Mitochondrial dysfunction in lung ageing and disease

Suzanne M. Cloonan [1,2], Kihwan Kim[1], Pauline Esteves[3,4], Thomas Trian[3,4] and Peter J. Barnes[5]

Affiliations: [1]Division of Pulmonary and Critical Care Medicine, Joan and Sanford I. Weill Dept of Medicine, New York, NY, USA. [2]School of Medicine, Trinity College Dublin and Tallaght University Hospital, Dublin, Ireland. [3]Univ-Bordeaux, Centre de Recherche Cardio-thoracique de Bordeaux, U1045, Dépt de Pharmacologie, CIC 1401, Bordeaux, France. [4]INSERM, Centre de Recherche Cardio-thoracique de Bordeaux, U1045, CIC 1401, Bordeaux, France. [5]National Heart and Lung Institute, Imperial College, London, UK.

Correspondence: Peter J. Barnes, National Heart and Lung Institute, Dovehouse St, London, SW3 6LY, UK. E-mail: p.j.barnes@imperial.ac.uk

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We provide an up-to-date synopsis of the diverse nature of the mitochondrion in maintaining cellular homeostasis in an array of lung cell types and what happens when these pathways become dysfunctional with ageing and with acute or chronic lung disease. https://bit.ly/30jNKjZ

Cite this article as: Cloonan SM, Kim K, Esteves P, *et al*. Mitochondrial dysfunction in lung ageing and disease. *Eur Respir Rev* 2020; 29: 200165 [https://doi.org/10.1183/16000617.0165-2020].

ABSTRACT Mitochondrial biology has seen a surge in popularity in the past 5 years, with the emergence of numerous new avenues of exciting mitochondria-related research including immunometabolism, mitochondrial transplantation and mitochondria-microbe biology. Since the early 1960s mitochondrial dysfunction has been observed in cells of the lung in individuals and in experimental models of chronic and acute respiratory diseases. However, it is only in the past decade with the emergence of more sophisticated tools and methodologies that we are beginning to understand how this enigmatic organelle regulates cellular homeostasis and contributes to disease processes in the lung. In this review, we highlight the diverse role of mitochondria in individual lung cell populations and what happens when these essential organelles become dysfunctional with ageing and in acute and chronic lung disease. Although much remains to be uncovered, we also discuss potential targeted therapeutics for mitochondrial dysfunction in the ageing and diseased lung.

Provenance: Commissioned article, peer reviewed

Received: 27 May 2020 | Accepted after revision: 25 Sept 2020

Introduction

Mitochondria are parasymbiotic organelles that contain their own DNA, metabolome, transcriptome and proteome, but rely on their host for replication, energy intermediates and proteins. Mitochondria supply energy and metabolites to the "host" cell by regulating the continuous aerobic oxidation of fatty acids, while at the same time consuming the end products of glucose, glutamine and amino acid degradation from the "host", generating energy (adenosine triphosphate; ATP) *via* the electron transport chain (ETC). Mitochondria form an interconnected intracellular network changing their shape, size and location inside the cell in response to a plethora of stimuli, including nutrient availability from the "host" [1, 2]. Such movement relies on a series of membrane remodelling events involving cycles of fusion and division (fission) which also allows for the generation of new mitochondria (biogenesis) to supply the "host" cell with a constant reserve of healthy mitochondria; processes reliant on both nuclear-encoded and mitochondrial-encoded proteins [3]. When a healthy pool of mitochondria is threatened, such as loss or damage of the mitochondrial genome, defective mitochondria are removed by the "host" through selective encapsulation into autophagosomes, that are in turn delivered to the lysosome for degradation, a process termed mitophagy. Under conditions of intense stress, mitochondria also play a central role in extrinsic/intrinsic apoptosis, necrosis/necroptosis and pyroptosis programmed cell death pathways [4, 5].

Mammalian mitochondrial DNA (mtDNA), which encodes merely 37 genes, is highly susceptible to oxidative DNA damage and acts as a damage-associated molecular pattern (DAMP) when found outside the mitochondrial compartment [6]. Cytosolic or extracellular oxidised, fragmented mtDNA is one of the most important mitochondrial DAMPs (mtDAMPs) associated with the regulation of innate immunity. N-formyl peptides, ATP, the mitochondrial transcription factor TFAM, the mitochondrial specific phospholipid cardiolipin, reactive oxygen species (ROS) and other mitochondrial derived messengers also behave as mtDAMPs. In moderation, these mitochondrial-derived factors also serve as important intracellular second messengers, signalling to the rest of the cell in a retrograde fashion to ensure survival and adaptation. Such processes have a symbiotic co-dependency on the nuclear genome and serve to regulate homeostatic processes from cell death and antioxidant signalling to inflammatory cascades.

Mitochondrial function and ageing are intricately linked; however, this relationship is complex. Mitochondrial function and ROS production are considered to be important modulators of lifespan and mitochondrial biogenesis and turnover are important for slowing down the ageing process. In general, with ageing, homeostatic mitochondrial processes decline or become dysfunctional; whereby mitochondria exhibit structural abnormalities, reduced biogenesis, increased mtDNA mutations and less ETC capacity and ATP production [7, 8]. Conversely, mild impairment of mitochondrial function may extend lifespan in yeast, worms and mice supporting a role for mitohormesis in longevity [9]. While the contribution of both mitochondria and ageing to the pathogenesis of acute and chronic lung diseases have been elegantly appraised by many before [7, 10–12], this review serves as an update for the lung community on the ever-increasing importance of the mitochondrion in lung ageing and disease. Lung ageing is accompanied by unique pathobiological changes that lead to functional, mechanical and structural alterations in the lung [13], yet how mitochondria contribute to these phenomena is an ever-evolving question. Importantly, lung diseases such as COPD, idiopathic pulmonary fibrosis (IPF), cancer or acute respiratory distress syndrome (ARDS) occur more frequently and with greater severity in older populations when compared to younger individuals [14–16].

To distinguish this review from others, we have focused our attention on the role of the mitochondrion in different sub-populations of cells in the lung and their related diseases. With the arrival of single cell sequencing and other sophisticated technologies, our characterisation and understanding of individual lung cell populations in the lung is just beginning. As the majority of mammalian cells rely on the mitochondrion for energy derivatives and/or metabolites, we will continue to uncover distinctive roles for this unfathomable organelle in ageing as well as in lung biology and disease. From ciliated epithelial cells to endothelial cells of the lung microvasculature, this review will discuss the importance of mitochondrial biology to specific specialised cellular functions (figure 1), as well as dissecting what ensues when mitochondrial-regulated critical processes inside these cells become dysfunctional.

