Mitochondrial quality control in pulmonary fibrosis

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ABSTRACT

Mechanisms underlying the pathogenesis of pulmonary fibrosis remain incompletely understood. Emerging evidence suggests changes in mitochondrial quality control are critical determinants in many lung diseases, including chronic obstructive pulmonary disease, asthma, pulmonary hypertension, acute lung injury, lung cancer, and in the susceptibility to pulmonary fibrosis. Once thought of as the kidney-bean shaped powerhouses of the cell, mitochondria are now known to form interconnected networks that rapidly and continuously change their size to meet cellular metabolic demands. Mitochondrial quality control modulates cell fate and homeostasis, and diminished mitochondrial quality control results in mitochondrial dysfunction, increased reactive oxygen species (ROS) production, reduced ATP, and fibroblasts within the context of pulmonary fibrosis.

1. Introduction

Mitochondrial quality control is maintained generally through three different mechanisms: (1) mitochondrial biogenesis; (2) mitochondrial dynamics (fusion and fission); and (3) mitophagy. Broadly, mitochondrial quality control also includes mitochondrial intracellular trafficking/migration and mitochondrial intracellular communication with the nucleus and other organelles, such as endoplasmic reticulum and Golgi apparatus. Mitochondrial dysfunction has been proposed to be a key player in pathogenesis of many pulmonary diseases, such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), pulmonary hypertension, asthma, acute lung injury, and lung cancer [1–9]. Many of these disease conditions, including IPF, are thought to be related to aging, and accumulation of dysfunctional mitochondria is considered a marker for the pathological conditions but is also the key factor that drives disease progression.

Evolutionally, mitochondria originated from the integration of an endosymbiotic alphaproteobacterium in host cells to facilitate a more efficient way of generating ATP through aerobic respiration [10,11]. The eventual transition to an intracellular organelle signifies the importance of mitochondrial quality control in maintaining cellular homeostasis. In the past years, in addition to their function as the powerhouse of the cell,” mitochondria have been shown to contribute profoundly to the regulation of signaling, metabolism, and cell death [12].

The complexity of mitochondrial quality control in pulmonary fibrosis is also related to various effector lung cells as the etiology of pulmonary fibrosis remains unknown and many hypotheses involving different cell types exist. While most of the research has been conducted on the three main cell types, namely alveolar epithelial cells (AEC), lung macrophages and fibroblasts, there are possible contributions from other cells such as vascular endothelial cells, smooth muscle cells, and fibrocytes. In this review, we concentrate on recent advances in mitochondrial quality control in AECs, lung macrophages, and fibroblasts; however, there have been studies suggesting that mitochondrial biogenesis is upregulated in vascular smooth muscle cells in both asbestos- and bleomycin-injured mice [13].

Given the evolution of the mitochondrion, it is not surprising that it is the only organelle, in addition to the nucleus, that contains its own DNA and transcription system. New mitochondria are not synthesized de novo but are generated through division from an existing mitochondrion. Mitochondrial biogenesis is a highly coordinated process utilizing both mitochondrial and nuclear encoded genes to increase mitochondrial size/mass. It requires synergistic efforts from mitochondria, nucleus, ER, and other organelles in the cell. The best documented regulator in mitochondrial biogenesis is PGC-1α, but other transcriptional factors, such as 5’ adenosine monophosphate-activated protein kinase (AMPK) and nuclear factor erythroid 2-related factor 1/2 (Nrf1/2) can also be involved [14]. Biogenesis is not only important during homeostasis and proliferation, but stress signals known to induce mitophagy can also promote biogenesis [15], suggesting biogenesis can serve as a possible rescue mechanism. However, dysregulated
biogenesis can also lead to increased mitochondrial ROS (mtROS) production and drive disease progression in pulmonary fibrosis [16,17].

Fusion (mitochondrial elongation) and fission (mitochondrial fragmentation) are not two separate processes but rather are interdependent. It has been hypothesized that mitochondrial dynamics are regulated in response to cellular stress. In mild to moderate stress conditions, cells mainly utilize fusion to combine damaged mitochondria with healthy mitochondria to offset the injuries. This will generate elongated mitochondria that can be spared from mitophagy. During severe stress conditions, normal or elongated mitochondria will undergo fission in which mitochondria will be separated into smaller compartments so that the diseased part will be split from the healthy part, limiting further damage. The fragmented and damaged mitochondria will eventually be removed by mitophagy. Regulatory proteins involved in fusion are optic atrophy 1 (OPA1) and mitofusin (MFN1 and MFN2). Proteins involved in fission are dynamin-related protein (Drp1) and its mitochondrial receptors, mitochondrial fission 1 (FIS1) and mitochondrial fission factors [18]. Increased numbers of mitochondria coordinate process involving both mitochondrial biogenesis and fission.

Mitophagy is a highly specialized form of autophagy called macropathology. The best-known regulators in mitophagy are PINK1 and PARK2. The canonical PINK1–PARK2-mediated mitophagy includes three steps: (1) PINK1 binds to mitochondrial outer membrane and recruits the E3 ligase PARK2; (2) PARK2 ubiquitinates mitochondrial proteins; and (3) SQSTM1/p62 binds ubiquitinated substrates to LC-3 ligands on autophagosomes. While this three-step process is the main pathway of mitophagy, PINK1 and PARK2 have additional functions in maintaining cellular homeostasis. PINK1/PARK2 have been shown to induce ubiquitination of fusion-related proteins, such as MFN1 and MFN2, as a mechanism to diminish the opposing process of mitophagy in neuroblastoma cells [19]. Interestingly, PARK2 binds to MFN2, but not MFN1, in HEK cells [20]. This binding is important for PINK1-mediated PARK2 mitochondrial recruitment, and PARK1/MFN2/PARK2 interaction is critical for the development of dilated cardiomyopathy.

All three mitochondrial quality control processes are considered catabolic and energy demanding. Several of the key proteins in mitochondrial dynamics are GTPases, such as MFN1/2 and Drp1, and mitochondrial receptors, mitochondrial mitofusin factors [18]. Increased numbers of mitochondria coordinate process involving both mitochondrial biogenesis and fission.

