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Mitochondrial network dynamics in pulmonary disease: Bridging the gap between inflammation, oxidative stress, and bioenergetics

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ABSTRACT

Once thought of in terms of bioenergetics, mitochondria are now widely accepted as both the orchestrator of cellular health and the gatekeeper of cell death. The pulmonary disease field has performed extensive efforts to explore the role of mitochondria in regulating inflammation, cellular metabolism, apoptosis, and oxidative stress. However, a critical component of these processes needs to be more studied: mitochondrial network dynamics. Mitochondria morphologically change in response to their environment to regulate these processes through fusion, fission, and mitophagy. This allows mitochondria to adapt their function to respond to cellular requirements, a critical component in maintaining cellular homeostasis. For that reason, mitochondrial network dynamics can be considered a bridge that brings multiple cellular processes together, revealing a potential pathway for therapeutic intervention. In this review, we discuss the critical modulators of mitochondrial dynamics and how they are affected in pulmonary diseases, including chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), acute lung injury (ALI), and pulmonary arterial hypertension (PAH). A dysregulated mitochondrial network plays a crucial role in lung disease pathobiology, and aberrant fission/fusion/mitophagy pathways are druggable processes that warrant further exploration. Thus, we also discuss the candidates for lung disease therapeutics that regulate mitochondrial network dynamics.

1. Introduction

Despite decades of research on pulmonary diseases such as pulmonary arterial hypertension (PAH), acute lung injury (ALI), idiopathic pulmonary fibrosis (IPF), and chronic obstructive pulmonary disease (COPD), clinical interventions remain primarily palliative, with multiple promising therapies failing clinically. This is likely due to the underlying complexity of these diseases. Studies often focus on one cell type or ignore interactions with other systems, how different cells actively respond to and interact with each other, and their environment is lost in most cell culture models. Therefore, to achieve effective therapies, we must examine these interactions and identify strategies to target multiple cell types. One such plausible strategy is targeting mitochondria, as aberrant mitochondria appear to be a critical contributor to disease progression in many cell types and various pulmonary diseases [1]. This review describes the emerging field of mitochondrial dynamics and the therapeutic potential of modulating mitochondrial dynamics in the context of pulmonary disease.

Gas exchange is unanimously accepted as the most essential function of the lung [2]. Many cell types tightly regulate this process and require that all these cells function properly; inadequate gas exchange may have disastrous consequences. The functional unit of the lungs is the alveolus, composed of epithelial cells and surrounded and influenced by various mesenchymal cell lineages, including endothelial cells, fibroblasts, and smooth muscle cells. Because of the tight interconnectedness of these different cell types, dysfunction in one pulmonary cell type may induce dysfunction in the surrounding cell types. For example, PAH is considered a pan-vasculopathy, in which dysfunction within each blood vessel layer contributes to the obstruction and constriction of the small pulmonary arteries, right ventricle hypertrophy, and ultimately, right heart failure [3].

It is widely accepted that PAH is accompanied by mitochondrial

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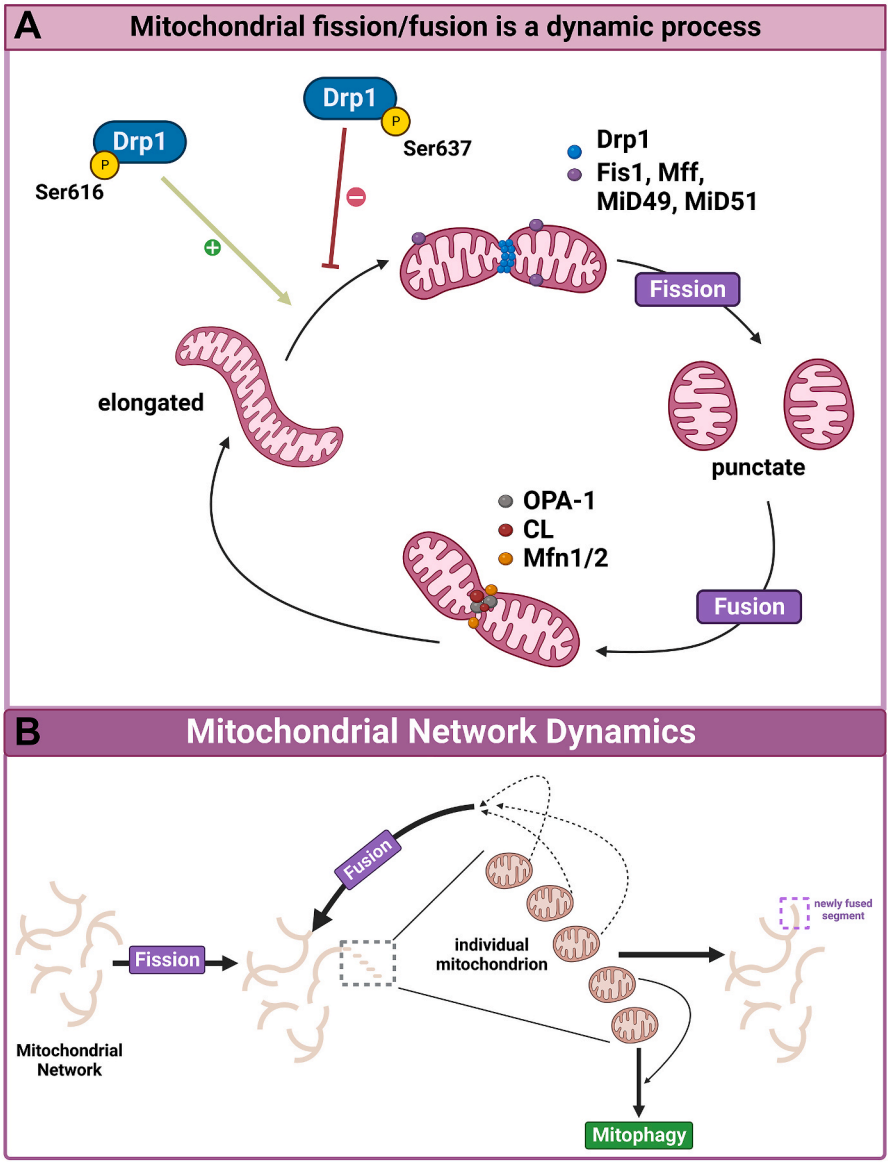

Fig. 1. Mitochondrial fission/fusion is a dynamic process. (**A**) Mitochondrial fission separates a mitochondrial network into two or more independent networks. Phosphorylation of Drp1 at site Ser^{616} promotes fission, while phosphorylation of Drp1 at site Ser^{637} inhibits fission. Fis1, Mff, MiD49, and MiD51 are other major regulators of fission. Mitochondrial fusion involves two or more mitochondrial networks joining to become an integrated unit. Significant regulators of fusion include OPA-1, CL, and Mfn1/2. Fused mitochondrial network morphology is often called elongated, while smaller mitochondrial networks are called punctate, indicative of a fission morphology. (**B**) Mitochondrial network dynamics refers to how mitochondria actively regulate their morphology, number, and size using fusion, fission, and mitophagy. Abbreviations: Drp1: dynamin-related protein 1, Fis1: mitochondrial fission protein 1, Mff: mitochondrial fission factor, MiD49 and MiD51: mitochondrial dynamics proteins of 49 and 51 kDa, OPA-1: optic atrophy protein 1, CL: cardiolipin, Mfn1/2: mitofusin 1/2.

dysfunction in many different cell types. With recent advances, specific mechanisms underlying mitochondrial dysfunction have been identified, offering a novel therapeutic strategy for pulmonary diseases, including PAH. Investigations on the role of mitochondria in pulmonary disease have primarily focused on their contribution to inflammation, bioenergetics, and oxidative stress. However, within the cell, mitochondria form a dynamic network that actively remodels in response to environmental changes. Mitochondrial network dynamics is thus a critical regulatory hub bridging inflammation, bioenergetics, and oxidative stress, and an excellent target for the development of therapeutic interventions for pulmonary disease. This review discusses the evidence for dysregulated mitochondrial dynamics in lung diseases and where further research is needed to develop effective treatments for these highly complex pulmonary diseases.

2. Mitochondria morphologically adapt to the needs of the cell using fission and fusion

Mitochondria are highly dynamic organelles that, in as little as a few seconds, can change size, shape, and position [4]. Mitochondria morphologically adapt to fit the needs of the cell. This adaptation allows the cell to respond to various cellular cues, including metabolism, calcium levels, apoptosis, and mitosis [5]. Mitochondrial function is necessary in all healthy tissues; however, the functions mitochondria play in the cell vary widely between cell types [6]. Therefore, it is critical to understand mitochondrial function during health to determine aberrant mitochondrial function during disease. Significantly, mitochondria do not form *de novo,* so the replication of pre-existing mitochondria mediates biogenesis [7]. This means that mitochondria must be highly adaptable and able to clear defective mitochondria to

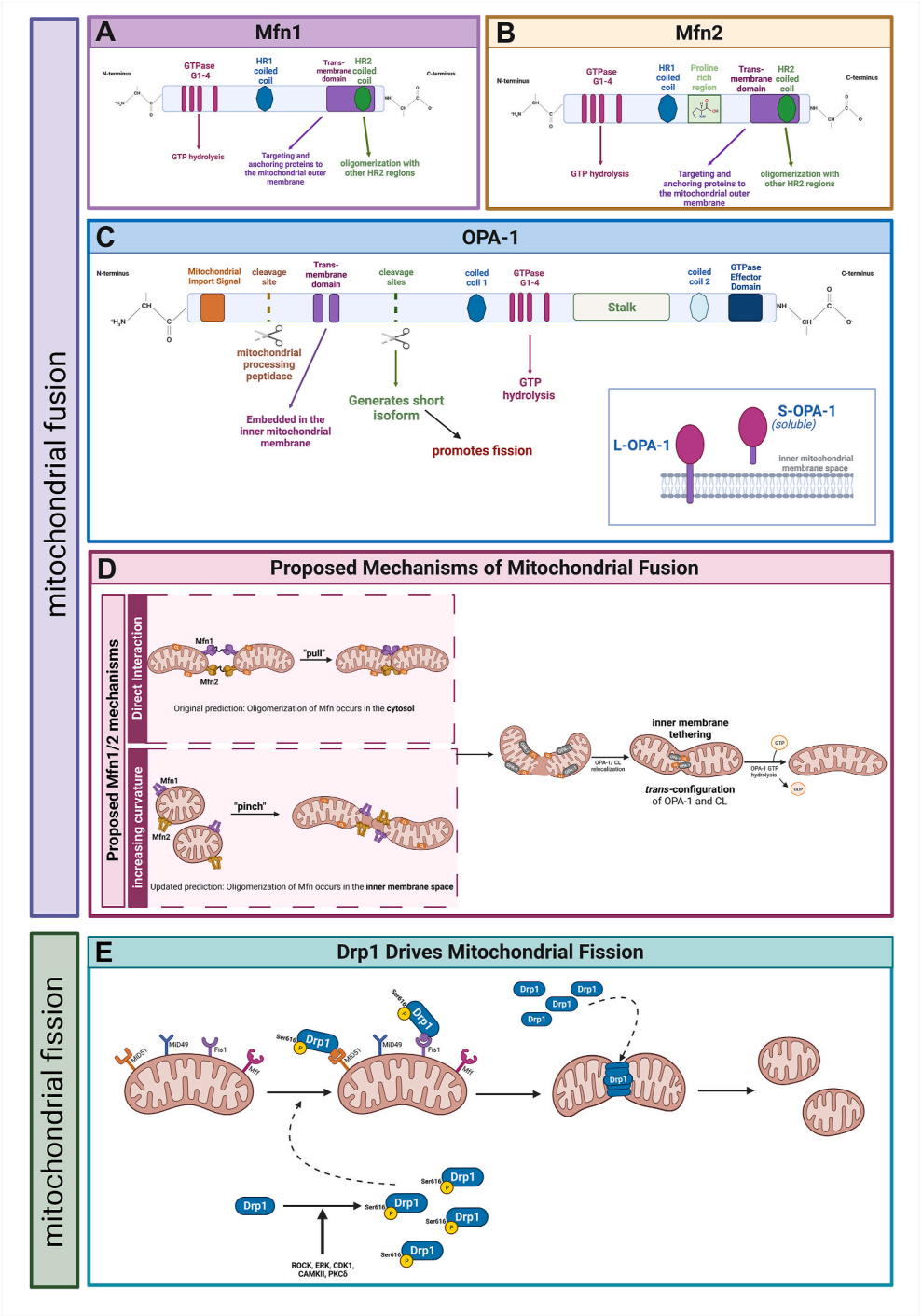

Fig. 2. Structure of the mitochondrial GTPases involved in fusion and fission. Three mitochondrial GTPases control mitochondrial fusion: (**A**) Mfn1, (**B**) Mfn2, and (**C**) OPA-1. The linear structural schemes of Mfn1, Mfn2, and OPA-1 are shown to highlight the critical sites and functional domains. (**D, left**) Mfn1 and Mfn2 regulate the fusion of the outer mitochondrial membrane. For fusion to occur, the two fusing mitochondrial networks must first be tethered together, requiring Mfn oligomerization. Two mechanisms for how this tethering event occurs have been proposed. If located in the cytosol, it is proposed that a direct interaction between Mfns results in a "pulling" event that brings the mitochondria together. If located in the inner membrane space, it is proposed that oligomerization of the Mfns results in a "pinching" event that increases the curvature of the mitochondria and the contact surface area between the two mitochondria. (**D, right**) OPA-1 regulates the fusion of the inner mitochondrial membrane. First, OPA-1 and CL interact in a *trans*-configuration between the two inner membranes, followed by GTP hydrolysis to complete fusing the inner membranes. (**E**) Phosphorylation of Drp1 at site Ser^{616} activates Drp1. Once activated, Drp1 is recruited to the outer mitochondrial membrane and is anchored to the OMM through interactions with Mff, Fis1, or MiD49/51. The anchored Drp1 then forms an oligomeric ring that wraps around the mitochondria and mechanically constricts and fragments the mitochondria into two independent networks. Abbreviations: Mfn1: mitofusin 1, Mfn2: mitofusin 2, OPA-1: optic atrophy protein 1, L-OPA-1: long OPA-1 isoform, S-OPA-1: short OPA-1 isoform, CL: cardiolipin, GTP: guanosine-5′-triphosphate, GDP: guanosine diphosphate, GTPase: guanosine triphosphatases, HR1 and HR2: heptad repeat domain 1 and 2. Drp1: dynamin-related protein 1, Fis1: mitochondrial fission protein 1, Mff: mitochondrial fission factor, MiD49 and MiD51: mitochondrial dynamics proteins of 49 and 51 kDa, ROCK: Rho-associated protein kinase, ERK: extracellular signal-regulated kinase, CDK1: cyclin-dependent kinase 1, CAMKII: calmodulin-dependent protein kinase II, PKCδ: protein kinase Cδ.

support cell survival. The adaptive nature of mitochondria is often attributed to their ability to perform highly coordinated fission and fusion processes. Fission divides a single organelle into two or more independent organelles, while fusion is the opposite process (Fig. 1A). Both these processes are continuously and simultaneously occurring in many cell types. The optimal balance of fission and fusion is critical for mitochondrial homeostasis [8–12].

Mitochondria dynamically undergo fusion, fission, and mitophagy to regulate their morphology and control their number and size, a process termed mitochondrial network dynamics [6] (Fig. 1B). Accumulating evidence has emphasized the importance of mitochondrial network dynamics in mitochondrial function. For example, mitochondrial fusion is associated with increased ATP production, while fusion inhibition is associated with impaired oxidative phosphorylation, mitochondrial DNA (mtDNA) depletion, and production of reactive oxygen species (ROS) [13]. Mitochondrial dynamics have been implicated in a variety of diseases, including cardiovascular [14,15] and metabolic disorders [16], and neurological diseases [17]. Interestingly, 10–14 days after fusing cells from patients with two different pathogenic mitochondrial mutations, the hybrid cells had normal mitochondrial morphology and respiratory activity [18]. As neither of the mutations had normal morphology or activity alone, this suggests that mitochondria perform complementation, in which they can transfer mtDNA from one mitochondrial network to another and perform mtDNA recombination. This has immense implications, as it suggests that mitochondria can transfer mtDNA between networks, with protective or deleterious consequences, depending on the specific mutations shared between mitochondria. Independently of the outcome, this complementation emphasizes the highly dynamic nature of the mitochondrial network, constantly adapting to the environment and adding to the complexity of dissecting the role mitochondria play in pathological conditions.

