Rejuvenating cellular respiration for optimizing respiratory function: targeting mitochondria

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Agrawal A, Mabalirajan U. Rejuvenating cellular respiration for optimizing respiratory function: targeting mitochondria. Am J Physiol Lung Cell Mol Physiol 310: L103–L113, 2016. First published November 13, 2015; doi:10.1152/ajplung.00320.2015.—Altered bioenergetics with increased mitochondrial reactive oxygen species production and degradation of epithelial function are key aspects of pathogenesis in asthma and chronic obstructive pulmonary disease (COPD). This motif is not unique to obstructive airway disease, reported in related airway diseases such as bronchopulmonary dysplasia and parenchymal diseases such as pulmonary fibrosis. Similarly, mitochondrial dysfunction in vascular endothelium or skeletal muscles contributes to the development of pulmonary hypertension and systemic manifestations of lung disease. In experimental models of COPD or asthma, the use of mitochondria-targeted antioxidants, such as MitoQ, has substantially improved mitochondrial health and restored respiratory function. Modulation of noncoding RNA or protein regulators of mitochondrial biogenesis, dynamics, or degradation has been found to be effective in models of fibrosis, emphysema, asthma, and pulmonary hypertension. Transfer of healthy mitochondria to epithelial cells has been associated with remarkable therapeutic efficacy in models of acute lung injury and asthma. Together, these form a 3R model—repair, reprogramming, and replacement—for mitochondria-targeted therapies in lung disease. This review highlights the key role of mitochondrial function in lung health and disease, with a focus on asthma and COPD, and provides an overview of mitochondria-targeted strategies for rejuvenating cellular respiration and optimizing respiratory function in lung diseases.

mitochondria; lung; chronic obstructive pulmonary disease; asthma; pulmonary hypertension

CELLULAR RESPIRATION IS INEXTRICABLY intertwined with functioning of the respiratory system. Aerobic cellular metabolic processes transfer the energy of carbon-hydrogen (C–H) bonds in nutrients to high-energy phosphate bonds of ATP, consuming oxygen and generating carbon dioxide (oxidative phosphorylation, OxPhos). Ventilation, gas exchange, and circulation are tightly coupled to the cellular OxPhos, delivering oxygen and removing carbon dioxide. Together, they determine the overall respiratory fitness of the organism, measured as maximal oxygen consumption (VO_{2max} during exercise (57, 83). These well-established principles explain the diminished exercise capacity in obstructive airway diseases (ventilation), lung fibrosis (gas exchange), heart failure (circulation), or deconditioning (cellular aerobic capacity) (42, 57). Mitochondria, often referred to as the powerhouse of the cell, are typically placed at the end of this hierarchy because they house the OxPhos reactions. However, the last two decades have seen an explosion of information pertaining to the multifaceted roles of mitochondria and their important roles in pathogenesis of lung diseases beyond generating energy (14, 30, 88, 124). Importantly, it has become evident that mitochondrial function is a direct determinant of lung health, and targeting mitochondria is a viable strategy for preventing or treating lung disease (110). The American Journal of Physiology Lung Cellular and Molecular Physiology has recently published a perspective by Schumacker and colleagues (103) highlighting the sentinel role of mitochondria in regulating cytotoxicity and the intricate mechanisms of mitochondrial quality control. Here, we will focus on the role of mitochondrial dysfunction in the pathogenesis of lung disease, especially asthma and chronic obstructive pulmonary disease (COPD), and discuss possible mitochondria-targeted therapeutic strategies for restoring optimal respiratory function. A brief introduction to the main actors is provided in the initial section.