Diseases of the trachea, bronchi and bronchioles

Luminal surfaces of the trachea, bronchi, and bronchioles are predominantly lined with a ciliated pseudostratified columnar epithelium intertwined with secretory goblet cells and lined by basal epithelial cells. Their combined mucociliary function is to secrete and move mucus along with foreign particles and pathogens out of the airways, a process that is sensitive to ageing [17–19]. Mitochondria with highly folded cristae and a dense matrix are abundant among the ciliary rootlets in apical regions of ciliated epithelial cells and are crucial for ciliary beating and mucociliary function [20]. Upon branching into the respiratory bronchioles, the epithelium acquires a simple cuboidal structure with the appearance of

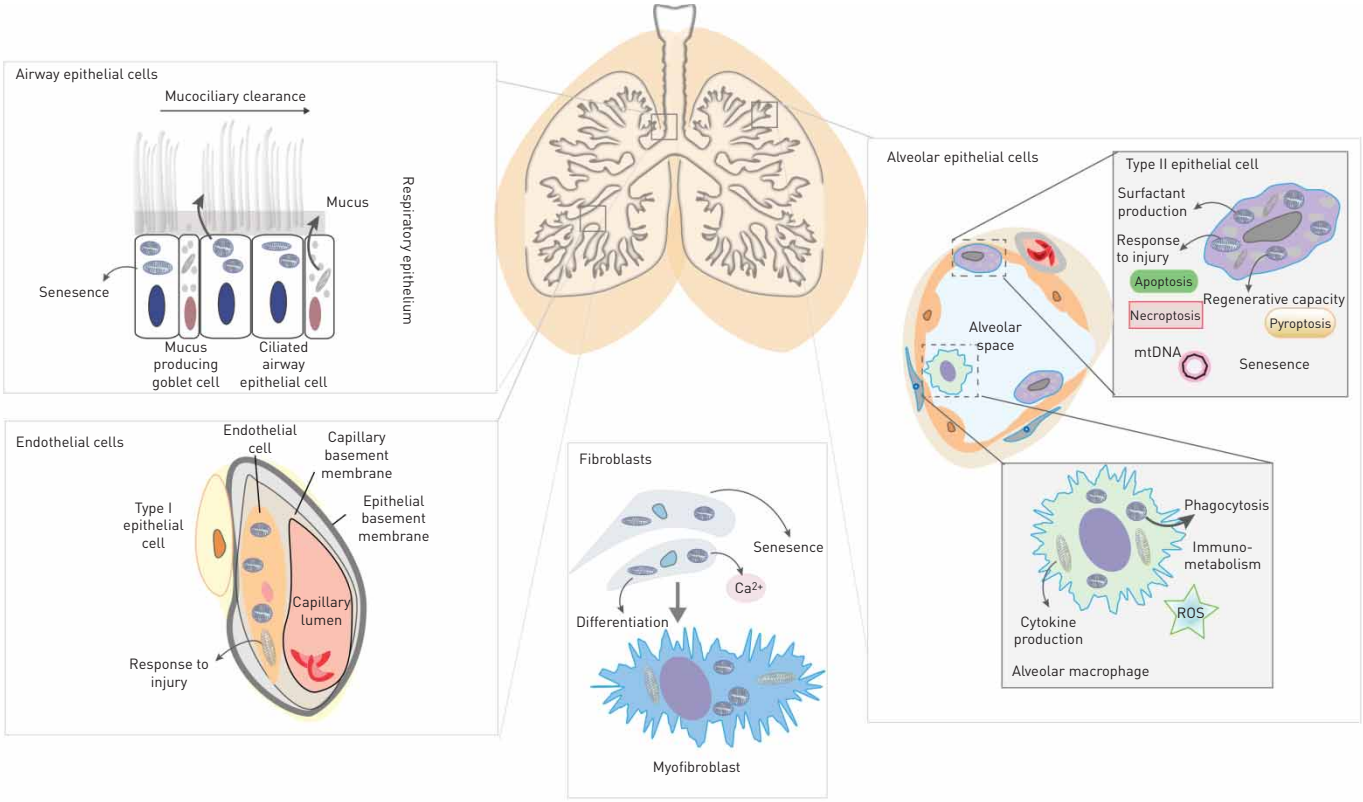

FIGURE 1 Mitochondria as regulators of cellular homeostasis in the lung. Mitochondria are important for the correct functioning of a number of well characterised and specialised lung cells. Mitochondria regulate mucociliary function, mucus secretion and senescence in airway epithelial cells. A number of important pathways in alveolar type two epithelial cells are regulated by the mitochondrion including surfactant production, senescence, programmed cell death and regeneration. Alveolar macrophages as well as other immune cells utilise the mitochondrion to respond to infection and release mitochondrial-related danger signals (damage-associated molecular patterns). Phagocytosis and immune-metabolic shifts in immune cell polarisation are also dependent on the mitochondrion. Differentiation of fibroblasts to myofibroblasts involves the up-regulation of a number of mitochondrial pathways and proteins and endothelial cells utilise the mitochondrion to responds to injury. ROS: reactive oxygen species.

non-ciliated club cells. Goblet, basal and club cells display relatively low densities of mitochondria distributed throughout the cytoplasm at baseline but this may change in response to injury and little is known about how mitochondria contribute to their specialised functions [21]. Similarly, ageing reduces tracheal epithelial thickness and decreases the number of tracheal basal stem cells which may be independent to the numbers of mitochondria in these cells [22]. Mitochondria in cells of the trachea, bronchi and bronchioles have been implicated in a number of diseases as discussed in detail below.

Asthma

Asthma is the most common chronic disease of the respiratory system and is characterised by inflammation, bronchial hyperresponsiveness (BHR) and remodelling of the bronchus [23]. Mitochondria have been associated with each of the above pathobiological features of asthma. Mitochondrial changes are observed in remodelled lung epithelium of a rat model of asthma [24] and in a murine ovalbumin model of asthma and have generally been associated with a loss in mitochondrial-related ATP production. However, the relationship between mitochondrial activity and BHR is debated with increased BHR correlating with a decline in the activity of cytochrome c oxidase (COX) subunit III and depletion of ATP [25], yet inhibition of COX4–2 subunit impairs ATP generation and reduces BHR [26]. ETC complex III dysfunction in mast cells also leads to enhanced histamine and serotonin secretion upon cell ragweed pollen extract exposure [27]. Conversely, mitochondrial dysfunction has largely and extensively been documented in bronchial smooth muscle (BSM) remodelling. The behaviour and proliferation of airway smooth muscle (ASM) cells are dependent on mitochondrial activity and contribute to the pathogenesis of asthma by secreting pro-inflammatory cytokines and other mediators [28]. In general, heightened mitochondrial mass and function associate with more BSM remodelling in asthma. Some specific examples include: increased expression of ATP5b of complex V of the ETC stimulates ASM cell proliferation and thickening in a murine model of asthma [29]; increases in mitochondrial mass are observed in the BSM of both severe [30] and mild asthmatic patients [31];