3. Regulation of mitochondrial fusion

During mild to moderate cellular stress, mitochondria may perform fusion to combine a damaged mitochondrion with a healthy mitochondrion to prevent damage to the cell. Fusion allows for the mixing of contents between mitochondria, mediating the redistribution of mitochondrial proteins and genes, which can protect against mitochondrial mutations [19]. Therefore, fusion is often considered a protective pathway [20]. Additionally, fusion is heavily implicated in maintaining the mitochondrial membrane potential. Support for this includes that hyperfused networks exhibit higher potentials [21] while silencing mitochondrial fusion mediators decreases the mitochondrial membrane potential [8,22]. Moreover, skeletal muscle mitochondria from a fusion-deficient mouse cannot maintain their membrane potential [23]. As fusion allows for complementation and generation of the mitochondrial membrane potential, hyperfused networks are generally considered more resilient to metabolic disturbances and thus preserve cellular integrity.

To prevent cellular stress, dysfunctional mitochondria undergo selective degradation by mitophagy, an autophagic clearance process [24,25]. Hyper-fused/elongated mitochondria cannot be cleared through the mitophagy pathway. Therefore, hyperfused mitochondrial networks can be protective during starvation by preventing mitophagy [26,27]. However, mitochondrial dynamics are intimately connected to mitophagy; mitophagy is necessary to prevent damage spreading from dysfunctional mitochondria to the entire mitochondrial network, maintaining mitochondrial and cellular homeostasis. Thus, both mitochondrial dynamics and mitophagy must be investigated to obtain an accurate picture of mitochondrial and cellular homeostasis. Furthermore, fission is also required to distribute mitochondria during mitosis [28], highlighting that fusion and fission are part of the dynamic system that mitochondria use to maintain overall mitochondrial and cellular homeostasis, function, and replication.

Mitochondrial dynamics are regulated by specific proteins, referred to as mitochondrial-shaping proteins. For fusion to occur, mitochondria must first fuse their outer mitochondrial membrane (OMM), followed by fusion of the inner mitochondrial membrane (IMM). Mitochondrial fusion is controlled by three mitochondrial guanosine triphosphatases (GTPases): the Mitofusin (Mfn) proteins, Mfn1 and Mfn2 [29], and optic atrophy protein 1 (OPA-1) [30] (Fig. 2A–C). Mfn1 and Mfn2 regulate the fusion of the OMM, while OPA-1 regulates the fusion of the IMM. Mfn1 and Mfn2 have a GTPase domain in the N-terminal region of the protein and a C-terminal transmembrane domain that anchors the protein to the OMM, flanked on either side by a heptad repeat domain with a coiled-coil structure, which is essential for protein-protein interaction (HR1 and HR2, Fig. 2A-B).

The oxidation of glutathione disulfide (GSSG) during cellular stress promotes the assembly of disulfide-mediated-Mfn oligomers [31,32]. Mfns were believed to have two transmembrane domains. However, in that case, the HR2 region containing the cysteine residues responsible for oligomerization would be exposed to the cytosol, a very reductive environment. Glutathione (GSH) is ubiquitously found throughout the cell and is considered the primary redox buffer. To maintain the overall cellular redox state, GSH reductase reduces GSSG to GSH. Only under oxidative stress conditions does GSSG accumulate sufficiently to interact with other proteins [32]. However, fusion does not only occur during oxidative stress, bringing into question how GSSG-induced oligomerization of Mfns can occur in a reductive compartment where levels of GSSG are likely too low to interact. New bioinformatic predictions and functional experiments suggest that human Mfns have only one transmembrane domain that places the HR2 region in the intermembrane space, which is more susceptible to GSSG accumulation [33]. Based on the previously predicted structure, it was suggested that Mfns have a U-turn topology that allows two mitochondrial membranes to be tethered by *trans*-interactions of their HR2 regions. GTP binding then induces a conformational change, leading mitochondria to dock and increase membrane contact [34,35]. However, if the HR2 region is in the intermembrane space as predicted [33], it needs to be clarified how Mfns are tethered. However, it has been suggested that Mfn oligomerization on the membrane could produce a “pinching” event that increases membrane curvature and increases the two membranes’ contact area [36] (Fig. 2D).

The HR2 region can form a stable complex with other HR2 regions in Mfn1 or Mfn2 [35], suggesting that Mfn-1/Mfn-2 can promote fusion independently or cooperatively [29,35]. Mfn1 overexpression induces a more interconnected mitochondrial phenotype, which we term “mt-fusion phenotype”. Mutations in the G1 motif of the GTPase region of Mfn1 reduce the Mfn1-induced mt-fusion phenotype after overexpression. Furthermore, mutations in the G2 motif of the GTPase region of Mfn1 induce punctate mitochondria, indicative of a mt-fission phenotype [19]. Ultimately, these mutation experiments demonstrate that the GTPase activity of Mfn1 directly influences the ability of mitochondria to perform fusion. Mfn2 overexpression also results in a mt-fusion phenotype. Interestingly, knockdown of Mfn1 results in punctate, short, and spherical mitochondria with uniform sizes. Conversely, Mfn2 knockdown results in less fragmentation and produces mitochondria of varying sizes [29,37]. Mfn1 has an 8-fold increase in GTPase hydrolysis activity compared to Mfn2, while Mfn2 has a higher GTP binding affinity than Mfn1 [38]. Although Mfn1 and Mfn2 can induce fusion, the varying presentation of these mediators’ knockdown suggests a functional difference we do not fully understand. Furthermore, Mfn1 can rescue fusion in $Mfn2^{-/-}$ cells, while Mfn2 cannot fully restore fusion in $Mfn1^{-/-}$ cells [29], further highlighting the functional differences between the two proteins.

While Mfn1 and Mfn2 regulate the fusion of the OMM, dynamin-related GTPase optic atrophy protein 1 (OPA-1) regulates the fusion of the IMM and other mitochondrial functions, including cristae formation and apoptosis [39]. OPA-1 contains an N-terminal mitochondria-import signal, a GTP-binding domain, a GTP-effector domain, and a middle/stalk domain [40]. The N-terminal sequence allows for the import of

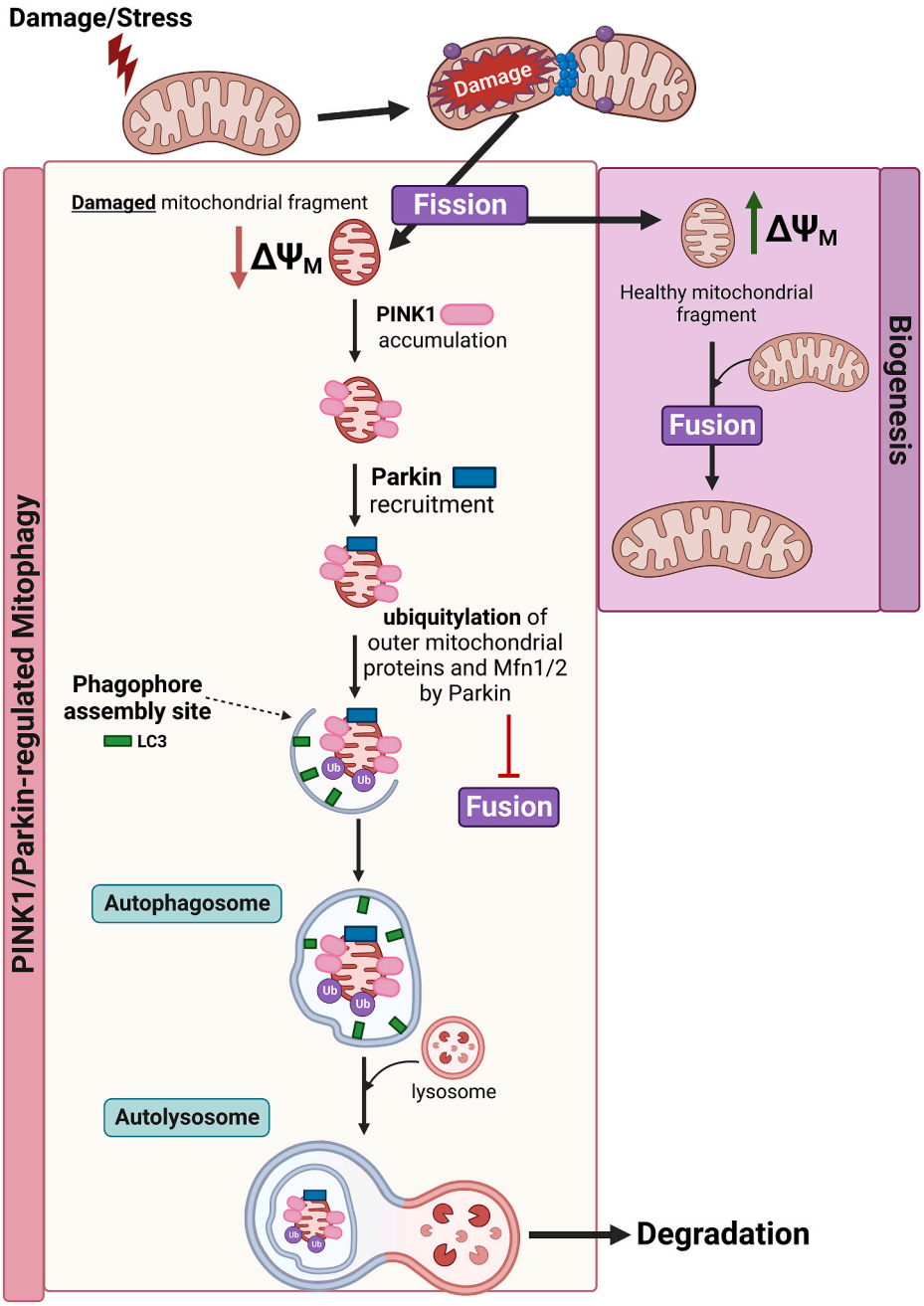

Fig. 3. PINK1/Parkin-regulated Mitophagy. Under normal conditions, PINK is imported to the IMM, where it undergoes degradation. During severe stress, mitochondrial networks undergo fission to separate the damaged portion of the network. The resulting damaged mitochondrial fragment has a decreased mitochondrial membrane potential, inhibiting PINK1 translocation to the IMM, and PINK1 accumulates on the OMM, and an auto-phosphorylation event activates its kinase activity. PINK1 phosphorylates ubiquitin, triggering the recruitment of Parkin. Parkin ubiquitinates OMM proteins, which interact with LC3 to induce phagophore assembly and autophagosome formation. The autophagosome fuses with a lysosome, leading to the autophagic elimination of the ubiquitinated mitochondrial fragment. The healthy mitochondrial fragment remains functional and can fuse with other mitochondrial networks. Abbreviations: $\Delta\Psi_M$: mitochondrial membrane potential, PINK1: PTEN-induced kinase 1, Mfn1/2: mitofusin 1/2, LC3: microtubule-associated protein 1A/1B-light chain 3, IMM: inner mitochondrial membrane, OMM: outer mitochondrial membrane. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the OPA-1 precursor protein into the mitochondria, where it is subjected to further processing by the mitochondrial processing peptidase (MPP) and subsequently embedded into the IMM by the transmembrane domain. Once tethered to the IMM, OPA-1 can be subjected to further proteolytic processing [41] (Fig. 2C). This proteolysis step is critical for OPA-1 regulation [42], resulting in either long (L-OPA-1) or short isoforms (S-OPA-1) of OPA-1 [41,43]. L-OPA-1 is membrane-bound, while S-OPA-1 is soluble.

Interestingly, L-OPA-1 is required for fusion, while S-OPA-1 limits fusion and promotes fission. Therefore, excessive processing of OPA-1 by the stress-activated inner-membrane peptidase, OMA1, is sufficient to induce mitochondrial fragmentation and, eventually, cell death [42]. Interestingly, the proteolytic processing of OPA-1 to S-OPA-1 is not required for fusion [44]. Thus, uncleaved L-OPA-1 is associated with fusion, while cleaved S-OPA-1 is linked to mitochondrial fission. OPA-1 is also implicated in regulating apoptosis, as it compartmentalizes

soluble cytochrome *c* within the cristae [45,46]. Therefore, OPA-1 is a critical modulator directly linking the fusion, fission, and apoptosis pathways. In addition to OPA-1, cardiolipin (CL) is also required for IMM fusion [47]. CL is primarily located on the IMM [48] and is known to stabilize the electron transport chain (ETC) [49] and regulate mitophagy [50]. Mechanistically, it appears that CL must react with L-OPA-1 for fusion to complete, with evidence for a *trans*-configuration between CL and L-OPA-1 before fusion (Fig. 2D). This tethering event between L-OPA-1 and CL appears to be independent of OPA GTP hydrolysis, which is needed for the successful completion of the fusion process [51].

4. Regulation of mitochondrial fission

In contrast to mitochondrial fusion, excessive mitochondrial fission induces mitochondrial fragmentation and is often associated with metabolic dysfunction and disease. However, it is essential to appreciate that fission is also involved in many critical cellular functions. For example, mitochondrial fission plays a pivotal role in cell division [52], regulated cell death [53], and mitochondrial quality control through mitophagy [54,55]. Therefore, dysfunctional fusion or fission can be detrimental to cell homeostasis. Under normal conditions, lung microvascular endothelial cells appear to have a predominately fission phenotype, indicated by a punctate mitochondrial pattern [52]. As discussed earlier, the fusion phenotype is more resilient against metabolic disturbances than the fission phenotype. Therefore, lung endothelial cells displaying a fission phenotype may be more sensitive to metabolic disturbances than cells with a fusion phenotype.

Mitochondrial fission is driven by the activity of dynamin-related protein 1 (Drp1, gene code *DNM1L*) [56]. Drp1 is a GTPase primarily located in the cytosol. Once activated, Drp1 is recruited to the mitochondria through interactions with multiple Drp1 receptors anchored to the OMM. These receptors include mitochondrial fission factor (Mff), mitochondrial fission protein 1 (Fis1), and mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51) [57–61]. Once Drp1 is recruited onto the OMM, it co-assembles with the receptors, forming an oligomeric ring that wraps around the mitochondrion, mechanically constricting and subsequently fragmenting the mitochondrion [61,62] (Fig. 2E).

The activity of Drp1 is predominantly regulated by post-translational modifications (PTMs). The most studied PTM of Drp1 is phosphorylation, which can activate or inhibit Drp1 activity based on the location of the phosphorylation. Multiple phosphorylation sites have been identified, including Ser^{40}, Ser^{44}, Ser^{579}, Ser^{585}, Ser^{592}, Ser^{600}, Ser^{616}, Ser^{637}, Ser^{656}, and Ser^{693} [63]. Of these, Ser^{616} and Ser^{637} are the most studied. Phosphorylation of the C-terminal residue Ser^{616} stimulates Drp1 localization to the OMM to promote mitochondrial fission and mitochondrial network fragmentation [64–68]. The phosphorylation at Ser^{616} can be catalyzed by several protein kinases, including Rho-associated protein kinase (ROCK) [69], protein kinase Cδ (PKCδ), cyclin-dependent kinase 1 (CDK1), extracellular signal-regulated kinases 1/2 (ERK1/2), and calmodulin-dependent protein kinase II (CaMKII) [70]. Drp1 activity is also regulated by other PTMs [71–73], including nitration [74]. Drp1 nitrated at tyrosine residues Y^{628} and Y^{665} has enhanced Drp1 oligomer assembly [74]. PTMs of Drp1 not only activates the pro-fission activity of Drp1, but it can also inhibit this activity. For example, phosphorylation of Drp1 at Ser^{637} by the cAMP-dependent protein kinase A (PKA) blocks Drp1 activity by decreasing its interaction with the OMM, reducing fission [75].

