, muscularization of distal pulmonary arteries, vascular occlusion, and complex plexiform lesions[4–6] . These histological changes are also observed in other groups of PH, although they are less prominent. Despite significant progress in the field, the molecular mechanisms underlying PH remain largely elusive. Currently, approved drug therapies for patients with PH mainly use non-specific vasodilators including phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, endothelin receptor antagonists, and prostacyclin analogs. When used alone or in combination, these agents can improve pulmonary vascular function and hemodynamics, as well as reduce the number of PH-related hospitalizations[2], However, these vasodilators do not specifically target the key pathogenic features of PH. Such interventions have not been proven to reduce mortality rates, with the five-year mortality rate remaining at approximately 50%[7]. Thus, lung transplantation remains the ultimate therapeutic option, and exploring the precise pathogenesis of PH and identifying novel therapeutic targets represent an urgent need and challenging resolution.

Mitochondria-associated endoplasmic reticulum membranes(MAMs) have been implicated in PH, heart failure, and other cardiovascular diseases[8]. these critical structural platforms bridges the two organelles mitochondria and ER to play a central role in maintaining cellular homeostasis[9,10]. Functional impairments of MAMs may contribute to the pathogenesis of PH through multiple pathological pathways, including Ca^{2+} homeostasis disorders, oxidative stress, ER stress, lipid metabolism dysregulation, and abnormal cell proliferation[11,12].

As the physical contact sites, MAMs are involved in controlling various physiological cellular processes. Their formation and function are modulated by a range of proteins and play key roles in cellular stress responses. Structural and functional alterations of MAMs are closely linked to diverse inflammatory and metabolic diseases, thereby leading to PH pathogenesis[13,14]. For instance, MAM formation is tightly associated with ER stress responses, accordingly disrupting intracellular Ca^{2+} homeostasis and impairing mitochondrial function[15,16]. During PH progression, MAM-mediated Ca^{2+} signaling and crosstalk with cellular metabolism may drive the proliferation and migration of PASMCs, exacerbating pulmonary vasoconstriction and vasoremodeling[17,18].

MAM plays a crucial role in PAECs as well. In PH, the regulatory mechanisms of MAMs have garnered increasing attention. Mitochondrial and ER dysfunction can alter MAM formation and stability, subsequently affecting cell survival and proliferation[19,20]. Disrupting MAMs in PAECs alleviates mitochondrial dysfunction, reduces cell apoptosis and inflammatory responses, and enhances nitric oxide (NO) release[21]. Knocking down phosphofurin acidic cluster sorting protein 2 (PACS2), a MAM tethering protein, suppresses ox-LDL-triggered cell apoptosis, along with the associated mitochondrial Ca^{2+} elevation, ROS generation, and cytochrome c release[21]. Hypoxia promotes the formation of MAMs, which directly cause a damage of EC mitochondria, resulting in

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increased ROS production and mitophagy. Moreover, increasing MAM formation is involved in oxidized low-density lipoprotein (ox-LDL)-induced PAECs apoptosis.

Additionally, nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the proliferation of vascular smooth muscle cells (VSMCs) not through their anti-inflammatory activity, but by depolarizing mitochondria, suppressing mitochondrial Ca^{2+} uptake, and thereby facilitating the Ca^{2+}-dependent inactivation of calcium release-activated calcium (CRAC/Orai) channels[22]. This mechanism blocks store-operated Ca^{2+} entry (SOCE) in VSMCs, providing a novel explanation for the therapeutic effect of NSAIDs in treating vascular proliferative disorders. These results underscore the significance of MAMs in controlling PAEC and PASMSC functions and their involvement in related vascular diseases. Dysregulated Ca^{2+} and lipid metabolism in MAMs may also exacerbate cardiovascular pathological changes[23,24], this is due to intimate crosstalk between lipid metabolism and Ca^{2+} signaling at the MAM interface—impaired lipid homeostasis compromises membrane integrity, perturbs oxidative phosphorylation, and elicits aberrant inflammatory cascades in cardiomyocytes and vascular smooth muscle cells, ultimately accelerating cardiac hypertrophy, myocardial fibrosis, and atherosclerotic lesion formation. These findings offer an innovative perspective on PH pathogenesis, suggesting that targeting MAM function could emerge as a new therapeutic strategy for PH.

Exploring the contribution of MAMs in PH not only deepens on our understanding of its pathogenic mechanisms but also offers potential directions for developing new treatments. Modulating MAM function to restore intracellular Ca^{2+} homeostasis and improve mitochondria-ER crosstalk may alleviate PH pathological processes, offering more effective therapeutic options for patients[16,25]. MAM proteins are critical for maintaining MAM structure and function. Their abnormalities further contribute to the onset and progression of PH and other related diseases. In this review, we elaborate MAM structure and function, as well as the associated biological cellular processes. Additionally, we discuss key insights into proteins that influence MAMs and thereby participate in the pathophysiology of PH and other related diseases. Clarifying how MAM proteins and protein complexes regulate MAMs may offer new strategies for drug intervention and disease management.

2. Composition and Structural Characteristics of MAMs

MAMs, the contact sites between mitochondria and SR in cells, exert vital biological functions. Mass spectrometry analysis of MAM-enriched fractions has identified 1052 proteins, which are known to participate in MAM communication and contribute to the pathogenesis of certain diseases(Figure 1)[26].

MAMs are composed of diverse protein complexes, including mitochondrial fusion protein 2 (MFN2), voltage-dependent anion channel 1 (VDAC1) on the outer mitochondrial membrane, and inositol triphosphate receptor 3 (IP3R3) on the ER membrane. These proteins are critical for MAM structure and function. MFN2 not only mediates mitochondrial fusion but also acts as a bridge in MAM formation[27]; VDAC1 is responsible for metabolite exchange between mitochondria and the cytoplasm [28], IP3R3 plays a key role in controlling intracellular Ca^{2+} concentrations [29]. A number of other proteins are localized to MAMs, such as the Sigma-1 receptor (Sig-1R). During stress responses, Sig-1R dissociates from binding immunoglobulin protein (BiP) and then binds to IP3Rs, enabling sustained Ca^{2+} entry into mitochondria via the IP3R-Grp75-VDAC1 complex. Subsequently, Sig-1R is released from MAMs and redistributed across the ER[30].

Currently, 1052 proteins have been identified as essential components of MAMs. These proteins either participate in MAMs formation or are localized to MAMs domains to regulate core biological processes. Some typical members include，the tethering structure formed by the interaction between vesicle-associated membrane protein B (VAPB) and mitochondrial protein tyrosine phosphatase-interacting protein 51 (PTPIP51)[31], receptor expression-enhancing protein 1 at the mitochondria-ER interface[32], tumor suppressor phosphatase and tensin homolog (PTEN) and PTEN-induced kinase 1 (PINK1)[33], ER molecular chaperone calnexin (CNX)[34], ACS2, which governs ER vesicle

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sorting[35], Beclin 1 and syntaxin 17 (STX17), involved in autophagosome formation and maturation [36,37], and ER stress-related proteins such as ATF6, XBP1, and GRP78[38].