Mitochondria

Mitochondrial origin and function. Mitochondria are semi-autonomous cell organelles, considered to be descendants of
endosymbiotic proteobacteria that, not only house metabolic processes of OxPhos and fatty acid metabolism, but also regulate cellular danger and damage responses. Positioned at the hub of cellular metabolic flux, mitochondria are uniquely adapted to communicate with the nucleus to bring about cellular as well as extracellular responses to perceived threats (88). These threats may range from altered availability of nutrients or oxygen, sensed through changes in electron flow, to presence of viral ds-RNA sensed through mitochondrial antiviral signaling protein (124). Mitochondria respond through release of reactive oxygen species (mtROS), changes in mitochondrial membrane polarity, and signaling through mitochondria-associated membranes and proteins. Initiation of stress response pathways, NF-κB, or interferon signaling cascades critically depends on such mitochondrial signals. Mitochondrial damage, with release of mitochondrial proteins or DNA, is a powerful activator of immune signaling as well as a trigger for apoptosis (30, 124). These innate cellular defense systems are well-preserved aspects of innate immunity in most species and are now well recognized to be associated with a full spectrum of health and disease (24, 30, 124). Of these, mtROS production is probably the best studied, especially in the context of respiratory diseases, and will be discussed in more detail. It should be emphasized here that the understanding of bidirectional mitochondrial communication with other cellular elements is still evolving, with mtROS as the most evident and targetable aspect of mitochondrial pathology (24, 30).

MtROS, ROS production is an unavoidable consequence of OxPhos. High-energy electrons pass through the mitochondrial electron transport chain (ETC), culminating in the reduction of oxygen to water in a reaction catalyzed by mitochondrial cytochrome c oxidase (63). Energy transfer from electrons is used to pump protons across the mitochondrial inner membrane into the intermembrane space, creating a measurable electrochemical proton gradient (ΔΨ), which is in turn used by ATP synthase to generate ATP (Fig. 1). Some electrons directly leak to oxygen at intermediary steps, resulting in formation of the superoxide anion radical (O₂⁻), which is further decomposed to hydrogen peroxide (H₂O₂) by superoxide dismutase that is present at very high concentrations within mitochondria (20, 109, 116). This is further decomposed by catalase to water and oxygen. Although most mtROS are thus scavenged within mitochondria, some leak to cytosol via poorly understood mechanisms (132). Mitochondrial ROS release is thus dictated by primary factors such as redox state, integrity of ETC components, rate of electron flow, and oxygen availability, among others, as well as secondary factors, such as local availability of antioxidants and influence of drugs or chemicals (118). It is important to emphasize here that low

![Fig. 1. Generation of reactive oxygen species (ROS) during electron transport. During aerobic respiration, electrons from NADH and FADH₂ are transferred to molecular oxygen via a chain of redox enzymes (Complex I–IV) embedded in the inner mitochondrial membrane (the dashed line indicates electron flux). The energy is used to pump protons across the inner membrane, as shown, generating an electrochemical proton gradient (ΔΨ). This proton gradient is converted to ATP by ATP synthase. Some electrons leak and react with molecular oxygen to generate superoxide (O₂⁻), especially at complexes I and III. The superoxide is further converted into hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD). H₂O₂ may give rise to hydroxyl radicals (OH⁻) or be decomposed to water and oxygen by catalase. It can be seen that electron flux in excess of available oxygen at complex IV or reduced integrity of ETC would promote electron leak/ROS.](http://ajplung.physiology.org/)
levels of mtROS are important in maintaining OxPhos and are considered normal (34). Balance between ROS production and antioxidant defense is a critical aspect of this healthy state.

**Mitochondrial quality control.** Excessive levels of ROS production coupled with depletion of ROS scavengers are associated with damage to the mitochondrial machinery, which further accelerates ROS production (34, 118). Left unchecked, this leads to degradation of mitochondrial function and fall in the electrochemical gradient, $\Delta \Psi$. Also, there is degradation of mitochondrial DNA (mtDNA), which is much more susceptible than nuclear DNA to oxidative damage and lacks sophisticated repair machinery (11, 12). Mitochondrial dynamics, referring to fission or fusion of mitochondria, enables mitochondria to maintain high functional capacity in adverse conditions. Fission leads to fragmented mitochondria that rapidly diverge in their mtDNA and respiratory capacity, whereas mitochondrial fusion promotes mixing and protects mtDNA function through several distinct mechanisms. It maintains mtDNA levels, preserves mtDNA fidelity, and enables cells to tolerate high levels of mtDNA mutations (26). When all fails, depolarized nonfunctional mitochondria are marked for selective degradation attributable to reduced $\Delta \Psi$-dependent translocation of PTEN-induced kinase 1 (PINK1) from the outer to inner mitochondrial membrane. This initiates a cascade of events, leading to arrest of mitochondrial motility and capture by a phagophore (mitophagy) (122). Thus an intricate system of homeostasis maintains optimal mitochondrial function in healthy cells, the failure of which leaves the cell susceptible to mtROS-mediated injury (Fig. 2).