Although Drp1 significantly promotes mitochondrial fission, both endoplasmic reticulum tubules and actin fibers are needed to provide the constrictive force to separate the OMM and IMM [76–79]. This suggests that many factors are required for fission that have yet to be uncovered. Furthermore, our understanding of mitochondrial fission is primarily restricted to the OMM. One possible factor involved in separating the IMM may be the inner mitochondrial membrane protein of 18 kDa (MTP18). MTP18 colocalizes with Drp1 to promote fission, and when MTP18 is silenced with siRNA, elongated mitochondrial networks are observed [80,81]. Furthermore, the cleaved isoform of OPA-1, S-OPA-1, has also been associated with promoting fission. S-OPA-1 colocalizes with Drp1 and the other fission factors on the OMM and has been shown to inhibit fusion [44,82]. However, both S-OPA-1 and MTP18 require Drp1 and other mitochondrial fission factors as they localize to the OMM. Thus, OMM fission may be sufficient to promote fission of both membranes at the same time, although additional research is needed to fully understand the fission process. Because of OPA-1's extensive role in fusion, S-OPA-1 appears to be a bridge between fusion and fission, and modulation of its proteolysis may be an unexplored target to modulate mitochondrial dynamics.

5. Regulation of mitophagy

Autophagy is a cellular process that degrades proteins and other cytoplasmic substances. Autophagy can compensate for nutrient depletion by degrading and recycling cellular components [83]. Additionally, autophagy protects the cell by degrading potentially harmful protein aggregates. It is well established that autophagy is necessary to maintain mitochondrial network homeostasis by eliminating old or damaged mitochondria [84,85]. Multiple mitophagy pathways have been identified (reviewed elsewhere [86,87]). Here, we will focus on PINK1/Parkin-regulated mitophagy, as this pathway is intimately linked with fission.

Under normal conditions, healthy mitochondria repress the kinase PINK1 (PTEN-induced kinase 1) by importing PINK1 to the IMM and degrading it via the protease PARL (presenilin-associated rhomboid-like protein). During severe stress conditions, mitochondrial networks undergo fission to separate the damaged portion of the network to prevent further damage. The damaged mitochondrial fragment has a decreased mitochondrial membrane potential, inhibiting PINK1 transportation into the IMM and PINK1 accumulation on the OMM. An autophosphorylation event activates PINK1 kinase activity, allowing PINK1 to phosphorylate ubiquitin, triggering the recruitment of Parkin, a ubiquitin ligase. Parkin then ubiquitinates OMM proteins, which interact with microtubule-associated protein 1A/1B-light chain 3 (LC3) to induce phagophore assembly. Once the autophagosome is formed, it can fuse with a lysosome, leading to the autophagic elimination of the ubiquitinated mitochondrial fragment [25,88]. The healthy mitochondrial fragment remains functional and can fuse with other mitochondrial networks (Fig. 3). Importantly, PINK1 accumulation only occurs with severely depolarized mitochondria. Therefore, mitochondrial depolarization stimulates PINK1/Parkin-induced mitophagy [89–91].

PINK1-mitophagy is negatively regulated by deubiquitinating enzymes, including multiple in the ubiquitin-specific protease (USP) subfamily of proteins. USP8, USP14, USP15, USP30, USP33, and USP35 have been shown to antagonize Parkin activity [92–97]. USP30 has emerged as a critical mitochondrial mediator and, as such, has been the deubiquitinating enzyme of interest [98]. Unfortunately, we still lack reliable methods to distinguish mitophagy from autophagy, *in vivo* or in patient samples [99], and conflicting reports of mitophagy during pulmonary disease exist, interpreting challenging [100]. Regardless, it is evident that the mitophagy pathway is critical to maintaining cellular health and function.

6. The interconnection of mitochondrial fission, fusion, and mitophagy

Mitophagy is heavily linked to mitochondrial fission and fusion. For example, inhibition of Drp1 reduces mitophagy levels and increases oxidized mitochondrial protein levels, suggesting a direct relationship between mitophagy, fission, and oxidative stress [101]. Specifically, Drp1 generates small mitochondria that allow autophagosomes to successfully engulf the mitochondria [54,101–103]. Additionally, Drp1 can

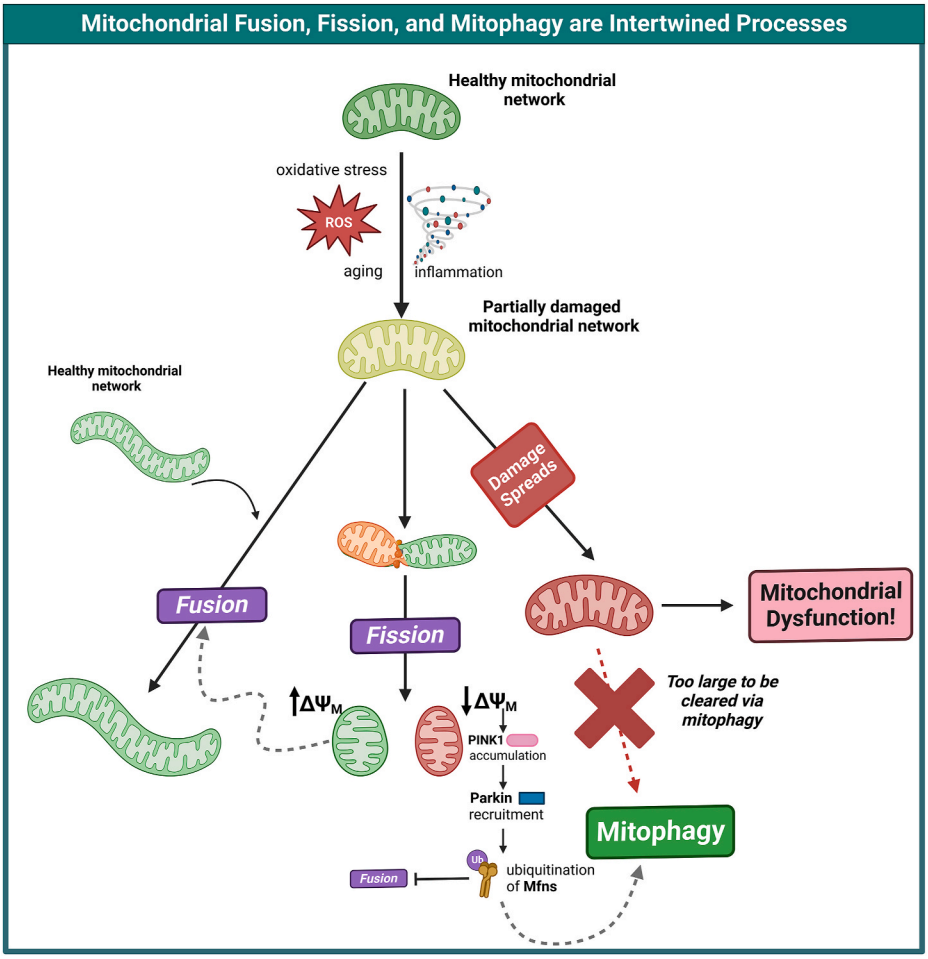

Fig. 4. Mitochondrial fusion, fission, and mitophagy are intertwined processes. Mitophagy is heavily linked to mitochondrial fission and fusion. Fission can produce two daughter mitochondrial fragments: one with a decreased membrane potential and another with an increased membrane potential. The mitochondrial fragment with a higher membrane potential can undergo fusion, while the fragment with a decreased membrane potential is susceptible to mitophagy. During PINK-mediated mitophagy, parkin ubiquitinates Mfns, directly inhibiting fission. Abbreviations: ROS: reactive oxygen species, $\Delta\Psi_M$: mitochondrial membrane potential, Mfns: mitofusins, PINK1: PTEN-induced kinase 1, Ub: ubiquitin. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

facilitate the segregation of damaged parts of the mitochondria and target this fragment to the PINK1/Parkin mitophagy pathway [25,54,55]. This selective targeting can be explained by mitochondrial fission producing two fragments with different mitochondrial membrane potentials. The mitochondrial fragment with a higher membrane potential will likely undergo fusion [101], while the mitochondrial fragment with decreased membrane potential recruits Parkin after PINK1 accumulation. Parkin ubiquitinates OMM proteins, including Mfn1/2. This way, the mitophagy pathway directly inhibits the fusion process [102] (Fig. 4) and eliminates the damaged mitochondria that might otherwise compromise the mitochondrial network and cause mitochondrial dysfunction. Additionally, mitochondrial proteins are constantly exposed to oxidative stress due to ROS produced via oxidative phosphorylation. Thus, mitophagy acts as a protective mechanism to isolate dysfunctional mitochondria that may have been oxidized or damaged. Furthermore, the healthy mitochondrial fragment can now fuse with other healthy mitochondrial fragments, a process termed mitochondrial biogenesis [104]. This process recycles and preserves resources by eliminating only the damaged mitochondria.

Further supporting the interconnectedness of mitophagy and mitochondrial dynamics, the knockdown of the deubiquitinating enzyme and antagonist of mitophagy, USP30, results in more fused and elongated mitochondrial networks [105,106]. Therefore, inhibiting USP30 promotes increased mitophagy associated with a fusion phenotype. Although it is unclear why increasing mitophagy leads to a fusion phenotype, one may speculate that increasing mitophagy promotes fusion indirectly. As shown in Fig. 4, mitochondrial fragments after mitophagy can fuse with the other mitochondrial networks. However, what is clear is that USP30 plays a regulatory role in mitochondrial fission and fusion [107], demonstrating that, as with metabolic pathways, mitochondrial dynamics and mitophagy are highly intertwined and should be interpreted together. Because USP30 is constitutively associated with the OMM and peroxisomes, USP30 may be a promising drug target that could limit the inhibition of ubiquitination in other parts of the cell to minimize the accumulation of other cytosolic proteins intended for degradation.

Another factor linking mitochondrial dynamics and mitophagy is cardiolipin (CL). CL is a dimeric phospholipid with four unsaturated fatty acid chains especially vulnerable to oxidation by ROS [48]. Under normal cellular conditions, unoxidized CL is primarily found in the IMM and its interactions with OPA-1 are essential for fusion [48]. During mild mitochondrial damage, CL can be translocated to the OMM to trigger mitophagy through interactions with LC3 [108,109]. Mounting evidence supports the conclusion that the oxidation state of CL affects its function [110]. Unoxidized forms of CL predominate in autophagosomes, while oxidized CL is involved in apoptosis [108,111,112]. This suggests functional differences between CL oxidation states could be how the cell differentiates between mild and severe stress [50]. During

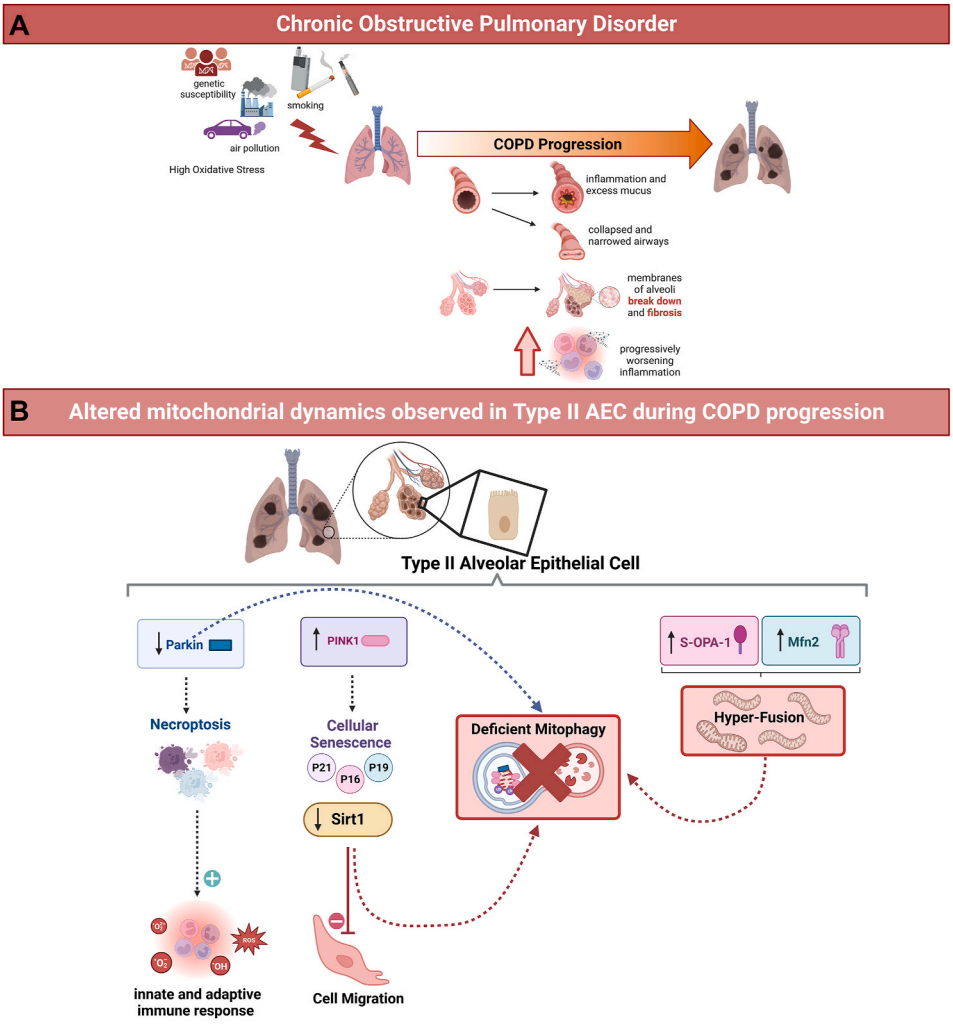

Fig. 5. Evidence of disrupted mitochondrial network dynamics in chronic obstructive pulmonary disorder. (**A**) COPD is characterized by irreversible airflow obstruction, collapse of alveoli, and chronic airway inflammation that progressively worsens. Oxidative stress is widely accepted as a significant contributor to COPD progression. (**B**) Pathways identified to produce altered mitochondrial dynamics in Type II AEC during the progression of COPD. Abbreviations: COPD: Chronic Obstructive Pulmonary Disorder, AEC: Alveolar Epithelial Cell, PINK1: PTEN-induced kinase 1, p21/p16/p19: CDK inhibitors, Sirt1: Sirtuin 1, Mfn2: mitofusin 2, S-OPA-1: short optic atrophy protein 1 isoform, ROS: reactive oxygen species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the early stages of apoptosis, CL is translocated from the IMM to the OMM. This process can be facilitated by scramblase-3, nucleoside diphosphate kinase, mitochondrial isoforms of creatine kinase, or t-Bid [113–115]. Once on the OMM, CL interacts with dephosphorylated cytochrome *c* to form a complex, resulting in the oxidation of CL [116]. Once oxidized CL accumulates on the OMM it recruits the Bcl-2 family protein, Bax, resulting in the release of cytochrome *c* into the cytosol, triggering apoptosis [108,111,112]. Intriguingly, CL has a strong binding affinity for Drp1 [117]. This interaction appears to be needed for Bax oligomerization, suggesting that CL binding to Drp1 promotes apoptosis [118]. Additionally, CL appears to be needed for Drp1 oligomerization, although the effects of CL oxidation status on these interactions are unclear [62,119,120]. As interactions between CL and Drp1 occur in both mitophagy and autophagy, the oxidation state of CL may be a critical determinant in which pathway is activated. However, more work is needed to elucidate how the cells differentiate these pathways and if changes in CL oxidation provide functional outcomes. Further, although much more effort is needed to understand CL's role fully, our present understanding shows how intertwined CL, mitophagy, fission, and fusion are.