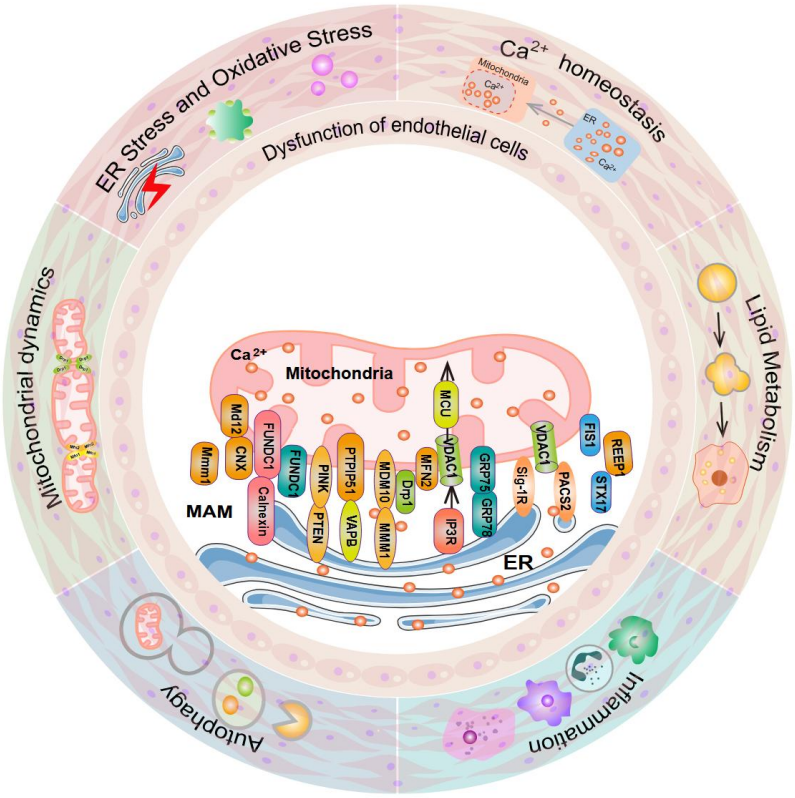

Figure 1. Function of MAM and its related proteins. The glucose regulatory protein 75 (Grp75), voltage dependent anion channel (VDAC), and inositol triphosphate receptor (IP3R) located in the mitochondrial associated endoplasmic reticulum membrane (MAM) connect the endoplasmic reticulum (ER) with the outer mitochondrial membrane (OMM) and participate in the transport of Ca^{2+} from the ER to the mitochondria. The Sigma-1 receptor (Sig-1R) serves as a dynamic multifunctional scaffold protein for ER mitochondrial communication, binding to the molecular chaperone protein immunoglobulin binding protein (BiP)/glucose regulatory protein 78 (Grp78) to form a complex at the MAM site. The binding of vesicle associated membrane protein B (VAPB) on MAM with protein tyrosine phosphatase interacting protein 51 (PTPIP51) forms a tethering structure. The ERMES complex consists of four core components: ER protein mitochondrial morphogenetic protein 1 (Mmm1), cytoplasmic protein mitochondrial distribution and morphogenetic protein 12 (Mdm12), and mitochondrial outer membrane barrel proteins Mdm10 and Mdm34. Mitochondrial fusion protein 2 (MFN2) connects ER and mitochondria through its homologous (MFN2-MFN2) or heterologous (MFN2-Mfn1) interactions, playing a crucial role in MAM, participating in mitochondrial fusion and division, and maintaining Ca^{2+} homeostasis. Protein 1 containing the FUN14 domain (FUNDC1) is enriched at the MAM site by binding to the ER membrane protein calnein.

One key function of MAMs is to serve as a bridge for Ca^{2+} transport, controlling dynamic changes in intracellular Ca^{2+} signaling[39]. In intracellular Ca^{2+} signaling, MAMs facilitate Ca^{2+} transfer between the ER and mitochondria, ensuring cells maintain metabolic homeostasis and normal signal transduction. Studies have demonstrated that MAM-mediated Ca^{2+} signaling pathways play crucial roles in various cellular physiological and pathological processes, particularly in neurodegenerative and cardiovascular diseases, where MAM dysfunction is closely linked to cell death and tissue damage[8,29].

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3. The Pathology of PH

3.1. Regulatory Mechanism of MAM in Mitochondrial Fission and Fusion

Mitochondrial fission and fusion are essential for preserving mitochondrial integrity, metabolic flexibility, and cellular homeostasis (Figure 2). Dysregulation of these processes has become recognized as a core pathological feature of PH, contributing to metabolic reprogramming, apoptosis resistance, and uncontrolled proliferation of PASMCs [40]. Central regulators including the fission mediator dynamin-related protein 1 (Drp1) and fusion proteins such as mitofusin-1/2 (MFN1/2) and optic atrophy protein 1 (OPA1) have been implicated in PH progression[41–43]. In PASMCs from patients with PH, Drp1 expression and activation are markedly increased, while levels of MFN1, MFN2, and OPA1 are reduced, resulting in fragmented mitochondrial networks, impaired oxidative metabolism, and enhanced proliferative and apoptosis-resistant phenotypes[44].

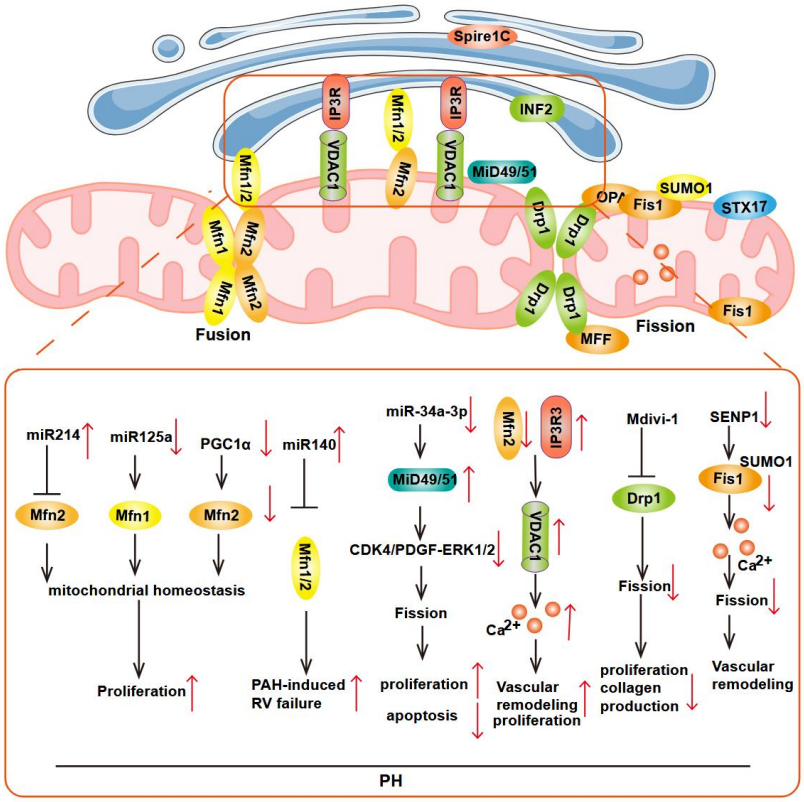

Figure 2. MAMs in mitochondrial dynamics. Mitofusin 2 (MFN2) localized at MAMs initiates mitochondrial fusion through physical interaction with Mfn1 on the outer mitochondrial membrane. Prior to mitochondrial fission, dynamin-related protein 1 (DRP1) and its adaptors, the mitochondrial dynamics proteins 49 and 51 (MiD49 and MiD51), are specifically recruited to mitochondria-ER contact sites. Inverted formin 2 (INF2) on the ER, together with the mitochondria-localized actin nucleation protein Spire1C, coordinately governs DRP1 recruitment at these contact sites and drives the contraction of mitochondrial tubules. miR-214, miR-125a, miR-140 and PGC1α regulate the expression of Mfn1/2 and affect mitochondrial homeostasis, leading to pulmonary arterial hypertension；Reduced expression of miR-34a-3p leads to upregulation of MiD. Increased expression of MiD in PASMCs with PH accelerates Drp1 mediated mitosis, increases cell proliferation, and reduces apoptosis；MFN2 binds to IP3R3 to mediate mitochondrial Ca2+transport, inhibit PASMCs proliferation and pulmonary vascular remodeling; Short-term hypoxia induces SENP1 translocation to endothelial mitochondria to regulate the reversible process of SUMOylation of mitochondrial fission protein FIS1, which protects against pulmonary arterial hypertension；Mitochondrial mitosis mediated by motor protein-related protein 1 allows

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for excessive proliferation of vascular smooth muscle cells. Both DRP1 inhibitors Mdivi-1 and siDRP1 can prevent mitosis and block PH PASMCs in the G2/M interphase.