mitochondrial mass positively correlates with BSM surface area and clinical outcomes such as rate of exacerbation in asthma patients [30, 31]; in severe asthmatic patients increases in mitochondrial mass are associated with increased mitochondrial biogenesis induced by abnormal calcium homeostasis [30]. In addition, Bnip3, a member of Bcl-2 family, which regulates mitochondrial function is overexpressed in asthmatic BSM cells and also participates in BSM cells proliferation [32, 33]. Similarly, increased myeloid cell mitochondrial fatty acid oxidation (FAO) modulates bronchial inflammation in asthma, whereby inhibiting FAO is associated with decreased inflammation and immune responses [34]. Whether or not these increased mitochondrial features are homeostatic and increased as a protective response or really driving disease pathogenesis remains to be fully determined. The role of mitochondria in the pathogenesis of asthma is therefore cell type specific and requires further thorough examination. Finally, the role of mitochondria in asthma may also implicate ageing mechanisms and accelerated cellular senescence. For example, increased number of senescence-associated-β-Gal positive fibroblasts has been observed [35] and could be targeted by senolytic drugs such as azithromycin [36] or metformin [37] that also improve major clinical features of asthma such as peak expiratory flow and severe asthma exacerbations. Likewise, restoring oxidative metabolism by stimulating more arginine metabolism in the bronchial epithelium may also have therapeutic relevance *via* inhibition of proinflammatory signalling and suppression of T-helper (Th)2 inflammation [38, 39].

Cystic fibrosis

Cystic fibrosis (CF) is a genetic pathology attributed to a mutation of the *CFTR* gene on chromosome 7 which results in an abnormality of chloride channels in mucus producing cells. This leads to chronic bacterial airway infection, prominent neutrophilic inflammation and excess thick mucus in the airways, which may lead to progressive bronchiectasis [40, 41]. Mitochondria play an important role in CF physiopathology. *CFTR*-mutated tracheobronchial gland epithelial cells display decreased ETC activity associated with dysfunction in complex I [42]. In fibroblasts isolated from patients with CF decreased activity of complex I is associated with an increase in oxygen consumption and mitochondrial calcium [43, 44] and in human and murine lung epithelial cells is associated with increased ROS [45, 46]. Increased ROS further decreases complex I activity and as a result, a vicious cycle may ensue [47]. Such increases in ROS may also facilitate *Pseudomonas aeruginosa* colonisation of the airway epithelium, as well as inflammatory responses [48].The precise source of ROS in these studies is yet to be attributed to the mitochondrion and alternative mechanisms may also contribute to the observed increases in ROS, including a loss in glutathione and superoxide dismutase pathways [49, 50], impairment of PTEN/CF transmembrane conductance regulator complex formation [51] and increased NADPH oxidase activity [52]. While ROS clearly play a role in the pathogenesis of CF, the source of this ROS and the cell-specific production of ROS and whether targeting ROS for therapeutic benefit in CF remains to be elucidated.

Diseases of the alveoli

Alveoli, the basic units for gaseous exchange are lined by a thin squamous epithelial cell layer surrounded by extracellular matrix, vascular endothelial cells and capillaries. The alveolar epithelium is composed of alveolar type 1 (AEC1) and alveolar type 2 (AEC2) epithelial cells. AEC2 cells are highly specialised, metabolically active secretory progenitor cells that contain significantly more mitochondria compared to their AEC1 counterparts [53–55]. AEC1 cells function as a gas exchange surface as well as maintaining the permeability barrier function of the alveolar membrane. Ageing is associated with a reduction in the lung elastic recoil, which may be associated with loss of alveolar epithelial surface area [56]. Alveolar size, widening of alveolar ducts and late alveolarisation all decline in murine ageing lungs; however, the absolute number of AEC2 cells in the alveolus appears to be relatively stable with ageing [13]. In lung parenchymal cells, ageing reduces mitochondrial number and function and increases ROS levels [22]. Similarly, the number of enlarged fused mitochondria increases with ageing in AEC2 cells [7] and AEC2 cells are predisposed to age-related mitophagy dysregulation which may directly affect their regenerative capacity [57]. In AEC2 cells mitochondrial biogenesis driven by the mammalian target of rapamycin/peroxisome proliferator-activated receptor-γ complex 1α/β (mTOR/PGC-1α/β) axis is upregulated in senescent lung epithelial cells [58]. Surfactant production and secretion by AEC2 cells is reliant on the mitochondrion [59, 60] and in response to injury, AEC2 differentiate into AEC1 cells concomitantly reducing the number and size of mitochondria [61, 62]. Similarly, while alveolar endothelial cells contain less mitochondria than AEC2 cells, they also require functional mitochondria to respond to injury [63, 64]. Mitochondria also play a key role in the correct functioning of immune cells in the alveoli, including alveolar macrophages, $CD4^+$ Th2 and $CD8^+$ (T-cytotoxic) cells [27, 65–69]. Mitochondria in cells of the alveoli have been implicated in a number of diseases as discussed in detail below.

COPD

COPD is a chronic inflammatory lung disease associated with cigarette smoking and other environmental exposures. Dysregulation of mechanisms controlling mitochondrial function are widely appreciated in COPD and have been comprehensively reviewed elsewhere [70, 71]. There is increasing recognition that

COPD represents accelerated ageing of the lung with an accumulation of senescent cells, including airway epithelial cells, fibroblasts, endothelial cells and AEC2 cells [72]. It is likely that oxidative stress from cigarette smoking or biomass smoke exposure, as well as ROS generated by activated inflammatory cells in the lungs, are the major drivers of senescence in COPD patients [73]. Senescent cells are metabolically active and release a variety of inflammatory proteins described as senescence-associated secretory phenotype (SASP), including proinflammatory cytokines (tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6), chemokines (CXCL1, CXCL8, CCL2), proteases (matrix metalloproteinase (MMP)2, MMP9) and growth factors (transforming growth factor (TGF)-β, insulin-like growth factor-binding protein, vascular endothelial growth factor), all of which are increased in COPD lungs. COPD is associated with a loss of several anti-ageing molecules, of which sirtuin-1 is predominant [74]. Reduced sirtuin-1 provides a mechanism for accelerated ageing in COPD by increasing cellular senescence through the acetylation of many key regulatory proteins that are linked to ageing.

This accelerated ageing process is associated with marked mitochondrial dysfunction (figure 2), which contributes to the pathophysiology of the disease in several ways [10]. Repeated cell divisions result in progressive shortening of telomeres, which eventually activates the DNA damage response, leading to activation of p53, resulting in cell cycle arrest or cellular senescence. Telomere shortening also leads to mitochondrial dysfunction through the activation of p53 and through mammalian target of rapamycin (mTOR) signalling [75]. Activated p53 and mTOR signalling inhibit PGC-1α, a transcription factor that is a key regulator of mitochondrial function, which is reduced in COPD [58, 76]. Dysfunctional mitochondria generate mitochondrial ROS (mROS), which may further damage telomeres to accelerate senescence [77]. When cells are depleted of mitochondria by Parkin-mediated mitophagy, there is a