7. Disruption of mitochondrial network dynamics during chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is the third-leading cause of death worldwide, causing more than 3 million deaths per year [121]. COPD is characterized by irreversible airflow obstruction, collapse of alveoli, and chronic airway inflammation that progressively worsens, leading to devastating systemic effects (Fig. 5A). Oxidative stress is widely accepted as a significant contributor to COPD progression, with both exogenous and endogenous sources of ROS being implicated in COPD development [122]. Inflammatory responses against cigarette smoke (CS) from leukocytes and macrophages have been considered a primary ROS source contributing to COPD. However, oxidative stress persists after CS cessation. Therefore, endogenous sources of ROS, such as the mitochondria, have begun to be investigated for their contribution to the pathobiology of COPD [122].

COPD is associated with skeletal muscle impairment, which significantly reduces the quality of life of COPD patients and contributes to premature mortality [123,124]. Mounting evidence suggests that mitochondrial function is a key determinant of skeletal muscle function during COPD progression. For example, mitochondrial dysfunction is

observed in skeletal and respiratory muscles of COPD patients, including those with early-stage COPD [125–127], suggesting that mitochondrial dysfunction is a systemic phenomenon during COPD progression, affecting many different types of cells. Here, we discuss mitochondrial function in the pulmonary vasculature during COPD progression, as these cells are the first cells affected at disease onset and the first cells subjected to oxidative stress. Therefore, targeting the pulmonary vasculature may allow for early intervention to prevent or alleviate systemic effects, such as muscle dysfunction.

The most common etiological factor for COPD is CS, with 15–20 % of smokers developing the disease [128,129]. CS is a perplexing mixture of thousands of harmful agents and reactive oxidants [130]. Because of the strong association between smoking and COPD development, ROS generation is heavily implicated in the pathogenesis of COPD, as CS is a known source of ROS [131,132]. A single puff of CS introduces more than 1×10^{15} oxidizing molecules, including nitrogen dioxide and carbon monoxide, to the lungs [133–136]. This large influx of oxidants induces oxidative stress, which can harm overall cell homeostasis. Mitochondria are especially susceptible to oxidative stress. Mitochondrial DNA is 30-fold more sensitive to oxidants than nuclear DNA [137–139]. Therefore, it is likely that the high oxidative stress environments that exist during COPD pathogenesis have immense effects on mitochondrial health and function. In support of this, oxidative DNA damage is primarily found in the mitochondrial genome in lung tissue from COPD patients [140].

In airway epithelial cells, long-term CS exposure induces mitochondrial morphological changes, altered mitochondrial dynamics, increased expression of mitochondrial complexes, and increased expression levels of the critical mitochondrial antioxidant enzyme, manganese superoxide dismutase (MnSOD) [141]. These mitochondrial morphological changes include abnormal branching, fragmentation, and decreased cristae, persisting even 3 months after CS exposure cessation. This suggests that CS exposure induces persistent functional and morphological mitochondrial changes. Translating these findings to clinical relevance, primary bronchial epithelial cells isolated from ex-smoking stage IV COPD patients display similar mitochondrial changes to those observed in the *in vivo* findings. Specifically, the mitochondria in the bronchial epithelial cells isolated from COPD patients are elongated, swollen, abnormally branched, and have little or no cristae. Importantly, cells isolated from ex-smoking stage IV COPD patients display more mitochondrial damage than the smoking controls [141]. This reiterates that COPD development is likely induced by various factors, with or without CS.

Short-term CS exposure also induces mitochondrial elongation in the mouse alveolar epithelial cell line, MLE12 [142]. These effects are observed as early as 6 h post-exposure. After 24 h of CS exposure, Mfn2 levels are increased, and by 48 h, Mfn2 levels of the CS-exposed cells revert to control levels. Fascinatingly, CS exposure increases the mitochondrial membrane potential after 6, 24, and 48 h of treatment, suggesting that CS directly promotes a hyper-fusion phenotype [142]. Evidence also supports S-OPA-1 upregulation during COPD pathogenesis, with CS promoting the conversion of L-OPA-1 to S-OPA-1 *in vitro* and increased levels of S-OPA-1 present in COPD lung tissues [143]. These isoforms likely have varying functions [144–146], warranting further investigation into the functional outcome of higher S-OPA-1 levels during COPD progression (Fig. 5B).

As discussed earlier, hyper-fused mitochondria cannot be cleared via the mitophagy pathway (Fig. 4). Therefore, the fact that CS-exposed cells display higher levels of fusion may indicate an environment that ultimately prevents mitophagy from proceeding, and this change may persist throughout COPD disease progression and result in the accumulation of damaged mitochondria, affecting overall cell function. Interestingly, cells isolated from ex-smoking COPD patients have increased mRNA levels of PINK1, when compared to healthy controls, regardless of smoking history [141]. In lung tissues from COPD patients, parkin levels are decreased [147]. Therefore, despite PINK1 levels being upregulated, the decreased parkin levels suggest that mitophagy is impaired during COPD, although additional studies at varying stages of COPD evaluating the changes in mitochondrial dynamics and mitophagy during COPD progression are needed to understand this mechanistic interplay.

Interestingly, increased mitochondrial fragmentation, increased levels of Drp1, and decreased levels of Mfn2 have also been reported in the epithelial cells of COPD lung tissue when compared to healthy smokers, and these results were mirrored in short-term exposure to CS extract *in vitro* [148]. The differences observed in short-term (24-h, mouse [142], 48-h, human [148]) and long-term (6 months, human [141]) *in vitro* CS exposure highlight that the mitochondrial network is genuinely dynamic and that at varying stages of COPD progression. Moreover, this indicates that different environmental or genetic factors may influence the mitochondrial morphology observed, complicating the interpretation of mitochondrial involvement during COPD progression. Supporting this concept, the mitochondria in bronchial epithelial cells isolated from ex-smoking COPD patients exhibit mitochondrial fragmentation and elongated, swollen mitochondria, suggesting that fusion and fission are likely perturbed during COPD development and progression. Therefore, it is likely that COPD patients exhibit different mitochondrial phenotypes that are not entirely hyper-fission or hyper-fusion but a mosaic of abnormal mitochondria.

On the other hand, alveolar type II cells isolated from the lungs of COPD patients receiving lung transplantation display high levels of mitochondrial superoxide generation and mtDNA damage compared to cells isolated from control non-smoker and smoker organ donors, confirming more significant mitochondrial dysfunction than solely CS-induced. Moreover, when COPD alveolar type II cells are compared to cells from smokers, the COPD alveolar type II cells exhibit lower levels of the fission mediators, $pSer^{616}$ Drp1 and Fis1, and lower levels of the fusion factors Mfn1/2 and OPA-1, indicative of overall mitochondrial dysfunction. Interestingly, the mitochondrial fraction from overall COPD lung tissue exhibits higher levels of Mfn1 than smoker and non-smoker controls [149]. These results suggest that overall mitochondrial function is lower, as indicated by lower fission and fusion levels. This is further supported by the fact that there is a correlation between disease severity and lower levels of fission and fusion, analyzed from both mild and severe emphysema regions isolated from the same lung [149]. Logically, more damaged areas will have higher levels of mitochondrial damage; thus, the mitochondria cannot perform the same volume of mitochondrial dynamic signaling. Furthermore, the COPD mitochondrial fraction from the entire lung matches the hyper-fusion state that mitochondria appear to undergo during COPD progression. This is indicative that a hyper-fusion mitochondrial state precedes overall mitochondrial dysfunction.

Another risk factor for COPD is air pollution, which, according to the World Health Organization, accounts for forty-three percent of COPD cases [150]. Mice continuously exposed to ozone exhibit a COPD-like phenotype in which lung inflammation, emphysema, and neutrophil infiltration of the lung are observed. In this model of COPD, anti-inflammatory and antioxidant molecules reduce inflammation and the severity of COPD symptoms [151–153]. Furthermore, lung inflammation and airway hyperresponsiveness are associated with mitochondrial dysfunction in these mice. Specifically, mitochondria exhibit decreased mitochondrial membrane potential, increased levels of mitochondrial oxidative stress, and reduced expression of mitochondrial complexes I, III, and V. Similarly, the smooth muscle cells of COPD patients exhibit reduced mitochondrial function, evidenced by decreased mitochondrial membrane potential, reduced basal and maximum respiration and ATP levels, and reduced electron transport chain complex expression, suggestive of overall mitochondrial dysfunction. In addition, levels of mitochondrial ROS are increased in COPD cells when compared to healthy controls, and exposure of healthy airway smooth muscle cells to the ROS, hydrogen peroxide (H_2O_2), results in mitochondrial dysfunction. However, no further dysfunction is observed when cells from COPD patients are exposed to oxidative stress

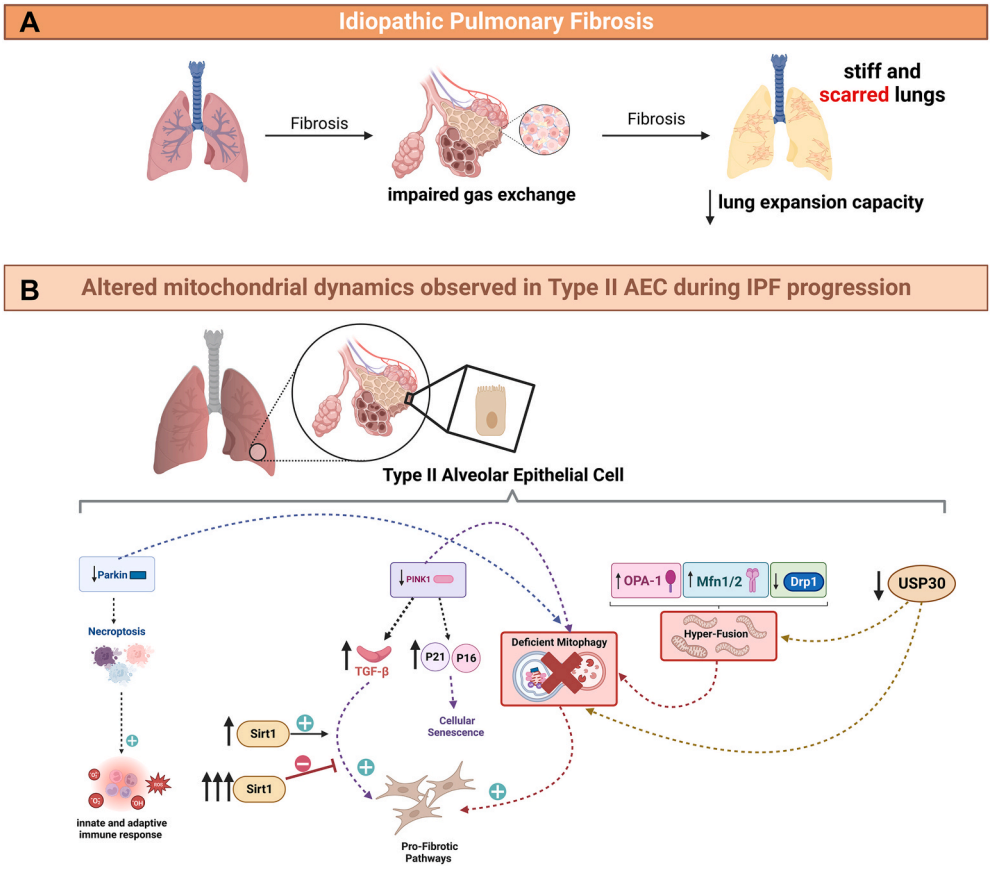

Fig. 6. Evidence of disrupted mitochondrial network dynamics in idiopathic pulmonary fibrosis. (**A**) IPF is a chronic fibrotic interstitial lung disease characterized by progressive scarring and stiffening of the lung that results in impaired diffusion capacity and ultimately requires lung transplantation. (**B**) Pathways identified to produce altered mitochondrial dynamics in Type II AEC during the progression of IPF. Abbreviations: IPF: Idiopathic Pulmonary Fibrosis, AEC: Alveolar Epithelial Cell, PINK1: PTEN-induced kinase 1, p21/p16: CDK inhibitors, Sirt1: Sirtuin 1, TGF-β: transforming growth factor β, Mfn1/2: mitofusin 1/2, OPA-1: optic atrophy protein 1, Drp1: dynamin-related protein 1, USP30: ubiquitin-specific protease 30, ROS: reactive oxygen species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[152], suggesting that an amount of oxidative stress may overwhelm the system and further addition of oxidative stress has no noticeable effect.

A significant component of air pollution is particulate matter (PM) that, even after short-term exposure, can increase circulating inflammatory molecules like IL-1β, IL-6, and TNF-α in the serum of humans [154]. Furthermore, PM exposure increases oxidative stress in macrophages and lung epithelial cells [155,156]. Excessive ROS activates the nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome, subsequently promoting inflammatory responses, an effect observed in bronchial epithelial cells among other cell types [157]. Both activation of the NLRP3 inflammasome and ROS are known to damage mitochondria, resulting in decreased mitochondrial respiration and function. Mice exposed to daily, low-dose PM for three weeks exhibit increased inflammatory markers in the bronchoalveolar lavage fluid (BALF). The lung tissue of these mice shows reduced levels of the mitochondrial antioxidant enzyme MnSOD and the pro-fusion marker OPA-1 and increased levels of the fission marker Drp1, the autophagy marker light-chain 3 microtubule-associated protein (LC3), and apoptotic markers [158].

Most of the research on COPD has focused on epithelial cells, as these are the cells that are directly exposed to the COPD risk factors, CS, and pollution. However, the epithelial layer is not in an isolated environment but is closely associated with the endothelial layer. Like the epithelial layer, the endothelial layer can be damaged by a high-oxygen environment and modulate inflammation. In COPD, airway remodeling results in thickening of the airway wall and airflow obstruction [159]. Because of this, it is logical that this remodeling also affects the structure and function of endothelial cells. However, very little is known about how endothelial cells are affected by the same morphological and functional changes observed in epithelial cells. There is some evidence that endothelial cells are damaged in patients with COPD. For example, increased levels of apoptosis in endothelial cells have been observed in COPD lung tissue [160–164] and emphysema patients exhibit higher levels of senescence markers than asymptomatic smokers and nonsmokers [165].

Evidence for endothelial cell involvement during COPD arises from the CS-induced increase in endothelial barrier permeability due to disruption of endothelial junctions [166]. Additionally, endothelial cells from emphysema patients exhibit higher levels of senescence markers than asymptomatic smokers and nonsmokers [165]. Moreover, CS induces apoptosis and mitochondrial ROS generation in pulmonary endothelial cells [167]. Despite both mitochondrial and endothelial cell dysfunction being implicated in COPD, very little is known about the role of mitochondria in endothelial cells during COPD, highlighting a significant knowledge gap that needs to be addressed. Furthermore, CS has been shown to alter mitochondrial morphology in rat endothelial cells. Mitochondria from *in vivo* and *in vitro* smoke-exposed endothelial cells have decreased Drp1 Ser^{637} phosphorylation and increased Drp1 Ser^{616} phosphorylation and Fis1 expression, all of which promote fission. These cells also exhibit aberrant mitophagy, increased mitochondrial oxidative stress, and reduced mitochondrial respiration. These effects are mitigated by the fission inhibitor Mdivi-1 and a mitochondria-targeted antioxidant (mitoTEMPO) [168].

Airway smooth muscle cells (ASMC) also significantly contribute to

the airway remodeling observed during COPD [169]. However, the effects of CS on ASMC remain largely unexplored [170], although initial investigations suggest that mitochondrial network dynamics are significantly altered in ASMC in COPD patients. For example, ASMC isolated from COPD patients exhibit persistent mitochondrial morphological changes, including fragmentation, high levels of mitophagy, and high levels of lysosome activity, compared to CS-exposed non-COPD cells [171]. Furthermore, ASMC cells from COPD patients not exposed to CS show increased amounts of fragmented mitochondria [171]. Studies with CS have shown that ASMC mitochondrial function and dynamics are affected by CS. For example, CS extract exposure induces mitochondrial fragmentation, upregulates the fission mediators Drp1 and Fis1, and downregulates the fusion mediators Mfn1/2 and OPA-1 in a dose-dependent manner. Inhibiting Drp1 is protective against CS-induced mitochondrial morphological changes, while inhibition of Mfn2 exacerbates the CS effect on the mitochondrial network [172]. These effects on mitochondrial dynamics have functional outcomes, such as altering energy metabolism, cell proliferation, and apoptosis [173]. However, it is essential to note that CS is only a component of COPD; thus, these studies provide an incomplete picture.