**Connecting Mitochondrial Dysfunction with Lung Disease**

Mitochondrial energy drives the functioning of more than 40 types of cells that compose the lung (70). Some of these, like type II alveolar epithelial cells that secrete surfactant or ciliated epithelial and Clara cells that line airways, are highly metabolically active and rich in mitochondria. Dysfunctional cellular bioenergetics leads to epithelial fragility, reduced barrier function, impaired secretion, and increased propensity to inflammation (121). Bronchopulmonary dysplasia, asthma,
COPD, and pulmonary hypertension have been strongly associated with mitochondrial dysfunction in multiple studies (14, 110). The mitochondrial defect is typically characterized by increased mtROS production, abnormal morphology, and disordered homeostatic processes. A recent review by Aravamudan et al. (14) describes this in greater detail. More recently, impaired mitochondrial homeostasis attributable to deficiency of PINK1 has been found to be associated with idiopathic pulmonary fibrosis (19). Defective mitochondria with impaired fatty acid oxidation have been associated with reduced lung compliance in mice, possibly attributable to long-chain acylcarnitine-mediated effects on lung surfactant (90). Furthermore, several genetic defects associated with impaired mitochondrial respiration are present with pulmonary hypertension (PH) (58, 108, 125). Thus the thread of mitochondrial dysfunction and altered mitochondrial homeostasis runs through a wide variety of lung pathologies. Asthma and COPD are common lung diseases in which the connection with mitochondria is best established. These are discussed in greater detail, with a focus on targetable pathology.

**Asthma.** A critical role for mitochondria in the pathogenesis of asthma emerges from observed genetic associations and experimental studies. The mitochondrial haplogroup U is associated with increased IgE (97) and asthma (131). There is also greater transmission of maternal asthma for some asthma phenotypes, and altered mitochondrial gene expression was reported in the placenta of mothers with asthma (31, 54). Sex-specific associations of polymorphisms in mitochondrially encoded genes, cytochrome B (males) and NADH dehydrogenase 2/16S RNA (females), have been seen in the only genome-wide study so far, conducted in a sample of 372 asthmatic children and 395 healthy children (40). These genes are strongly associated with ROS production. Notably, this study typed 16,158 mitochondrial single-nucleotide polymorphisms using the DNA-pooling technique for reducing costs. More than 30 very highly significant associations were seen despite stringent statistical correction and modest sample size. Because mitochondria lack the sophisticated nuclear DNA repair system, some of these may be a consequence of increased ROS production in asthma, rather than a cause. In more precise experimental systems, mitochondrial dysfunction, induced by deficiency of UQRC2 in airway epithelium of mice, potentiated allergic airway inflammation, mucin secretion, and bronchial hyperresponsiveness (4). Together, this fits a model in which genetic predisposition toward increased mtROS production or increased susceptibility to oxidative damage increases asthma risk. Histological or biochemical evidences of mitochondrial dysfunction have been consistently reported in airway biopsies or cultured airway epithelial cells (AEC) from patients with asthma, as well as in experimental models (73, 79). However, the etiology of such mitochondrial dysfunction and its role in the key cellular mediators of asthma pathogenesis, namely airway epithelium, smooth muscle, and immune cells, is less clear.