Growing experimental evidence further supports the pathogenic role of altered mitochondrial dynamics in PH. Excessive mitochondrial fission promotes PASMCs proliferation by ensuring sufficient bioenergetic support and sustaining pro-survival signaling pathways, whereas reduced fusion facilitates vascular remodeling and disease progression[16]. Regulatory pathways controlling these dynamics have also been identified. MicroRNAs (miR-125a and miR-140) reduce MFN1 expression, contributing to hypoxia-induced PASMC proliferation and right ventricular remodeling in SuHx models[45,46]. Upregulation of the Drp1-interacting proteins MiD49/51 drives pathological mitochondrial fission, enhancing PASMC proliferation and apoptosis resistance[47]. Reduced MFN2 and PGC-1α expression contributes to mitochondrial fragmentation in both human and experimental PH models, and restoring MFN2 improves disease manifestations[48]. Inhibiting Drp1, via Mdivi-1 or siDrp1, blocks pathological fission and induces cell-cycle arrest at G2/M, suppressing PASMCs proliferation[49]. Moreover, sentrin-specific protease 1 (SENP1) preserves endothelial mitochondrial morphology via modulation of FIS1 SUMOylation, protecting against hypoxia-induced PH[50]. These studies support mitochondrial dynamics, particularly enhancing fusion and limiting Drp1-dependent fission, as a promising therapeutic avenue in PH.

Beyond mitochondria-intrinsic mechanisms, emerging work demonstrates that the ER is an upstream coordinator of mitochondrial fission and fusion. Prior to Drp1 recruitment, ER tubules encircle mitochondria to pre-define and constrict future division sites[51]. ER-associated inverted formin-2 (INF2) facilitates this process by regulating actin polymerization, providing mechanical support for membrane constriction[52]. The positioning of mitochondrial fission sites is further influenced by mtDNA replication and mitochondrial metabolic status, suggesting that ER–mitochondria coupling integrates mitochondrial morphology with bioenergetic demand and genome maintenance[53]. ER signaling also modulates mitochondrial fusion by regulating MFN1/2 conformation and oligomerization. Under stress conditions such as unfolded protein response activation, ER stress suppresses MFN and OPA1 expression, impairing mitochondrial fusion and exacerbating dysfunction[53].

These regulatory interactions are spatially organized at MAMs, specialized ER–mitochondria contact domains enriched with proteins governing mitochondrial dynamics, calcium transfer, and metabolic signaling. Disruption of ER–mitochondria communication, particularly MAM dysfunction, extends pathological consequences to the right ventricle (RV). In SuHx-induced PH, cardiomyocytes exhibit reduced MFN2 and OPA1 expression, accompanied by mitochondrial fragmentation, hypertrophy, and impaired RV performance[43]. These findings position ER–mitochondria communication and MAM integrity as central determinants of mitochondrial dynamics in PH. Dysregulated Drp1-dependent fission, impaired MFN/OPA1-mediated fusion, and stress-driven disruption of ER–mitochondria signaling synergistically promote metabolic reprogramming, apoptosis resistance, and proliferative remodeling in both the pulmonary vasculature and right ventricle. Restoring mitochondrial dynamic balance, particularly through modulation of MAM-associated pathways, therefore represents a compelling therapeutic direction for both vascular and cardiac manifestations of PH.

3.2. Mechanisms of Ca^{2+} Homeostasis Imbalance

The Ca^{2+} signaling not only serves as central regulators of cellular metabolism, but also is essential for maintaining normal cellular functions, most notably in the proliferation and apoptosis of PASMCs[54,55]. In PH, the disruption of Ca^{2+} homeostasis represents a key pathophysiological mechanism. MAMs, the specialized membrane contact sites between mitochondria and the ER, exert a critical influence on Ca^{2+} signaling(Figure 3)[56,57]. These structures are intimately involved in Ca^{2+} transport and signal transduction processes. Studies have identified several key molecules in this context, ER Ca^{2+} release channels such as inositol triphosphate receptor 3 (IP3R3)[58] (inositol

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triphosphate receptor 3), VDAC1(Voltage-dependent anion channel 1)[59] , and MFN2(Mitofusin-2)[60]. IP3R3 activation facilitates Ca^{2+} release from the ER, while VDAC1 is critically involved in controlling mitochondrial Ca^{2+} influx and efflux[44]. MFN2, on the other hand, modulates mitochondrial morphology and function, thereby impacting Ca^{2+} signaling between the ER and mitochondria[61]. Beyond these MAM-localized molecules, the ER-resident transmembrane protein TMCO1 is also pivotal for ER Ca^{2+} homeostasis: it acts as a "Ca^{2+} load-activated Ca^{2+} (CLAC) channel" that senses ER Ca^{2+} overload, forms a Ca^{2+}-selective channel via reversible homotetramerization, and mediates ER Ca^{2+} release to prevent excessive store filling[62]. This evolutionarily conserved protective mechanism, when lost, causes severe ER Ca^{2+} mishandling and is linked to developmental disorders like cerebrofaciothoracic (CFT) dysplasia spectrum[62]. By maintaining ER Ca^{2+} within a physiological range, TMCO1 indirectly stabilizes MAM-mediated Ca^{2+} transfer, avoiding ER Ca^{2+} overload that would disrupt ER-mitochondria Ca^{2+} signaling precision at MAMs. Thus, by governing Ca^{2+} signaling to influence PASMC proliferation and apoptosis, MAMs act as a critical bridge linking Ca^{2+} homeostasis imbalance to the progression of PH.