8. Disruption of mitochondrial network dynamics during idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrotic interstitial lung disease with no known cause and varying patient presentation and progression (Fig. 6A). In contrast to COPD, IPF is characterized by progressive scarring and stiffening of the lung that results in impaired diffusion capacity and ultimately requires lung transplantation [174,175]. Initially considered a chronic inflammatory process, IPF is now widely accepted as a fibrotic response confined to the lungs by abnormally activated airway epithelial cells (AEC, Fig. 6B). There are two subtypes of AEC: type I and type II AEC. Type I AEC are large, thin cells that cover most of the alveolar surface, forming a barrier that is intimately involved with gas exchange and inflammation. Type II AEC cover less alveolar surface than type I; however, they are exceptionally immunologically active and are responsible for repairing the epithelium. Overactivation of type II AEC has been linked to many pulmonary diseases, including IPF [176,177]. Once diagnosed with IPF, patients have a median survival of 2.5–5 years after diagnosis, highlighting the devastating progression of this disease [178]. However, median survival strongly depends on the phenotype of IPF [179], contributing to IPFs unpredictability as the disease progresses. Currently, only two anti-fibrotic therapies, pirfenidone and nintedanib, have been shown to improve survival rates. However, these drugs do not offer a cure and only slow the disease progression [180].

Because the currently available IPF therapies are inadequate, it is imperative to identify alternative strategies to treat the disease. The mitochondrial network is a promising therapeutic target. For example, the lung cells of IPF patients have decreased levels of both PINK and parkin, suggesting that these cells have reduced levels of mitophagy [181–183]. Furthermore, PINK1-deficient mice are more prone to developing pulmonary fibrosis and exhibit mitochondrial dysfunction [183,184], suggesting that promoting the mitophagy pathway may have some therapeutic benefit. PINK1-mitophagy is negatively regulated by USP30 and is associated with increased fusion [95,105,106,185], suggesting that inhibiting USP30 may be therapeutically beneficial for IPF treatment by stimulating mitophagy and restoring the mitochondrial fission/fusion balance by promoting fission (Fig. 6B).

However, conflicting reports discuss PINK1 expression during IPF. For example, in contrast to the studies mentioned above, increased levels of PINK1 have been observed in IPF lung tissues [184]. In addition to increased PINK1 levels, the IPF lung tissues from this study also contained a higher number of damaged mitochondria when compared to the controls. Furthermore, TGF-β1 stimulation resulted in mitochondrial depolarization, mitochondrial ROS production, and increased PINK1 [184]. An explanation for these seemingly paradoxical results is that IPF is not a static disease; it progresses, and as the disease progresses, more damage to both the lungs and mitochondria occurs. If damage is widespread throughout the lungs, the maximal mitochondrial function decreases as there are fewer functional mitochondria. Alternatively, another pathway, such as fibrotic pathways, may become upregulated during IPF progression and aging, ultimately downregulating PINK1. In support of this, PINK1 expression in IPF lungs decreases with age, and old lungs have increased expression of the pro-fusion mediators OPA-1 and Mfn1 and reduced levels of fission-promoting Drp1. Moreover, PINK1 deficiency promotes profibrotic responses [186], possibly unveiling a pathological mechanism that deviates from COPD progression [183]. Therefore, these results are not necessarily paradoxical but imply a dynamic disease state. Because Type II AEC from IPF lungs exhibit mitochondria with enlarged, dysmorphic structures with severely ruptured cristae [187], mitophagy is likely decreased during IPF, as these mitochondria would usually be cleared from the cell. A plausible disease progression may be that early-stage IPF is characterized by increased fusion, leading to a hyperfused phenotype that decreases the mitochondrial network's susceptibility to mitophagy. As the disease progresses, mitochondrial damage accumulates and negatively affects cell and tissue function, ultimately triggering fission and mitophagy. However, at this point, mitophagy cannot reduce the accumulated damage as it is too extensive, and other cell types, such as immune and endothelial, are dysfunctional and contribute to the disease progression. However, additional studies are needed to monitor the progress of IPF through all stages of the disease to capture how mitochondria change during disease progression.

Cellular senescence is a key feature of IPF, with mitochondria playing an important role via mtROS production and activation of the p21 or p16 pathways [188]. PINK1-deficiency in Type II AEC is associated with upregulation of the senescence markers p16 and p21 and with increased levels of TGF-β expression, which is known to be a key mediator of fibrotic processes during IPF [183,189]. Mitophagy impairment induced by PINK1 deficiency is associated with increased extracellular matrix deposition, implicating mitochondria in the fibrogenesis process of IPF [190]. Sirtuin 1 (Sirt1) is a central epigenetic regulator whose upregulation is implicated in many diseases, including cardiovascular disease, neurodegeneration, and obesity [191], and is implicated in regulating mitochondrial biogenesis and dynamics [192]. Sirt1 is upregulated in the lungs of IPF patients and bleomycin-induced lung fibrosis. Intriguingly, activation or overexpression of Sirt1 attenuates TGF-β-mediated fibroblast differentiation and reduces the severity of lung fibrosis in mice [193,194]. It is interesting to speculate on the presence of a potential feedback system that in IPF may not be able to reach high enough concentrations of Sirt1 to end the pro-fibrosis processes.

It is currently hypothesized that local micro-injuries to AECs in the lungs induce AEC dysfunction, ultimately resulting in AEC senescence [195]. Various factors can induce these microinjuries, including CS, environmental toxins, gastroesophageal reflux, microbes, and viruses [196]. When AECs are senescent, they overproduce cytokines, chemoattractants, and growth factors [197]. This creates a pro-fibrotic environment that activates tyrosine kinase and serine–threonine kinase pathways that initiate fibroblast proliferation and induce differentiation into myofibroblasts [198]. Additionally, this environment induces an abnormal phenotype in myofibroblasts in which these cells become anti-apoptotic and pro-fibrotic [196]. The overstimulated fibroblasts and myofibroblasts create an environment that significantly disturbs the extracellular matrix (ECM) homeostasis, leading to lung fibrosis and lung damage [199]. However, the molecular events that lead to this phenotype remain largely unknown, although emerging evidence suggests that mitochondria actively interact with the extracellular matrix and promote fibrosis during IPF [200].

Further evidence for mitochondrial dynamics involvement in IPF progression includes that type II AEC from IPF patients display higher levels of mitochondrial fusion, as indicated by increased Mfn2 mRNA

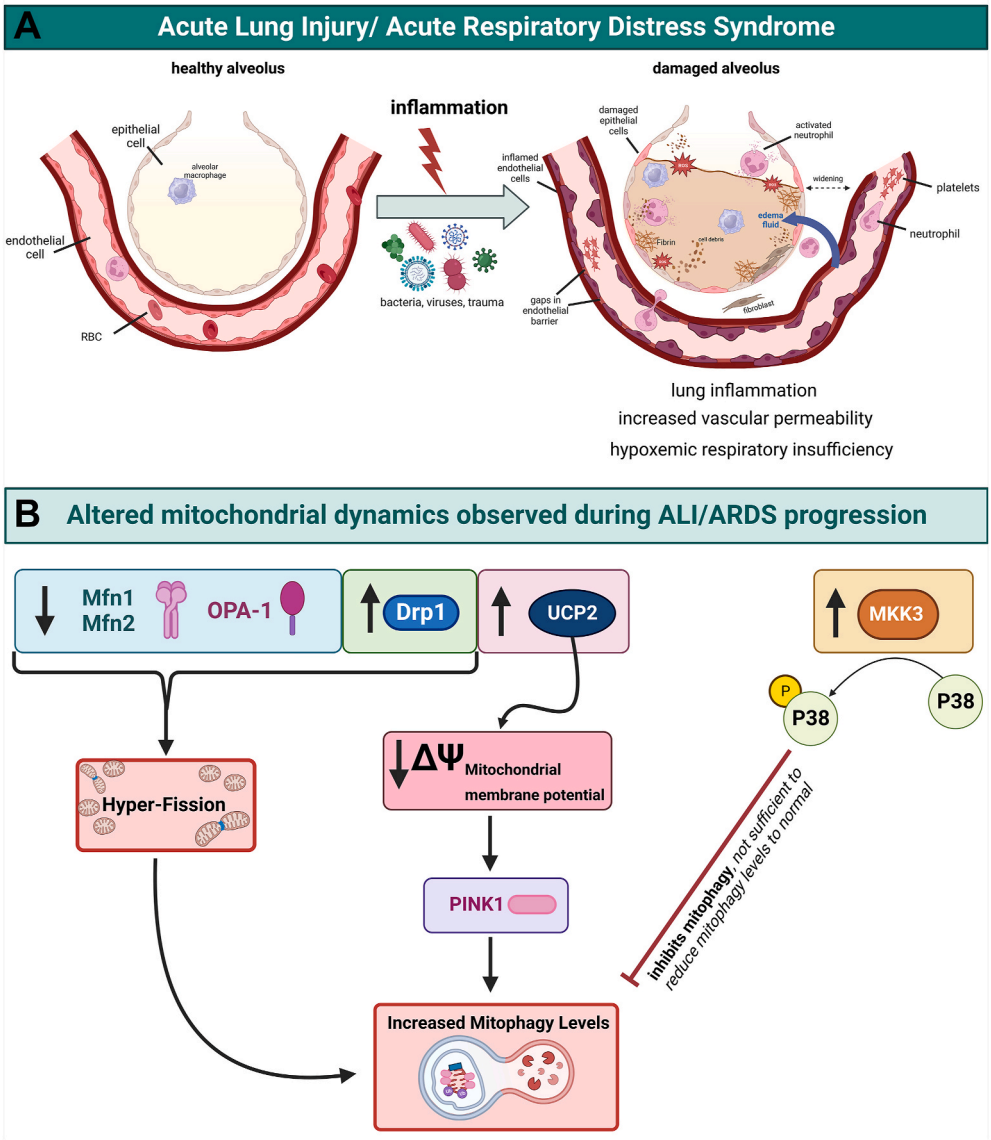

Fig. 7. Evidence of disrupted mitochondrial network dynamics in acute lung injury/acute respiratory distress syndrome. (**A**) ALI/ARDS are life-threatening syndromes defined by lung inflammation, increased vascular permeability, and hypoxemic respiratory insufficiency. Systemic inflammation (for example, sepsis) or lung inflammation (for example, severe pneumonia induced by SARS-CoV-2 or other bacteria or viruses) can cause ALI/ARDS. (**B**) Pathways identified to produce altered mitochondrial dynamics during the progression of ALI/ARDS. Abbreviations: ALI/ARDS: acute lung injury/acute respiratory distress syndrome, RBC: red blood cell, Mfn1/2: mitofusin 1/2, OPA-1: optic atrophy protein 1, Drp1: dynamin-related protein 1, UCP2: uncoupling protein 2, PINK1: PTEN-induced kinase 1, MKK3: mitogen-activated protein kinase 3, p38: p38 mitogen-activated protein kinase. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

expression [201]. In support of this, when the genes *Mfn1* or *Mfn2* are inducibly deleted from murine Type II AEC, these mice develop pulmonary fibrosis [202]. Interestingly, *Mfn1* deletion results in mitochondrial fragmentation, while *Mfn2* deletion results in swollen mitochondria [184]. Furthermore, type II AEC from old lungs exhibit enhanced expression of OPA-1 and Mfn1 with inactivation of Drp1, impaired mitophagy, and a decline in mitochondrial function. These studies imply that mitochondrial dynamics involve multiple pathways that create high-oxidative stress and the pro-fibrotic environment.

9. Disruption of mitochondrial network dynamics during acute lung injury

Acute lung injury (ALI) and a more severe form, acute respiratory distress syndrome (ARDS), are life-threatening syndromes defined by lung inflammation, increased vascular permeability, and hypoxemic respiratory insufficiency (Fig. 7A). ALI/ARDS includes a wide range of pulmonary damage, from airway inflammation to extensive pulmonary fibrosis. Causes of ALI/ARDS include systemic inflammation (for example, sepsis) or lung inflammation (for example, severe pneumonia induced by SARS-CoV-2 or other bacteria or viruses). Sepsis is a life-threatening condition where the overactivation of the immune system causes tissue damage. A common cause of sepsis is the excessive release of lipopolysaccharide (LPS), a significant component of the outer membrane of the gram-negative bacteria that acts as a strong endotoxin [203]. After LPS exposure, subsequent disruption of the alveolar-capillary barrier leads to alveolar flooding, where the alveoli fill rapidly with fluid. This accumulation of fluid and proteins in the alveolar space impairs gas exchange and causes respiratory distress. Sepsis syndrome with multiple organ failure remains the most common cause of death in ALI/ARDS patients, responsible for 30–50 % of all ALI deaths [204].

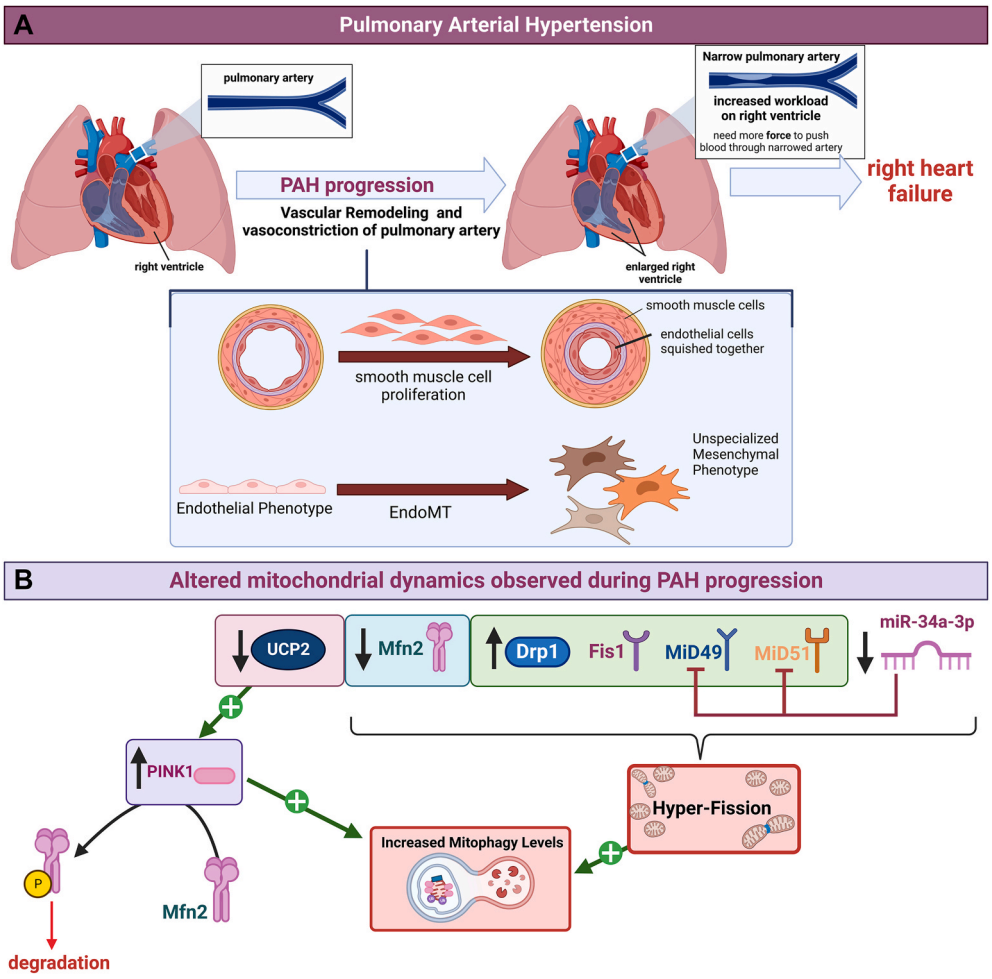

Fig. 8. Evidence of disrupted mitochondrial network dynamics in pulmonary arterial hypertension. (**A**) PAH is characterized by right heart dysfunction and eventually leads to right heart failure and death. Right heart failure results from the need of the right ventricle to increase its workload to compensate for the enhanced resistance of the pulmonary artery due to constriction driven by vascular remodeling. (**B**) Pathways identified to produce altered mitochondrial dynamics during the progression of PAH. Abbreviations: PAH: Pulmonary Arterial Hypertension, EndoMT: endothelial to mesenchymal transition, UCP2: uncoupling protein 2, Mfn2: mitofusin 2, Drp1: dynamin-related protein 1, PINK1: PTEN-induced kinase 1, Fis1: mitochondrial fission protein 1, Mff: mitochondrial fission factor, MiD49 and MiD51: mitochondrial dynamics proteins of 49 and 51 kDa. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Broad classification of targets being clinically tested in pulmonary disease. Abbreviations: ARDS: acute respiratory distress syndrome, COPD: Chronic Obstructive Pulmonary Disorder, IPF: Idiopathic Pulmonary Fibrosis, PAH: Pulmonary Arterial Hypertension.

