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REVIEW

Mitochondria: at the crossroads of regulating lung epithelial cell function in chronic obstructive pulmonary disease

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Aghapour M, Remels AH, Pouwels SD, Bruder D, Hiemstra PS, Cloonan SM, Heijink IH. Mitochondria: at the crossroads of regulating lung epithelial cell function in chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 318: L149–L164, 2020. First published November 6, 2019; doi:10.1152/ajplung.00329.2019.—Disturbances in mitochondrial structure and function in lung epithelial cells have been implicated in the pathogenesis of various lung diseases, including chronic obstructive pulmonary disease (COPD). Such disturbances affect not only cellular energy metabolism but also alter a range of indispensable cellular homeostatic functions in which mitochondria are known to be involved. These range from cellular differentiation, cell death pathways, and cellular remodeling to physical barrier function and innate immunity, all of which are known to be impacted by exposure to cigarette smoke and have been linked to COPD pathogenesis. Next to their well-established role as the first physical frontline against external insults, lung epithelial cells are immunologically active. Malfunctioning epithelial cells with defective mitochondria are unable to maintain homeostasis and respond adequately to further stress or injury, which may ultimately shape the phenotype of lung diseases. In this review, we provide a comprehensive overview of the impact of cigarette smoke on the development of mitochondrial dysfunction in the lung epithelium and highlight the consequences for cell function, innate immune responses, epithelial remodeling, and epithelial barrier function in COPD. We also discuss the applicability and potential therapeutic value of recently proposed strategies for the restoration of mitochondrial function in the treatment of COPD.

COPD; cigarette smoke; lung epithelial cells; mitochondrial dysfunction

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease caused mainly by cigarette smoking and characterized by chronic inflammation, airway obstruction, and emphysema, culminating in airway obstruction and a progressive decline in lung function (50). Despite the fact that smoking is the most important risk factor for the development of COPD, 10–30% of COPD patients have never smoked (112), indicating that environmental factors distinct from cigarette smoke (CS) contribute to COPD, including occupational exposures and indoor and outdoor air pollution (6, 71). Furthermore, only 20% of smokers develop COPD, which indicates that, in addition to environmental factors, genetic factors may also be involved (112). A wide variety of cells have been implicated in the complex pathogenesis of COPD. In particular, epithelial cells are considered a central player (47, 60). Lung epithelial cells, extending from the upper airways to the terminal alveoli, act as the first line of protection against pathogens and noxious particles in the inhaled air (123). Chronic exposure of lung epithelial cells to these toxicants, in particular CS, triggers oxidative stress, inflammation (116), cell death, cellular remodeling, cellular senescence, and cellular dysfunction (84),

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all of which are key processes involved in the pathogenesis of COPD. Emerging evidence indicates a regulatory role for mitochondria in these cellular processes alongside with their traditional role as the cell's powerhouse (24).

Disturbances in mitochondrial morphology and function have been reported in airway epithelial cells of both non-COPD smokers as well as COPD patients, with more pronounced changes in COPD (40, 49). Impaired mitochondrial function in in these cell types may have severe consequences for cell fate and functionality, particularly in cells with high energy demands, including ciliated cells of the airway epithelium and type II alveolar epithelial cells (AECII) that need substantial levels of ATP for ciliary beating and surfactant production, respectively (24). In addition to energy depletion, mitochondrial dysfunction leads to excessive production of mitochondrial reactive oxygen species (mtROS), stress, and cell death, which ultimately can contribute to tissue injury. In this review, we provide a comprehensive overview of the potential links between CS exposure, mitochondrial dysfunction, and cardinal cellular responses involved in the pathophysiology of COPD, including loss of epithelial barrier integrity, innate immunity, disturbed redox balance, autophagy, and cellular senescence.

MITOCHONDRIA IN LUNG EPITHELIAL CELLS DURING HOMEOSTASIS

Mitochondria are double-membrane organelles with prokaryotic ancestors that are thought to have been incorporated into the eukaryotic cell cytoplasm during evolution (136). As a result, mitochondria have preserved their own specific structures, such as CpG-rich DNA, membrane lipids (in particular cardiolipin), N-formylated peptides, and bioenergetic pathways, while functioning as a part of an integrated intracellular system predominantly regulated by genomic DNA. Mitochondrial DNA (mtDNA) encodes 37 genes, most of which encode for redox active proteins located in the inner mitochondrial membrane (IMM) that are involved in oxidative phosphorylation (OXPHOS), a process involving the transfer of electrons through a chain of enzymatic complexes (I–IV) leading to the production of ATP (Fig. 1*A*) (24). Generation of ATP by the mitochondrion provides the energy required for a wide variety of cellular processes. Interestingly, different subtypes of airway epithelial cells, including ciliated cells, secretory goblet and club cells, and progenitor basal cells, as well as type I alveolar (AECI) and AECII, contain a distinct number of mitochondria with different intracellular organization to optimally facilitate energy demand and cellular function. For instance, in ciliated airway epithelial cells, mitochondria with highly folded cristae and a dense matrix are concentrated mostly apically (125), whereas in airway goblet cells and AECII mitochondria reside around the nucleus (17, 18). In basal and club cells of the airway epithelium, mitochondria are found scattered throughout the cytoplasm (Fig. 1*B*) (92, 97). This cell-specific distribution of mitochondria affects ATP and metabolite levels that are subsequently used for specific functions of these different cell types (ciliary beating or mucus secretion in differentiated airway epithelial cells and surfactant production in AECII). In addition, different epithelial cell subsets vary in their sensitivity to mitochondrial damage. For example, mitochondria in club cells are more susceptible to metabolic changes compared with other cell types within the airway epithelium (63), which is not surprising given recent evidence from single-cell transcriptomics demonstrating that human club cells are metabolically active and contain a large number of functional mitochondria (137), rendering them highly sensitive to oxidative damage.

Disturbances in the production of reactive oxygen species (ROS) by the mitochondrion contribute to cellular injury. Under normal physiological conditions, ROS released from mitochondria act as a second messenger to maintain cellular homeostasis. Several mitochondrial proteins, in particular those in the electron transport chain (ETC), contribute to ROS formation, including complex I and III of the ETC, ubiquinol-cytochrome *c* reductase core II (UQCRC2) (4), mitochondrial uncoupling protein 2 (UCP2) (73), and the adaptor protein $P66^{shc}$ (54). When generated in excessive amounts by damaged mitochondria, ROS contribute to cellular injury, further propagating cellular stress response or programmed cell death pathways (16). Besides being major endogenous producers of ROS, mitochondria are also the main targets of ROS, resulting in oxidative damage to mitochondrial proteins, mtDNA damage, and mutations with excessive electron leakage, inflicting further oxidative stress in a vicious cycle.

The mitochondrial network is highly dynamic, allowing adaptation to changes in homeostatic conditions and cellular responses to damage. Processes involved in remodeling of the mitochondrial network include fusion and fission events, e.g., to exchange mtDNA during repair processes, the creation of new mitochondria (biogenesis), or the removal of damaged mitochondria by mitochondrial-specific autophagy (mitophagy) (Fig. 1*C*). The biogenesis of mitochondria is regulated largely by peroxisome proliferator-activated receptor-γ coactivator-1 molecules (PGC-1α and PGC-1β), with mitochondrial transcription factor A (TFAM) and nuclear respiratory factors 1 and 2 (NRF1/2) acting as key downstream regulators (87). Activation of several kinases can contribute to mitochondrial biogenesis via regulation of PGC-1α. Among these, protein kinase C, mitogen-activated protein kinases (MAPK), and AMP-activated protein kinase (AMPK), an upstream regulator of mechanistic target of rapamycin (mTOR) (46), have been implicated in CS-induced epithelial cell dysfunction (7). Although increased mitochondrial biogenesis is expected to be beneficial for cellular functions, increased ROS production as a result of an increase in the number or activity of mitochondria may render the cells more vulnerable to deleterious processes such as irreversible growth arrest or senescence (41), especially when accompanied by an imbalance between oxidants and antioxidant capacity, as observed in COPD (72).