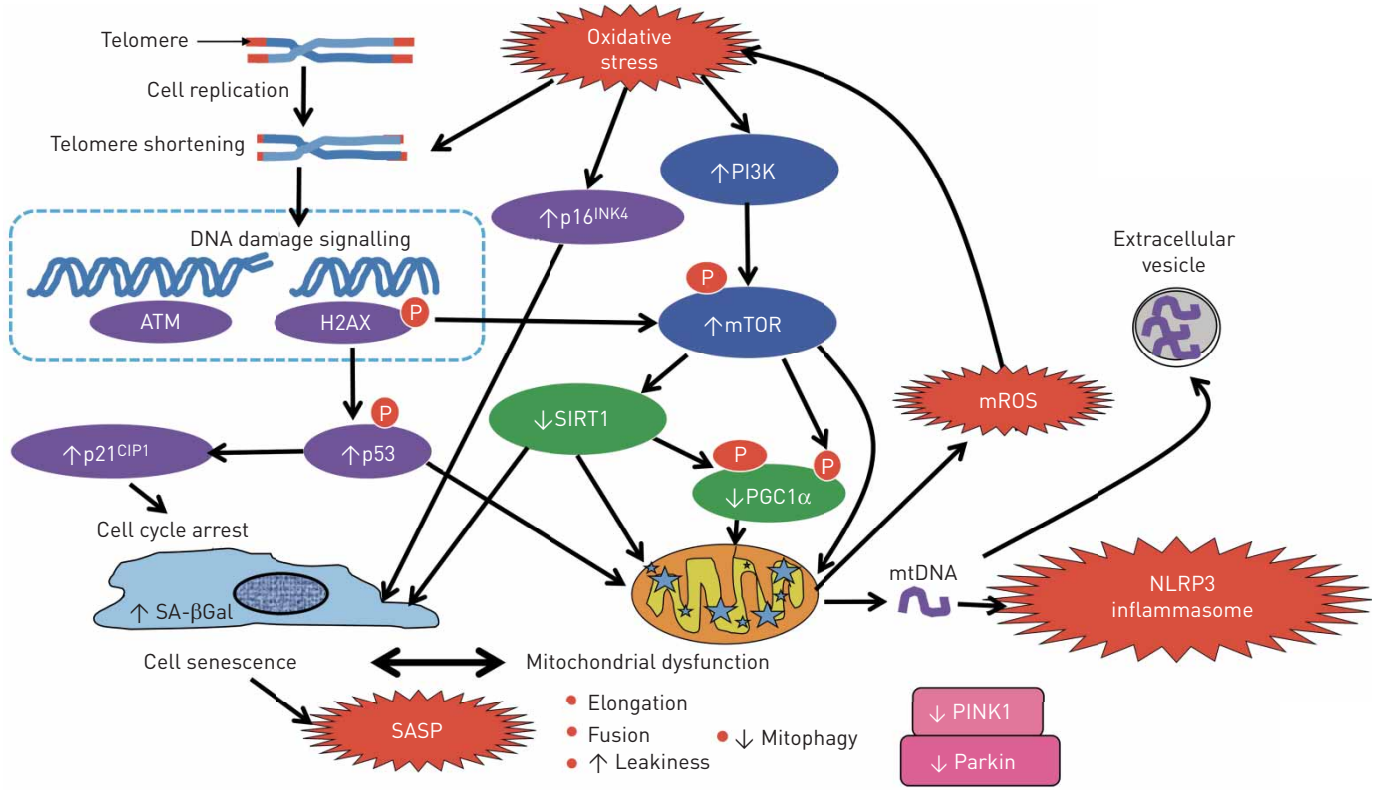

FIGURE 2 Mitochondrial dysfunction in COPD. Repeated cell division causes progressive telomere shortening which activates the DNA damage response, resulting in activation of p53, which activates the cyclic kinase inhibitor $p21^{CIP1}$, which puts the cell into cell cycle arrest or cellular senescence and also induces mitochondrial dysfunction. In COPD these effects are accelerated as oxidative stress causes further shortening and damage of telomeres, activates $p16^{INK4a}$, which increase cellular senescence. Oxidative stress also activates phosphoinositide 3-kinase (PI3K), leading to activation of the key molecule mammalian target of rapamycin (mTOR), which further increases mitochondrial dysfunction by inhibiting peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α), a key regulator of mitochondrial biogenesis and function. mTOR also leads to a reduction in the anti-ageing molecule sirtuin-1 (SIRT1), which further inhibits PGC-1α and mitochondrial function. Mitophagy is impaired in COPD so that damaged mitochondria accumulate in the cell as a result of reduced PTEN-inducible putative kinase-1 (PINK-1) and Parkin due to mTOR activation. Leaky mitochondria release mitochondrial reactive oxygen species (mROS), which are a major source of oxidative stress in COPD. Leaky mitochondria also release mitochondrial DNA (mtDNA), which may activate the NLRP3 inflammasome and also be released from the cell in extracellular vesicles. Senescent cells secrete a senescence-associated secretory phenotype (SASP) with multiple inflammatory proteins that are characteristically increased in COPD.

reduction in cellular senescence and release of SASP mediators, indicating that mitochondria are important in driving senescence [78].

Mitochondria may be a major source of endogenous oxidative stress in COPD and therefore play a key role in driving its pathophysiology. ASM cells from COPD patients show abnormal metabolic activity with increased mROS generation at baseline and after exposure to inflammatory stimuli such as TNF-α and IL-1β and a reduction in ATP generation [79]. Similarly, ASM cells treated with cigarette smoke extract have impaired mitochondrial fission/fusion which may control ASM proliferation [80, 81]. Mitochondria show abnormal morphology in COPD cells, with increased mitochondrial mass, fragmentation, branching and loss of cristae [76]. This is mimicked in airway epithelial cells by cigarette smoke exposure *in vitro*, which also increased markers of senescence and SASP secretion [5, 76, 82]. These morphological mitochondrial abnormalities may reflect a reduction in PGC-1α, a key regulator of mitochondrial biogenesis, which is regulated by sirtuin-1 and may also be due to dysregulation of mitophagy [5, 83–85]. Importantly, impaired mitophagy is linked to senescence in small airway epithelial cells and fibroblasts [84].

Increased mitochondrial iron may also contribute to mitochondrial dysfunction in COPD patients [86]. Defective mitochondrial function in macrophages from COPD patients may contribute to the characteristic defect in bacterial phagocytosis that may underlie bacterial colonisation of the lower airways in COPD patients [65–67]. Mitochondrial damage may release mtDNA, which is able to activate the NLRP3 inflammasome and release IL-1β, which is increased in COPD [87–89] and mtDNA also activates the cytosolic DNA sensor cyclic guanosine monophosphate–adenosine monophosphate synthase, which results in activation of innate immunity and the secretion of interferons, which are also increased in COPD [90]. In an epithelial cell line, oxidative stress releases mtDNA in extracellular vesicles which may be taken up by untreated epithelial cells resulting in the release of IL-6 [91]. Increased mtDNA has been detected in the urine of COPD patients and correlates with some markers of disease severity [92]. Mitochondrial dysfunction may also contribute to the skeletal muscle weakness that is commonly found in COPD patients [93]. Overall there is accumulating evidence that mitochondrial dysfunction plays a key role in the pathogenesis of COPD and therefore may be a target for future therapies [70].