Targets tested or being tested in clinical trials					
	COPD	IPF	ARDS	PAH	% of drugs tested from this class
immune modulation	26	24	28	12	45 %
bronchodilation or vasodilation	15	9	6	11	21 %
other	6	27	23	12	34 %
Successful Phase 3 targets or currently in phase 3 testing					
	COPD	**IPF**	**ARDS**	**PAH**	**% of successful or ongoing drugs tested from this class**
immune modulation	4	2	7	2	17 %
bronchodilation or vasodilation	12	3	3	7	61 %
other	0	4	5	3	18 %

According to an international, multicenter, prospective cohort study, ALI/ARDS is devastating in intensive care units (ICU), accounting for 10 % of ICU admissions. ARDS represents 0.42 cases per ICU bed, placing severe strain on the ICU due to extended hospital stays and the requirement for mechanical ventilators, as well as limiting the ability of the medical center to treat other patients. The prevalence of mild, moderate, and severe ARDS in the ICU is 30.0 %, 46.6 %, and 23.4 %, respectively [205], suggesting that even mild cases of ARDS contribute to the strain ARDS causes on hospitals' resources. These studies were conducted before the SARS-CoV-2 outbreak. Because the significant mortality rate of SARS-CoV-2 is often attributed to the development of ALI/ARDS, the SARS-CoV-2 outbreak only accentuated the strain ALI/ARDS represents for the healthcare system [206–208].

Currently, there are no effective treatments for ALI/ARDS [209,210]. The molecular events that lead to fibrogenesis and disease severity are largely unknown. A hindrance in ALI/ARDS therapy development stems from different types of pulmonary cells actively responding to and interacting with their environment and other systems. Signaling pathways differ from one cell type to another [211–213]. Because the different cell types that compose the lung establish dynamic interactions, it is essential to understand how these cells interact with one another to identify a therapeutic target that, if common to multiple cell

Table 2

Completed Clinical Trials Evaluating Efficacy of Immunomodulatory Drugs in Pulmonary Diseases. List of all completed clinical trials testing for the efficacy of an immunomodulatory target for treatment of ARDS, COPD, IPF, or PAH. FDA approved drugs for each respective disease were excluded. Highlighted in green indicate studies with a positive, primary clinical outcome with no increase in serious adverse events. Abbreviations: RV: right ventricle, LV: left ventricle, IL: interleukin, GM-CSF: granulocyte-macrophage colony stimulating factor, MARCKS: The myristoylated alanine-rich C-kinase substrate, JAK: janus kinase, HMT: histone methyltransferase enhancer, TRPC6: transient receptor potential channel C6, KCNN4: potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4, TNF: Tumor necrosis factor, LOX: lipoxygenase, MMP: matrix metalloproteinases, Nrf2: Nuclear factor-erythroid factor 2-related factor 2, GPR: G-protein-coupled receptor, CSF1R: Colony stimulating factor 1, PDGFRα/β: platelet-derived growth factor alpha and beta receptors, BMPR4: bone morphogenetic protein 4, ARDS: acute respiratory distress syndrome, COPD: Chronic Obstructive Pulmonary Disorder, IPF: Idiopathic Pulmonary Fibrosis, PAH: Pulmonary Arterial Hypertension.

Table 2: Completed Clinical Trials Evaluating Efficacy of Immunomodulatory Drugs in Pulmonary Diseases

	Trial ID	Target/Action	Result of Study
ARDS	NCT03111212, NCT00314548, NCT01467076, NCT02895373, NCT02370095	Prostaglandin	No significant improvement; NCT00314548 showed significant improvement in diastolic dysfunction
ARDS	NCT04382755, NCT04369469, NCT04395456, NCT04402060	Complement system inhibition	No significant improvement
ARDS	NCT00004494, NCT04360096, NCT04311697, NCT04843761	Vasoactive Intestinal Polypeptide	No significant improvement
ARDS	NCT01659307, NCT01504867, NCT00000574	Nonsteroidal anti-inflammatory drug	No significant improvement
ARDS	NCT00351533, NCT00789685, NCT02622724, NCT04576728, NCT04350580, NCT04475588, NCT04412668, NCT04570254, NCT03096314, NCT04525820, NCT04357782, NCT04443673	Broad anti-inflammation	No significant improvement; NCT00351533 reports reduced mortality in hospitalized patients with early sepsis, NCT04475588 reports survival and recovery-benefit
ARDS	NCT04315298	IL-6 antagonist	No significant improvement
ARDS	NCT04326920	GM-CSF	No significant improvement
ARDS	NCT03202394	MARCKS inhibitor	Phase II Studywith 38 subjects reports Reduced all-cause mortality from ARDS at 28 days. Completed in 2020, no update since.
ARDS	NCT04412057	Cytokine neutralization	Phase II trial with 88 subjects; reports possible reduction of respiratory failure and death
ARDS	NCT04565249	Integrin inhibitor	Safety Concerns
ARDS	NCT00036062	Neutrophil elastase inhibitor	No significant improvement
ARDS	NCT00201409	Promotes macrophage differentiation	No significant improvement
ARDS	NCT04402866, NCT04359290, NCT04377620	JAK inhibitor	No significant improvement
ARDS	NCT05018975	HMT inhibitor	Safety Concerns
ARDS	NCT04579393	Spleen tyrosine kinase inhibitor	Phase II trial with 59 subjects; reports improved clinical outcomes
ARDS	NCT04678739	Antiviral and immunosuppression	No significant improvement
ARDS	NCT01335932	Anti-viral	No significant improvement
ARDS	NCT04604184	TRPC6 inhibitor	Safety Concerns
ARDS	NCT04594668	KCNN4 inhibitor	Worse outcome in treatment group
COPD	NCT01448850, NCT00581945	IL-1 antagonist	No significant improvement
COPD	NCT01463644, NCT01227278	IL-5 inhibitor	No significant improvement
COPD	NCT00035828	IL-8 inhibitor	No significant improvement
COPD	NCT02546700	IL-13 inhibitor	No significant improvement
COPD	NCT01966549	IL17A protein inhibitor	No significant improvement
COPD	NCT03615040	IL-33 inhibitor	Phase II trial with 81 subjects; reports improved health status
COPD	NCT00056264	TNF-α inhibitor	No significant improvement
COPD	NCT03170232, NCT03250689, NCT03034967	CXCR2 antagonist	No significant improvement
COPD	NCT01108913	Pan-selectin antagonist	Favorable anti-inflammatory effect, no further update since 2011
COPD	NCT01054170	Neutrophil elastase inhibitor	No significant improvement
COPD	NCT00690482	Prostaglandin antagonist	No significant improvement
COPD	NCT00418613	5-LOX inhibitor	No significant improvement
COPD	NCT00758706	MMP-9/12 inhibitor	No significant improvement
COPD	NCT01335971	Nrf2 stimulator	No significant improvement
COPD	NCT00929734, NCT01944176, NCT01061671, NCT01151306	Statins	No significant improvement. Mild clinical benefit in non-primary outcomes (NCT00929734 reports reduced systemic inflammation and improved endothelial-dependent vascular function, NCT01944176 reports reversal of IL-17A/IL-10 imbalance in airways and reduced sputum macrophage counts, but not neutrophils. NCT01151306 reports Reduced aortic pulse wave velocity in patients with higher baseline aortic stiffness)
COPD	NCT00974142	Calcineurin inhibitor	Safety concerns

(*continued on next page*)

Table 2 (*continued*)

Table 2: Completed Clinical Trials Evaluating Efficacy of Immunomodulatory Drugs in Pulmonary Diseases

	Trial ID	Target/Action	Result of Study
IPF	NCT04594707	inhibits monocyte differentiation to profibrotic fibrocytes	No significant improvement
IPF	NCT02345070	IL-4 and IL-13 inhibitor	No significant improvement and increased serious adverse effects
IPF	NCT01629667, NCT01872689	IL-13 antagonist	No significant improvement
IPF	NCT01442779	Interferon α	Possibly beneficial, larger studies needed, 18 subjects in study, study completed 2007, no update given
IPF	NCT00076635, NCT00075998, NCT00047645, NCT00047658, NCT00052052	Interferon gamma-1b	No significant improvement
IPF	NCT00063869	TNF antagonist	No significant improvement
IPF	NCT03725852	GPR84 antagonist	Phase II trial with 68 subjects; reports reduced FEV decline
IPF	NCT03573505	αvβ6 integrin inhibitor	Serious adverse effects
IPF	NCT04396756	αvβ6 and αvβ1 integrin inhibitor	Phase II trial with 120 subjects; reports reduced lung function decline
IPF	NCT00786201	CCL2 antagonist	No significant improvement
IPF	NCT02503657	leukotriene receptor antagonist	No significant improvement
IPF	NCT02460588	immunosuppressant	Increased 3-month mortality
IPF	NCT02707640, NCT00650091	General antioxidant and anti-inflammation	No significant improvement and health concerns
IPF	NCT02759120	Antimicrobial Therapy	No significant improvement
PAH	NCT03657095, NCT00120380, NCT01908699	Prostaglandin	No significant improvement
PAH	NCT02279160	non-prostanoid prostaglandin receptor agonist	Phase II trial with 61 subjects; reports decreased pulmonary vascular resistance
PAH	NCT04456998, NCT01392495, NCT00477269, NCT00902174, NCT01179737	small-molecule kinase inhibitor that targets PDGFRα/β, CSF1R, and c-KIT, and modulates BMPR4	Frequent serious adverse events in all studies (NCT04456998 and NCT00477269: decreased pulmonary vascular resistance, NCT01392495: improved exercise capacity/ hemodynamics, NCT00902174: improvement in RV function, LV size and LV early diastolic relaxation)
PAH	NCT04576988, NCT03496207	activin signaling inhibitor	Phase III trials report greater improvement in exercise capacity and reduced pulmonary vascular resistance in patients receiving background therapy

Table 2 (*continued*)

Table 2: Completed Clinical Trials Evaluating Efficacy of Immunomodulatory Drugs in Pulmonary Diseases

	Trial ID	Target/Action	Result of Study
PAH	NCT02676947	IL-6 antagonist	No significant improvement
PAH	NCT02736149, NCT02664558	inhibitor of aminopeptidases and leukotriene hydrolases	No significant improvement
PAH	NCT02234141	Apoptosis Signal-Regulating Kinase 1 inhibitor	No significant improvement
PAH	NCT00384865, NCT00615823	Statins	No significant improvement
PAH	NCT03554291	Histamine H2 antagonist	Phase II trial with 80 subjects; reports lower RV mass and smaller RV end-diastolic volume with treatment
PAH	NCT01647945	Calcineurin inhibitor	Positive trend, no significance, larger trials needed
PAH	NCT00942708, NCT03638908	selective serotonin reuptake inhibitor	No significant improvement

types, would render maximal therapeutic benefits. Recently, mitochondrial dysfunction and altered mitochondrial network dynamics have been implicated in ALI development [214], offering a new avenue for the development of potentially effective therapeutic strategies to treat this devastating disease. Unfortunately, the mechanisms triggering and supporting mitochondrial dysfunction in ALI/ARDS are poorly understood. Here, we will highlight the research implicating mitochondrial involvement in ALI/ARDS development.

ALI/ARDS is associated with the presence of nitrosative and nitrative stress. LPS is a known stimulator of endothelial ROS generation and protein nitration [215–217]. We and others have shown that LPS can generate endothelial ROS by activating NADPH oxidase and xanthine oxidase, uncoupling the endothelial isoform of nitric oxide synthase (eNOS), and inducing mitochondrial dysfunction, which are all implicated in ALI [218–221]. All these systems contribute to the LPS-induced overproduction of nitric oxide (NO) and superoxide ($O_2^{\cdot-}$). The increased levels of NO and superoxide overwhelm the cellular antioxidant defenses and lead to the formation of peroxynitrite ($ONOO^-$), the most potent biological oxidant. The radical products of peroxynitrite decomposition react with tyrosine residues in proteins to form 3-nitrotyrosine (3-NT). Depending on the location of the tyrosine residue in the protein, nitration may affect protein function and alter cellular signaling and homeostasis [222]. Elevated levels of 3-NT have been observed in the lung and BAL fluid in ALI mice [219,223,224]. Importantly, 3-NT has been shown to impair vascular endothelial function and increase oxidative stress in developing sheep lungs [225]. The production of NO and downstream peroxynitrite impacts mitochondrial function. For example, NO can regulate mitochondrial respiration during metabolic stress or disease through reversible inhibition of mitochondrial complex IV. When peroxynitrite is formed, mitochondrial complexes I and II can be inactivated by tyrosine nitration and S-nitrosation, further decreasing the antioxidant defenses; MnSOD can be inactivated by nitration of a critical tyrosine residue located in its active site [226,227]. An additional mechanism by which peroxynitrite regulates mitochondrial activity is nitration of the pro-survival molecular chaperone heat shock protein 90 (Hsp90). Nitration of Hsp90 at tyrosine 33 has been shown to decrease mitochondrial activity [228]. Ultimately, peroxynitrite can affect mitochondrial function and trigger cell death by promoting oxidation and nitration reactions.

Our published studies have highlighted the importance of protein tyrosine nitration in regulating endothelial barrier dysfunction during sepsis-mediated ALI/ARDS by modulating the activity of the critical

Table 3

Status of COPD Clinical Trials Evaluating Drugs with evidence of mitochondrial dynamics modulation. List of completed and ongoing clinical trials evaluating drugs with indirect evidence of mitochondrial dynamics modulation in other diseases or cell types. Abbreviations: COPD: Chronic Obstructive Pulmonary Disorder, mt: mitochondrial, p38 MAPK: p38 mitogen-activated protein kinase, Mfn1/2: mitofusin 1/2, LABA: long-acting bronchodilators, LAMA: long-acting muscarinic and anticholinergic antagonists, FEV: Forced Expiratory Volume.