Mitochondrial fusion and fission events are regulated primarily by specialized proteins located on the outer and inner mitochondrial membrane. These proteins belong to the guanosine triphosphatase (GTPases) family of dynamin proteins, which are regulated chiefly by the proteolytic machinery (77). During fission, the outer mitochondrial membrane (OMM) is segregated by dynamin-related protein 1 (DRP1) upon recognition by the OMM receptors fission protein 1 (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamic protein 49 (MID49), and MID51, resulting in a fragmented mitochondrial network (78). On the other hand, during fusion, the OMM proteins mitofusin 1 (MFN1) and MFN2, as well as the IMM

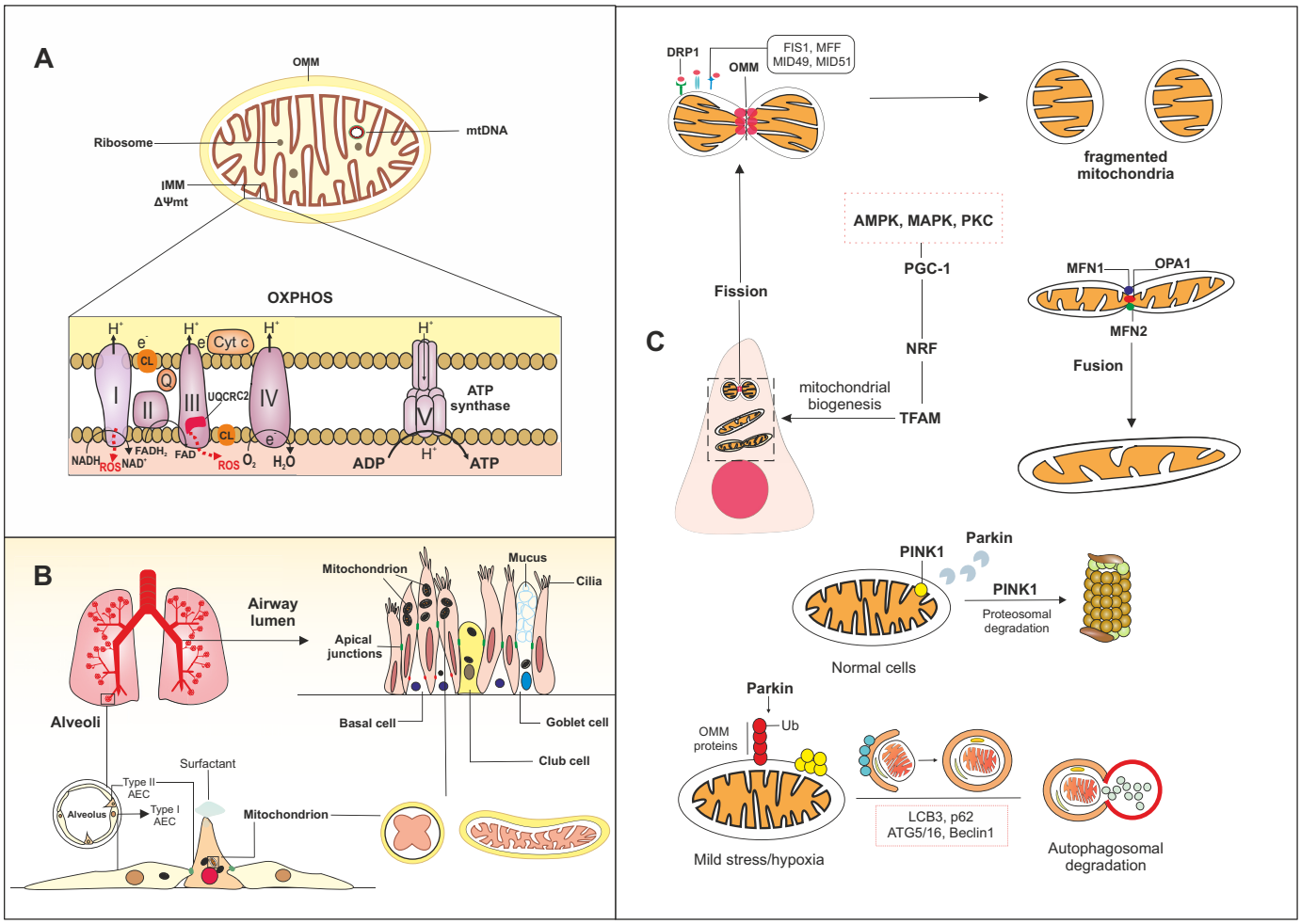


Fig. 1. Schematic of mitochondrial function and localization in various lung epithelial cells during homeostasis. *A*: within the mitochondria, the double membrane with a folded inner membrane (IMM) forms cristae. The mitochondrial matrix contains circular mitochondrial DNA (mtDNA). Oxidative phosphorylation (OXPHOS), which provides the ATP required for cellular functions, occurs in this hyper-folded IMM. During OXPHOS, a proton gradient is established over the IMM through the flow of electrons through chains of enzymatic complexes (I–V), which leads to the production of reactive oxygen species (ROS), H_2O, and as ATP. Cytochrome *c* and coenzyme Q facilitate the transfer of electrons during OXPHOS. Complexes I and III are the major sites of ROS production in the electron transport chain (ETC). Ubiquinol-cytochrome *c* reductase core II (UQCRC2) is a damage-sensitive protein that contributes to ROS generation in the complex III of ETC. Oxidation of cardiolipin (CL), the mitochondrial-specific lipid in the IMM, can induce oxidative damage. *B*: in the upper respiratory tract, the airway epithelium constitutes the first physical defensive barrier against inhaled pathogens and toxic gases and particles, whereas the type I and type II alveolar epithelial cells (AEC) that cover the alveolar surface facilitate gas exchange. *C*: the mitochondrial network is highly dynamic in lung epithelial cells. Various processes contribute to generation and degradation of mitochondria in the cells. These processes include fusion, fission, mitochondrial-specific autophagy (mitophagy), and mitochondrial biogenesis. During fission, recognition of activated dynamin-related protein 1 (DRP1) by surface receptors (FIS1, MFF, MID49, and MID51) leads to fragmentation of the mitochondrial outer membrane (OMM) and formation of new mitochondria. Mitochondria can form an elongated shape by fusion, in which mitochondrial membranes tether through fusion proteins in the IMM such as optic atrophy 1 (OPA1) and outer membranes mitofusin 1 (MFN1) and MFN2. Mitophagy is regulated by several proteins, including PTEN-induced kinase 1 (PINK1) and Parkin. In homeostatic conditions, translocation of PINK1 to the IMM triggers recruitment of E3 ligase Parkin, which leads to proteasomal degradation of PINK1. Upon mild stress mitochondria can undergo complete degradation by mitophagy, in which accumulation of PINK-1 in the OMM and subsequent ubiquitination of several proteins at the OMM by Parkin lead to engulfment of the damaged mitochondrion by autophagy compartments. Autophagy proteins LCB3, p62, ATG5/16, and beclin 1 facilitate the generation of phagophore and autophagosomes. Formation of new mitochondria can be triggered by activation of peroxisome proliferator-activated receptor-γ coactivator1α (PGC-1α) and downstream transcription factors, nuclear respiratory factors 1 and 2 (NRF1/2), and mitochondrial transcription factor A (TFAM). Activation of several proteins, including AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK), and protein kinase C (PKC), may activate the PGC-1α signaling pathway and in turn lead to synthesis of mitochondria.

protein optic atrophy 1 (OPA1), tether mitochondrial membranes, encouraging fusion, allowing neighboring mitochondria to exchange genetic material as well as promoting OXPHOS capacity (77). Silencing of fusion factors OPA1 and MFN leads to mitochondrial fragmentation and subsequent irreversible cell growth arrest in airway epithelial cells, whereas inhibition of FIS1 and DRP1 does not impact cell growth (40), suggesting an essential role of especially mitochondrial fusion in the regulation of cell growth during homeostasis.