AEC from mouse models of asthma and human biopsies typically show mitochondrial swelling and fragmentation, increased oxidative damage, and activation of apoptotic pathways (73, 79). Cultured AEC from patients with asthma exhibit preserved total cellular mitochondrial respiration through increased mitochondria with abnormal morphology and high ROS production. The mitotoxic effects of metabolic products such as 13-S-hydroxyoctadecadienoic acid and asymmetric dimethyl arginine (ADMA) appear to be critical in epithelial injury (5, 6, 77, 78). These may be endogenously generated in response to inflammation or be exogenously delivered in the case of systemic metabolic diseases that precipitate asthma (2, 3, 117). Overall, most lines of data converge to show that mitochondrial dysfunction is an important part of epithelial fragility in asthma. Multifarious mechanisms, as shown in Fig. 2, are implicated.

The evidence for an important role of mitochondrial abnormality in altered airway smooth muscle (ASM) function is more conflicted. Trian et al. (112) reported about a twofold increased mitochondrial number in ASM of patients with severe persistent asthma, presumably attributable to increased biogenesis. However, a more recent study, looking mostly at fatal young asthma cases, did not find such an increase, even in ASM surrounded by inflammatory cells (112). In cultured human ASM, inflammation alters mitochondrial calcium flux and inhibits calcium buffering, which raises cytoplasmic calcium, promotes ASM contraction, and may alter mitochondrial metabolism or biogenesis (35). The Ca\(^{2+}\)-channel blocker gallopamil was found to decrease the proliferation of ASM cells from patients with severe asthma and also reduced their exacerbations (49). Thus ASM contractility, inflammation, calcium flux, and mitochondrial function are intricately interlinked, and different subtypes of asthma may show different patterns. Furthermore, based on the possibility of mitochondrial transfer in vivo, another possibility is that increased mitochondrial biogenesis in ASM is a compensatory response to epithelial mitochondrial loss. In support, we have shown the ability of smooth muscle cells or fibroblasts to donate mitochondria to AEC with mitochondrial dysfunction (8). Although we have not visualized such a transfer in vivo, peri-bronchial increase in myofibroblasts and mitochondrial biogenesis is attenuated by exogenous mitochondrial delivery. Although much remains to be done, cross talk between AEC and ASM mitochondrial function is an intriguing possibility.

Data on mitochondrial function of immune cells in asthma are largely lacking. These cells may use alternative pathways such as glycolysis or NADPH oxidases for sudden bursts of energy requirement or ROS production, and the exact role of mitochondria is highly variable. Eosinophils, the prototypic inflammatory cells of allergic asthma, are thought to have relatively few mitochondria, which are possibly unimportant in cellular bioenergetics but critical for apoptosis regulation with a delicately poised balance of proapoptotic Bid/Bax and antiapoptotic Mcl-1. The exquisite efficacy of glucocorticoids in eosinophilic allergic asthma may be due to their ability to inhibit Mcl-1 while accelerating Bid processing, causing eosinophil apoptosis (59). Although a general sentinel apoptotic role of mitochondria may be common to other short-lived granulocytes, the finer details vary greatly. In neutrophils, glucocorticoids induce Mcl-1 expression, which may prolong neutrophil survival, as seen in neutrophilic steroid-resistant asthma (101). In contrast to granulocytes, mitochondria are likely to be a primary energy source in lymphocytes. However, the relative contribution of mitochondrial respiration vs. glycolysis is variable and critically influences T cell lineage specification, especially along the Th17/Treg axis (17). Dominance of Th17 over Treg is associated with glycolytic dominance and is seen in severe asthma. Systemic metabolic factors
like nutritional state, redox balance, hypoxic response, and insulin, among others, are expected to modulate shifts between glycolytic and mitochondrial respiration. Of these, hypoxic response is known to be important in Th17 specification, and strong induction of the hypoxic response led to a severe phenotype of asthma in mice, characterized by increased inflammatory cell infiltrate and remodeling (7). Importantly, induction of hypoxic response does not require actual cellular hypoxia and can be induced by mtROS or metabolic products like methyl-arginines. These have potentially important roles in the emerging link between the cardiometabolic syndrome and asthma and are reviewed elsewhere (3). However, despite these tantalizing possibilities that may link mitochondrial respiration of inflammatory cells to asthma and associated comorbidities, little is actually known at an experimental or clinical level. This has important global implications, beyond asthma, and should be an important avenue of research in coming years.