Lung cancer

Lung cancer is the most common cause of cancer-related death in men and the second most common in women. Smoking is the causal mechanism in 85% of lung cancers. It has long been recognised that cancer cells preferentially produce ATP by glycolysis rather than by mitochondria-mediated oxidative phosphorylation as in normal cells (Warburg effect). This metabolic shift leads to increased glucose uptake which leads to increased DNA and protein synthesis though increased generation of nucleic acids and amino acids. This suggests that mitochondrial dysfunction may play an important role in carcinogenesis and somatic mutations of mitochondrial genes are common in many types of cancer, including lung cancers [94]. Mutations of mtDNA are reported in lung cancer [95–97]. Cigarette smoke exposure may lead to damage and mutation of mtDNA, which seems to be more susceptible to oxidative damage than genomic DNA [98].

There is a greatly increased incidence of lung cancers in patients with COPD and this may reflect common molecular pathways [99]. Mitochondrial dysfunction in COPD may be an important factor increasing the development and spread of lung cancers [100]. Cigarette smoke exposure may cause damage and mutations in DNA, including mtDNA, but this is normally repaired by different DNA repair mechanisms. However, in COPD patients some of these repair mechanisms may be defective, as a result of decreased sirtuin-1 [101]. Dysfunctional mitochondria may also contribute to the acceleration of lung cancer because of chronic inflammation induced by mROS release from damaged mitochondria and due to the SASP as a consequence of cellular senescence. The hypoxia in severe COPD patients activates hypoxia-inducible factor-1α, which is overexpressed in a high proportion of nonsmall cell lung cancer patients, potentially increasing the risk of metastasis through promotion of increased epithelial-mesenchymal transition factors [102]. Activation of NLRP3 inflammasome as a consequence of mitochondrial dysfunction may also promote the growth and spread of lung cancers [103]. Mitochondria have a clear pathogenic role in the development and progression of lung cancer; however, the mechanism surrounding this relationship remains to be extensively determined. For example, does mtDNA sequence contribute to tumorigenicity or will targeting mROS or other mitochondrial related SASP processes hold therapeutic promise?

Pneumonia

Bacterial and viral community-acquired pneumonia (CAP) is a leading cause of morbidity and death worldwide. CAP is a syndrome in which acute infection of the lungs involves fever, cough, sputum production, shortness of breath, physical findings of consolidation, and leukocytosis [104]. *Streptococcus pneumoniae* remains the most commonly identified bacterial source of CAP with *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa* also being causative. During

influenza outbreaks, influenza virus becomes the principal cause of CAP increasing susceptibility to secondary bacterial infection. Respiratory syncytial virus, parainfluenza virus, human metapneumovirus, adenovirus, coronavirus and rhinovirus have also been implicated in CAP [104]. Pneumonia, both bacterial and viral, is a leading cause of morbidity and age-adjusted death and accounts for the majority of hospitalisations in patients aged >65 years, suggesting ageing plays an important role in the progression of CAP [105].

Mitochondria have an important role to play in the above bacterial and viral infections. Loss of mitochondrial-derived ATP, dysregulated mitochondrial complex expression, enhanced oxidative stress, diminished antioxidant responses, and decreased numbers of healthy mitochondria have been observed in response to *S. pneumoniae* [106]. More intriguingly, mitochondria are often targeted by virulence factors during infection to promote bacterial or viral replication [107, 108]. Pneumolysin, a major virulence factor of *S. pneumoniae* targets the mitochondria of AEC cells leading to metabolic and morphological changes involving host mitochondrial calcium influx, loss of mitochondrial membrane potential and release of mtDNA [109]. Similarly, *S. pneumoniae*-derived hydrogen peroxide promotes mtDNA leakage and mediates interferon-β expression in lung macrophages [109, 110]. *S. aureus* infection increases the mitophagy protein PINK1 and decreases the availability the mitochondrial specific phospholipid cardiolipin [111]. A number of influenza A virulence factors translocate to the mitochondrial inner membrane, interact with mitochondrial antiviral-signalling protein, regulate fission/fusion processes, accelerate mitochondrial fragmentation, induce mitophagy and hijack mitochondrial-derived NADPH, ultimately resulting in suppression of innate immune signalling pathways [112–117]. Finally, age-associated deterioration in mitochondrial ATP, oxidative stress and antioxidant responses in macrophages also increase susceptibility to *S. pneumoniae* infection [106]. From the above studies it is clear that mitochondrial responses are essential for the response of the host to infection but how to therapeutically support and sustain mitochondrial function in the context of infection remains an open-ended question.

Tuberculosis

Tuberculosis is the leading cause of death in the world among infectious diseases [118]. *Mycobacterium tuberculosis*, the aetiologic agent, is transmitted *via* inhalation of droplets which are readily phagocytised by innate immune cells in the lung, yet only a fraction of infected persons show disease symptoms [119, 120]. Aging is a major risk factor for *M. tuberculosis* infection [121]. Similar to *S. pneumoniae*, *M. tuberculosis* targets mitochondria to enhance bacterial replication. One way is altering cellular death pathway in alveolar macrophages. Mitochondria play a vital role in apoptosis. With unclear mechanism, *M. tuberculosis* promotes necrosis and inhibits apoptosis upon infection [122]. *M. tuberculosis* contains tuberculosis necrotising toxin (TNT), a secreted nicotinamide adenine dinucleotide (NAD+) glycohydrolase, that induces necrosis in infected macrophages. TNT has been shown to activate key mediators of necroptosis RIPK3 and MLKL pathway [123]. In addition, *M. tuberculosis* damages the mitochondrial membrane upon infection and promotes release of cytochrome c from the intermembrane space, further promoting necrosis. Virulent *M. tuberculosis* inhibits crosslinking of annexin 1 and inhibits the formation of the apoptotic envelope [124]. Ageing inhibits the normal response of host alveolar macrophages to *M. tuberculosis* [125] and *M. tuberculosis* also induces a shift from oxidative phosphorylation to aerobic glycolysis and depresses the rate of mitochondrial respiration in macrophages. It also depresses the rate of glycolysis and OXPHOS to enter a state of metabolic quiescence and consequently decreases the rate of ATP production of the macrophage [126, 127]. In a similar manner to other bacteria, *M. tuberculosis* manipulates host mitochondrial homeostasis for its own benefit; how to therapeutically support and sustain mitochondrial function in the context of *M. tuberculosis* infection requires further attention.

Critical care illness

Acute lung injury (ALI), ARDS and sepsis are all associated with high morbidity and mortality in the critically ill patient population [67]. ALI is associated with the rapid onset of bilateral pulmonary infiltrates and hypoxaemia of noncardiac origin and is associated with sepsis, hyperoxia, trauma, pharmacological or xenobiotic exposures, and mechanical ventilation [128]. ALI and ARDS result from an aberrant inflammatory response of the lung with older patients at a significantly greater risk [129]. Diffuse alveolar damage, capillary congestion, atelectasis, intra-alveolar haemorrhage, alveolar oedema, hyaline-membrane formation, epithelial-cell hyperplasia, and interstitial oedema are all common histopathological features of ARDS [130, 131].