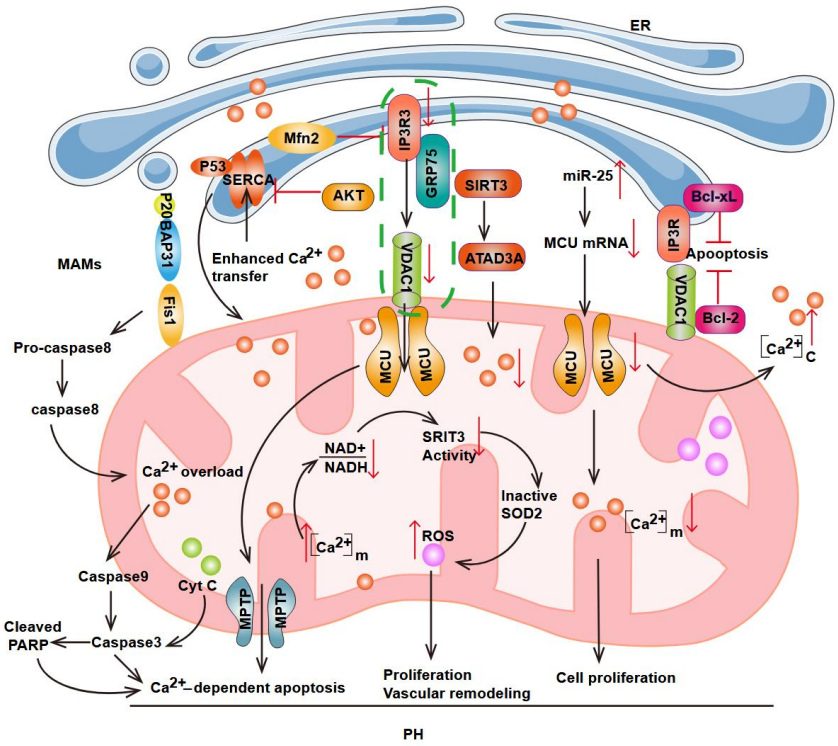

Figure 3. MAMs in Ca^{2+} homeostasis. Mitochondrial Ca2+ elevation increases the activity of Ca2+-sensitive dehydrogenases, which diminishes the NAD+/NADH ratio. The NAD+-dependent deacetylase activity of sirtuin 3 (SIRT3) deacetylates superoxide dismutase 2 (SOD2), and the deacetylated and active form of SOD2 decreases ROS levels. Low levels of NAD+, caused by Ca2+-sensitive dehydrogenases, inhibit SIRT3 activity, and the inactive form of SOD2 allows accumulation of ROS, which activates the c-Jun N-terminal kinase pathway; IP3R3 bind Bcl-xL inhibiting apoptosis; VDAC1 bind Bcl-2 inhibiting apoptosis; MiR-25 binding to MCU mRNA promotes a decrease in mitochondrial Ca^{2+}, an increase in cytoplasmic Ca^{2+} ions, and promotes cell proliferation; The Fis1-Bap31 complex (ARCosome), which spans the mitochondria-ER junction, acts as a platform for activating the initiator procaspase-8, thus connecting these two key organelles in apoptotic signaling. Caspase-3 activation and induction of PARP cleavage than induce apoptosis.

Interaction between Bap31 and Fis1 mediates the formation of the Bap31–Fis1 complex localized to MAMs[63–65]. Simmen et al. demonstrated that another protein called PACS2 modulates the

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contribution of Bap31 in tethering the two organelles. However, depletion of PACS2 was reported to cause Bap-31-dependent mitochondrial fragmentation and uncoupling from the ER along with inhibition of Ca^{2+} signal transmission[66]. The role of miR-25 as a mediator of apoptosis through the MCU has been confirmed in PH[67]. Bcl2 and Bclextra large (Bcl-XL) proteins exert part of their anti-apoptotic functions by directly binding to ER localized IP3Rs and to OMM localized VDAC1, respectively. The joining of Bcl-2 and Bcl-XL to these Ca^{2+} transport systems will negatively affect Ca^{2+} transfer and apoptosis[68].

Aberrant Ca^{2+} signaling drives the imbalance between excessive proliferation and insufficient apoptosis of PASMCs in PH. Studies have revealed that under hyperoxic or other pathological conditions, the intracellular Ca^{2+} concentration in PASMCs rises markedly; The following Ca^{2+} overload pushes PASMCs into a proliferative state, ultimately contributing to pulmonary artery remodeling[69]. Moreover, disrupted Ca^{2+} homeostasis can activate multiple intracellular signaling pathways including p38 MAPK and PI3K/Akt cascades, which are closely tied to cell proliferation and survival. For instance, enhanced Ca^{2+} signaling promotes PASMC proliferation and suppresses apoptosis by activating these pathways, a phenomenon particularly prominent in PH patients[70]. This process is further amplified by abnormal activation of the extracellular calcium-sensing receptor (CaSR), a G protein-coupled receptor that senses extracellular Ca^{2+} ($[Ca^{2+}]_0$) and modulates intracellular Ca^{2+} homeostasis[71]. In PAH-PASMCs, CaSR expression is upregulated by hypoxia (via HIF-1α) and inflammation (via NF-κB), and its activation by elevated $[Ca^{2+}]$ triggers Gq/11-PLCβ-mediated ER Ca^{2+} release. Moreover, CaSR interacts with IP3R3 at MAMs, enhancing Ca^{2+} transfer to mitochondria and further exacerbating Ca^{2+} overload. This sustained Ca^{2+} signal activates CaMKII and ERK pathways, promoting Cyclin D1 expression and inhibiting mitochondrial apoptosis, thereby driving PASMCs hyperplasia[71]. Additionally, Ca^{2+} signaling abnormalities are strongly linked to cellular stress responses, reducing cells' ability to survive adverse environments like hypoxia[12]. Thus, aberrant Ca^{2+} signaling not only triggers excessive PASMCs proliferation but also disrupts apoptotic balance, collectively exacerbating PH pathogenesis.

A case in point is that MFN2 mediates mitochondrial Ca^{2+} transport via IP3R3, suppressing PASMCs proliferation and pulmonary vascular remodeling[72]. Researchers have found that inhibiting IP3R3 expression or restoring MFN2 levels significantly improves vascular function in PH models, suggesting that restoring Ca^{2+} homeostasis could serve as an effective therapeutic strategy for PH[9]. The mitochondria-ER axis also exerts a significant regulatory effect on the function of pulmonary artery endothelial cells. Studies have confirmed that Ca^{2+} signaling mediated by inositol triphosphate receptor 3 (IP3R3) can regulate the proliferation and migration of endothelial cells[73].

3.3. ER Stress and Oxidative Stress in PH

A close interplay exists between ERS and oxidative stress. Studies have demonstrated that in PH, forming a reciprocal vicious cycle that exacerbates vascular pathology. ERS can amplify cellular oxidative stress responses, further triggering inflammation and apoptosis. Conversely, oxidative stress increases ER load, forming a vicious cycle that ultimately leads to PASMCs dysfunction and vascular remodeling[20].

As a tightly interconnected pathogenic process with oxidative stress, ER stress in PH arises when the ER's protein-folding capacity is overwhelmed, activating the unfolded protein response (UPR) via the PERK, IRE1, and ATF6 signaling pathways (Figure 4). These adaptive branches transiently reduce protein translation, increase molecular chaperone synthesis, and restore proteostasis. However, in PH, ER stress becomes chronic rather than adaptive. Hypoxia, inflammation, and viral infection, during PH development and progression, robustly activate ER stress in pulmonary artery endothelial cells (PAECs) and smooth muscle cells (PASMCs). Persistent UPR signaling induces CHOP-mediated apoptosis, disrupts endothelial barrier integrity, and promotes inflammatory cytokine expression (e.g., IL-6, TNF-α), contributing to vascular dysfunction and remodeling[9,15,74]. Notably, the ATF6–Nogo-B signaling axis has been selectively implicated in pulmonary vascular smooth muscle proliferation, emphasizing vascular-bed–specific stress responses[75,76].

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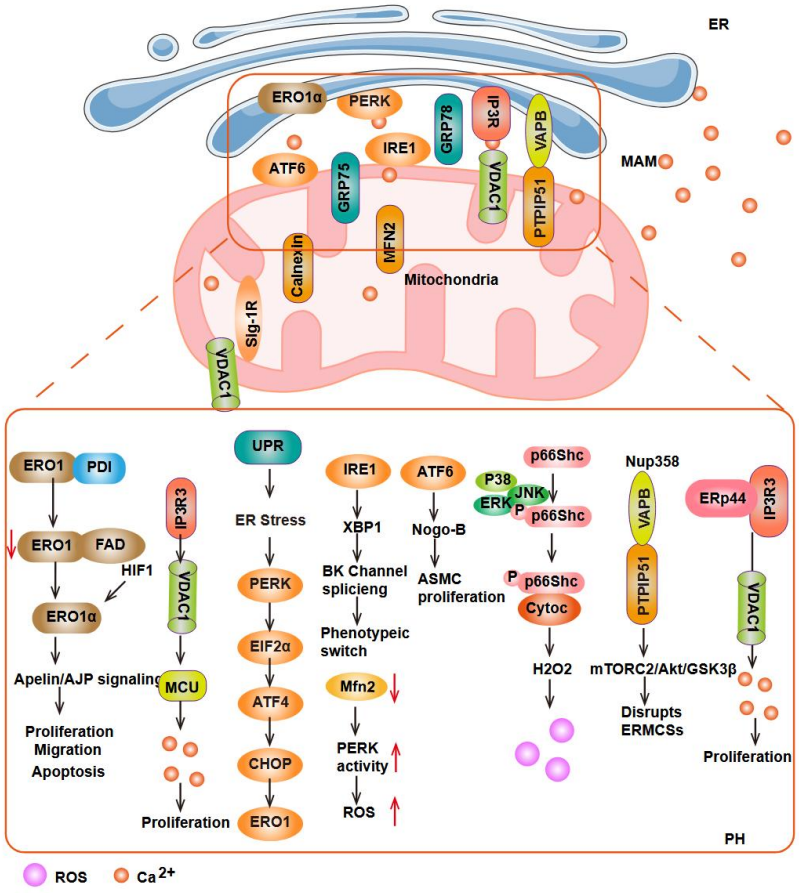