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All three mitochondrial quality control processes are considered catabolic and energy demanding. Several of the key proteins in mitochondrial dynamics are GTPases, such as MFN1/2 and Drp1, and ubiquitination of mitophagy-related proteins requires ATP. Mitochondria supply energy for these processes through the highly efficient oxidative phosphorylation (OXPHOS). However, emerging evidence suggests that cellular bioenergetics also change during mitochondrial quality control. It is generally accepted that glycolysis, particularly aerobic glycolysis, is elevated in fibroblasts during pulmonary fibrosis [21–23]. Changes in fatty acid oxidation also have been observed in pulmonary fibrosis, particularly in lung macrophages [17]. Lung macrophages have augmented fatty acid oxidation that facilitates their profibrotic polarization.

Mitochondria contain DNA and their own transcriptional mechanism. Traditionally considered as a surrogate of oxidative injuries during pulmonary fibrosis, mitochondrial DNA (mtDNA) is emerging as an important topic in understanding the pathogenesis of IPF, predicting prognosis, and determining effectiveness of anti-fibrotic drugs. Due to its proximity to the origin of ROS production and lack of the sophisticated mechanisms of protection and repair, mtDNA are prone to sustained injuries [24]. Many studies have evaluated mtDNA, particularly in its oxidized form, as an indicator of oxidative stress in AECs. Animal models with defects in mtDNA repair have exacerbated pulmonary fibrosis [25]. Recent studies have highlighted the biological relevance of mtDNA more than a mere indicator of excessive ROS. Release of mtDNA can serve as a Damage-Associated Molecular Pattern (DAMP) and bind to toll-like receptor 9 (TLR9), which leads to a cascade of intracellular responses involving TGF-β1 production, release and activation [26,27]. Moreover, extracellular mtDNA may be generated in an active production/secretory fashion related to mitochondrial biogenesis or mitophagy and have an autocrine or paracrine effect on fibroblasts to promote their transition into myofibroblasts. Translationally, mtDNA is increased in BAL fluid and serum of various fibrotic lung diseases, including IPF, and is correlated with increased mortality. Moreover, elevated expression of TLR9, the proposed mtDNA receptor, is correlated with rapid progression in IPF [28]. In this review, we will focus on recent advances in mitochondrial quality control in AECs, lung macrophages, and fibroblasts to distinguish their differences during pulmonary fibrosis, a devastating chronic lung disease.

2. Alveolar epithelial cells

The current and long-standing dogma is that recurrent epithelial cell injury and subsequent apoptosis are required for the development of lung fibrosis [29–31]. In response to normal injury, type II AECs differentiate into type I AECs to promote re-epithelialization; however, in pulmonary fibrosis, AECs undergo apoptosis. The injury impairs the ability of the lung to replace type I cells, and type II cells undergo hyperplasia inducing ineffective re-epithelialization. Additionally, the loss of alveolar epithelium basement membrane integrity in the fibrotic lung promotes alveolar collapse. Data supporting this shows that type II AECs from IPF subjects stain positive for pro-apoptotic markers and show a reduction in anti-apoptotic markers compared with control subjects [32]. Using experimental mouse models, the induction of type II AEC apoptosis promotes a fibrotic phenotype [33] and inhibition of apoptosis attenuates pulmonary fibrosis [34–36].

Emerging evidence indicates that mitochondrial damage occurs in IPF type II AECs. These cells from IPF subjects are increased in number and have enlarged and swollen mitochondria with disrupted cristae [34,35]. Mitochondrial mutations in IPF subjects are correlated with increased mtROS levels compared to wild type mice [39]. Nrf2 is an oxidant-sensitive transcription factor promoting antioxidant and detoxification genes with a fundamental role in regulating mitochondrial function [40,41]. AECs isolated from Nrf2-deficient mice exhibit sensitivity to oxidant-induced cell death, dysregulated type II AEC proliferation [42], and exaggerated fibrosis.

Fig. 1. Schematic of mitochondrial quality control in fibrotic type II alveolar epithelial cells.

(A) The production of mtROS promotes mtDNA damage by reducing the mitochondrial expression of SIRT3 and OGG1 to mediate AEC apoptosis. (B) Fibrotic type II AECs show increased ER stress that inhibits PINK1-mediated mitophagy to promote apoptosis. (C) Reduced PGC-1α expression leads to increased mitochondrial fusion (MFN1 and 2) and the accumulation of swollen and elongated mitochondria in fibrotic type II AECs. Abbreviations: LC3 = microtubule-associated protein 1A/1B-light chain 3; MFN = mitofusin; mtDNA = mitochondrial DNA; mtROS = mitochondrial reactive oxygen species; PINK1 = PTEN-induced putative kinase 1; PGC-1α = peroxisome proliferator-activated receptor-γ coactivator 1-α; OGG1 = 8-oxoguanine DNA glycosylase 1; SIRT3 = sirtuin 3.
Mitochondrial DNA (mtDNA) damage is linked to oxidative injury and pulmonary fibrosis. The DNA base excision repair enzyme, 8-oxoguanine DNA glycosylase 1 (OGG1), is critical for repair of type II AEC mtDNA oxidative damage [44]. Type II AECs from IPF subjects show increased levels of DNA oxidation [45], and Ogg1−/− mice are more susceptible to type II AEC mtDNA damage, oxidant-induced apoptosis, and asbestos-mediated lung fibrosis [25]. Mitochondrial sirtuin 3 (SIRT3), a member of the sirtuin family of NAD-dependent deacetylases, has been implicated in regulating lung fibrosis. SIRT3 function is decreased in type II AECs from IPF patients [46], and Sirt3−/− mice spontaneously develop lung fibrosis [47] (Fig. 1A). Moreover, SIRT3 modulates mtDNA damage by regulating OGG1 and manganese superoxide dismutase (MnSOD) acetylation [46], suggesting that SIRT3 deficiency leads to the acetylation and inactivation of mitochondrial proteins that augment oxidant-induced type II AEC mtDNA damage and apoptosis, thereby promoting pulmonary fibrosis.