**COPD.** The susceptibility of mtDNA to environmental oxidative damage, such as by cigarette smoke (CS), is thought to be an important aspect of COPD pathogenesis. It has been shown that primary bronchial epithelial cells, from ex-smokers with COPD, have highly abnormal mitochondrial morphology, similar to cultured bronchial epithelial cells (BEAS2B) exposed to CS extract. This suggests lasting mitochondrial damage from CS (56). Wood smoke, an important cause of non-smoking COPD, has been similarly shown to be toxic to mitochondria (51, 102). CS induces mitochondrial fragmentation through increased expression of dynamin-related protein 1 (Drp1) and decreased expression of mitofusin-2 (15). In lung epithelial cells, CS induced phosphorylation of Drp1 appears to be a critical step toward mitochondrial fragmentation, accelerated mitophagy, and cellular necroptosis (15).

The effect of CS is not restricted to epithelial cells because ASM from patients with COPD also show reduced mitochondrial respiration and attenuated reserve capacity (126). Such ASM cells have higher levels of mtROS than healthy smokers or normal controls, secrete greater quantities of proinflammatory cytokines, and exhibit greater proliferation in response to transforming growth factor-β (TGF-β). These changes persisted undiminished through several passages in culture but could be attenuated by a mitochondria-targeted antioxidant (MitoQ) or a mitochondria-localized antioxidant (Tiron). Contemporary work from the Choi laboratory (84) found similar changes in lung epithelial cells and further showed that CS stress-mediated effects on mitochondrial respiration are aggravated, rather than rescued, by activation of mitophagy. They posit that activation of mitophagy in this context leads toward necroptosis of lung epithelial cells and eventual emphysema. Conflicting data from the Rahman laboratory (8), however, shows that defective mitophagy leads to CS stress-induced lung cellular senescence, and restoring mitophagy delays cellular senescence. Importantly, mitochondria-targeted ROS scavengers, such as MitoQ or Mito-Tempo, inhibited the effects of CS stress in both studies.

The role of mitochondria in COPD pathogenesis may extend beyond the lung. COPD is sometimes considered a systemic disease. Limb and respiratory striated muscle dysfunction is commonly seen in COPD exacerbations and is thought to be an important part of the reduced maximal respiratory capacity and exercise tolerance in COPD (45). Gene profiling in vastus lateralis muscle of hospitalized patients with COPD showed that several genes involved in the mitochondrial respiration chain were downregulated, whereas apoptosis pathway genes were upregulated (33). Skeletal muscle from patients with COPD has decreased mitochondrial content, decreased capacity to generate new mitochondria, reduced activity and coupling of the ETC, and increased production of ROS. Weakness of inspiratory muscles-like diaphragm, which may precipitate respiratory failure, is also associated with mitochondrial dysfunction and attributed to hypoxia, hypercapnia, inflammation, and acidosis (82).

Genetic associations of COPD with mitochondria are not well described, but common polymorphisms in the isocitrate dehydrogenase (IDH) gene family and peroxiredoxins (PRDX) interacted with smoking to predict FEV1/FVC in both African Americans and European Americans (18). Many of these, namely IDH2, IDH3, and PRDX5, are localized to mitochondria and are critical regulators of redox function. The effect sizes were almost 5%, which is sizable. Interestingly, hypercapnia induces miR-183, which represses mitochondrial IDH2, reducing α-ketoglutarate and OxPhos (120). Blood levels of miR-183 are more than threefold elevated in patients with COPD (22). We are not aware of mitochondrial genome-wide association studies for COPD, but the copy number of mitochondrial genome is almost 50% lower in leukocytes of patients with COPD, compared with healthy smokers or controls (72). This is further associated with reduced serum glutathione and oxidative stress. Genetic variations in response to oxidative stress may be additionally important because the rs1806649 minor allele of nuclear factor erythroid 2-related factor 2 (Nrf2), the master antioxidant regulator, is associated with a marked reduction in COPD mortality (39).