Figure 4. MAMs in ER stress and oxidative stress (ROS production). The three ER transmembrane proteins involved in UPR including ATF6, PERK, and IRE1 are all distributed in MAMs. Oxidative conditions induce serine 36 phosphorylation of p66Shc, prompting its translocation to MAMs and subsequent ROS production. This triggers ER stress signaling via the PERK-EIF2α-ATF4-CHOP pathway. Notably, the interaction between sigma-1 receptor (Sig-1R) and IRE1 at MAMs can mitigate this stress state. Nup358 in controlling ERMCSs through the modulation of the mTORC2/Akt/GSK3β axis; Elevating VAPB-PTPIP51 integration repairs damaged mitochondria-associated ER membranes and inhibits lung fibroblasts activation. Enhancing the interaction between VAPB and PTPIP51 repairs impaired mitochondria-associated ER membranes and suppresses the activation of lung fibroblasts. Under ERS, ERO1α oxidizes IP3R1, resulting in the dissociation of ERP44. ERP44, which is present in MAMs, can bind to IP3R1 to inhibit Ca^{2+} transfer from IP3R1 to mitochondria. This process promotes Ca^{2+} release from the ER, ultimately leading to excessive mitochondrial reactive oxygen species (mtROS) production. ERMCSs: ER–mitochondria contact sites; PDI: protein disulfide isomerase.

Oxidative stress is tightly coupled to ER stress in this setting. Reactive oxygen and nitrogen species (ROS/RNS) intensify UPR activation, while unresolved ER stress increases ROS production, forming a feed-forward loop that promotes endothelial apoptosis, PASMC hyperproliferation, and inflammation[77,78]. Pharmacologic attenuation of ER stress, such as with the chemical chaperone 4-phenylbutyrate, has shown benefit in experimental PH, underscoring its therapeutic relevance[79,80]. Emerging evidence indicates that this interaction is spatially coordinated by mitochondria–ER contact sites, known as mitochondria-associated membranes (MAMs). Structural modules within MAMs, particularly the IP_3R–GRP75–VDAC1 axis, regulate Ca^{2+} transfer from the ER to mitochondria. In PH, enhanced ER stress tightens MAM coupling and increases Ca^{2+} flux, driving mitochondrial Ca^{2+} overload and excessive mitochondrial ROS (mtROS) generation (Figure 4). Elevated mtROS further disrupts ER proteostasis and alters Ca^{2+} channel function, thereby

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reinforcing MAM tethering and amplifying ER stress. This bidirectional amplification loop, ER stress, increased Ca^{2+} transfer, mtROS generation, and intensified ER stress, is a key driver of endothelial apoptosis and PASMCs hyperproliferation, central pathological features of PH vascular remodeling. Similar stress-coupling mechanisms have been reported in diabetic vasculopathy, liver fibrosis, and neurodegeneration, suggesting that this MAM-dependent circuit represents a conserved cellular stress program. Targeted disruption of this axis through modulation of MAM structure, inhibition of mitochondrial Ca^{2+} uptake, or activation of antioxidant programs such as the SIRT3/SOD2 pathway, reduces mitochondrial ROS, restores endothelial function, and attenuates vascular remodeling in PH models[81].

In summary, these findings position the mitochondria-ER axis, but not ER stress or oxidative stress in isolation, as a central pathogenic mechanism in PH. Therapeutically interrupting this MAM-driven stress–ROS–Ca^{2+} feedback loop represents a promising strategy now supported by emerging preclinical evidence.

3.4. Involvement of MAM in Inflammation, Autophagy and Lipid Metabolism in PH

Inflammation plays a pivotal role in the progression of PH, particularly in pulmonary vascular remodeling. Evidence indicates that inflammatory mediators drive PASMC proliferation and fibrosis through multiple mechanisms, exacerbating vascular remodeling. For instance, in chronic PH animal models, elevated levels of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) correlate closely with PASMC proliferation and phenotypic transition toward a synthetic state[82,83]. Mitochondria act as central regulators of immune responses, and several mitochondria-associated membrane (MAM) proteins participate in this regulation (Figure 5). MAM-associated proteins are critical for initiating interferon and other inflammatory responses upon pathogen exposure or cellular stress[26,84,85]. While inflammation is a key defense mechanism against ischemia, hypoxia, excessive immune activation, and aging, it also constitutes a core pathological process in cardiovascular and cerebrovascular diseases, cancer, and metabolic disorders[86,87]. The nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) functions as a sensor of microbial infection and cellular damage, forming multi-protein inflammasomes to initiate inflammatory signaling[88]. MAMs not only facilitate inflammatory activation but also serve as a platform for NLRP3 inflammasome assembly. NLRP3 is widely expressed, with macrophages exhibiting the highest levels. In its inactive state, NLRP3 resides in the ER membrane and cytoplasm; upon activation, NLRP3 and its adaptor ASC translocate to MAMs to detect ROS from damaged mitochondria[89]. Respiratory chain inhibitors such as rotenone (complex I inhibitor) can activate the inflammasome, linked to mitochondrial membrane potential ($\Delta\Psi m$) loss and ROS accumulation[90]. Excessive mitophagy, ER stress (ERS), and aberrant ER–mitochondria Ca^{2+} transfer can further impair mitochondrial function, increase ROS, and release mitochondrial DNA (mtDNA), collectively activating NLRP3 inflammasomes[26]. MAM-resident VDAC proteins are essential in this process; VDAC1 mediates mitochondrial Ca^{2+} uptake and ROS production, and VDAC knockout markedly suppresses IL-1β inflammasome formation[90]. In PH, ERS activation and the interplay of inflammatory signaling pathways, particularly NF-κB and NLRP3 inflammasomes, drive disease progression[91]. Activated NF-κB promotes transcription of pro-inflammatory cytokines such as TNF-α and IL-6, amplifying inflammation and inducing pulmonary artery endothelial cell apoptosis, ultimately contributing to vascular remodeling and hypertension[92–95]. NLRP3 inflammasomes thus act as a mechanistic bridge between ERS and PAH, suggesting that targeting ERS-related pathways could provide novel therapeutic strategies. Mitochondrial dysfunction, a hallmark of PH, disrupts cellular energy homeostasis and elevates oxidative stress, contributing further to inflammation. mtDNA and ROS serve as potent inflammatory triggers[96,97]. Impaired mitochondria release mtDNA into the cytoplasm, activating downstream interferon signaling via the cGAS-STING pathway and promoting inflammatory factor production[98]. Elevated mitochondrial ROS, observed in PH patients, is associated with pulmonary

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endothelial dysfunction and inflammatory responses[99]. Moreover, imbalances in mitochondrial fission and fusion exacerbate oxidative stress and inflammation[40].