The accumulation of dysfunctional mitochondria that is seen in IPF type II AECs is related to the downregulation of mitophagy [48]. In type II AECs from the IPF lung, PINK1 expression is decreased [5]. Impaired mitophagy is associated with enhanced AEC apoptosis and bleomycin-induced lung fibrosis in PINK1−/− mice [5,37] (Fig. 1B). Moreover, the pro-fibrotic cytokine TGF-β1 may protect lung epithelial cells by inducing PINK1 expression and attenuating AEC apoptosis, while silencing PINK1 in bronchial epithelial cells promotes TGF-β1-mediated apoptosis and mitochondrial depolarization [5,37]. Although Kobayashi et al. observed enhanced bleomycin-induced lung fibrosis in Park2−/− mice, no difference in type II AEC apoptosis was detected in these mice [49]. The ER stress protein, ATF3 (activating transcription factor 3), is a negative regulator of PINK1 gene expression (Fig. 1B). Among the three superoxide dismutase (SOD) enzymes, Cu,Zn-SOD (SOD1), which is located in the cytosol and mitochondrial intermembrane space, is increased in asbestosis lung macrophages and pulmonary fibrosis [66,67]. Lung macrophages from IPF subjects generate increased mROS [6,68], similar to the increase seen in lung macrophages from asbestos subjects [69,70]. Levels of mROS can be reduced in fibrotic macrophages by silencing the iron-sulfur protein, Rieske, in complex III of the mitochondrial ETC [67,71]. The increased ROS levels in IPF lung macrophages is due to the increased recruitment of monocyte-derived macrophages [72]. The increase in ROS levels is lung specific as this increase was not detected in blood monocytes isolated from IPF subjects.

Among the three superoxide dismutase (SOD) enzymes, Cu,Zn-SOD (SOD1), which is located in the cytosol and mitochondrial intermembrane space, is increased in asbestosis lung macrophages and contributes to the increased mROS production by the dismutation of superoxide to hydrogen peroxide [66] (Fig. 2A). In addition, mitochondrial SOD1 accelerates the development of pulmonary fibrosis by augmenting the profibrotic polarization of lung macrophages via redox regulation of the Jumonji domain-containing protein 3 [70,73].

Mitochondrial quality control in lung macrophages, including processes such as mitophagy and mitochondrial biogenesis, are critical determinants in pulmonary fibrosis. Diminished mitochondrial quality control results in augmented mitochondrial dysfunction and increased mROS that leads to decreases ATP production and promotes intrinsic apoptosis [74–76]. Lung macrophages polarize to a profibrotic phenotype and are resistant to apoptosis in IPF subjects secondary to enhanced mitophagy [6] (Fig. 2B). Furthermore lung macrophage mitochondria must undergo mitophagy to protect TGF-β1, as Park2−/− mice fail to secrete TGF-β1 and are protected from bleomycin-induced fibrosis [6]. Translational support of the importance of mitophagy in fibrosis progression showed that IPF subjects taking the rapamycin analog, everolimus, which increases mitophagy in macrophages, had response to bleomycin [43].
exposed to ± mice [85]. During ER stress, calcium is released from exposed WT mice showed a loss in mtROS and biogenesis are critical for the maintenance of the pro-

The removal of dysfunctional mitochondria by mitophagy in fibrotic lung macrophages is associated with increased mitochondrial biogenesis in these cells. Levels of PGC-1α are increased in lung macrophages from IPF and asbestosis subjects [16,17] (Fig. 2C). NOX4, which is also present in mitochondria [78], has been shown to regulate mitochondrial biogenesis in fibrotic macrophages [16]. Moreover, NOX4 regulated PGC-1α expression, and PGC-1α is required for NOX4-mediated mitochondrial biogenesis in fibrotic macrophages (Fig. 2C). The observed increase in mitochondrial biogenesis appears to be critical for sustaining the profibrotic phenotype of macrophages. NOX4−/− macrophages fail to polarize to the profibrotic phenotype and show reduced mtROS production [16], suggesting that mtROS and biogenesis are critical for the maintenance of the profibrotic macrophage phenotype.

Metabolic reprogramming that entails fatty acid oxidation (FAO) and OXPHOS is a key feature of profibrotic macrophages [79,80]. Recent evidence indicates that metabolic reprogramming shifts glycolysis to FAO in macrophages from bleomycin- or asbestos-injured mice [16,17]. The metabolic reprogramming in profibrotic macrophages is necessary to support long-term cellular activities (i.e., lung remodeling), as well as to promote the apoptotic resistance that is seen in these cells [6,81–83]. Studies indicate that ATP levels are increased in BAL fluid and within lung macrophages from subjects with lung fibrosis [84,85], and PGC-1α is required for ATP production in lung macrophages [85] (Fig. 2C). Moreover, extracellular ATP has been shown to promote mitochondrial dysfunction and mtDNA oxidation in mouse macrophages [86].

Calcium homeostasis plays a critical role in mitochondrial quality control. IPF and asbestosis subjects have higher mitochondrial calcium levels in lung macrophages compared with normal subjects [17,85]. The mitochondrial calcium unipporter (MCU), a highly selective ion channel transporting calcium into the mitochondria, is also increased in the lung macrophages from fibrosis subjects (Fig. 2A). An increase in mitochondrial calcium levels is associated with a loss of mitochondrial membrane potential (ΔΨm). Lung macrophages isolated from asbestos-exposed WT mice showed a loss in ΔΨm, which was not seen in asbestos-exposed MCU−/− mice [85]. During ER stress, calcium is released from the ER and enters mitochondria. ER stress has been reported in macrophages isolated from IPF and asbestosis subjects and in bleomycin and asbestosis-exposed mice [87,88] (Fig. 2A). Calcium influx into the mitochondria is suggested to regulate OXPHOS and may promote an adaptive response to acute ER stress [85,89]. Moreover, MCU regulates PGC-1α expression (Fig. 2C) to mediate metabolic reprogramming in fibrotic lung macrophages [17].

Mitochondrial-derived ROS have been linked to inflammasome activation [90], and asbesstop silica, which can cause pulmonary fibrosis, are known to activate the NLRP3 inflammasome [91,92]. Likewise, lung macrophages from IPF subjects show increased NLRP3 inflammasome expression [93]. Vimentin intermediate filaments are known to modulate mitochondrial motility [94], and lack of vimentin results in increased ROS production [95]. Furthermore, mtROS is required for inflammasome priming [96], and vimentin is critical for NLRP3 inflammasome assembly and activation in pulmonary fibrosis [97].