Table 3: COPD Clinical Trials Potentially Modulating Mitochondrial Dynamics

Trial ID	Drug Name	Target/Action	Possible effect on mitochondrial network dynamics	Result of Study
NCT0121812, NCT02299375	Losmapimod	p38 MAPK inhibitor	Linked to decreasing mt-fission [287]	No significant improvement
NCT00559910	PH-797804			Phase II trial report improved lung function, completed trial in 2009. No update provided
NCT00144859	Dilmapimod			Results not posted
NCT00423137	BIBW 2948	Epidermal growth factor receptor inhibitor	Likely increases mt-fusion via Mfn1 [291]	Safety concerns and unable to reach effective dose
NCT00929708	AZD3199	β2-adrenergic agonists	Likely increases mt-fusion via Mfn1 [292]	Phase II trial with 329 subjects; report bronchodilation. Completed in 2010, no update
NCT00808288	PF00610355			Results not posted
NCT00206167	Symbicort	inhaled corticosteroid/LABA	Downregulates Mfn1/2 and suppresses mitophagy [289]	Studies demonstrate clinical benefit. FDA approved treatments for COPD.
NCT01691885	RELOVAIR			
NCT00633217	Advair HFA			
NCT0246556, NCT02497001	PT010	inhaled corticosteroid/LAMA/LABA		
NCT0319781, NCT01911364	CHF 5993			
NCT02696564	Losartan	Angiotensin II receptor type 1 antagonist	Angiotensin II upregulates Mfn2, therefore inhibition likely increases mt-fission [293]	No significant improvement
NCT04072887	Icenticaftor	activates cystic fibrosis transmembrane conductance regulator	Likely decreases mt-fission and increases mt-fusion [294]	Phase II trial with 974 subjects; Did not improve trough FEV at 12 weeks (primary outcome) but positive findings at 24 weeks
NCT00000621	Retinoic Acid	Retinoic acid receptor agonist	Likely increases fission by increasing Drp1 levels [295]	No significant improvement

Table 4

Status of IPF Clinical Trials Evaluating Drugs with evidence of mitochondrial dynamics modulation. List of completed and ongoing clinical trials evaluating drugs with indirect evidence of mitochondrial dynamics modulation in other diseases or cell types. Abbreviations: IPF: Idiopathic Pulmonary Fibrosis, mt: mitochondrial, PF: pulmonary fibrosis, LPA1: lysophosphatidic acid receptor 1, PARK: parkin, PINK: PTEN-induced kinase, STAT3: Signal transducer and activator of transcription 3, TGF: transforming growth factor, Mfn2: mitofusin 2, FVC: Forced Vital Capacity.

Table 4: IPF Clinical Trials Potentially Modulating Mitochondrial Dynamics

Trial ID	Drug Name	Target/Action	Possible effect on mitochondrial network dynamics	Result of Study
NCT0066203, NCT0008022, NCT0320893, NCT0136620, NCT0028772, NCT00287716	pirfenidone	collagen-production inhibition	Likely increases PARK2/mitophagy [288]	Studies demonstrate clinical benefit. FDA approved treatment for IPF
NCT05497284	LTP001	STAT3 inhibitor	induces PINK1-dependent mitophagy [296,297]	ongoing, recruiting, est. completion May 2025
NCT05671835	TTI-101			ongoing, recruiting, est. completion March 2025
NCT03832946	GB0139	galectin-3 inhibitor	likely increases mitochondrial fission [298]	No significant improvement and increase of adverse events
NCT0225717, NCT03832946	TD139			Phase II trial with 60 subjects; reports decreased plasma biomarkers associated with IPF progression. A larger study with 426 subjects completed in 2023, awaiting results (NCT03832946)
NCT01262001	FG-3019	connective tissue growth factor inhibitor	likely decreases TGF-β1-induced mitophagy [299]	Phase II trial with 60 subjects; reports promising changes in FVC and radiographic pattern of PF
NCT01890265				Phase II trial with 160 subjects; reports reduced progression of IPF
NCT05722964	SHR-1906			ongoing, recruiting, est. completion May 2024
NCT04419558	Pamrevlumab			ongoing, est. completion April 2025
NCT03955146				ongoing, est. completion May 2024
NCT05951296	Leramistat	mitochondrial complex 1 inhibitor	increases mitophagy [300]	ongoing, recruiting, est. completion September 2024
NCT04968574	taladegib	Smoothened, a key Hh pathway component	increase mt-fission and mt-membrane hyperpolarization (prevents mitophagy) [301]	ongoing, recruiting, est. completion December 2023
NCT01766817	BMS-986020	LPA1 antagonist	Linked to increasing mt-fission [302]	Reduced FVC decline but increased risk of serious adverse events
NCT06025578	BMS-986278			not yet recruiting, est. completion December 2027
NCT06003426				ongoing, recruiting, est. completion October 2026
NCT04308681				ongoing, not recruiting
NCT05032066	HZN-825			ongoing, est. completion July 2025
NCT04533022	C21	Angiotensin II type 2 receptor agonist	Likely upregulates Mfn2/mt-fusion [293]	ongoing, est. completion March 2024

Table 5
Status of ARDS Clinical Trials Evaluating Drugs with evidence of mitochondrial dynamics modulation. List of completed and ongoing clinical trials evaluating drugs with indirect evidence of mitochondrial dynamics modulation in other diseases or cell types. Abbreviations: ARDS: acute respiratory distress syndrome, mt: mitochondrial, mAb: monoclonal antibody, eNAMPT: extracellular nicotinamide phosphoribosyltransferase, LPA1: lysophosphatidic acid receptor, p38 MAPK: p38 mitogen-activated protein kinase, HIF-PH: hypoxia-inducible factor–prolyl hydroxylase, Mfn1/2: mitofusin 1/2, PPARα: Peroxisome proliferator-activated receptor-α, PARK: parkin.

Table 5: ARDS Clinical Trials Potentially Modulating Mitochondrial Dynamics

Trial ID	Drug Name(s)	Target/Action	Possible effect on mitochondrial network dynamics	Result of Study
NCT04842747	Sabizabulin	Microtubule disruptor	Decreases mt-fission and fusion [303]	Phase III study with 204 subjects reports reduced mortality and lowered incidence of adverse events in hospitalized patients with high risk for ARDS/death
NCT05938036	ALT-100 mAb	eNAMPT antagonist	Decreases mt-fission [304]	Not yet recruiting, est. completion December 2024
NCT05135624	SP16	LPA1 inhibition	Linked to increasing mt-fission [302]	Ongoing, recruiting, Est completion March 2024
NCT05795465	GEn-1124	p38 MAPK inhibitor	Linked to decreasing mt-fission [287]	Ongoing, recruiting, Est completion July 2024
NCT04478071	vadadustat	HIF-PH inhibitor	Linked to decreasing mt-fission [305]	Clinical Benefit by Day 14
NCT04545242	Dexamethasone	Glucocorticoid	Downregulates Mfn1/2 and suppresses mitophagy [289]	Recruiting, Est completion December 2023
NCT01731795				reduced mechanical ventilation time and overall mortality in moderate-to-severe ARDS patients
NCT01284452	Hydrocortisone			Significant improvement in pulmonary physiology, no significant survival benefit
NCT00562835	Methylprednisolone			Improved pulmonary/organ dysfunction and reduced mechanical ventilation time and ICU stay length
NCT04355936	Telmisartan	Angiotensin 2 receptor blockers	Angiotensin II upregulates Mfn2, therefore inhibition likely increases mt-fission [293]	Reduced median discharge time and 30-day mortality
NCT04606563	Losartan, Valsartan, Azilsartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan			Terminated: DSMC recommendation due to futility
NCT04312009	Losartan			No significant improvement
NCT04977960	Potassium Canrenoate	Aldosterone receptor antagonist	Likely decreases mt-fission [306]	Not yet recruiting, Est completion December 2023
NCT00434993	Albuterol	β2-adrenergic Receptor Agonist	Likely increases mt-fusion via Mfn1 [292]	Terminated: Stopped for futility by DSMB
NCT05527704				Ongoing, recruiting, Est completion September 2026
NCT05241067	Centhaquine			Not yet recruiting, Est completion December 2025
NCT05847517	Metoprolol	Cardioselective Beta Blocker	Likely increases mt-fission [292]	Not yet recruiting, Est completion December 2027
NCT06013319	Esmolol			Recruiting, Est completion October 2026
NCT04115514	Liothyronine	Thyroid Hormone	Likely increases mitophagy [307]	Ongoing, recruiting, Est completion October 2023
NCT04725110				Not yet recruiting, Est completion 2028
NCT05075161	Pirfenidone	Anti-fibrotic drug	Likely increases PARK2/ mitophagy [288]	Ongoing, recruiting, Est completion October 2025
NCT04661930	Fenofibrate	Activates PPARα	Likely upregulates Mfn1/2 [308]	Phase III study with 15 subjects; Rapid reduction in inflammation and faster recovery

Table 6
Status of PAH Clinical Trials Evaluating Drugs with evidence of mitochondrial dynamics modulation. List of completed and ongoing clinical trials evaluating drugs with indirect evidence of mitochondrial dynamics modulation in other diseases or cell types. Abbreviations: PAH: Pulmonary Arterial Hypertension, mt: mitochondrial, Mfn2: mitofusin 2, PINK: PTEN-induced kinase, STAT3: Signal transducer and activator of transcription 3, RV: right ventricle.

Table 6: PAH Clinical Trials Potentially Modulating Mitochondrial Dynamics

Trial ID	Drug Name	Target/Action	Possible effect on mitochondrial network dynamics	Result of Study
NCT0045455, NCT0200762, NCT0081069, NCT00863681	Riociguat	soluble guanylate cyclase stimulator	Likely inhibits mt-fission and maintain mt-membrane potential (decreases mitophagy) [290]	Studies demonstrate clinical benefit. Approved treatments for PAH.
NCT04732221	MK-5475			Ongoing, recruiting, est. completion January 2028
NCT06053580	Valsartan	Angiotensin II receptor type 1 antagonist	Angiotensin II upregulates Mfn2, therefore inhibition likely increases mt-fission [293]	ongoing, recruiting, est. completion July 2027
NCT05764265	LTP001	STAT3 inhibitor	Induces PINK1-dependent mitophagy [296,297]	Ongoing, recruiting, est. completion September 2025
NCT05135000				Ongoing, recruiting, est. completion July 2024
NCT0344952, NCT04053543	CXA-10	nitro-fatty acid	Likely increases fission [309]	No significant improvement
NCT0210267, NCT03273387	Trimetazidine	fatty acid beta-oxidation inhibitor	Likely decreases fission [310]	Phase II/III studies with 25 subjects; increased 6MWT and minor improvement in RV remodeling (NCT02102672), improved RV ejection fraction (NCT03273387)

regulators of the actin cytoskeleton, small GTPases of the Rho protein family, RhoA, and Rac1. RhoA/Rac1 are extensively involved in forming and stabilizing the endothelial barrier [229]. We showed that peroxynitrite-mediated nitration of RhoA at tyrosine 34 constitutively activates RhoA to promote an endothelial contractile phenotype, leading to barrier disruption [230]. In addition, we have shown that peroxynitrite-mediated nitration inhibits Rac1, destabilizing endothelial junctions and disrupting the barrier [231]. It is known that RhoA also

regulates the activity of Drp1 by promoting phosphorylation at Ser^{616} (pS^{616}Drp1) through its downstream effector, ROCK. Once phosphorylated, pS^{616}Drp1 translocates to the OMM, where mitochondrial fission is subsequently promoted, disrupting mitochondrial dynamics, which can affect overall cell homeostasis [66]. Additionally, we have shown that an antioxidant specifically targeting the mitochondria decreases the levels of LPS-induced mitochondrial ROS generation *in vitro*, and significantly suppresses the LPS-induced inflammatory response in mouse lungs exposed to LPS [232]. Overall, these studies provide substantial evidence that mitochondrial dysfunction in endothelial cells is involved and contributes to the severity of ALI. Therefore, mitochondrial dynamics in ALI development should be thoroughly explored to identify potential therapeutic targets that could potentially offer a different, more efficient therapeutic approach to treat the disease.

Since RhoA is a modulator of Drp1 activity and LPS can induce activation of RhoA by nitration, LPS exposure may alter the mitochondrial network dynamics in ALI. Mice exposed to LPS exhibit decreased Mfn1, Mfn2, and OPA-1 levels and increased levels of Drp1 [233,234], suggesting that LPS promotes a fission phenotype (Fig. 7B). Moreover, LPS-exposed mice exhibit increased mitophagy, as observed using transmission electron microscopy. Relatedly, LPS-challenged A549 lung cancer cells show increased Drp1 levels and mitochondrial ROS and decreased Mfn2 levels, mitochondrial membrane potential, and ATP production. These cells also exhibited increased PINK1 expression in the mitochondria after LPS exposure [233]. Collectively, this data suggests that an LPS-mediated switch in mitochondrial phenotype towards fission, associated with increased PINK1-mediated mitophagy, alters mitochondrial function and network dynamics, leading to mitochondrial dysfunction.

It has been proposed that mitophagy is a protective pathway for ALI, as enhanced mitophagy can remove dysfunctional mitochondria from the system [235–239]. However, other studies provide evidence supporting a deleterious role for mitophagy, as it promotes more cell death and subsequently contributes to the pathogenesis of ALI [240,241]. When interpreting these studies and evaluating potential therapeutic approaches, it is essential to recognize that the etiology of ALI is very complex and involves many critical pathways essential for overall cellular homeostasis. One hindrance to therapeutic success for ALI is the aggregation of all ALI patients. ALI can develop in response to many stimuli, including pneumonia, sepsis, surgery, and trauma [242]. From a mechanistic point of view, the dysfunctional pathways may vary depending on the ARDS cause. Initial investigations reveal two sub-phenotypes within ARDS. Notably, one of these sub-phenotypes is characterized by more severe inflammation, shock, metabolic acidosis, and worse clinical outcomes [243]. Given this evidence, mitophagy is likely altered to varying degrees depending on ALI cause and cell type. Therefore, we must develop clinical diagnostic markers to successfully restore mitophagy homeostasis and determine a patient's mitophagy state. Moreover, the currently available methods to measure mitophagy are suboptimal, and different tools have different assumptions they rely on [99]. Therefore, more thorough analysis and studies are needed to fully understand mitophagy states during ALI. Here, we focus on studies around PINK1 or parkin, as these are highly associated with mitochondrial function and not directly tied to general autophagy.

Earlier, we discussed how LPS-exposed mice exhibit higher levels of mitophagy. However, others report that ALI/ARDS is associated with decreased mitophagy [235–239]. One such study took peripheral blood mononuclear cells isolated from septic patients and found that the septic patients exhibited higher levels of mitogen-activated protein kinase (MAPK) kinase 3 (MKK3) activity when compared to non-septic patients [244]. LPS activates MKK3, a major upstream kinase of the p38 MAPK. Once MKK3 is activated, it phosphorylates and activates p38, where it then directly phosphorylates and inactivates Parkin, leading to decreased mitophagy [245,246]. Moreover, MKK3-deficient endothelial cells have a larger pool of healthy mitochondria and exhibit higher levels of PINK1-mitophagy and mitochondrial biogenesis, supporting that MKK3 activation decreases mitophagy. This robust mitochondrial network appears to be protective, as MKK3 deficiency reduces lung and mitochondrial injury in septic mice while also reducing ROS generation. Furthermore, PINK1-knockout mice exhibit worse survival rates during LPS-induced sepsis [244]. These studies suggest that increasing levels of mitophagy is protective and that decreasing MKK3/p38 signaling may be a potential strategy to upregulate mitophagy. However, other studies report increased mitophagy in ALI/ARDS patients [240,241,247]. A plausible explanation for these seemingly paradoxical findings may be that the studies in MKK3-deficient mice do not accurately represent direct clinical relevance, as these mice are born with an abnormally active mitochondrial pool and a high capacity to perform mitophagy, which may offer protection as they can clear the mitochondrial damage before it progresses to ALI/ARDS. However, in most clinical cases, when a patient seeks treatment for ALI/ARDS, increased mitophagy may not be sufficient to prevent the disease's progression. However, more studies are needed to fully understand the role of mitophagy in different disease severity/stages.