Bioenergetic dysfunction of mitochondria in the lung and skeletal muscle are a component of ARDS development [132]. mtDAMPs are higher in the bronchoalveolar lavage fluid (BALF) and circulation of individuals with sepsis and ARDS, as well as in experimental models [133–135]. Mitochondrial dysfunction is implicated in a number of animal models of ARDS (*e.g.* ventilator-induced lung injury (VILI) or hyperoxic-induced lung injury) and sepsis. Animals exposed to chronic hyperoxia have an

increased number of swollen mitochondria with abnormal cristae [136]. Targeted depletion of fatty acid synthase FASN, a key enzyme in the synthesis of fatty acids, in AEC2 cells resulted in altered mitochondrial bioenergetics and more severe lung injury with hyperoxic exposure [137]. Similarly, VILI is associated with mitochondria damage, mitophagy, release of mtDNA and impaired FAO [138–140]. Mitochondrial dysfunction along with increased release of mtDAMPS, mitophagy and increased biogenesis have also been observed in a number of experimental sepsis models including *Staphylococcus aureus*- and LPS-induced sepsis [141–145]. Mitochondrial regulation in macrophages also plays an important role in the pathobiology of ALI, ARDS and sepsis with mitochondrial proteins regulating innate immune responses, including caspase-1 activation and phagocytic activity [146–148]. To summarise, loss of mitochondrial integrity and function is a common pathobiological theme in critical care illness. Whether or not this loss of integrity and consequent release of mtDAMPs into the extracellular space and /or circulation is a selective and programmed mechanism or merely a by-product of cell death/injury remains to be fully evaluated.

Cells of the interstitium

The pulmonary interstitium predominantly consists of the alveolar epithelium, pulmonary capillary endothelium, basement membrane, fibroblasts and perivascular and perilymphatic tissues. Quiescent elongated fibroblasts found in the interstitium regulate extracellular matrix remodelling and the repair following injury through agonist-dependent (*e.g.* TGF-β) transformation into myofibroblasts. Contractile myofibroblasts produce extracellular matrix proteins and can remodel tissues through the expression of α-smooth muscle actin (SMA). Cellular senescence, a classic signature of the ageing process, plays an important role in lung fibroproliferative disorders. Disease-related changes in fibroblast behaviour rely on the upregulation of a number of key mitochondrial processes and the transformation of fibroblasts to myofibroblasts is accompanied by an increase in functioning mitochondrial content, activation of the mitochondrial stress response pathway *via* the transcription factor ATF4, as well as increases in glycolysis and mitochondrial biogenesis [149–152].

IPF

IPF is an irreversible progressive interstitial lung disease characterised by excessive formation of scar tissue in lungs and destruction of alveolar structure and pulmonary interstitium [153]. The exact aetiology remains unknown, however it is considered to be a disease of ageing whereby a genetically susceptible individual incurs AEC injury or damage, which in turn results in the activation and proliferation of myofibroblasts that secrete excessive extracellular matrix, driving abnormal lung architecture and gaseous exchange [154]. Age-associated changes in mitochondrial function may promote IPF development and progression [7] and fibroblasts and AT2 cells from IPF patients have senescent like phenotypes [155, 156].

Dysregulation of mechanisms controlling mitochondrial function are widely appreciated in IPF and have been comprehensively reviewed recently elsewhere [7, 157]. Briefly, mitochondrial dysfunction is evident in AEC2 cells of individuals with IPF [57, 59, 158] and extracellular mtDNA is detected in the BALF and plasma of these individuals [159]. Experimental models of IPF demonstrate extensive mitochondrial-related in injury in AEC2 cells of the lung [57, 59, 158, 160, 161]. Targeted induction of mitochondrial damage in AEC2 cells *via* depletion of mitochondrial fusion results in spontaneous fibrosis, loss of the mitophagy regulator PINK1 or the mitochondrial-regulating transcription factor NRF2 promotes experimental fibrosis which may be age dependent, whereas loss of the mitochondrial protein phosphoglycerate mutase family member 5 (PGAM5) reduces lung fibrosis [57, 59, 158, 160, 162]. Inhibition of the mTORC1 complex with rapamycin restores mitochondrial homeostasis and reduces cellular senescence to bleomycin in lung epithelial cells [58].

Whether or not mitochondrial-associated injury in AEC2 cells directly regulates the activation and proliferation of myofibroblasts remains to be determined. However, a recent study has suggested that extracellular mtDNA which is found to be higher in the BALF of individuals with IPF may play a role in α-SMA expression and metabolic changes in response to TGF-β1 in normal human lung fibroblasts [159]. Mitochondrial related calcium changes may also regulate fibroblast to myofibroblast differentiation [163], which is consistent with fibroblasts from IPF lung fibroblasts having reduced mitochondrial mass, disrupted membranes, and altered cristae and oxygen consumption [155]. The master mitochondrial biogenesis regulator PGC1α is stably repressed in IPF fibroblasts and in fibroblasts isolated from mice treated with bleomycin; an effect that is restored prior to fibrosis resolution in young but not aged mice [164]. TGF-β-induced glycolysis and mitochondrial oxygen consumption in human lung fibroblasts requires mTORC1 [165]. Finally, mitochondria in alveolar macrophages from IPF patients display prominent morphological defects with lower expression of PINK1, PARK2 and NRF1, however loss of mitophagy in macrophages in murine models of fibrosis is anti-fibrotic [166, 167]. The above studies demonstrate that a number of these highly conserved homeostatic mitochondrial pathways have distinctive