Lung macrophages contribute to the pathogenesis of pulmonary fibrosis by initiating an immune response and by generating mtROS. The increase in mtROS promotes mitophagy to clear the dysfunctional mitochondria. Mitochondria from fibrotic lung macrophages maintain functionality through increased biogenesis and fission. The impact of the increased mitochondrial turnover leads to enhanced mitochondrial ATP content. The increased mtROS that is seen in these cells also promotes ER stress and an influx of calcium into the mitochondria. Lung macrophages in pulmonary fibrosis exhibit apoptosis resistance, and their prolonged survival is associated with disease progression. Fibrotic lung macrophages also undergo metabolic reprogramming to FAO, which is regulated by mtROS, to support the long-term cellular activities necessary for lung remodeling.

4. Lung fibroblasts

IPF lung fibroblasts have reduced mitochondrial mass, disrupted membranes, and altered cristae compared with lung fibroblasts from normal subjects, suggesting changes in mitochondrial homeostasis [98]. Studies concentrating on the three different aspects of mitochondrial homeostasis (biogenesis, dynamics, and mitophagy) provide many potential mechanisms of how fibroblast mitochondrial quality control actively participate in the pathogenesis of pulmonary fibrosis.

Much of the research on mitochondrial biogenesis focuses on PGC-1α, although contradicting data exist. TGF-β1 has been shown to activate PGC-1α, and induce mitochondrial biogenesis and bioenergetics in fibroblasts in vitro [99,100]. This augmented mitochondrial biogenesis is required to maintain the differentiated status of myofibroblasts. Interestingly, increasing mitochondrial biogenesis does not increase fibronectin and collagen Iα1 expression, suggesting a multi-latitude of regulatory mechanisms for matrix protein production or degradation. In contrast, another study showed that PGC-1α level is suppressed in primary IPF fibroblasts, and overexpression PGC-1α can reverse the profibrotic phenotype of IPF fibroblasts with reduction of collagen production [101]. Inhibiting PGC-1α in normal primary lung fibroblasts and IMR90 lung fibroblasts leads to augmented collagen protein production. Activation of PGC-1α with a PPARγ activator, rosiglitazone, can induce PGC-1α expression and reduce collagen gene expression. A PGC-1α-independent mechanism of mitochondrial biogenesis was also reported [41]. TGF-β1 induces NOX4 expression in fibroblasts and subsequent mtROS production [102] (Fig. 3A). NOX4, which is increased in IPF fibroblasts and promotes pulmonary fibrosis, suppresses mitochondrial biogenesis, and reduces OXPHOS. NOX4 modulates mitochondrial biogenesis by regulating Nrf2 expression and its nuclear translocation (Fig. 3B). Pharmacological inhibition or genetic silencing
of NOX4 promotes mitochondrial biogenesis in fibroblasts.

The AMPK pathway plays a critical role in myofibroblasts differentiation and autophagy. Early research has shown that despite AMPK pathway activation in IPF lungs, there is no change of autophagy [103]. To note, these were measured in whole lung homogenates that lack specificity to cell types. TGF-β1 inhibits autophagy in human lung fibroblasts. Moreover, inhibition of autophagy by silencing LC3 increases α-SMA expression in fibroblasts. A recent study addressed AMPK activities in different cells types in pulmonary fibrosis. While AMPK activities remain unchanged in airway epithelial cells between normal and IPF lungs, AMPK activities are significantly reduced in the fibrotic foci where active myofibroblasts are located [104]. IPF fibroblasts have reduced AMPK activity and reduced autophagy, which is linked to mitochondrial dysfunction and metabolic reprogramming. IPF myofibroblasts have more fragmented mitochondria secondary to a defective mechanism involving mitophagy (Fig. 3C). Activation of AMPK pathway with a pharmacological activator, metformin, reversed established pulmonary fibrosis in an animal model of pulmonary fibrosis [104]. AMPK activation also transposed the apoptosis-resistant phenotype observed in IPF fibroblasts. One of the proposed mechanisms of fibroblast reversal from apoptotic resistance is through increasing AMPK-activated mitochondrial biogenesis.

Several recent studies have specifically evaluated mitophagy in pulmonary fibrosis by focusing on mitophagy-specific proteins, such as PINK1 and PARK2. TGF-β1 treatment inhibits PINK1 and PARK2 expression in lung fibroblast cell lines [105] (Fig. 3C). Targeting PINK1 and PARK2, Kobayashi et al. showed that silencing either gene is able to induce α-SMA expression in lung fibroblasts with a greater extent seen in PARK2−/− fibroblasts [49]. Although no significant mitochondrial damage was observed by electron microscopy, increased mtROS production was detected biochemically, which can be attenuated by either N-acetylcysteine (NAC) or Mito-TEMPO. Surprisingly, no changes in both fusion-related (OPA1 and MFN1/2) and fission-related (Drp1 and FIS1) proteins were observed in PARK2 null fibroblasts compared with wildtype fibroblasts. Additionally, no differences were observed in ATP production between wildtype and PARK2−/− fibroblasts. Silencing PARK2 resulted in increased phosphorylation of Akt1 and PDGF, and the Akt1/2 inhibitor blocked α-SMA expression and PDGF phosphorylation in PARK2−/− fibroblasts, suggesting an important role of Akt in mitochondrial dynamics and fibrosis progression [106]. Using a PDGF inhibitor, AG1296, the authors demonstrate that mice are protected from developing pulmonary fibrosis and conclude that in fibroblasts, PARK2-mediated mitophagy mainly regulates fibroblast differentiation through PDGFR/Akt signaling pathway. The same authors further demonstrate that pifirfenidine, one of the two FDA-approved anti-fibrotic medications, induce PARK2 but not PINK1 expression and promote mitophagy in fibroblasts. Mechanistically, pifirfenidine inhibits ROS production and abrogates Akt activation in PARK2 null fibroblasts. Further studies are needed to address the knowledge gap of mitophagy-specific genes in different types of fibrosis-promoting cells during fibrogenesis.