Under conditions of mitochondrial injury, damaged or dysfunctional mitochondria are removed by mitophagy, the selective removal of mitochondria by autophagy. PTEN-induced kinase 1 (PINK1), a master regulator of mitophagy (41), undergoes proteasomal degradation in healthy cells via recruitment of E3 ligase PARK2-encoded Parkin upon translocation

to the IMM (130). Under stressful conditions (e.g., hypoxia, oxidative stress) this proteasomal degradation is impaired, leading to accumulation of PINK1 on the OMM and subsequent recruitment of Parkin. Parkin then ubiquitinates several proteins in the OMM, acting as a tagging signal for the engulfment of stressed mitochondria into a double-membrane autophagosome. Autophagosomal degradation of mitochondria is mediated by several proteins, including autophagy-related genes (ATGs), beclin-1, microtubule-associated protein 1 light chain 3 (LCB3), and p62 (99). Additionally, PINK1-independent dephosphorylation of the OMM receptor FUN14 domain containing 1 (FUNDC1) and PINK1-dependent phosphorylation of BLC2-interacting protein 3 (BNIP3), BNIP3-like (BNP3L) proteins, optineurin (OPTN), and nuclear dot protein 52 (NDP52) may act as adaptors for the formation of the autophagosome around mitochondria, facilitating mitophagy (129).

MITOCHONDRIAL ABNORMALITIES IN LUNG EPITHELIAL CELLS EXPOSED TO CIGARETTE SMOKE

Several studies have provided evidence for mitochondrial abnormalities in lung epithelial cells of subjects with COPD or emphysema (79). These studies reported loss of cristae, abnormally branched, swollen, and fragmented organelles in primary airway epithelial cells isolated from COPD patients (40, 49), and mitochondrial swelling in primary AECII from patients with emphysema (62). In support of a causative role of CS exposure in mediating these morphological abnormalities, long-term exposure of human airway epithelial cells to CS extract (CSE) recapitulates observations in COPD subjects, with CSE inducing similar aberrations in mitochondrial morphology (24, 40, 49). Given that the above studies utilized airway epithelial cells (from both COPD and non-COPD subjects) in submerged cultures instead of cells differentiated in an air-liquid interface, the main cell type investigated in these studies is most likely a basal-like undifferentiated cell. Further studies are needed to assess whether exposure of differentiated cultures of human airway epithelial cells containing ciliated cells, goblet, and club cells to CS in vitro results in similar changes in mitochondrial morphology.

In line with the above-mentioned in vitro studies using human cells, 3 months in vivo exposure of mice to CS also resulted in mitochondrial damage in both the airway and alveolar compartment (79). Suggestive of a potential link between these morphological changes and impairments in mitochondrial function as well as aberrations in cellular functionality in response to CS exposure, abnormalities in mitochondrial morphology were associated with a lower mtDNA content, decreased oxygen consumption rates, reduced ATP levels, decreased activity of the ETC, loss of mitochondrial membrane potential, increased levels of mtROS, loss of ciliary function, and decreased production of surfactant (Fig. 2) (1, 24, 40, 49, 62, 74, 79, 114, 116). Decreases in cellular energy production in response to airway epithelial exposure to CS in these studies likely originated from impairments in the ETC, as exposure of airway epithelial cells to CS has been shown to result in decreased activity levels of ETC complexes I–III (114, 116) as well as increased autophagosomal breakdown of ETC complexes III and V (79). Taken together, these studies convincingly demonstrate that CS exposure results in aberrations in mitochondrial morphology and function in lung epithelial cells, which is in line with observations in primary lung epithelial cells from COPD patients.

Interestingly, in apparent contrast to these studies, Ballweg et al. (11) reported hyperfusion of mitochondria and increased metabolic activity in primary mouse AECII in response to low and non-toxic doses of CSE, suggestive of mitochondrial adaptation. More specifically, these authors observed that exposure of these cells to 25% CSE resulted in hyperfused mitochondria as early as 6 h and persisting to 48 h postexposure, whereas increased cellular metabolic activity was observed with concentrations of CSE of $\leq$10–25%, with the effects being most pronounced 24–48 h postexposure. In line with this, 8 wk of exposure of mice to CS increased expression of genes encoding ETC complexes II, III, IV, and V as well as activity levels of ETC complexes II, IV, and V and upregulated genes involved in metabolism, mitochondrial transport, and dynamics in lung tissue (2). The increased expression of subunits of ETC complexes II, III, and VF1α was also reported in immortalized human bronchial BEAS2-B epithelial cells exposed to 10% CSE for 6 mo, whereas complex VF1α was higher in COPD-derived airway epithelial cells compared with control-derived cells, and may be illustrative of a compensatory mechanism (49). These apparently conflicting results in the effect of CSE on mitochondrial function are most likely attributed to the different concentrations of CSE used and possibly relate to an initial adaptation response by mitochondria (mitohormesis) at lower concentrations, whereas higher concentrations or prolonged exposure may result in mitochondrial dysfunction (19).

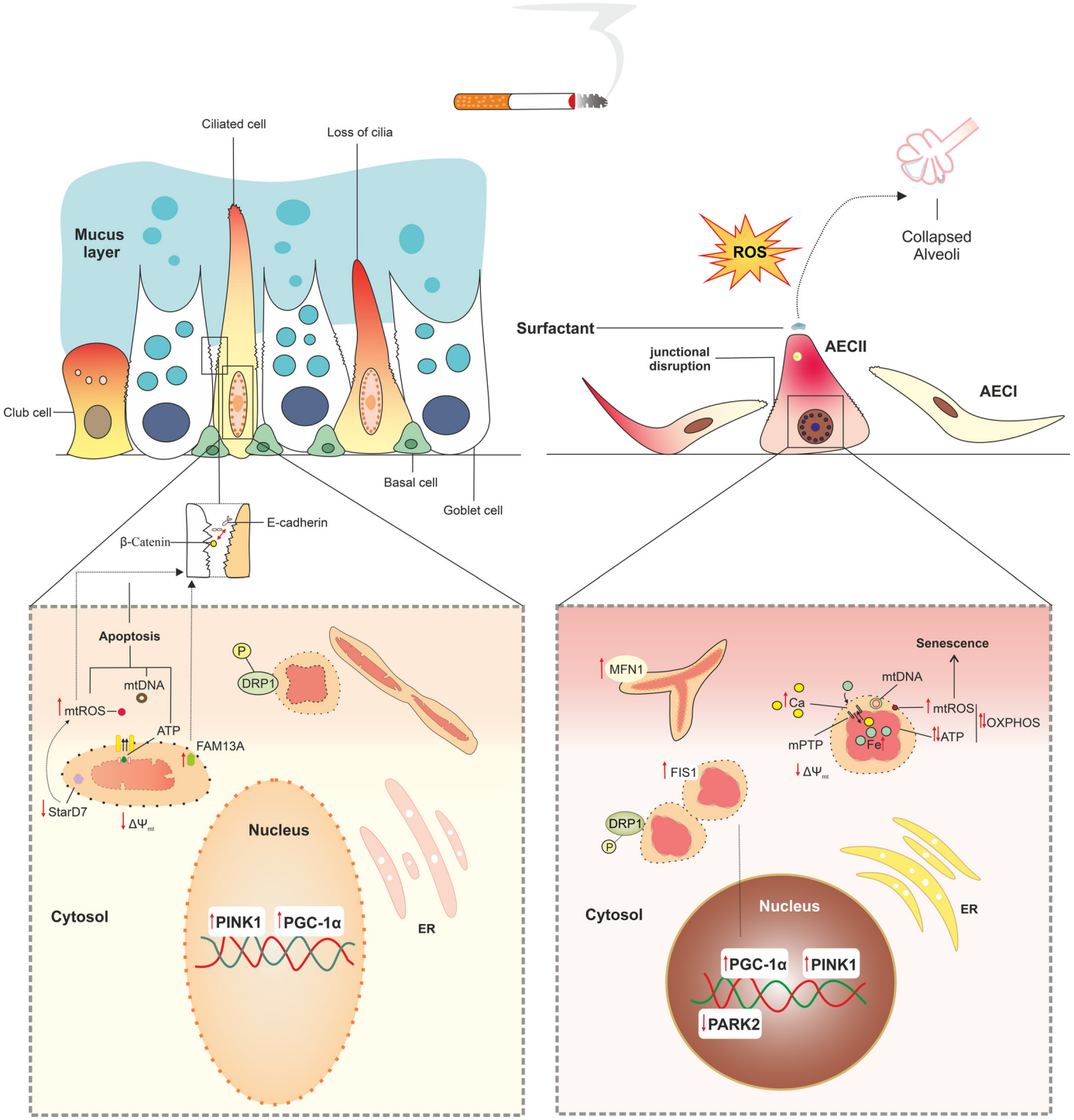
In addition to deteriorative effects of CS on mitochondrial morphology and function in lung epithelial cells, studies have also reported similar detrimental effects of CS in other cells of the airways, including human airway smooth muscle cells and lung fibroblasts, indicating that detrimental effects of CS components on mitochondrial function likely extend beyond the epithelium (5, 8). Collectively, these studies indicate that CS alters mitochondrial structure and function in various cells of the airway walls and lung parenchyma.