Together, these data strongly support a central role for mitochondria in the pathogenesis of asthma and COPD, most likely through chronically increased mtROS production that persists beyond the initiating environmental triggers. The mechanisms for such mitochondrial phenotypes merit exploration. At a mitochondrial level, this may be because of genetic variations or low mtDNA copy number. Importantly, such mtDNA defects can be inherited or acquired through chronic ROS-mediated damage. This is especially relevant in the context of a reemerging view that asthma and COPD represent different phenotypes of a continuum of processes, with overlapping features common and severe chronic asthma very similar to COPD (89). The asthma-COPD overlap syndrome (ACOS) is now a distinct clinical entity with clinical features of both asthma and COPD. It is expected that, in coming years, we will see greater emphasis on systematic characterization of patients at various levels, rather than COPD vs. asthma label generation (96). We believe that mitochondrial dysfunction is part of the continuum of processes that manifest as asthma or COPD or ACOS and expect that mitochondrial function assessment will contribute toward better characterization of subjects with suspected respiratory disease.

**Targeting Mitochondria**

Preceding sections define the important role of mitochondrial dysfunction in the genesis of lung diseases and support...
mitochondria-targeted therapeutic strategies in patients with lung disease. Such strategies can be broadly considered in a 3R model: repair via scavenging harmful ROS, reprogramming via regulatory pathways, and finally replacement by exogenous healthy mitochondria (Fig. 3).

**Repair.** MtROS have a physiological as well as pathological role. Clinical trial data suggest that untargeted antioxidants such as α-tocopherol (vitamin E) lack beneficial effects and may worsen the disease (98). MitoQ, developed by Murphy and Smith (86), is the prototypical mitochondria-targeted form of ubiquinol or CoQ10, which is a component of the ETC and can therefore exist in both fully oxidized and reduced states. Although CoQ10 is a powerful mitochondrial antioxidant, a very small fraction of CoQ10 supplements will reach the mitochondria. In MitoQ, CoQ10 is conjugated to a lipophilic triphenylphosphonium cation group that leads to mitochondrial accumulation and can more efficiently prevent mitochondrial oxidative damage and mitochondrial dysfunction (86). Mito-TEMPO is a similar triphenylphosphonium mitochondria-targeted antioxidant that has also been extensively validated. MitoQ and MitoTEMPO have both been found to be effective in protecting mitochondrial function during CS exposure (9, 84). MitoQ and Tiron, another mitochondria-targeted antioxidant, were effective in reversing defective mitochondrial function in ASM cells from patients with COPD and ozone-exposed mice (126). MitoTEMPO was effective in attenuating mtROS, TGF-β, and collagen deposition in cultured human airway cells or mouse models of allergic inflammation (61). Although these appear translatable to bedside and MitoQ is now sold over the counter as a dietary supplement, some caution is warranted because even vitamin E and other untargeted antioxidants were found to be beneficial in experimental models (74, 75, 80). Nevertheless, some clinical data exist for CoQ10 that can be reasonably extrapolated to MitoQ, its more efficient form (113). In an open-label crossover study of 41 steroid-dependent patients with asthma, supplementation with a daily antioxidant cocktail, consisting of CoQ10 (120 mg) + 400 mg α-tocopherol + 250 mg vitamin C, was associated with a reduction in steroid usage (46, 52). CoQ supplementation has also been variably found to be beneficial in other diseases characterized by mitochondrial dysfunction (16). However, supplementation with other nonspecific antioxidants like α-tocopherol and vitamin C may interfere with mitochondrial signaling and biogenesis (50). An important alternate approach toward optimizing oxidant-antioxidant balance is to enhance Nrf2, the master antioxidant defense transcription factor that has been shown to be cytoprotective in lungs (111). The prototypical Nrf-stimulating molecule, sulforaphane, is found in natural dietary products such as broccoli, and other analogs are in development (37, 62, 64). Sulforaphane has been