One proposed mechanism of regulating PARK2-mediated mitophagy in pulmonary fibrosis is through miR-1224-5p [105]. TGF-β1 treatment inhibits PARK2 mitochondrial translocation, which is rescued by a miR-1224–5p inhibitor in vitro. miR-1224–5p binds to the promoter region of becn1 gene and inhibits its expression. Immunoprecipitation experiments further verified that beclin-1, which can also induce autophagy/mitophagy, binds to PARK2. In an experimental silicosis model, miR-1224–5p levels are increased, and the expression of beclin-1 is reduced in mice exposed to silica [107]. Similarly, these authors showed that silencing PARK2 leads to activation of Akt/PDGFR pathways and increased α-SMA expression in fibroblasts.

Limited studies have investigated the role of mitochondrial fusion and fission in fibroblasts during pulmonary fibrosis. One study showed that astaxanthin, a carotenoid usually used as dietary supplement, can induce Drp1 expression and mitochondrial translocation that leads to enhanced fission and apoptosis in human fibroblasts and bleomycin-injured mice. However, TGF-β1 treatment does not alter mitochondrial fission [108]. The authors proposed that the protective mechanism of astaxanthin in bleomycin-injured mice is secondary to fission and apoptosis of myofibroblasts; however, additional experiments utilizing mice harboring a selective deletion of fission-associated genes, such as Drp1, are required to validate these observations.

Mitochondrial DNA is an emerging research area due to its translational significance as a biomarker of mitochondrial homeostasis and cellular injuries in various disease conditions, such as ARDS, COPD and various fibrotic lung diseases, including IPF. Studies have shown that IPF fibroblasts produce increased amount of mtDNA in vitro [23]. Similarly, normal lung fibroblasts have increased mtDNA production upon stimulation of TGF-β1. Furthermore, mtDNA is elevated in bronchoalveolar lavage fluid and plasma of IPF subjects (Fig. 3A). From a biomarker standpoint, elevated serum mtDNA is correlated with increased all-cause mortality in IPF, and mtDNA is reduced in IPF subjects that respond to pirfenidone treatment [23]. Although the authors did not specifically address whether increasing mtDNA is a direct result of either mitochondrial biogenesis or mitophagy, they showed that TGF-β1-stimulated fibroblasts have reduced mitochondrial mass, elongation and enlargement, and maintenance of ΔΨM. The authors postulate that elevated extracellular mtDNA is not related to cell viability (i.e., not secondary to increasing apoptosis or necrosis), but rather is the result of a potential active releasing/production that may enhance fibroblasts differentiation into myofibroblasts (Fig. 3A). In contrast, Bueno et al., showed that there are no differences in mtDNA production by IPF fibroblasts compared with age-matched normal fibroblasts [109]. Given mtDNA is found to be elevated in various fibrotic lung diseases, including IPF, sarcoidosis, hypersensitivity pneumonitis, and ILD caused by connective tissue diseases, it is critical to further delineate the role(s) and mechanism(s) that lead to increased extracellular mtDNA given its translational importance in determining drug efficacy and predicting prognosis, at least, in IPF.

During fibrosis, fibroblasts have augmented mitochondrial dysfunction and generate increased mtROS while having suppressed mitophagy and mitochondrial biogenesis that promotes differentiation into myofibroblasts. The suppression of mitophagy and biogenesis also contributes to apoptosis resistance. The evidence supporting the role of mtDNA as a biomarker and prognostic indicator has translational and
Mitochondrial dynamics (control including mitophagy, mitochondrial biogenesis, and mitochondrial organization) are critical for driving the profibrotic behaviors and contribute to the development and progression of lung fibrosis. A randomized clinical trial using NAC failed to improve clinical outcomes (changes in lung function, rate of death, or acute exacerbation) in patients with IPF [114]. Although attempts to alter ROS with general antioxidants have been largely unsuccessful, the failure of the NAC trial may have resulted from an incomplete understanding of role of mtROS and IPF development. Strategies to target mitochondria with organelle specific drugs are being developed. MitoQ, a mitochondrial targeted antioxidant, has been shown to attenuate expression of TGF-β1 and NOX4 in IPF lung fibroblasts [115]. In addition, transgenic mice expressing mitochondrial catalase are protected from asbestos- and bleomycin-induced lung fibrosis and had lower levels of mtDNA damage [39]. Furthermore, using lung tissue and/or cells from patients undergoing biopsy or lung transplantation to develop 3D lung organoids (pulmospheres) may aide in the ability to target cell-specific mitochondria for a personalized medicine approach [116]. The growing interest in mitochondrial biology may provide new avenues to identify novel therapeutics.

5. Conclusion

The most common form of pulmonary fibrosis, idiopathic pulmonary fibrosis is a devastating disease affecting approximately 3 million people worldwide and has a high mortality rate [110]. The currently approved therapies, pirfenidone and nintedanib, have limited efficacy in that neither drug is associated with improvements in quality of life or mortality and only show modest improvements in disease symptoms [111,112]. Emerging evidence suggest that the contribution of mitochondrial dysfunction is a key player in the pathophysiology of lung diseases, such as IPF.

Mitochondrial quality control is an important determinant for bioenergetic changes that occur in response to metabolic stress. Disrupted mitochondrial quality control is associated with changes in mitochondrial morphology, OXPHOS, excessive mtROS production, decline in mitochondrial membrane potential, altered energy production, modulated intracellular calcium flux, and activation of cell death mechanisms/apoptosis (Table 1). Changes in mitochondrial quality control including mitophagy, mitochondrial biogenesis, and mitochondrial dynamics (fission and fusion) are critical to maintain cell homeostasis. A recent study highlighted a core set of genes that changed in the IPF lung before fibrosis was histologically evident and continued to change with disease progression [113]. Systems biology analysis revealed that genes related to mitochondrial transport, mitochondrial trafficking, and mitochondrial organization were found to be altered with advanced fibrosis, potentially explaining the loss of mitochondrial quality control that occurs during fibrogenesis.