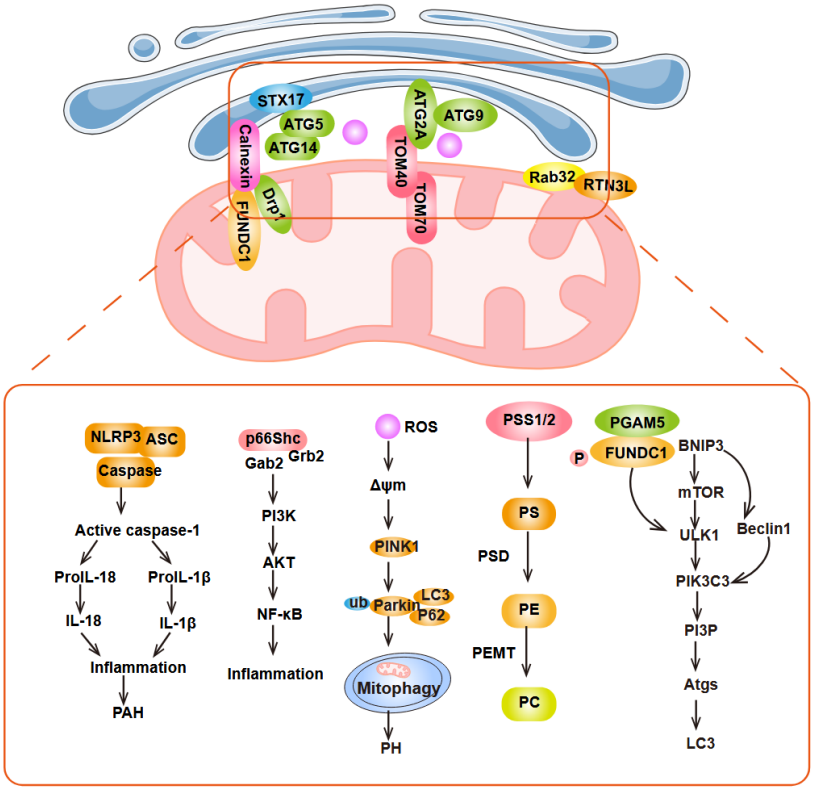

Figure 5. Role of MAMs in inflammation, autophagy and Lipid Metabolism. In response to ROS stimulation, NLRP3 translocates from the ER to MAMs, where it interacts with its adaptor ASC. This interaction activates caspase-1, leading to the generation of mature IL-1β and IL-18 and initiating inflammasome formation; p66Shc-mediated inflammation via Gab2-PI3K signaling; Within the ER, phosphatidic acid (PA) is catalyzed into phosphatidylserine (PS) by the enzymes PSS1 and PSS2. Subsequently, PS is transported to mitochondria, where it is converted to phosphatidylethanolamine (PE) via phosphatidylserine decarboxylase (PSD). The PE synthesized in mitochondria is then transported back to the ER, where phosphatidylethanolamine N-methyltransferase 2 (PEMT2) catalyzes its conversion to phosphatidylcholine (PC); PC is ultimately transported from the ER to mitochondria. Furthermore, acyl-coenzyme cholesterol acyltransferase 1 (ACAT1), which is localized in MAMs, catalyzes the conversion of intracellular free cholesterol to cholesterol esters, thereby maintaining the dynamic balance between bound and free cholesterol under resting conditions. Under physiological conditions, syntaxin 17 (STX17) interacts with dynamin-related protein 1 (DRP1) to regulate mitochondrial fission. During starvation, the STX17-DRP1 interaction becomes abnormal, leading to mitochondrial elongation. STX17 then binds to ATG14 and recruits it to MAMs to participate in autophagosome formation. The TOM70-TOM40 complex recruits ATG2A to MAMs, which further binds to ATG9 to promote membrane structure expansion and autophagy progression. In mitophagy, PINK1 and Parkin are enriched at MAMs, facilitating mitochondria-ER contact and autophagosome formation. FUN14 domain-containing protein 1 (FUNDC1) binds to calnexin and localizes to MAMs. PGAM5-mediated dephosphorylation at Ser13 and ULK1-mediated phosphorylation at Ser17 facilitate the binding of FUNDC1 to LC3, thereby triggering the initiation of mitophagy. During mitophagy, the interaction between FUNDC1 and calnexin is weakened, and FUNDC1 recruits DRP1 to MAMs through direct interaction, thereby mediating mitochondrial fission. Additionally, Rab32 interacts with the ER-phagy receptor reticulon-3 (RTN3L), both localized at MAMs to induce ER phagy.

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MAMs are increasingly recognized for their roles in autophagy, with several autophagy-related proteins localized to MAMs[35]. In PINK/Parkin-dependent mitophagy, PINK accumulates on the outer mitochondrial membrane (OMM), phosphorylates ubiquitin at Ser65, recruits Parkin, and activates polyubiquitination of proteins such as VDAC1 and p62/SQSTM1[100,101]. MAMs serve as initiation sites for this mitophagy. Notably, PINK depletion hinders BECN1 accumulation independently of Parkin, indicating a unique regulatory role for PINK[33]. Parkin overexpression strengthens ER–mitochondria contacts, enhances ER-to-mitochondria Ca^{2+} transfer, and increases mitochondrial ATP production[102]. Similarly, under hypoxia, FUNDC1 accumulates in MAMs, highlighting MAM involvement in mitophagy regulation[103].

MAMs are integral to lipid metabolism. Lipid synthesis—including triacylglycerol (TAG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE)—requires enzymatic activity spanning both the ER and mitochondria. Phosphatidylserine (PS), produced from PC by PSS1 in MAMs, is converted to PE by mitochondrial PS decarboxylase, and phosphatidylethanolamine N-methyltransferase 2 (PEMT2) completes PC synthesis[104]. In idiopathic PAH (IPAH), TAG, LPS, PA, PG, PE, PI, PS, SS, and St lipid levels are significantly elevated, suggesting potential pathogenic roles[105]. Cellular metabolic reprogramming is critical in PAH progression[106,107]. Mitochondria–ER crosstalk influences PASMC proliferation and apoptosis resistance, enabling survival under hypoxia and promoting vascular remodeling[108]. Hypoxia-inducible factor-2α (HIF-2α) is a key transcriptional regulator of EndMT in severe PAH: MAM dysfunction–induced Ca^{2+} imbalance activates CaMKII-mediated HIF-2α phosphorylation, enhancing its stability and nuclear translocation even under normoxic conditions. HIF-2α binds Snai1 and Twist1 promoters, suppressing endothelial markers (VE-cadherin) and upregulating mesenchymal markers (α-SMA), exacerbating remodeling[109]. Therapeutically, combining dichloroacetic acid (DCA) and atorvastatin alleviates PH by modulating mitochondrial metabolism[110–113], highlighting metabolic reprogramming as a potential therapeutic target.

Pulmonary vascular remodeling in PH involves PASMC proliferation, endothelial apoptosis, and small vessel restructuring, regulated by both growth factors and immune cells. Macrophages and neutrophils secrete inflammatory mediators that promote PASMC proliferation and migration. Oxidative stress further amplifies these effects by impairing endothelial function and enhancing smooth muscle cell migration and proliferation, partly via NF-κB signaling. Endothelial VEGFR2–VE-cadherin–PECAM-1 complexes respond to shear stress through p66Shc-mediated Gab2–PI3K signaling[114]. Epigenetic modifications of p66Shc (e.g., decreased methylation and increased acetylation) enhance its transcription, acetylation, and phosphorylation, promoting mitochondrial translocation and ROS production[115,116]. Mitochondrial dysfunction–induced lipid metabolism disorders exacerbate inflammation: chronic inflammation impairs fatty acid oxidation, generating excessive ROS and stimulating release of TNF-α and IL-1β[116]. Therapeutically, anti-inflammatory agents such as glucocorticoids alleviate PH symptoms by suppressing inflammatory mediator production, although current treatments cannot fully reverse vascular remodeling. Collectively, mitochondrial dysfunction and MAM dysregulation emerge as key contributors to PH pathogenesis, highlighting the potential of strategies targeting mitochondrial function, ER–mitochondria communication, and inflammatory signaling. A deeper understanding of MAM-mediated interactions among inflammation, autophagy, and lipid metabolism may inform more effective therapeutic approaches.