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**Fig. 3.** The 3 Rs of mitochondria-targeted therapeutic strategies. Failing mitochondrial respiration may be restored through 1) repair via scavenging harmful ROS, 2) reprogramming via regulatory pathways, and 3) replacement by exogenous healthy mitochondria. MitoQ and MitoTEMPO are triphenylphosphonium-tagged antioxidants that target mitochondrial dysfunction. Sulforaphane, found in broccoli, stimulates nuclear factor erythroid 2-related factor 2 (Nrf2). Vanadia nanowires act as the cytoprotective antioxidant enzymes as shown. Microbial molecules that activate Nrf2 or pyrroloquinoline quinone (PQQ), a powerful antioxidant that also stimulates mitochondrial biogenesis, seem to be important. Mitochondrial biogenesis may also be promoted by reprogramming through diet, exercise, metformin (Metf), L-arginine (L-Arg)/nitric oxide (NO) or antagonists to mitotoxic miRNAs. Finally, mitochondria can be replaced by microinjection of isolated mitochondria, peptide tagging of naked mitochondria, tunnelling nanotubes (TNT)/microvesicle-mediated transfer of mitochondria from donor cells. PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; TFAM, mitochondrial transcription factor A.
shown to protect against COPD (44, 55). Although mitochondrial function was not specifically looked for in the COPD studies, sulforaphane has been shown to enhance mitochondrial antioxidant defense and protect against a panel of oxidant stressors (129). However, the enthusiasm for activating Nrf2 as a protective strategy is somewhat dampened by concerns about mitochondrial stress. Exercise, diet, and possibly metformin remain the most reliable methods of reprogramming mitochondria. Increased mitochondrial biogenesis is a well-known consequence of exercise. Exercise has been found to be beneficial in reducing asthma exacerbations and is a cornerstone of pulmonary rehabilitation to improve overall respiratory performance in COPD (43). Chemical stimulators of mitochondrial biogenesis include resveratrol and nitric oxide. Resveratrol or L-arginine supplementation improved mitochondrial function and attenuated asthmatic features in experimental models of allergic inflammation (10, 76). Similar effects were obtained by inhibition of arginase or ADMA (5). Metformin shows anti-asthma potential in experimental models and also attenuates the increased allergic inflammatory tendencies of obese mice (21). These approaches may be particularly relevant in targeting mitochondrial dysfunction in patients with obese-asthma or COPD (3). Clinical trials of metformin in COPD show some promise toward reducing dyspnea and increasing exercise capacity, but results of more comprehensive studies are awaited (104).

Emerging molecular strategies for metabolic reprogramming also include manipulating the let-7/film28 axis that has been shown to critically determine cellular OxPhos capacity and is shown to enhance tissue repair or reduce aging (106). Let-7 miRNAs have been implicated in asthma pathogenesis (68, 69, 95). Other miRNAs also appear to modulate mitochondrial function, especially two classes, hypoxamirs and mitomirs. Hypoxamirs are, as the name suggests, induced by hypoxic response (32). This is relevant to both asthma and COPD. MiR-210 is one of the best understood hypoxamirs, with a highly conserved hypoxia response element in its promoter. It is also inducible by nuclear NF-kB, which is known to be increased in severe asthma and COPD. The iron-sulfur (Fe-S) cluster assembly homologue (ISCU) 1/2, which enables redox reactions in the mitochondrial ETC, is one of the direct targets of miR-210 (23). Foxp3, the master transcription factor for lineage specification of the anti-inflammatory regulatory T cells, is also targeted by miR-210, supporting a proinflammatory role of chronic hypoxic response (128). Modulation of miR-210 is not yet attempted in COPD but has been shown to attenuate pulmonary hypertension that can secondarily complicate COPD or severe asthma.