CHANGES IN MITOCHONDRIAL BIOGENESIS AND MITOPHAGY IN LUNG EPITHELIAL CELLS IN RESPONSE TO CIGARETTE SMOKE

Given that mitochondrial function and mitochondrial content (i.e., mtDNA copy number) in airway epithelial cells are significantly impacted by exposure to CS, it is not surprising that the pathways controlling mitochondrial biogenesis and mitophagy are also affected in these cell types in response to CS and in the airways of COPD patients. Indeed, Li et al. (65) demonstrated that PGC-1α protein levels were higher in peripheral lung tissue from mild COPD patients compared with controls, with levels progressively decreasing with increasing disease severity to levels comparable with the controls. In addition, Hoffmann et al. (49) reported significantly higher transcript levels of PGC-1α in primary airway epithelial cells from patients with advanced COPD compared with those from control subjects. Consistent with these observations, short-term exposure (24 h) of human bronchial epithelial cells (16HBE) to CSE increased PGC-1α transcript levels (Fig. 2) (118). Because PGC-1α is also known to control the expression of genes

involved in mitochondrial fusion, the observation that hyperfusion of mitochondria in primary mouse AECII exposed to CSE was associated with increased expression of the mitochondrial fusion protein MFN-1 is also in line with this (11). In this context, increased levels of PGC-1α may reflect an adaptive cellular mechanism in response to reductions in mitochondrial content and function, whereas decreased levels in more advanced disease stages or in response to longer exposure or a higher dose of CS may reflect an inability to compensate for these changes in a chronic setting. In line with this notion, in rats exposed to CS for 30 days, pulmonary PGC-1α protein levels were significantly reduced (122). Besides smoke exposure, rats were also challenged twice (on *days 1* and *14*) with 200 μg of LPS instilled intratracheally. Because intratracheal LPS instillation has been shown to suppress PGC-1α expression in AECII (27), it cannot be excluded that reduced levels of PGC-1α in this model may be the result of LPS instillation instead of smoke exposure.

With regard to mitophagy, Mizumura et al. (79) showed that PINK1 protein levels were significantly higher in airway epi-



thelial cells of COPD patients compared with controls. In line with this, Hoffmann et al. (49) showed increased levels of PINK1 mRNA in primary airway epithelial cells of COPD patients compared with controls. In the study from Mizumura et al. (79), higher PINK1 protein levels in COPD lung tissue were associated with increased abundance of the activated form of the fission protein DRP1. This likely resulted from exposure of lung epithelial cells to CS components, as in the same study CSE exposure of BEAS-2B cells resulted in significant mtROS-mediated increases in both PINK1, and phosphorylated DRP1 protein abundance and smoke exposure (3 wk) of mice similarly increased the abundance of phosphorylated DRP1 protein in the lung (79). In line with this, protein levels of phosphorylated DRP1 have been shown to be increased in AECII of smokers compared with nonsmokers (62). These changes were associated with CS-induced activation of mitophagy, as evidenced by enhanced mitochondrial clearance, and were directly linked to CS-induced mtROS production, as the mitochondrial-targeted antioxidant molecule Mitoquinone prevented these changes (79). Other studies confirmed this by demonstrating increases in PINK1 protein levels, increased abundance of activated fission proteins, and decreased mRNA levels of fusion genes in airway epithelial cells in response to CS in vitro (49, 106) and in vivo (5). In addition to increased mRNA and protein abundance of PINK1, decreased protein levels of PARK2 have also been reported in COPD lung tissue (55). In another study, PARK2-deficient mice demonstrated enhanced airway wall thickening with aggravated emphysematous changes following CS exposure in comparison with wild-type mice. AECII of CS-exposed PARK2-knockout animals also displayed an accumulation of damaged mitochondria, increased oxidative modifications and accelerated cellular senescence compared with wild-type animals (Fig. 2) (9). These results collectively suggest that disturbances in the PINK/PARK2 pathway may play a pivotal role in (CS-induced) COPD pathogenesis by regulating mitophagy. The lower levels of PARK2 observed in COPD (55) could lead to the accumulation of damaged mitochondria, and PARK2 induction may mitigate the progression of COPD. Nevertheless, whether mitophagy in the context of COPD and smoking is mainly protective or contributes to epithelial injury remains controversial. This controversy is illustrated, for example, by the observations that blocking mitophagy prevented CS-induced airspace enlargement and inhibition of ciliary function in mice (79), whereas activation of mitophagy prevented smoke-induced cellular senescence of cultured small airway epithelial cells (5). Further studies assessing the cell-specific function of mitophagy and mitochondrial fission/fusion in the pathogenesis and progression of COPD will help shed light on these outstanding questions.

CONTRIBUTION OF CIGARETTE SMOKE-INDUCED MITOCHONDRIAL DYSFUNCTION TO CELLULAR SENESCENCE IN LUNG EPITHELIAL CELLS

Cellular senescence has been implicated in the complex pathogenesis of COPD (13), and recent studies have suggested that mitochondrial dysfunction may act as a driver of cellular senescence in both human and murine lung cells. Several studies have shown that CS exposure leads to the activation of senescence in structural cells of the airways (40, 55, 94, 119, 128).

Interestingly, in vivo overexpression of sirtuin1 (SIRT1), an activator of mitochondrial biogenesis, protected against CS-induced senescence of airway epithelial cells in mice via SIRT1-mediated deacetylation of stress response transcription factor forkhead box O3 (FOXO3) (127). FOXO3 activation has previously been shown to result in enhancement of mitochondrial mass, increased ATP production, and clearance of defective mitochondria, likely via transcription of key anti-oxidant enzymes, thereby reducing ROS production and oxidative damage to the mitochondria and limiting cellular senescence (39, 96). However, SIRT1 also has other intracellular targets independent of the pathways regulating mitochondrial biogenesis, and whether or not the induction of senescence involved mitochondrial dysfunction was not assessed in the above-mentioned study (127). Recent studies provided further evidence directly linking impaired mitochondrial quality control processes and mitochondrial dysfunction to cellular senescence in structural cells of the airways. More specifically, several studies suggest a causal involvement of the PINK1/PARK2 pathway in CS-induced senescence in airway epithelial cells (5, 9, 55). For example, CSE-induced mitochondrial damage in primary human airway epithelial cells was associated with increased ROS production and cellular senescence, and knockdown of PINK1 and PARK2 aggravated both mtROS production as well as senescence (55). More recently, airway epithelial cells from PARK2-deficient mice displayed a greater accumulation of damaged mitochondria and accelerated senescence in response to CS exposure compared with wild-type animals. In the same study, these authors also showed that in vitro overexpression of PARK2 in airway