During oxidative phosphorylation, electrons are transferred along the electron transport chain. During this electron transfer, protons are pumped into the mitochondrial intermembrane space, generating a robust electrochemical gradient. This gradient is then used by ATP synthase to generate ATP by transporting the protons along their gradient back to the mitochondrial matrix [248]. However, in some instances, the electron transfer can continue to reduce oxygen but does not maintain the proton gradient. This process is termed mitochondrial uncoupling and broadly refers to when electron transport is not used for ATP synthesis; the dissipation of the proton gradient is a common cause of uncoupling. Mitochondrial uncoupling can rapidly deplete levels of ATP; therefore, it is considered pathological. However, cells endogenously express uncoupling proteins (UCPs), suggesting other biologically relevant reasons to uncouple. One such theory is that it provides a mechanism to alleviate oxidative stress and stabilize membrane potential [249]. UCP2 is widely expressed in the lungs and can be a negative regulator of mitochondrial ATP production and protective against oxidative stress in vascular cells [250–252]. Despite this, UCP2 overexpression has been shown to decrease mitophagy and contribute to mitochondrial dysfunction and ROS generation [253,254]. Moreover, overexpressing UCP2 in mice increases LPS-induced pathological changes, exhibited by increased lung permeability, inflammation, and lower survival rates. Furthermore, ATP levels and mitochondrial membrane potential decrease, while ROS production increases [255]. These LPS effects can be mitigated by pretreatment with Jun N-terminal kinase (JNK) or p38 MAPK inhibitors [255]. This suggests that LPS-induced mitochondrial dysfunction may be influenced by UCP2 activation of p38 MAPK and/or JNK signaling. UCP2 overexpression has clinical relevance regarding sepsis-induced ALI, as UCP2 levels are higher in the blood of septic patients [256,257]. High levels of UCP2 are also observed with aging and IPF and have been shown to reprogram metabolism to induce oxidative stress [258]. Additionally, in acid-induced ALI, UCP2 knockdown or deletion is protective against acid-induced mitochondrial depolarization, endothelial-barrier disruption, and pulmonary edema [259]. Therefore, excessive UCP2 could contribute to these diseases by uncoupling the mitochondrial membrane and decreasing mitophagy, leading to the accumulation of dysfunctional mitochondria and insufficient energy production.

10. Disruption of mitochondrial network dynamics during of pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a devastating and incurable disease that is characterized by right heart dysfunction and eventually leads to right heart failure and death [260] (Fig. 8A). Right heart failure results from the need of the right ventricle to increase its workload to compensate for the enhanced resistance of the pulmonary artery due to constriction driven by vascular remodeling. Unfortunately, there

is no cure for PAH, with vasodilators being the only available therapy; however, this only results in temporary relief of symptoms, and eventually, lung transplantation becomes the only option for PAH patients [261]. The etiology of PAH is highly complex and involves inflammation, endothelial cell dysfunction, de-differentiation to mesenchymal cells (endothelial to mesenchymal transition), and excessive proliferation of pulmonary artery smooth muscle cells (PASMC). Recently, mitochondria have been recognized as a critical mediator of oxidative stress, bioenergetics, and the vascular remodeling that is observed during PAH (reviewed here [262]). However, the regulation of mitochondrial dynamics remains largely unexplored. Most PAH research has focused on PASMC; as such, most of the knowledge we have about mitochondrial dynamics is in this cell type, which may not be directly comparable to other cell types. However, the studies discussed in this section highly suggest that mitochondria are integral in developing PAH and warrant further exploration.

One of the most fascinating aspects of PAH is the many characteristics shared with cancer. Notably, PAH vascular cells exhibit an apoptosis-resistant, hyperproliferative phenotype and a Warburg shift in their metabolism, common to cancer cells [263]. The Warburg effect describes the phenotypic switch of cells from using mitochondrial oxidative phosphorylation to generate the majority of their energy to using aerobic glycolysis [264]. Additionally, mitochondria from PAH vascular cells share a similar phenotype as mitochondria from cancer cells. These mitochondria exhibit enhanced fragmentation from increased Drp1-mediated mitochondrial fission and have impaired fusion capabilities [265,266], while normal PAMSC under normal conditions display a more fusion-phenotype [266]. Drp1 has emerged as a critical player in PAH progression and is specifically being explored for its role in vascular remodeling. For example, lung tissue from PAH patients has more blood vessels that stain positive for phosphorylated Drp1 at serine 616, heavily implicating Drp1 and mitochondrial fission in the PAH pathobiology. Furthermore, in PASMCs isolated from PAH patients, the mitochondria exhibit more fragmentation, increased mRNA levels of Drp1 and Fis1, and decreased Mfn2 mRNA levels. Moreover, PAH PASMCs exhibit increased Drp1 protein levels and increased levels of phosphorylated Drp1 at serine 616, indicating that these cells exhibit a hyper-fission state (Fig. 8B). When these cells are administered Mdivi-1, an inhibitor of Drp1 GTPase activity, it reverses the fragmentation phenotype, reduces their proliferation rate, and is protective in animal models of PAH, suggesting that inhibitors of Drp1 have therapeutic potential for PAH patients [265]. However, Mdivi-1 is a suboptimal drug for clinical use, as it mainly acts on complex I instead of the Drp1 GTPase activity and has many other cellular targets that are not Drp1-associated [267]. Despite the pitfalls of Mdivi-1, inhibiting Drp1 appears to still be a promising target, as another inhibitor of Drp1 that targets the Drp1-Fis1 interaction, P110, improves mitochondrial function and is cardioprotective in mice with monocrotaline (MCT)-induced PAH [268].

Other mitochondrial dynamic mediators are dysregulated in PAH patients. For example, in the lung tissue of PAH and PAH PASMCs, the fission-promoting receptors, MiD49 and MiD51, are upregulated and contribute to the hyper-fission and hyperproliferative, apoptosis-resistant phenotype of PAH PASMCs. In PASMC isolated from PAH patients, silencing MiD49 and MiD51 reduces mitochondrial fission and Drp1 phosphorylation levels at Ser^{616}. Moreover, miR-34a-3p, which binds and inhibits MiD49 and MiD51, is downregulated in PAH PASMC. miR-34a-3p clinical relevance was validated in the whole blood and plasma of PAH patients. PAH patients have reduced miR-34a-3p levels, and restoration of miR-34a-3p levels in rodent PAH models is protective [269]. This implies that PAH patients have lower inhibitory regulation of MiD49 and MiD51, contributing to the hyper-fission state.

Contributing to the hyper-fission state observed in PAH PASMCs is the downregulation of Mfn2. PASMC from patients with PAH exhibit decreased levels of Mfn2 and display fragmented mitochondria. Viral overexpression of Mfn2 in chronic hypoxia + Sugen-5416 rats improved lung functionality, and these cells had increased levels of fusion, reduced levels of proliferation, and increased levels of apoptosis [270], indicative of a therapeutic potential of augmenting Mfn2 expression in those with, or susceptible to, PAH. Moreover, Mfn2 inhibits smooth muscle cell proliferation [8,271]. Therefore, targeting Mfn2 may also alleviate the hyper-proliferative phenotype associated with PAH. In PAH PASMCs and PAH lung tissue, levels of $pSer^{616}$ Drp1 are elevated, alongside mitochondrial fragmentation [265], supporting upregulation of fusion as a protective strategy for PAH treatment. Interestingly, Mfn2 also appears to have a connection to the mitophagy pathway. In PAH, Mfn2 is phosphorylated at serine 442 by PINK1 and subsequently degraded [272]. This suggests that increased mitophagy through PINK1 activity regulates fission and fusion by downregulating Mfn2, contributing to the hyper-fission state observed in PAH. Moreover, hypoxia, one of the main features of PAH, has been shown to stimulate PINK1 mitophagy and promote vascular remodeling [273], further supporting the idea that both fission and mitophagy play a vital role in PAH development.

UCP2 is also involved in the regulation of mitophagy in PAH. UCP2 regulates hypoxia-induced PAH in mice [274,275]. When UCP2 is deleted in the pulmonary endothelial cells of mice, these mice exhibit excessive levels of PINK1-mitophagy and a more severe intermittent hypoxia-induced PAH phenotype, highlighting the inverse relationship between mitophagy and UCP2 expression. Ablation of PINK1 in the endothelial-UCP2-knockout mice prevents intermittent hypoxia-induced PAH development, suggesting that mitophagy plays a direct role in PAH initiation. Moreover, endothelial cells isolated from PAH patients exhibit increased levels of PINK1 and decreased levels of UCP2 [274], suggesting that augmenting UCP2 levels may be beneficial for PAH patients, as it may decrease excessive levels of mitophagy. Supporting this idea, PASMC-UCP2-knockdown in mice results in reduced calcium levels, mitochondrial hyperpolarization, and increased resistance to apoptosis, mimicking the hypoxia-phenotype observed in PAH PASMC [275], suggesting that insufficient amounts of UCP2 are involved in the development of PAH.

Current debate surrounds mitophagy, fission, and AMP-activated protein kinase (AMPK) activation during PAH, as both activation and inhibition of AMPK are protective against developing PAH [276]. AMPK is widely regarded as a central regulator of energy homeostasis and mitochondrial health and is activated by multiple stimuli, including hypoxia and starvation [277]. AMPK has been suggested to increase mitophagy and endothelial cell proliferation [278]. This is supported by an AMPK-binding screening that shows that AMPK phosphorylates Mff to promote fission, leading to mitophagy activation [279,280]. Interestingly, AMPK activation promotes mitophagy independently of the PINK1/parkin pathway, as AMPK enhances mitochondrial fission through phosphorylation of Mff and TANK-binding kinase 1 (TBK1), which activates autophagosomal engulfment, independent of PINK1/-parkin [281]. This reinforces the idea that multiple pathways converge together, such as mitophagy, which makes interpreting pulmonary vascular diseases complicated. This may be a source of classification for different forms of PAH, as some may have higher or lower AMPK activity, and this may explain the conflicting reports of both inhibition and activation of AMPK being protective. For example, persistent pulmonary hypertension of the newborn (PPHN) is a highly fatal PAH condition where, at birth, the pulmonary vascular resistance fails to decline and results in extrapulmonary right-to-left shunting (where blood enters the aorta without first passing through the pulmonary circulation) and severe hypoxemia [282]. In a fetal lamb model of PPHN, AMPK function is decreased in the PPHN PAEC. Mitochondrial ROS activate AMPK, shift cellular metabolism towards catabolism, and activate fission and mitophagy [277,283,284]. Administration of an AMPK agonist increases mitochondrial complex protein levels and angiogenesis *in vitro* [285], implicating AMPK signaling as protective for PPHN. However, since AMPK promotes fission and mitophagy and multiple reports of PAH indicate increased fission and PINK1-mediated mitophagy levels, increasing AMPK activity may not be therapeutically beneficial in these

cases. However, AMPK may provide insight into identifying different forms of PAH in patients and how their mitochondria differ, possibly guiding therapeutic approaches.

11. Therapeutic targeting of mitochondrial network dynamics

Despite the recent advancements in understanding the etiology of pulmonary vascular diseases, no progress has been made clinically, and treatment options for these patients remain primarily palliative. For example, there are still no effective treatments for ALI/ARDS [209,210]. The only option for physicians is to place these patients on mechanical ventilation and hope they can improve gas exchange while attempting to minimize the many complications and further harm to the lungs the ventilator can cause [286]. The COVID-19 pandemic resulted in an influx of research efforts to identify treatments for ARDS secondary to Sars-CoV2 infection. Unfortunately, as the Sars-CoV2 infection became accepted as endemic and testing for infection decreased, many ongoing clinical trials have been withdrawn or terminated due to a lack of recruitment or loss of funding/business interest. This means that some promising targets from pre-clinical studies will remain untested. Sadly, ALI/ARDS remains a devastating disease even after the influx of COVID-19-related funding, with an ICU mortality rate that remains between 30 and 50 % [205].

To evaluate the progress of therapeutic development for pulmonary diseases, we examined the targets tested in the clinical setting or are currently being tested for ARDS, COPD, IPF, and PAH. We excluded studies that only evaluated safety without measuring clinical outcomes. Once the trial information was collected, we could broadly classify the targets into the following categories: immune modulation, bronchodilation or vasodilation, and other (Table 1). 45 % of the drugs tested are related to immune modulation. Of these drugs, only 17 % of the targets have had a phase 3 trial that was safe and effective in reducing disease progression or are still being evaluated in phase 3. Compiling a table of the 108 completed clinical trials evaluating immunomodulatory targets (Table 2), only 10 demonstrated clinical efficacy in the tested primary outcome without serious adverse events, and only one of these studies was a phase 3 trial. This implies that solely targeting the immune system to reduce inflammation is insufficient for pulmonary vascular diseases, and other strategies are needed. Using the master list of all clinically tested targets, we evaluated the effects of agents targeting the mitochondria. Unfortunately, only one phase 2 clinical trial directly targets mitochondrial function (NCT05951296) by assessing the clinical effect of inhibiting mitochondrial complex 1 in IPF patients. This implies that targeting the mitochondrial network dynamics remains an unexplored clinical strategy.

Next, we examine whether any trials utilized agents that there was indirect evidence that mitochondrial dynamics could be modulated. This included evidence from studies in different disease states and cell types. Using these criteria, we were able to evaluate targets tested in the clinical setting or currently being tested for COPD (Table 3), IPF (Table 4), ARDS (Table 5), and PAH (Table 6). Although this is indirect evidence, these links to mitochondrial dynamics may shed light on the reasons behind the therapeutic failure of current strategies. For example, p38 MAPK inhibitors have mostly failed to demonstrate efficacy for COPD (Table 3). p38 MAPK inhibition has been linked to reducing fission levels [287]. As discussed earlier (Fig. 5B), COPD is characterized by a hyper-fusion phenotype; therefore, p38 MAPK inhibition may exacerbate these abnormal mitochondrial dynamics. Although this remains speculative, it supports the idea that mitochondrial dynamics should be investigated during COPD progression and drug development.

Currently, only two anti-fibrotic therapies (pirfenidone and nintedanib) have been shown to improve survival rates in patients with IPF; however, these only slow down the disease progression [180]. When evaluating pirfenidone's effect on mitochondrial dynamics, it appears that pirfenidone augments deficient mitophagy via increases in PARK2 expression [288]. This is likely a beneficial effect, albeit insufficient to entirely halt disease progression, as IPF is characterized by deficient mitophagy (Fig. 6B). Increasing mitophagy likely restores part of the imbalanced mitochondrial dynamics. In support of this, in ARDS (Table 5), multiple angiotensin II receptor type 1 antagonists have failed to improve clinical outcomes in ARDS patients. Importantly, angiotensin II receptor type 1 antagonists likely increase levels of fission. ARDS is characterized by increased fission levels (Fig. 7B); therefore, these drugs will only exacerbate the mitochondrial dysfunction and may explain their inefficacy.

Moreover, glucocorticoids have both efficacy and safety concerns for ARDS patients (NCT04327401, NCT04347980, NCT00295269). However, some studies report positive clinical outcomes (NCT01731795, NCT00562835). In neurons, glucocorticoids have been shown to downregulate fusion by decreasing Mfn1/2 levels and suppressing mitophagy [289]. This could explain the mixed results from these trials, as downregulating Mfn1/2 likely decreases fusion even further, though suppressing mitophagy may benefit mitochondrial health and dynamics. Furthermore, riociguat, a soluble guanylate cyclase stimulator, is one of the few treatments for PAH that has established some clinical efficacy (Table 6). In myogenic precursor cells, soluble guanylate cyclase likely inhibits fission and maintains the mitochondrial membrane potential [290], thus decreasing mitophagy. Both counteract the increased fission and mitophagy levels observed during PAH (Fig. 8B). Although no conclusions may be drawn by these indirect comparisons of mitochondrial dynamics and drug targets being tested clinically, they do support mitochondrial dynamics as an essential component of pulmonary vascular diseases and thus warrant further investigation.

12. Conclusions

Mitochondrial network dynamics are a critical signaling hub for multiple cellular processes in several pulmonary diseases. As such, it is an attractive clinical target. However, its therapeutic potential is limited by our incomplete understanding of the cellular processes that lead to dysregulation of the fission/fusion cycle in an individual disease. Further, the therapeutic regulators of mitochondrial network dynamics are, at present, suboptimal. Thus, basic biological investigations and optimized drugs will be needed to exploit the therapeutic potential of mitochondrial network dynamics fully.

Declaration of competing interest

None

Data availability

No data was used for the research described in the article.

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