TABLE 1 Potential mitochondrial targeting treatments for lung diseases

Disease	Cell target	Therapeutic compounds	Mitochondrial target	Model	Main finding
Asthma					
	Smooth muscle cell	Gallopamil	Mitochondrial biogenesis	Human	Decrease of SMC mass and exacerbation rate
	Macrophage	Etomoxir	Fatty acid oxidation pathway	Mouse	Decreased TH_2 inflammation
	Fibroblast	Azithromycin	Senescence	Human	Improved peak expiratory flow, symptoms and quality of life
	NA	Metformin	ROS production	Human	Decreased severe exacerbations
	NA	Roxithromycin	Senescence	Rat	Decreased airway remodelling
	NA	Rapamycin	Senescence	Mouse	Decreased eosinophilic airway inflammation
	NA	Resveratrol	Senescence	Mouse	Decreased fibrotic responses and airway inflammation
	NA	L-arginine	NO pathway	Mouse	Decreased AHR and inflammation
PAH					
	NA	Metformin	Senescence	Rat	Increased endothelial function and decrease arterial remodelling
	NA	Rapamycin	Senescence	Rat	Decreased pulmonary artery smooth muscle cell proliferation
	NA	MitoQ	ROS production	Mouse	Decreased right ventricular hypertrophy
Acute lung injury					
	NA	NA	Adaptive mitochondrial biogenesis and mitophagy stimulation	Mouse	Removes damaged mitochondria and increases tissue repair and cell survival
	Alveolar epithelial cell	Bone marrow–derived mesenchymal stem cells mitochondria transfer	NA	Mouse	Restoration of alveolar bioenergetics and protection against LPS-induced ALI
	NA	Fusion protein targeting the DNA repair enzyme 8-oxoguanine-DNA glycosylase 1 to the mitochondria	Mitochondrial ROS	Mouse	Enhanced survival under ventilator-induced lung injury
	Epithelial cell	Mildronate	Carnitine synthesis	Mouse	Improves lung function
	NA	MitoTEMPO	Mitochondrial ROS	Influenza A virus infected mouse	Reduction in lung inflammation, neutrophil infiltration, viral titre and mortality
	Epithelial cell	MitoQ	Mitochondrial ROS	Human Epithelial cells and mouse	Reduced epithelial RSV production and inflammation
	Epithelial cell	Targeting dynein motor protein	Mitochondrial ROS	Human Epithelial cells	Reduced epithelial RSV production and inflammation
IPF					
	Alveolar epithelial cell	Thyroid hormone mimetics Sobetirome	Mitochondria biogenesis	Mouse	Decreased bleomycin-induced pulmonary fibrosis
	Alveolar epithelial cell	Genetic over expression of mitochondrial-targeted catalase	Mitochondrial ROS	Mouse	Decreased asbestos or bleomycin-induced pulmonary fibrosis
	Fibroblast	Carbon monoxide	Heme oxygenase	Mouse	Decreased bleomycin-induced pulmonary fibrosis
	Alveolar epithelial cell	Rapamycin	Mitochondrial biogenesis	Epithelial cells	Reduces mitochondrial biogenesis and cellular senescence in cultured alveolar epithelial cells

Continued

TABLE 1 Continued

Disease	Cell target	Therapeutic compounds	Mitochondrial target	Model	Main finding
	Myofibroblast	Pirfenidone	PARK2-mediated mitophagy	Mouse and cells	Inhibits lung fibrosis development in the setting of insufficient mitophagy
COPD					
	Macrophage	Carbon monoxide	Heme oxygenase	Human	Reduced sputum eosinophilia and improved methacholine responsiveness
	NA	SkQ1	Mitochondrial ROS	Rats	Prevent and partially reverse age-related decline
	Ciliated epithelial cell	Mdivi-1, a pharmacological inhibitor of Drp1	Mitophagy	Mice	Restores mucociliary dysfunction
	Epithelial cell	MitoTEMPO	Mitochondrial ROS	Human epithelial cells	Inhibition of cigarette smoke extract induce mitochondria fragmentation
	Smooth muscle cell	MitoQ	Mitochondrial ROS	Human smooth muscle cells	Decrease TGF-β induce ASM cell proliferation and CXCL8 release in HSMC
	Smooth muscle cell	MitoQ	Mitochondrial ROS	Mouse	Reduced inflammation and airway hyperresponsiveness in ozone treated mouse
	Endothelial cell	Mitochondria-targeted H_2S donors AP39, AP123 and RT01	Mitochondria proteins persulfidation	Human endothelial cells	Decrease cellular senescence
	Mononuclear blood cell	Rapamycin	mTOR pathway	Human peripheral blood mononuclear cells	Restoring corticosteroid sensitivity
	NA	Deferiprone	Mitochondrial iron redistribution	Mouse	Restoring mucociliary function, inhibiting BAL infiltrates
	NA	Resveratrol	Mitochondrial ROS	Mouse	Delayed the loss of lung compliance, maintained lung structure and blocked parenchymal cell DNA damage
Lung cancer					
	Epithelial cell	Mitochondria-targeted mito-lonidamine	Oxidative phosphorylation	Human epithelial cell line	Improved mitochondrial function and restores autophagy Metastasis reduction in experimental lung tumours

PAH: pulmonary arterial hypertension; IPF: idiopathic pulmonary fibrosis; SMC: smooth muscle cell; TH: T-helper; NA: not addressed; ROS: reactive oxygen species; NO: nitric oxide; AHR: airway hyperreactivity; LPS: lipopolysaccharide; ALI: acute lung injury; RSV: respiratory syncytial virus; TGF: transforming growth factor; ASM: airway smooth muscle; HSMC: human smooth muscle cells; mTOR: mammalian target of rapamycin.

functions that are cell type specific highlighting the complexities of targeting these pathways for therapeutic benefit in IPF.

Vascular endothelial cells

The lung microvasculature is an evolutionarily conserved system consisting of a dense network of capillaries that line the branched airways, alveolar ducts and alveoli. This expansive capillary surface is covered by a thin layer of capillary endothelial cells. Delicate alveolar–capillary membrane interactions involving precise epithelial-endothelial crosstalk mediate gaseous exchange, delivery of nutrients and regulation of immunosurveillance. Lung capillary endothelial cells are embedded within grooves of AT1 cells producing paracrine factors to stimulate the propagation of alveolar progenitor and mesenchymal cells, ultimately guiding regeneration and repair of the lung [168–170]. Ageing results in a loss in the density of pulmonary capillaries [171] and cellular senescence-elevated oxidative stress plays a role in age-associated vascular endothelial dysfunction [172]. Despite their close proximity to circulating oxygen

and nutrients, endothelial cells oxidise only a minor fraction of glucose in their mitochondria, metabolising 90% of cellular glucose anaerobically to produce lactate, a phenomenon that appears to facilitate expansion of vascular networks during organ growth [173–175]. Endothelial cells have active mitochondria (5% of the cellular volume) and rely on their mitochondria for essential functions as hubs for second messenger signalling and in response to injury, however, the role of mitochondria in pulmonary capillary endothelial cells has not been extensively investigated [63, 64, 176].

Pulmonary arterial hypertension

Mitochondrial dysfunction is associated with pulmonary arterial hypertension (PAH), an incurable disease characterised by pulmonary arterial endothelial cell apoptosis, decreased microvessels and occlusive vascular remodelling [177]. PAH is characterised by increased pulmonary arterial pressure associated with remodelling of the pulmonary arteries that may in turn lead to right ventricular hypertrophy increasing the risk of right heart failure and death [178]. Mitochondrial dysfunction has been described in endothelial cell and pulmonary arterial smooth muscle cells (PASMC) of individuals with PAH and mitochondrial ETC dysfunction associated with a metabolic shift towards increased glycolysis has also been observed [179–181]. PAH-PASMCs display increased expression of ENO1, a key enzyme in glycolysis, leading to a shift away from mitochondrial oxidative phosphorylation in favour of glycolysis [182]. Similarly, PAH-endothelial cells have increased glucose uptake in parallel to diminished oxygen consumption [179]. PDH, the enzyme permitting the entry of pyruvate into mitochondria, is decreased in PAH-endothelial cells and PAH-PASMC [179, 183]. Metabolic shifts toward increased glycolysis in PAH-PASMC are also associated with a loss in activity of