Fig. 2. Cigarette smoke (CS) induces abnormalities in mitochondrial morphology and function in lung epithelial cells. CS exposure of lung epithelial cells results in swollen and branched mitochondria with a condensed matrix. These morphological features are accompanied by functional alterations in mitochondria, including altered oxidative phosphorylation (OXPHOS) with higher reactive oxygen species (ROS) production and lower ATP generation, depolarization of mitochondrial membrane, and impaired mitophagy, all of which cause subsequent changes at the cellular level. These changes include loss of ciliary function, increase in mucus production in the airway epithelium, and impaired production of surfactant in type II alveolar epithelial cells (AECII), leading to alveolar collapse. CS increases the permeability of the mitochondrial membranes and opens ion channels such as mitochondrial permeability transition pore (mPTP) in the inner membrane, leading to overload of iron in mitochondria and cytoplasmic accumulation of mitochondrial danger-associated molecular patterns (mtDAMPs), including Ca^{2+}, ATP, mitochondrial ROS (mtROS), mtDNA, cardiolipin, and N-formylated peptides, further inducing oxidative damage and cell death. CS-induced mitochondrial dysfunction may contribute to a leaky manifestation of the airway epithelium by increased mtROS and subsequent weakening cellular junctions. Furthermore, increase in FAM13A has been associated with disrupted airway epithelial barrier by increasing mtROS. Excessive mtROS has also consequences for cell fate and growth, inducing permanent damage to mtDNA, leading to increased mitophagy and cell apoptosis, and irreversible arrest in cell growth. Short-term CS exposure may enhance mitochondrial biogenesis via increase in PPAR-γ coactivator1α (PGC-1α) transcript levels, whereas longer exposure times suppress this process. CS induces an imbalance in fusion/fission process by more trends to fission, leading to fragmentation of mitochondria in lung epithelial cells. CS enhances clearance of fragmented mitochondria by mitophagy through increase in PTEN-induced kinase 1 (PINK1) mRNA and protein levels in both airway epithelial cells and AECII.

epithelial cells was sufficient to attenuate smoke-induced mtROS production and cellular senescence (9). The link between the PINK1/PARK2 pathway and CS-induced senescence was further confirmed by another study demonstrating that impaired mitophagy leads to CS-induced senescence in primary human airway epithelial cells (5). The direct link between mitochondria and cellular senescence in the airways is further illustrated by a recent study showing that the pathways involved in mitochondrial biogenesis can contribute to cellular senescence, as activation of the mTORC1/PGC1 axis promoted mitochondrial biogenesis and enhanced OXPHOS as well as mtROS production and induced cellular senescence in rat AECII (111). Although this provided more evidence for the link between mitochondrial function and cellular senescence, understanding the complex link between mitochondrial dysfunction and cellular senescence requires further mechanistic studies. Collectively, these studies suggest that mitochondrial function, and alterations in homeostatic response to CS exposure, may contribute to cellular senescence in lung epithelial cells and as such can contribute to COPD pathogenesis.

LINKS BETWEEN MITOCHONDRIAL FUNCTION AND LUNG EPITHELIAL INTERCELLULAR JUNCTIONS

In the airways, epithelial cell contacts such as tight junctions and adherens junctions, as well as the trapping function of the mucociliary system, constitute a robust physical barrier against pathogens and inhaled toxicants such as CS (3). Interestingly, mitochondrial function has been shown to modulate epithelial integrity in various organs (52, 100, 113, 120, 126). More specifically, ATP starvation as well as high levels of mtROS and mitochondrial calcium overload disrupt tight junctions in airway and renal epithelial cells (20, 102, 113). Moreover, increased mtROS disrupts airway epithelial barrier integrity in response to the viral mimic poly (I:C) as well as rhinovirus (113), and loss of steroidogenic acute regulatory protein D7 (STARD7), a lipid transfer protein, disrupts airway epithelial tight and adherens junctions by enhancing mtROS and subsequent mtDNA damage in vitro and in vivo (126) (Fig. 2). This may contribute to COPD pathogenesis, since the lipid metabolism is dysregulated in airway epithelium of COPD subjects (115). Although CS was shown to disrupt apical and basal junctions between airway epithelial cells, to our knowledge there is no direct evidence for a causal link between smoking, mitochondrial dysfunction, and disruption of the lung epithelial barrier. However, studies in the airway and intestinal epithelium highlight the concept that mitochondrial dysfunction may contribute to the breakdown of the epithelial barrier in a mtROS-dependent mechanism (53, 102, 120).

An excessive oxidative burden is considered one of the underlying mechanisms of epithelial barrier disruption in COPD (3, 44), and mitochondrial dysfunction induces oxidative stress by overproduction of mtROS, which increases permeability of the epithelial barrier (120). Indeed, the CS-induced increase in mtROS has been shown to dissociate tight junctions through activation of epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK) signaling (3). Thus, mitochondrial dysfunction may lead to increased permeability of the lung epithelial barrier. In support of this, the barrier-disruptive effect of CS was reversible by mitochondrial-targeted antioxidant therapy (126). In addition, mtDNA damage, ATP depletion, and oxidative stress enhance apoptosis (68), which may eventually lead to epithelial barrier disruption (Fig. 2). For example, the CS-induced increase in FAM13A, a reported COPD susceptibility gene (82), causes a shift in mitochondrial metabolic activity in AECII and increases mtROS production from the fatty acid β-oxidation by interaction with SIRT1 in damaged mitochondria (43, 81). This in turn may lead to mtROS-mediated epithelial barrier impairment and apoptosis (Fig. 2). Therefore, regulation of FAM13A may have implications for mitochondrial-mediated epithelial barrier impairment in COPD in part by the ability of FAM13A to regulate mitochondrial fatty acid oxidation and thus mtROS levels in lung epithelial cells.

Furthermore, mitochondrial regulation of the epithelial barrier is thought to be important for polarization and differentiation of airway epithelial cells (98) and, for example, could impact mucociliary function. Indeed, it was shown that CS-induced increases in mtROS mediate autophagy-dependent overexpression of mucin 5AC (MUC5AC) in an immortalized bronchial epithelial cell line (CRL-2741) (134). In addition, mtROS promote mitophagy and_mitochondrial fission by upregulation of PINK1 and phosphorylation of DRP1 (79). Inhibition of mitochondrial fission and mitophagy by a pharmacological inhibitor and genetic deletion of PINK1, respectively, was shown to protect against CS-induced reduction in mucociliary clearance in the airways of mice (79), suggesting a contribution of mitochondria to mucociliary function.

Together, the aforementioned studies suggest that mitochondrial dysfunction may induce oxidative damage that may in turn disrupt cell-cell contacts in lung epithelial cells. Epithelial damage not only may lead to increased susceptibility to environmental factors such as smoke and pathogens but is thought to contribute to proinflammatory and remodeling processes as well (3).

MITOCHONDRIAL DAMPS: POTENT INDUCERS OF LUNG INFLAMMATION IN COPD

In addition to the activation of proinflammatory responses through ROS production, the accumulation of damaged mitochondria may contribute to lung inflammation by the release of so-called damage-associated molecular patterns (DAMPs). Besides infectious agents originating from "strangers" that carry pathogen-associated molecular patterns (PAMPs), endogenous danger signals originating from damaged or necrotic cells ("dangers"; DAMPs) are able to induce innate immune signaling pathways (76). A DAMP is defined as a molecule that has no proinflammatory function under physiological conditions but after being released during cellular damage or immunogenic cell death elicits a proinflammatory response by activating pattern recognition receptors (PRRs) (89). A wide variety of mitochondrial-derived molecules, which at normal physiological concentrations act as second messengers, can also behave as mitochondrial damage-associated molecular patterns (mtDAMPs) when produced in surplus or in an alternative cellular compartment (38). Upon cellular damage or necrotic cell death, released mtDAMPs are highly abundant and are potent activators of proinflammatory responses, which is partially due to their high level of homology to PAMPs. This may be explained by the endosymbiotic theory (95), as described earlier. Homology is evident by the high number of unmeth-