The mitochondrial quality control system plays various roles in different cell types within the context of pulmonary fibrosis. While type II AECs, lung macrophages, and fibroblasts all show increased mtROS production in fibrotic lungs, their response to the excessive ROS differs. Type II AECs undergo apoptosis in response to increased mtROS; however, lung macrophages and fibroblasts display apoptosis resistance. Mitochondria from fibrotic type II AECs become swollen and enlarged, but mitophagy and biogenesis are reduced. In contrast, dysfunctional mitochondria from fibrotic lung macrophages are removed via mitophagy, subsequently biogenesis increases and mitochondria undergo fission. Fibroblasts and fibroblasts show reduced mitophagy and mitochondrial biogenesis, but enhanced fission/fragmentation is seen, leading to a pool of dysfunctional mitochondria. In response to increasing oxidative stress, all three cell types undergo a dramatic metabolic reprogramming (Table 1). A loss in lipid synthesis in type II AECs leads to impaired production of cholesterol and phospholipids required for surfactant synthesis. Fatty acid oxidation and OXPHOS in fibrotic lung macrophages promote the sustained profibrotic phenotype that potentiates dysregulated repair. Many rate-limiting glycolytic enzymes are upregulated in fibrotic lung fibroblasts promoting an increase in glycolytic flux. These metabolic reprogramming events are critical for driving the profibrotic behaviors and contribute to the development and progression of lung fibrosis.

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The growing interest in mitochondrial biology may provide new avenues to identify novel therapeutics.

Declaration of competing interest

The authors have declared that no conflict of interest exists.

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Appendix A. Supplementary data

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References

[1] K. Mirumura, S.M. Ciofano, N. Nakahara, A.R. Bhattacharya, M. Cervo, T. Kitada, K. Glass, C.A. Owen, A. Mahmood, G.R. Washko, S. Hashimoto, S.W. Ryter, A.M. Choi, Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD, J. Clin. Investig. 124 (2014) 3987–4003.
[2] W. Xu, T. Kreck, A.R. Lara, D. Neumann, F.P. DiFilippo, M. Koo, A.J. Janocha, F.A. Masi, A.C. Arrolliga, C. Jennings, R.A. Dweik, R.M. Tuder, D.J. Stuehr, S.C. Erzurum, Alterations of cellular bioenergetics in pulmonary artery endothelial cells, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 1342–1347.
[3] U. Mahalirajan, A.K. Dinda, S. Kumar, R. Risban, P. Gupta, S.K. Sharma, B. Ghosh, Mitochondrial structural changes and dysfunction are associated with experimental allergic asthma, J. Immunol. 181 (2008) 3540–3548.
[4] T. Tran, G. Benard, H. Begaert, R. Rossignol, P.O. Girodet, B. Ghosh, O. Ousova, J.M. Vernejoux, B. Marthan, J.M. Tunon-de-Lara, P. Berger, Bronchial smooth muscle remodeling involves calcium-dependent enhanced mitochondrial...
biogenesis in asthma, J. Exp. Med. 204 (2007) 3713–3718.

[5] M. Bueno, Y.C. Lai, Y. Romero, J. Brands, C.M. Si Croix, C. Kang, A. Corey, J.D. Herazo-Maya, H. Zhang, P. Toth, G. Marsboom, Z. Hong, R. Salgia, H. Yanagisawa, M. Hashimoto, H. Wakui, J. Kojima, K. Shimizu, T. Numata, K. Nakayama, K. Kuwano, Involvement of PARK2-mediated mitophagy in idiopathic pulmonary fibrosis, J. Immunol. 197 (2016) 516–528.

[6] J.L. Larson-Casey, J.S. Deshane, A.J. Ryan, V.J. Thannickal, A.B. Carter, Mitochondrial calcium uniporter regulates PGC-1alpha expression to promote culling damaged mitochondria, Science 340 (2013) 471–474.

[7] B.B. Chen, T.A. Coon, J.R. Glasser, C. Zou, B. Ellis, T. Das, A.C. McKelvey, J.L. Larson-Casey, J.S. Deshane, A.J. Ryan, V.J. Thannickal, A.B. Carter, Mitochondrial-derived free radicals mediate asbestos-induced alveolar epithelial cell apoptosis, Am. J. Respir. Crit. Care Med. 181 (2010) 254–263.

[8] J. Rehman, H.J. Zhang, P.T. Toth, Y. Zhang, G. Marsboom, Z. Hong, R. Salgia, A.N. Husain, C. Wietholt, S.L. Archer, Inhibition of mitochondrial cell cycle progression in lung cancer, FASEB J. 26 (2012) 2175–2186.

[9] M. Bueno, Y.C. Lai, Y. Romero, J. Brands, C.M. Si Croix, C. Kang, A. Corey, J.D. Herazo-Maya, H. Zhang, P. Toth, G. Marsboom, Z. Hong, R. Salgia, H. Yanagisawa, M. Hashimoto, H. Wakui, J. Kojima, K. Shimizu, T. Numata, K. Nakayama, K. Kuwano, Involvement of PARK2-mediated mitophagy in idiopathic pulmonary fibrosis, J. Immunol. 197 (2016) 516–528.

[10] R.M. Schwartz, M.O. Dayho, G.W. Dorn 2nd, PINK1-phosphorylated mitofusin 2 is a Parkin receptor that promotes mitophagy, Am. J. Respir. Crit. Care Med. 178 (2008) 838–846.

[11] J.L. Larson-Casey, J.S. Deshane, A.J. Ryan, Y.V. Thannickal, A.B. Carter, Macrophase Akt1 kinase-mediated mitophagy modulates apoptosis resistance and pulmonary fibrosis, Immunity 44 (2014) 582–596.

[12] R. Chen, G. Marshbom, Y.H. Fang, P.T. Toth, E. Morrow, N. Loo, P. Liao, Z. Hong, K. Ericson, H.J. Zhang, M. Han, C.R. Haney, C.T. Chen, W.W. Sharp, S.L. Archer, C.G. Lee, S. Bindu, V.B. Pillai, S. Samant, Y. Pan, J.Y. Huang, M. Gupta, J. Rehman, H.J. Zhang, P.T. Toth, G. Marsboom, Z. Hong, R. Salgia, H. Yanagisawa, M. Hashimoto, H. Wakui, J. Kojima, K. Shimizu, T. Numata, K. Nakayama, K. Kuwano, Involvement of PARK2-mediated mitophagy in idiopathic pulmonary fibrosis, J. Immunol. 197 (2016) 516–528.

[13] J. Rehman, H.J. Zhang, P.T. Toth, Y. Zhang, G. Marsboom, Z. Hong, R. Salgia, A.N. Husain, C. Wietholt, S.L. Archer, Inhibition of mitochondrial fission prevents cell cycle progression in lung cancer, FASEB J. 26 (2012) 2175–2186.