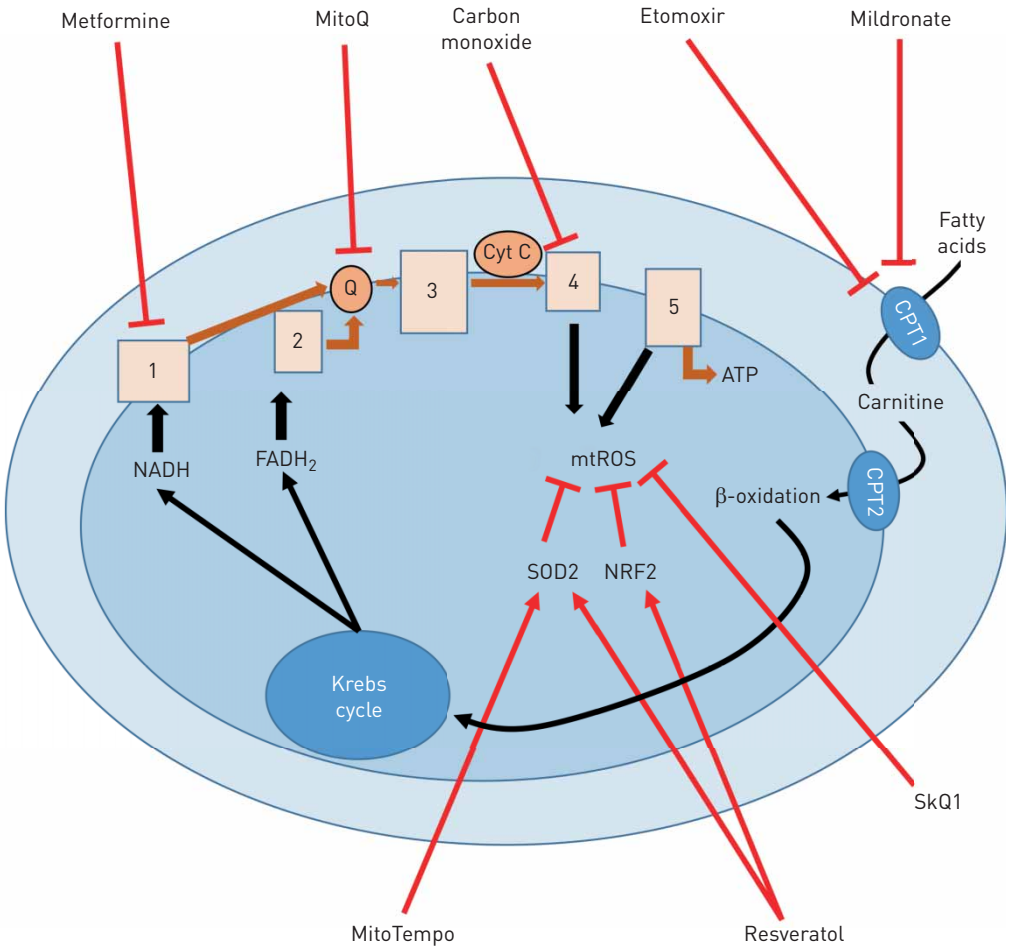

FIGURE 3 Schematic diagram of drugs targeting mitochondria developed in lung disease. Red arrows/lines represent the activation/inhibition site of action of drugs. NADH: nicotinamide adenine dinucleotide; FADH: flavin adenine dinucleotide; ATP: adenosine triphosphate; SOD2: superoxide dismutase 2; NRF2: nuclear factor erythroid-2-related factor; mtROS: mitochondrial reactive oxygen species; CPT: carnitine palmitoyltransferase; Q: ubiquinone/ubiquinol; Cytc: cytochrome c.

complex I and III of the ETC [184–186]. Since disruption of ETC is an important contributor to mROS release, the absence of oxygen consumption may lead to a retrograde accumulation of electrons and generation of superoxide production in PAH-PASMC [187, 188]. Such an increase of mROS also activates ageing pathways. Ageing is an important risk factor for cardiovascular disease and senescence has been implicated in the development or aggravation of PAH [189]. ROS production is associated with DNA damage leading to PAH-endothelial cell and PAH-PASMC cell cycle arrest [190]. Moreover, pro-inflammatory SASP is increased in PAH [189]. Interestingly, modulation of SASP expression with mitochondrial drugs such as metformin has been shown to inhibit SASP expression and prevent PAH [191, 192]. Also, rapamycin, interferes with the development of PAH reducing SASP and PAH-PASMC remodelling [193]. To summarise metabolic switching to glycolysis has been identified as an important pathobiological feature in PAH-endothelial cells and PAH-PASMCs; however, whether or not this process is a pathogenic or protective response and how this relates to the generation of ROS and mROS remains to be investigated.

Conclusion

Mitochondrial dysfunction is a hallmark of ageing and is observed in the majority of acute and chronic lung diseases. However, as this review highlights, a significant amount of these ground-breaking studies infer conclusions from whole lung experimental systems (global deletion of a gene rather than targeted deletion in cell types) or *in vitro* models. In the past 5 years we are beginning to appreciate the opposing and diverse responsibilities that many central mitochondrial proteins have in specialised cells of the lung and this is a rapidly evolving field. Fortunately, as the awareness of this essential organelle in disease pathology has increased, a demand for new approaches to measure mitochondrial function and metabolites has resulted in the development of new forms of microscopy and spectroscopy that open windows into previously unknown aspects of mitochondrial biology. Marrying these technologies with the isolation of pure and distinct lung cell populations will expand our knowledge even further, allowing us to better explore the feasibility of targeting the mitochondrion for therapy in lung disease. At the time of writing, a number of potential mitochondrial pathways have already been proposed for promising therapeutic exploration (table 1). From the use of senolytics to reverse senescence, to stimulating mitochondrial biogenesis and mitophagy [143], to mitochondrial transplantation approaches to repair the injured alveolar epithelium [194], to targeting mROS [195, 196] or stimulating mitohormesis with carbon monoxide (figure 3) [197], an array of promising pre-clinical studies may provide opportunities to deliver mitochondrial-based therapeutics to the bedside to treat acute and chronic lung disease. However, until we uncover more about the distinctive functions of mitochondria in individual lung cell populations, we must proceed with cautious enthusiasm.

Conflict of interest: S.M. Cloonan has nothing to disclose. K. Kim has nothing to disclose. P. Esteves has nothing to disclose. T. Trian has nothing to disclose. P.J. Barnes reports grants and personal fees from AstraZeneca and Boehringer Ingelheim, during the conduct of the study; and personal fees from Novartis, Pieris and EpiEndo, outside the submitted work;.

Support statement: The authors acknowledge support from the US National Institute of Health grant R00-HL125899 (S. M. Cloonan), Science Foundation Ireland grant FRL4862 (S.M. Cloonan), the Fondation pour la Recherche Médicale grant DEQ20170336706 (T. Trian and P. Esteves.) and the Agence Nationale pour la Recherche grant ANR, ROSAE project CE14-0015-01 (T. Trian and P. Esteves).

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