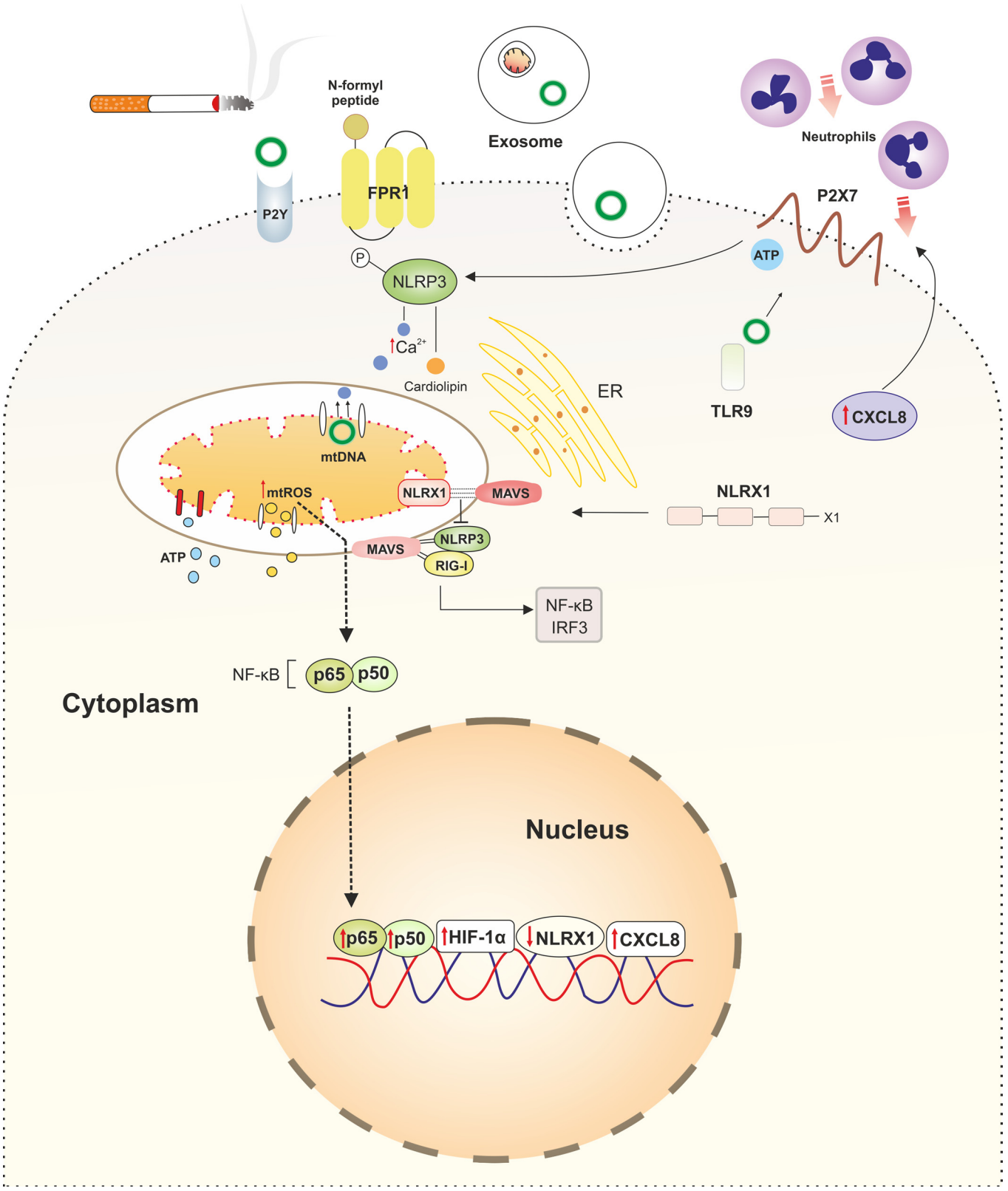
ylated CpG repeats in mtDNA compared with genomic DNA and the fact that peptides encoded by mtDNA are N-formylated, which is a characteristic of bacterial proteins (42). Such formyl peptides act as DAMPs by activating the formyl peptide 1 receptor (FPR1) (57). In mice, genetic ablation of the *FPR1* gene, encoding the receptor for N-formyl peptides, resulted in a significant protection against CS-induced emphysema and airway inflammation (21). In line with this, treatment of wild-type mice with the FPR1 antagonist cyclosporin H also provided protection against CS-induced acute airway inflammation (21). Although it is technically challenging to measure extracellular levels of N-formyl peptides, various studies suggest that N-formyl peptides are important in the pathophysiology of COPD (10, 21, 75). The best-studied mtDAMP is mtDNA, which can activate Toll-like receptor 9 (TLR9) as well as multiprotein complexes that mediate inflammatory responses termed inflammasomes, including the nuclear oligomerization domain (NOD), leucine-rich repeats, and pyrin domain-containing protein 3 (NLRP3) inflammasome (89). MtDNA can be passively released upon immunogenic cell death and actively secreted via exosomes or from neutrophils as neutrophil extracellular traps (NETs) (57). Next to mtDNA and N-formyl peptides, other mitochondrial molecules can act as DAMPs, including ATP, TFAM, cardiolipin, carbamoyl phosphate synthetase, and cytochrome *c* (38). MtDAMPs are involved in the pathophysiology of various diseases, including sepsis, trauma, and autoimmune diseases (28). However, their role in COPD is less well characterized. Few studies have assessed the extracellular role of mtDAMPs. Nonetheless, it was shown that exposure of airway epithelial cell lines to CSE induces necroptotic and necrotic cell death, followed by the release of mtDNA alongside other non-mitochondrial-derived DAMPs (91). Furthermore, it was shown that 37 wk of CS exposure significantly increased serum mtDNA levels in vivo (131). Additionally, primary airway epithelial cells isolated from either healthy controls or COPD patients exposed to CSE released significant levels of mtDNA (45, 90). Stimulation of airway epithelial cells from either healthy controls or COPD patients with mtDAMPs induced a strong CXCL8 response (90). Acute exposure to CS in BALB/c mice resulted in significant mtDNA release in their bronchoalveolar lavage (BAL) fluid compared with air-exposed mice, leading to higher levels of the CXCL8 analog KC and neutrophilic infiltration (90). Next to mtDNA, other mtDAMPs have been studied in the context of COPD. ATP, which can activate the proinflammatory purinergic P2Y and P2X receptors, is produced by the mitochondrion and can be released both actively and passively from multiple subcellular compartments, including mitochondria, endoplasmic reticulum, and the cytoplasm (Fig. 3) (12). Extracellular ATP levels are increased in COPD patients compared with non-COPD smokers and showed a positive correlation with disease severity (33, 69). Together, mtDAMPs may invoke and perpetuate the inflammatory reaction as well as induce lung tissue damage, e.g., by attraction of neutrophils and subsequent release of neutrophil elastase during COPD.

INTERPLAY BETWEEN MITOCHONDRIAL FUNCTION AND INNATE IMMUNE RESPONSES IN LUNG EPITHELIUM

Besides providing a physical barrier against environmental insults, the lung epithelium is important for the production of antimicrobial peptides, antioxidants, and proinflammatory mediators, which are able to attract and activate cells of both the innate and adaptive immune systems (47). Lung epithelial cells express a wide array of proinflammatory PRRs, including TLRs, NOD (nucleotide-binding oligomerization domain)-like receptors (NLRs), RIG (retinoic acid-inducible gene)-I-like receptors (RLRs), and the receptor for advanced glycation end products (RAGE), which can be activated by PAMPs and DAMPs, including mtDAMPs. Next to mtDAMPs, it has been shown that mtROS are able to induce a proinflammatory response by directly activating the NF-κB pathway and hypoxia-inducible factor-1α (HIF-1α) (12). mtROS also regulate inflammation through activation of inflammasomes, and selected NLR family members oligomerize to form inflammasomes. The best-studied NLR inflammasome is NLRP3, which has a strong link to mitochondrial dysfunction. NLRP3 can be activated by various mtDAMPs either indirectly by extracellular ATP, which activates the P2X7 receptor, or directly by mtDNA and cardiolipin (56, 103). Furthermore, activated NLRP3 inflammasomes colocalize with mitochondria, which may act as a scaffold for NLRP3 assembly and activation (32, 135). Activation of NLRP3 inflammasomes has been reported in lung epithelium of COPD patients undergoing an exacerbation (35). Several studies have shown that the NLRP3 inflammasome anchors at the mitochondria via mitochondrial antiviral signaling protein (MAVS), whereas cardiolipin and the antiapoptotic c-FLIP protein have also been suggested as mitochondrial anchors (12, 34, 110, 124). Activation of NLRP3 induces mitochondrial damage and the subsequent release of mtROS and vice versa (83, 135). In line with this, dysfunctional mitophagy augments the activation of