3.5. MAM-Mediated Apoptosis in PH

Apoptosis, a form of programmed cell death (PCD) that maintains cellular homeostasis by regulating cell proliferation, development, and renewal, is tightly controlled in pulmonary vascular cells, with mitochondria-associated membranes (MAMs) serving as central hubs that mediate apoptotic signaling. Both mitochondria and the ER contribute to apoptotic regulation, and MAMs integrate multiple molecular cues to control cell fate.

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The mitochondrial fission protein Fission 1 (Fis1) interacts with B cell receptor-associated protein 31 (Bap31) on the ER, cleaving Bap31 into the pro-apoptotic fragment p20Bap31. This Fis1–Bap31 complex is essential for activating pre-caspase-8, highlighting how MAMs mediate the transmission of apoptotic signals[65,117] (Figure 5).

ER-to-mitochondria Ca^{2+} transfer is another critical determinant of apoptosis. PTEN, an antagonist of Akt signaling, interacts with IP3R1 to enhance ER-to-mitochondria Ca^{2+} flux, thereby increasing cellular susceptibility to apoptosis[118]. Conversely, Bcl-xl, an anti-apoptotic Bcl-2 family member, translocates to MAMs during thapsigargin (Tg)-induced apoptosis, strengthening its interaction with IP3R3. Bcl-xl modulates mitochondrial Ca^{2+} uptake, limits cytosolic Ca^{2+} overload, supports cellular metabolism, and ultimately prevents apoptosis following Tg treatment[119]. Similarly, the translocation of Bcl2 from the ER to MAMs and then to mitochondria depends on MAM integrity and the Bcl2–TOM20 interaction, enhancing its anti-apoptotic function[120].

Pro-apoptotic Bax also relies on MAMs for translocation to the mitochondrial membrane, where it triggers cytochrome c release and apoptosis through mitochondrial Ca^{2+} accumulation and opening of the mitochondrial permeability transition pore (MPTP)[121]. Additional molecules localizing to MAMs mediate ER stress-induced apoptosis, including mitochondrial E3 ubiquitin ligase MITOL[122] , transcription factor C/EBP homologous protein (CHOP) [123], and FK506 binding protein 8 (FKBP8) [124].

Emerging evidence highlights the role of promyelocytic leukemia protein (PML) in apoptosis regulation via MAMs. PML modulates the ER machinery at ER–mitochondria contact sites, regulating Ca^{2+} signaling from ER to mitochondria and cytosol, thereby influencing cell survival and sensitivity to apoptotic stimuli[125]. Moreover, MAM-resident proteins such as p66Shc and tumor suppressor p53 can modulate ROS production and ER–mitochondria Ca^{2+} flux, amplifying apoptotic responses under stress conditions[126]. Structural dynamics of MAMs, including tethering protein expression and contact site density, further influence cellular susceptibility to apoptosis, highlighting MAMs as a highly dynamic signaling platform in PH.

These mechanisms underscore MAMs as central regulators of apoptosis, integrating Ca^{2+} signaling, pro- and anti-apoptotic protein interactions, ER stress, and oxidative cues to modulate vascular cell fate in PH.

4. Therapeutic Strategies Targeting Mitochondria-ER Axis

4.1. Mitochondrial Dynamics as A Potential Therapeutic Target

In recent years, a growing body of research has identified mitochondrial dynamics as a promising therapeutic target for PH. By targeting inhibitors of the mitochondrial fission protein Drp1 such as Mdivi-1. Researchers have observed symptom improvements in PH animal models. This inhibitory effect alleviates excessive mitochondrial fission, restores mitochondrial function and morphology, and thereby improves the proliferation and apoptosis balance of PASMCs[49]. Additionally, strategies to enhance mitochondrial fusion including the use of agonists for MFN1 or OPA1have also shown potential in ameliorating PH. Recently, researchers discovered that Drpitor1, a specific Drp1 GTPase inhibitor, reduces proliferation, induces apoptosis in PH human PASMCs (hPASMCs), and reverses monocrotaline-induced PH [41]. By controlling mitochondrial dynamic balance, researchers aim to reverse pulmonary artery remodeling and offer new therapeutic options for PH patients[16,127].

Activating mitochondrial fusion is equally crucial. MFN2, a crucial outer mitochondrial membrane fusion protein, can lead to mitochondrial fragmentation and dysfunction when functionally impaired. In PH, MFN2 activation promotes mitochondrial fusion, improving mitochondrial morphology and function. Research indicates that upcontrolling MFN2 enhances mitochondrial interactions, facilitates intracellular energy transport, and improves cellular metabolic status[43]. Targeted activation of MFN2 can effectively promote mitochondrial health, thereby countering PH progression. FUN14 domain-containing protein 1 (FUNDC1), an integral outer

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mitochondrial membrane protein, mediates the formation of MAMs. Silencing FUNDC1 reduces MAMs formation, which inhibits angiogenesis by decreasing VEGFR2 expression[128], suggesting that targeting FUNDC1-dependent MAMs could be a promising approach for PH treatment.

Together, mitochondrial dynamics regulators such as the Drp1 inhibitor Mdivi-1 and MFN2 activation strategies offer new targets and approaches for PH treatment. By modulating mitochondrial fission and fusion, these interventions can significantly improve mitochondrial function, reduce cellular oxidative stress, and slow PH pathological progression. Future research should further explore the clinical potential of these modulators to offer more effective therapeutic options for PH patients[16,127].

4.2. Antioxidant and Metabolic Regulating Drugs

The combination of antioxidant and metabolic regulatory drugs has shown significant potential in treating PH. Notably, the combination of dichloroacetic acid (DCA) and atorvastatin (ATO) is thought to synergistically improve the pathophysiological status of PH patients through distinct mechanisms.

DCA, a well-known metabolic regulator, activates pyruvate dehydrogenase (PDH) to promote aerobic metabolism, reduce lactate production, and enhance cellular energy metabolism. This is particularly critical for PH patients, especially those with hypoxia-induced metabolic disorders, since improving energy metabolism can alleviate right heart burden and enhance cardiac function[111].

Atorvastatin, a statin drug, primarily acts by lowering cholesterol levels and improving endothelial function. Studies have revealed that statins not only benefit cardiovascular health but also exhibit anti-inflammatory and antioxidant properties. Atorvastatin can slow PH progression by inhibiting endogenous oxidative stress and protecting endothelial function; additionally, it governs lipid metabolism to further promote pulmonary artery relaxation and reduce pulmonary arterial pressure[113].

The combination of DCA and ATO exerts positive effects on PH through multiple pathways. Firstly, their synergistic action effectively enhances cellular energy status, mitigates hypoxia-induced metabolic disturbances, and reduces oxidative damage. Moreover, atorvastatin's anti-inflammatory effects complement DCA's metabolic regulatory role, collectively alleviating PH pathological progression at the molecular level. Clinical data indicate that DCA-ATO combination therapy significantly improves heart function and quality of life in PH patients[129].

Noticeably, DCA-ATO combination therapy offers an innovative therapeutic strategy for PH, highlighting the importance of combining antioxidant and metabolic regulatory drugs in improving PH patient prognosis. However, further clinical trials are needed to verify its long-term efficacy and safety, laying a more robust theoretical foundation for clinical application.

The application prospects of antioxidant therapy in PH have garnered growing attention, particularly in the context of controlling ER-mitochondria crosstalk. Recent studies have highlighted that mitochondria-ER interactions play a pivotal role in cellular metabolism and oxidative stress, the processes closely linked to PH pathogenesis[130]. Since oxidative stress is recognized as a key pathological mechanism in PH, driving endothelial dysfunction and vascular remodeling, antioxidant-based interventions may offer innovative therapeutic insights for PH[130,131].