[14] R.M. Schwartz, M.O. Dayho, G.W. Dorn 2nd, PINK1-phosphorylated mitofusin 2 is a Parkin receptor that promotes mitophagy, Am. J. Respir. Crit. Care Med. 178 (2008) 838–846.
[51] G. Yu, A. Toureuleakis, R. Wang, J.D. Herazo-Mayo, G.H. Ibarra, A. Srivastava, J.P.W. de Castro, G. Deluiti, A. Pangari, T. Woluare, N. Euldrid, E.D.R. Arrojo, Y. Gan, M. Graham, X. Liu, R.J. Homer, T.S. Scanlan, P. Mannam, P.J. Lee, L.E. Herzog, A.C. Bianco, N. Kaminski, Thyroid hormone inhibits lung fibrosis in mice without modifying mitochondrial function, Nat. Med. 24 (2018) 38–49.

[52] Y. Xu, M. Mizuno, A. Sridharan, Y. Du, M. Guo, J. Tang, K.A. Wikenheiser-Brokamp, A.T. Perl, V.A. Funari, J.J. Goyke, R.R. Stripp, J.A. Whisett, Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis, JCI Insight 4 (2019) e90558.

[53] K.P. Chung, C.L. Hu, L.C. Fan, Z. Huang, D. Batista, Y.J. Chen, S. Hsiatu, S.J. Chao, K. Nakahira, M. Imamura, M.E. Choi, C.J. Yu, S.M. Clemons, A.M.K. Choi, Mitofusin regulates lipid metabolism to mediate the development of lung fibrosis, Nat. Commun. 10 (2019) 3399.

[54] P.C. Robinson, L.C. Watters, T.E. King, R.J. Mason, Idiopathic pulmonary fibrosis. Abnormalities in bronchovascular laval fluid phospholipids, Am. Rev. Respir. Dis. 137 (1988) 585–595.

[55] Y.D. Zhao, L. Yin, S. Archer, C. Lu, G. Zhao, Y. Yao, L. Wu, M. Hsin, T.K. Waddell, S. Yona, K.W. Kim, Y. Wolf, A. Mildner, D. Varol, M. Breker, D. Strauss-Ayali, J. Strausz, J. Muller-Quernheim, H. Steppling, R. Ferlinz, Oxygen radical production by bronchoalveolar lavage fluid macrophages and neutrophils in idiopathic pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 197 (2018) 1333–1343.

[56] J.L. Larson-Casey, M. Vaid, L. Gu, C. He, G.Q. Cai, Q. Ding, D. Davis, T.F. Berryhill, C. He, S. Murthy, M.L. McCormick, D.R. Spitz, A.J. Ryan, A.B. Carter, Cu,Zn-superoxide dismutase-mediated redox regulation of Junonii domain containing 3 modulates macrophage polarization and pulmonary fibrosis, Am. J. Respir. Cell Mol. Biol. 55 (2016) 58–66.

[57] A.C. Kim, E. Trease, E.M. Kolatia, A. Mollies, R.E. Thomas, N.H. Alami, B. Wang, A. Joshi, R.B. Smith, G.P. Ritson, B.J. Winborn, J. Moore, J.Y. Lee, T.P. Yao, L. Pallanich, M. Kundi, J.P. Taylor, VCP is essential for mitochondrial quality control by PINK1/Parkin and this function is impaired by VCP mutations, Neuron 78 (2013) 65–80.

[58] M. Song, Y. Chen, G. Gong, E. Murphy, P.S. Rabinovich, G.W. Dorn 2nd, Superoxide-mediated matrix metalloproteinase 9 expression signaling compensates autophagy in primary cardiomyocyte, Circ. Res. 115 (2014) 348–353.

[59] K.F. Winklhofer, Parkinson and mitochondrial quality control: toward assembling the puzzle, Trends Cell Biol. 24 (2014) 352–361.

[60] M.A. Maluf, P. Hopkins, G. Spinell, A.R. Glanville, Eulovismus in IPSI. An investigator-driven study of eulovismus in surgical lung biopsy confirmed idiopathic pulmonary fibrosis, Respirio 16 (2011) 776–783.

[61] K.A. Graham, M. Kulawiec, K.M. Owens, X. Li, M.M. Desouki, D. Chandra, K.K. Singh, NADPH oxidase 4 as an oncprotein localized to mitochondria, Cancer Biol. Ther. 10 (2010) 223–231.

[62] S.C. Huang, B. Everts, Y. Ivanova, D.O. Sullivan, M. Nascimento, A.M. Smith, W.T. Love, L. Gregory, W.Y. Lam, C.M. O'Neill, C. Yan, H. Du, N.A. Abumrad, J.R.G.M. Pearse, Mitochondrial respiratory capacity is a critical regulator of T cell memory development, Immunity 36 (2012) 68–76.

[63] J.L. Larson-Casey, S. Murthy, A.J. Ryan, A.B. Carter, Modulation of the mevalonate pathway by Akt regulates macrophage survival and development of pulmonary fibrosis, J. Biol. Chem. 289 (2014) 36204–36219.

[64] N. Riteau, P. Gasse, L. Faussconier, A. Gombault, M. Gouegnot, L. Fick, J. Kanellopoulos, V.F. Quesniaux, S. Marchand-Adam, B. Crestani, B. Rylef, L. Coulon, Extracellular ATP is a danger signal activating P2X7 receptor in lung inflammation and fibrosis, Am. J. Respir. Cell Med. 184 (2011) 547–554.

[65] A.T. Phythian-Adams, N. van Rooijen, C. Haslett, S.E. Howie, A.J. Simpson, N. Subbotina, K. Browning, Y. Lin, R.E. Morey, J.K. Dayrit, J.C. Horowitz, E.L. Herzog, A.C. Bianco, N. Kaminski, Thyroid hormone inhibits lung fibrosis, JCI Insight 1 (2016) e90558.
[96] F. Bauerenfeind, E. Bartok, A. Bieger, L. Franchi, G. Neumann, V. Hornung. Cutting edge: reactive oxygen species inhibitors block priming, but not activation, of the NLRP3 inflammasome, J. Immunol. 187 (2011) 613–617.