Fig. 3. Links between cigarette smoke (CS)-induced mitochondrial dysfunction and altered innate immune responses in chronic obstructive pulmonary disease (COPD). Damaged mitochondria as a result of CS exposure release their contents into to the cytoplasm, acting as damage-associated molecular patterns (DAMPs) and subsequently activating the innate immune system. Cytoplasmic levels of mitochondrial danger-associated molecular patterns (mtDAMPs), including mitochondrial reactive oxygen species (mtROS), mtDNA, ATP, cardiolipin, and Ca^{2+}, are increased in lung epithelial cells in COPD. Increased levels of cytoplasmic mtDNA, mtROS, Ca^{2+}, and cardiolipin activate innate immune responses by stimulation of intracellular pattern recognition receptors (PRRs), in particular the NLRP3 inflammasome. mtDNA acts as a ligand for Toll-like receptor 9 (TLR9), further activating NLRP3. mtDNA can be transferred to the neighboring cells via exosomes. N-formyl peptides encoded by mtDNA are released upon cellular damage, acting as DAMPs by activating formyl peptide receptor 1 (FPR1), which induces attraction of neutrophils to the site of damage. Extracellular ATP also activates NLRP3 via purinergic receptors P2X7 and P2Y2R. Activated NLRP3 translocates to mitochondria, which subsequently induces more damage by enhancing mtROS production. This increase in mtROS levels directly induces proinflammatory responses by increasing nuclear translocation of p65 and activating hypoxia-inducible factor-1α (HIF-1α) transcription factor. Furthermore, mtDAMPs elicit proinflammatory responses by inducing strong CXCL8 responses in airway epithelial cells, recruiting neutrophils to the site of damage. Another PRR, NLRX1, is also localized into mitochondria and interacts with mitochondrial antiviral signaling (MAVS), exerting anti-inflammatory responses by precluding interaction of NLRX1 with NLRP3 and retinoic acid-inducible gene-I (RIG-I) and subsequent activation of NF-κB and interferon regulatory transcription factor 3 (IRF3). CS reduces the expression of NLRX1 in the lung at both gene and posttranscriptional levels, perpetuating inflammation.

NLRP3 (70). The correlation between mitochondrial dysfunction and NLRP3 activation may be caused by high cytosolic calcium levels, which are needed for NLRP3 activation and which may induce damage to the mitochondria (12). Another NLR family member, NLRX1, also colocalizes with mitochondria by binding to MAVS (80). Therefore, NLRX1 acts as an anti-inflammatory decoy receptor, limiting the binding and activation of NLRP3 and RIG-I, which subsequently results in



decreased NF-κB and interferon regulatory factor 3 (IRF3) activation (124). However, recently, it was shown that NLRX1 may also have proinflammatory functions under specific conditions e.g., viral infections (36), suggesting that NLRX1 may have pro- as well as anti-inflammatory function and is thus important for the modulation of inflammatory responses. NLRX1 expression is lower in CS-exposed mice as well as in severe stages of COPD compared with healthy controls at both gene and protein levels (58). Interestingly, it was shown that loss of NLRX1 levels induces mtROS-mediated oxidative stress in ischemia-reperfusion injury model in renal epithelial cells by interacting with UQCRC2 (109), thus, acting as a mediator between innate immune compartments and mitochondrial ETC. Collectively, dysfunctional mitochondria as a result of CS exposure can potentially affect innate immune responses by the release of both mtDAMPs and mtROS and by anchoring and modulating the proinflammatory inflammasomes in lung epithelial cells, as depicted in Fig. 3.

Whereas these observations clearly link dysfunctional mitochondria to inflammation, mitochondrial dysfunction may also contribute to increased susceptibility to infection by changing the energy balance in affected cells as well as through direct involvement of mitochondria in antimicrobial activity of cells. Mitochondria have been shown to contribute to antibacterial activity through various mechanisms, including the aforementioned regulation of innate immune responses but also through direction of mitochondria toward the phagolysosome and subsequent delivery of mtROS, which in concert with phagosomal NADPH-oxidase-derived ROS contribute to bacterial killing within the phagolysosome (reviewed in Ref. 86). Such studies help to explain the observed link between mitochondrial dysfunction and decreased antibacterial activity of macrophages in COPD (14, 15). In addition, mitochondria contribute to antiviral responses in cells by facilitating RLR signaling, including that of the aforementioned mitochondria-associated MAVS, thus regulating the production of antiviral type I and type III interferons and proinflammatory cytokines following viral infection (reviewed in Refs. 59 and 88). This was first shown in studies demonstrating that mitochondrial fragmentation and loss of mitochondrial membrane potential, as observed in cells exposed to CS (40, 49), result in a marked impairment of MAVS-mediated antiviral immunity (22, 61). Viruses exploit this mechanism by inducing mitochondrial dysfunction (e.g., by increasing mitophagy), thus escaping efficient antiviral host defense. These observations may help to explain how exposure of cultured airway epithelial cells to CS increases infection by, e.g., rhinoviruses (30, 93), as well as the observed antiviral immunity in, e.g., COPD patients with frequent exacerbations (105). Collectively, these studies show that mitochondrial dysfunction as observed in smokers with and without COPD may contribute to their increased susceptibility to respiratory infections. Conversely, these findings suggest that such infections may contribute to the observed mitochondrial dysfunction.

RESTORATION OF MITOCHONDRIAL FUNCTION IN COPD: FROM BASICS TO CLINIC

Emerging mitochondrial-specific therapeutics may assist in alleviating COPD symptoms or even improve pathophysiological features of the disease. To restore the impaired function of lung epithelial cells in COPD, strategies have been developed to specifically target mitochondria in these particular cells. These strategies are aimed at amelioration of impaired mitochondrial bioenergetics and biogenesis, improved regulation of mitophagy, and inhibition of excessive mtROS generation, as summarized in Table 1.

Another interesting approach involves the transfer of mitochondria from mesenchymal stromal cells to damaged lung epithelial cells. This approach ameliorates the imbalance in oxidative respiration, improves energy levels, and enhances mitochondrial biogenesis in damaged epithelial cells (85). This transfer can be mediated by various cellular dynamic compartments, including gap junctions, microvesicles, and nanotube structures (85), and has proven to be efficient in mediating protection against LPS-induced acute lung injury as well as in smoke-exposed airway epithelial cells (67). Interestingly, exosomes in airway cells were shown to carry mtDNA to neighboring cells (51), making these vesicles potential factors for reversing CS-induced mtDNA damage. Replacement of CS-induced damaged mitochondria by healthy mitochondria through nanotube structures from human pluripotent stem cells accelerates lung repair upon damage in vivo (67). Additionally, coculture of CS-stimulated airway epithelial cells with mesenchymal stromal cells (MSCs) reduces apoptosis by interfering with mitochondrial cytochrome *c* release (66). This type of intervention not only reduces cell death but may also be able to rescue mtDNA damage in lung epithelial cells, as transfer of healthy mitochondria from wild-type MSCs was shown to recover oxidative respiration in mtDNA-damaged AECII (108).