In antioxidant therapy research, activation of the SIRT3/SOD2 pathway has attracted widespread interest. SIRT3, a mitochondrial deacetylase, governs the activity of various antioxidant enzymes, most notably superoxide dismutase 2 (SOD2) via deacetylation, thereby reducing mitochondrial ROS levels. Studies have demonstrated that SIRT3 activation significantly lowers pulmonary artery pressure and alleviates right ventricular hypertrophy in PH model mice, underscoring its potential as a therapeutic target in PH[132].

The utility of natural antioxidants extends beyond direct free radical scavenging; they also modulate cellular signaling pathways and enhance cellular function. For instance, β-carotene has been shown to alleviate oxidative stress and ER stress in PH mouse models by controlling Ca^{2+} signaling between the ER and mitochondria, thereby exerting a protective effect against PH

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development[133,134]. Furthermore, some antioxidants can enhance cellular tolerance to oxidative stress by modulating autophagy and apoptosis pathways, which may positively impact PH pathophysiology.

Obviously, antioxidant therapy holds broad application prospects in PH, particularly regarding SIRT3/SOD2 pathway activation and natural antioxidant research. Future studies should further clarify the mechanisms of action of antioxidants and explore their efficacy and safety in clinical settings to offer more effective treatments for PH patients. A deeper understanding of Mitochondria-ER interactions and their regulatory roles in PH will also lay a crucial theoretical foundation for developing innovative antioxidant therapies.

4.3. Ca^{2+} Signaling Regulation-Related Functional Roles and Their Therapeutic Targets

Ca^{2+} signaling is critically involved in diverse cellular functions including cell proliferation, migration, and apoptosis, all of which are critical to PH pathogenesis. For instance, the application of Ca^{2+} channel inhibitors or specific signaling pathway antagonists reduces PASMC proliferative capacity under hyperoxic conditions[135]. Additionally, research has demonstrated that therapeutic strategies focusing on Ca^{2+} homeostasis such as agents that restore Ca^{2+} balance can significantly ameliorate PH in animal models, highlighting substantial therapeutic potential[17]. Thus, agents that modulate Ca^{2+} signaling pathways are regarded as promising therapeutic targets. In recent years, research on modulators of endogenous signaling molecules has grown, with a particular focus on inositol triphosphate receptor 3 (IP3R3) inhibitors and Ca^{2+} channel modulators.

As a key channel for intracellular Ca^{2+} release, abnormal IP3R function is closely linked to the development of various diseases. In PH, altered expression and activity of IP3R lead to excessive Ca^{2+} release in smooth muscle cells, driving their proliferation and migration, and exacerbating pulmonary artery remodeling and hypertension. Studies have demonstrated that IP3R3 lacking can effectively reduce migration, proliferation and mesenchymal transition of PAECs induced by hypoxia and improve physiological indicators in PH animal models, offering new insights for PH treatment[73].

Notably, while these targeted drugs have exhibited favorable efficacy in experimental and clinical studies, their long-term safety and effectiveness require further validation. Future research should focus on optimizing their administration and exploring combinations with other therapies to achieve better outcomes. Moreover, the identification of new biomarkers may enable more precise personalized strategies for Ca^{2+} signaling-targeted therapy.

Understandably, these findings not only offer critical insights into deepening our understanding of PH pathogenesis but also expand options for clinical intervention. IP3R3 inhibitors and Ca^{2+} channel modulators offer new targets and approaches for PH treatment. Ongoing research will further advance their clinical application, contributing to improved prognosis and quality of life for PH patients.

4.4. Autophagy Regulation-Related Functional Roles and Their Therapeutic Targets

Autophagy has been recognized as a crucial mechanism that sustains energy metabolism, thereby fueling cell proliferation, supporting cell survival, and suppressing apoptosis. This regulatory role of autophagy is not only well-documented in cancer[136,137], but also implicated in PH [77]. Study showed that treatment with ROC-325 was found to suppress autophagy in the lung tissues of rats exposed to monocrotaline (MCT). This inhibitory effect is supported by the observation that ROC-325 reversed the MCT-induced upregulation of LC3B and p62 expression levels[138]. The clinical application prospects of autophagy regulators in the treatment of PH are remarkably broad, with rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), standing out as a particularly notable candidate. Rapamycin has been extensively studied and confirmed to promote autophagy by inhibiting the mTOR pathway, effectively improving pulmonary arterial hypertension and vascular remodeling in PH models[139]. Additionally, Research has shown that metformin

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promotes autophagy by activating the AMPK signaling pathway, thereby improving metabolic disorders and related cardiovascular pathological changes[140,141].

The treatment of PH targeting autophagy is a crucial breakthrough direction from "relieving symptoms" to "reversing pathological remodeling". Among them, AMPK activators (such as metformin) are currently the most promising candidate drugs for conversion due to their high safety and solid clinical foundation; Lung targeted mTOR inhibitors and Beclin-1 agonists are expected to achieve more efficient treatment by precisely controlling autophagy.

5. Conclusions

A deeper understanding of mitochondria-ER crosstalk, particularly MAMs as a critical cellular structure, has gradually emerged as an innovative therapeutic target in the treatment of PH. Evidently, MAMs not only play pivotal roles in Ca^{2+} signaling transduction, lipid metabolism, and autophagy, but also exert significant impacts on controlling cellular stress responses, cell survival, and cell death. Studies have confirmed that MAMs dysfunctions are closely linked to the pathogenesis of PH, other cardiovascular disorders, metabolic syndrome, and neurodegenerative diseases[128,142].

The role of MAMs in PH pathogenesis is increasingly recognized. PH development is closely associated with mitochondrial dysfunction and ER stress, which exacerbate the disease by impairing MAMs structure and function. Specifically, MAMs integrity is critical for maintaining cellular Ca^{2+} balance and energy metabolism. Its disruption may enhance ER stress responses, thereby promoting PASMC proliferation and migration, ultimately contributing to PH development. Innovative therapeutic strategies targeting MAMs primarily by modulating IP3R, GRP75, and VDAC can improve mitochondria-ER interactions, thereby restoring normal cellular function. In support, enhancing the expression and activity of these proteins effectively alleviates mitochondrial dysfunction and ER stress, ameliorating PH pathology[128,143]. Progress has been made in developing new drugs that act on MAMs. For instance, natural products and synthetic compounds have been found to mitigate intracellular stress responses by controlling MAMs formation and function. Their underlying mechanisms may involve in MAM-related signaling pathways, thereby improving cellular metabolic status and survival capacity[144]. Combining with other therapies (e.g., immunotherapy or gene therapy), MAM-based targeted strategies can substantially enhance therapeutic efficacy. Furthermore, combining MAM-function-improving agents with traditional PH drugs such as prostaglandins or endothelin receptor antagonists yields synergistic effects, thereby boosting therapeutic outcomes[145].

Manifestly, innovative MAM-targeted therapeutic strategies offer new insights for PH treatments. These approaches not only improve mitochondria-ER interactions, but also alleviate PH pathological processes by controlling intracellular Ca^{2+} balance and energy metabolism. Future research should further explore MAM roles in PH and other related diseases to offer new ideas and directions for their novel and more effective clinical treatments and improve patient prognosis[16]. It is hoped that this intensive review article will shed new lights on the functional importance of mitochondria and ER interactions in PH and the discovery of new PH drugs, ultimately contributing to human health.

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Abbreviations

The following abbreviations are used in this manuscript:

ER	Endoplasmic reticulum
ERS	Endoplasmic Reticulum Stress
IP3Rs	Inositol triphosphate receptors
MAMs	Mitochondria-associated endoplasmic reticulum membranes
MCU	mitochondrial Ca^{2+} uniporter
NO	Nitric oxide
PAH	Pulmonary arterial hypertension
PASMCs	pulmonary artery smooth muscle cells
PAECs	Pulmonary artery endothelial cells
ROS	Reactive Oxygen Species
VDAC1	Voltage-dependent anion channel 1

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