[97] G. dos Santos, M.R. Rogel, M.A. Baker, J.K. Troken, D. Urich, L. Morales-Nebrada, J.A. Sennello, M.A. Kutzover, A. Szikov, J.M. Davis, A.P. Lam, P. Cheshire, D. Kemp, D.K. Shumaker, G.R. Budinger, K.M. Ridge. Vimentin regulates activation of the NLRP3 inflammasome, Nat. Commun. 6 (2015) 6754.

[98] D. Alvarez, N. Cardenes, J. Sellares, M. Bueno, C. Corey, V.S. Hanamanthu, V. Peng, H. D’Cunha, J. Sembrat, M. Nouria, S. Shanker, C. Caufield, S. Shiva, M. Armanios, A.L. Mora, M. Rojas, IFP lung fibroblasts have a senescent phenotype, Am. J. Physiol. Lung Cell Mol. Physiol. 313 (2017) L1164–L1173.

[99] Q. Sun, L. Fang, X. Tang, S. Lu, M. Tamam, D. Zhou, S. Roth. TGF-beta upregulated mediates mitochondrial damage to a human pulmonary fibroblast model, Nat. Med. 24 (2018) 1121.

[100] J.W. Zmijewski, K. Mitra, G. Liu, V.M. Darley-Usmar, V.J. Thannickal. Metabolic bleomycin model, Nat. Med. 24 (2018) 1121.

[101] M.L. Locy, S. Ravi, J. Deshane, R.B. Mannon, E. Abraham, V. Darley-Usmar, M. Bueno, D. Zank, I. Buendia-Roldan, K. Fiedler, B.G. Mays, D. Alvarez, J. Sembrat, B. Kimball, J.K. Bullock, J.L. Martin, M. Nouria, B.A. Kaufman, M. Rojas, A. Pardo, M. Selman, A.L. Mora. PINK1 attenuates miRNA release in alveolar epithelial cells and TLR9 mediated proinflammatory responses, PLoS One 14 (2019) e0218003.

[102] J.E. McDonough, I.A. Zmijewska, S. Jiang, D.W. Park, K. Bernard, A.S. Patel, L. Lin, A. Geyer, J.A. Haspel, C.H. An, J. Cao, I.O. Rosas, D. Morse, L. Hecker, R. Vittal, T. Jones, R. Jagirdar, T.R. Luckhardt, J.C. Horowitz.

[103] Y. Peng, H. D'Cunha, J. Sembrat, M. Nouria, S. Shanker, C. Caufield, S. Shiva, M. Armanios, A.L. Mora, M. Rojas, IFP lung fibroblasts have a senescent phenotype, Am. J. Physiol. Lung Cell Mol. Physiol. 313 (2017) L1164–L1173.

[104] J.W. Zmijewski, K. Mitra, G. Liu, V.M. Darley-Usmar, V.J. Thannickal. Metabolic bleomycin model, Nat. Med. 24 (2018) 1121.

[105] M.L. Locy, S. Ravi, J. Deshane, R.B. Mannon, E. Abraham, V. Darley-Usmar, M. Bueno, D. Zank, I. Buendia-Roldan, K. Fiedler, B.G. Mays, D. Alvarez, J. Sembrat, B. Kimball, J.K. Bullock, J.L. Martin, M. Nouria, B.A. Kaufman, M. Rojas, A. Pardo, M. Selman, A.L. Mora. PINK1 attenuates miRNA release in alveolar epithelial cells and TLR9 mediated proinflammatory responses, PLoS One 14 (2019) e0218003.

[106] F.J. Martinez, H.R. Collard, A. Pardo, G. Bagh, L. Richeldi, M. Selman, J.J. Swigris, H. Taniguchi, A.U. Wells. Idiopathic pulmonary fibrosis, Nat. Rev. Dis. Prim. 3 (2017) 17074.

[107] T.E. King Jr., W.Z. Bradford, S. Castro-Bernardini, E.A. Fagan, I. Glaspole, M.K. Glassberg, E. Gerina, P.M. Hopkins, D. Kardatzke, L. Lancaster, D.J. Lederer, S.D. Nathan, C.A. Pereira, S.A. Sahn, R. Susman, J.J. Swigris, P.W. Noble, A.S. Group. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis, N. Engl. J. Med. 370 (2014) 2083–2092.

[108] L. Richeldi, R.M. du Bois, G. Bagh, A. Azuma, K.K. Brown, U. Costabel, V. Cottin, K.R. Flaherty, D.M. Hansell, Y. Inoue, D.S. Kim, M. Kohlb, A.G. Nicholson.

[109] Y. Surolia, F.J. Li, Z. Wang, H. Li, G. Liu, Y. Zhou, T. Luckhardt, S. Bae, R.M. Liu, J. Zhang, P. Xu, Y. Wang, M. Wang, H. Li, S. Lin, C. Mao, B. Wang, X. Song, C. Lv. Astaxanthin prevents pulmonary fibrosis by promoting myofibroblast apoptosis dependent on Drp1-mediated mitochondrial fission, J. Cell Mol. Med. 19 (2015) 2215–2231.

[110] M. Bueno, D. Zank, I. Buendia-Roldan, K. Fiedler, B.G. Mays, D. Alvarez, J. Sembrat, B. Kimball, J.K. Bullock, J.L. Martin, M. Nouria, B.A. Kaufman, M. Rojas, A. Pardo, M. Selman, A.L. Mora. PINK1 attenuates miRNA release in alveolar epithelial cells and TLR9 mediated proinflammatory responses, PLoS One 14 (2019) e0218003.

[111] J. Zhang, P. Xu, Y. Wang, M. Wang, H. Li, S. Lin, C. Mao, B. Wang, X. Song, C. Lv. Astaxanthin prevents pulmonary fibrosis by promoting myofibroblast apoptosis dependent on Drp1-mediated mitochondrial fission, J. Cell Mol. Med. 19 (2015) 2215–2231.

[112] M. Bueno, D. Zank, I. Buendia-Roldan, K. Fiedler, B.G. Mays, D. Alvarez, J. Sembrat, B. Kimball, J.K. Bullock, J.L. Martin, M. Nouria, B.A. Kaufman, M. Rojas, A. Pardo, M. Selman, A.L. Mora. PINK1 attenuates miRNA release in alveolar epithelial cells and TLR9 mediated proinflammatory responses, PLoS One 14 (2019) e0218003.