Correction of the imbalance in oxidant/antioxidant by mitochondrial-targeted antioxidants that specifically target mtROS or pathways involved in generation of mtROS may also have potential as a therapeutic strategy for the restoration of mitochondrial function in COPD. Indeed, it has been shown that mtROS scavengers counteract CS-induced cellular dysfunction in airway epithelial cells in vitro (5, 40). Furthermore, studies demonstrated that mitochondrial-targeted antioxidants such as MitoTEMPO, Quercetogetin and Resveratrol reduce mucus overproduction, cellular senescence, and apoptosis in CS-exposed airway epithelial cells (79, 106, 107, 134). Despite all advances in preclinical development of this strategy, serious complications may limit the clinical applications of mtROS scavengers in COPD patients. MtDNA damage induced by short-lived molecules such as ROS is thought to be irreversible, particularly upon persistent oxidative damage, when repair mechanisms of mtDNA also become impaired (117). Because ROS levels are essential for the adequate activation of immune compartments in COPD (15), systemic inhibition of mtROS may interfere with innate immune cell function. Furthermore, mitochondrial antioxidants not only protect healthy cells but may also promote the survival and migration of cells undergoing malignant changes (64, 101).

Although it is not completely clear whether mitophagy is protective in COPD, regulation of mitophagy by inhibition of mitochondrial degradation via blocking fission or enhancing OXPHOS capacity by activation of fusion factors, particularly in the early stages of the disease, may improve mitochondrial function and subsequently restore cellular functionality in lung epithelial cells. Indeed, inhibition of fission improves mitochondrial respiration as well as mtROS production. Mdivi-1, a

Table 1. *Potential therapeutic targets for regulation of mitochondrial-mediated cellular dysfunction in cigarette smoke-exposed lung epithelial cells*

Therapeutic Strategies	Drug/Inhibitor/Activator	Lung Epithelial Cells	Therapeutic Effects	Potential Adverse Effects	Ref. No.
Mitochondrial transfer	Stem cells (iPSC-MSCs)	HBECs HBECs in vitro and in vivo	• Inhibited CSE-induced apoptosis and cellular damage • Restored ATP levels and improved lung repair in CS-induced cellular damage	Not reported	66, 67
Mitophagy inhibitors	Mdivi1	HBECs Murine BECs in vitro and in vivo	• Reduced CSE-induced decrease in $\Delta\psi_{mt}$ • Prevented apoptosis emphysema and MCC dysfunction	Not reported	79, 106, 133
	Quercetogetin	HBECs	• Inhibited CSE-induced mitophagy and apoptosis		
Mitochondrial ROS scavengers	MitoTEMPO	HBECs HBECs and mouse airways HBECs	• Inhibited PINK1 stabilization • Reduced DRP-1 phosphorylation • Inhibited CSE-induced autophagy and muc5AC mRNA and protein expression • Inhibited CSE-induced mitochondrial fragmentation • Suppressed CSE-induced cellular senescence	• Global reduction of ROS in immune cells • Enhanced proliferation of malignant cells	5, 40, 79, 106, 134
Iron chelators	Deferiprone	In vivo	• Improved MCC • Reduced lung inflammation	• Nephrotoxicity • Low bioavailibilty • Exacerbating preexisting anemia	25
Mitochondrial biogenesis stimulators	AMPK activators	HBECs and mouse airways	• Decreased cellular senescence • Lowered inflammatory responses • Reduced emphysema	Not reported	23, 127
	SIRT1 overexpression/activation	Mouse airways	• Attenuated CS-induced cellular senescence		

AMPK, AMP-activated protein kinase; CSE, cigarette smoke extract; DRP1, dynamin-related protein 1; HBEC, human bronchial epithelial cells; iPSC, induced pluripotent stem cells; MCC, mucociliary clearance; MSC, mesenchymal stem/stromal cells; PINK1, PTEN-induced kinase 1; SIRT1, sirtuin1; $\Delta\psi_{mt}$: mitochondrial membrane potential.

well-known fission inhibitor that has been broadly used in experimental studies, was shown to inhibit fission-related protein DRP1 and reverse CS-induced decrease in mitochondrial integrity in airway epithelial cells (79). Interestingly, Mdivi-1 was also able to inhibit cathepsin-E/parkin-mediated apoptosis and emphysema as well as mucociliary dysfunction in lung epithelial cells of CS-exposed mice (79, 133). On the other hand, diminished mitophagy acts as an upstream regulator of cellular senescence in response to CSE through increased oxidative burden, as demonstrated both in vitro and in vivo (9, 55). Thus, enhancement of mitophagy may be able to inhibit cellular senescence induced by CS. Other therapeutics that were reported to promote mitochondrial turnover include mitochondrial-targeted antioxidants, SIRT1 activators, transcription factor nuclear factor E2-related factor 2 (Nrf2) inducers, mTORC1/2 inhibitors, and iron chelators (37, 48). Together, strategies based on recovery of factors involved in mitophagy, fission, and fusion in lung epithelial cells may be effective in alleviation of COPD progression.

Iron also contributes to the regulation of the redox balance in lung epithelial cells, and its metabolism has been shown to be disturbed in COPD (26), which may have implications for mitochondrial function (132). Smokers exhibit higher levels of iron in the lung, and CS-induced increase in cellular load of iron was shown to induce mitochondrial dysfunction in lung epithelial cells (132). Cloonan et al. (25) showed that iron binding protein 2 (IRP-2), which is a susceptibility gene for COPD (29), regulates iron uptake to the mitochondria, leading to increased cytochrome *c* oxidase (COX) in complex IV of the ETC and subsequent mitochondrial dysfunction. This may lead to an imbalance in the oxidant/antioxidant responses in lung epithelial cells as one of the main features of COPD. Thus, iron chelators (deferiprone and other siderophores) that specifically reduce the uptake of iron could restore mitochondrial function as well as mucociliary clearance in COPD (25). However, because many COPD patients suffer from anemia in late stages (104), systemic administration of iron chelators may worsen the condition. Alternatively, delivery of aerosolized forms of

these compounds directly to the lung using nebulizers may circumvent the disadvantage of systemic therapy, providing beneficial effects.

Other strategies that could potentially be used to counteract mitochondrial dysfunction in COPD include reducing excessive mtROS production by blocking calcium and iron flux channels in mitochondrial membrane and stimulating mitochondrial biogenesis by AMPK and SIRT1 activators, which was already reported to reverse CS-induced cellular senescence in airway epithelial cells (23, 127).

CONCLUSIONS AND FUTURE DIRECTION

The contribution of mitochondrial dysfunction to the pathophysiology of lung diseases is an emerging area of research with many unknown aspects. More specifically, in COPD patients, there is evidence for abnormalities at the level of the mitochondrion in various cell types of the airway and alveolar epithelium. CS likely contributes to these mitochondrial changes observed in lung epithelial cells by interfering with OXPHOS, mitophagy, and mitochondrial membrane integrity and mediating mtDNA damage. COPD patients may be more susceptible to the development of severe smoking-induced mitochondrial abnormalities than smokers with normal lung function. These abnormalities may eventually lead to alterations in epithelial cell growth, innate immunity, and barrier function. Although several studies have highlighted the potential contribution of CS to progression of mitochondrial dysfunction in COPD, there are still many unanswered questions. For instance, there are no studies comparing the effects of CS on mitochondrial function in different lineages of differentiated lung epithelium. It is important in this regard to carefully consider cell-specific abnormalities in the mitochondria of various lung epithelial cells during COPD. Single-cell sequencing may help uncover the susceptibility of distinct lineages of lung epithelial cells to develop mitochondrial abnormalities in COPD. Furthermore, it is not clear whether current COPD therapeutics such as corticosteroids, inhaled bronchodilators (β2-agonists, anticholinergics and theophyllin), and antibiotics alter mitochondrial function in lung epithelial cells. From a therapeutic perspective, mitochondria targeting strategies such as increasing ETC function, antioxidant therapy, and inducing mitochondrial biogenesis have displayed beneficial effects in several clinical trials in aging-related diseases (31). Future experimental developments in therapeutic approaches based on similar strategies as well as other strategies aiming to promote mtDNA repair in COPD will stimulate progress of these strategies to the clinical phase.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.A. prepared figures; M.A., A.H.V.R., and S.D.P. drafted manuscript; A.H.V.R., S.D.P., D.B., P.S.H., S.M.C., and I.H.H. edited and revised manuscript; M.A., A.H.V.R., S.D.P., D.B., P.S.H., S.M.C., and I.H.H. approved final version of manuscript.

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