Mitomirs are a specific set of miRNAs present in the mitochondria that are closely linked to mitochondrial functions like energy metabolism and mitochondrial dynamics (38). They are more extensively reviewed elsewhere (38), but, briefly, miR-149 increases mitochondrial biogenesis through poly(ADP-ribose) polymerase-2, Sirt-1, and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (85). MiR-761 and miR-30 members modulate mitochondrial fission (38). MiR-183, which is discussed in greater detail in the section on COPD, inhibits OxPhos by targeting IDH2, is elevated in COPD, and may be a relevant therapeutic target. The effects of mitomirs are not restricted to mitochondria and may be relevant to comorbidities of asthma/COPD. For example, miR-183 downregulates the expression of large-conductance, Ca²⁺-activated K⁺ channel (BKCaβ1) that determines arterial tone, possibly increasing risk for systemic and pulmonary hypertension (22).

Because the lungs are easy to target specifically through aerosol delivery, antagonists to miR-210 and miR-183 may be specifically valuable for treating pulmonary hypertension or hypercapnic lung disease, respectively, as discussed previously. Extensive preclinical testing across multiple models will be necessary because these miRNAs have multiple targets and functions. For example, miR-210 is associated with increased stem cell survival in hypoxic conditions and may mediate other cytoprotective functions of the hypoxic response.

Replacement. There is now extensive evidence of mitochondrial transfer as a mechanism to replace defective mitochondria. Methods for mitochondrial transfer to cells are diverse, including microinjection (29, 36, 65, 92), incubation with mitocytoplasts or intact purified mitochondria (66, 67), gap junctional channel-mediated cell attachments (60), and direct transfer from donor cells like mesenchymal stem cells (MSC) to recipient cells via cytoplasmic bridges called tunneling nanotubes (TNT) (8, 48, 93, 94, 100). Each of these methods has its own advantages and disadvantages. Microinjection of isolated mitochondria was pioneered by Tatum’s group (36) and was later used by many other groups to generate transmitchondrial cell lines that have provided significant insights into the survival of foreign mitochondria in recipient cells (29, 65, 87). Clark and Shay (29) demonstrated the spontaneous incorporation of isolated mitochondria into recipient cells. Delivery of isolated autologous mitochondria through injection into ischemic tissue was able to improve tissue ATP content and postinfarct cardiac function (81). Further work from the same group found this to be an actin-dependent uptake, presumably endocytosis, but how mitochondria escape the endosome remains unclear (91). Although this avoids the unknown long-term effects of MSC therapy and benefits from the relatively short time to isolate the organelles than to grow stem cells, others have been less successful with this approach (107). Cell-penetrating peptide tags for efficient cell penetration of mitochondria may represent the next generation of naked mitochondrial transfer (25). However, mitochondria are also powerful triggers of the innate immune response, and the risk of damaged mitochondria enhancing inflammation should be carefully excluded. MSC are found in the connective tissue of multiple organs and have limited stem potential, earning them an alternate name, mesenchymal stromal cells. Prockop’s group (107) showed for the first time that mitochondria or mtDNA shuttled from human MSC to neighboring rodent cells with nonfunctional mitochondria, leading to a bioenergetic rescue. This discovery extended the potential clinical application of MSC to a wide array of disease associated with mitochondrial dysfunction (1, 8, 27, 53, 60, 71, 94, 114, 130). Coculture of MSC or fibroblast cells with mitochondria-depleted (ρ0) lung alveolar epithelial cells (A549) showed rescue of proliferative capacity, even in restricted media. Genetic screening of both the mitochondrial and nuclear DNA from the revived cells confirmed that the mitochondria in the revived cells were derived from either of the donor cells (human MSC...
Nanozyme that penetrates cells and bolsters defense against ROS. Mugesh and colleagues (119) found that ROS bursts without altering the basal antioxidant system. Although these remain untested outside cell lines and the ability to penetrate mitochondria is unclear, this represents an exciting new frontier in the field.

Summary

Cellular respiration is a reflection of global respiratory health, and disordered mitochondrial homeostasis confers increased risk for lung diseases. Mitochondrial dysfunction initiates cytotoxic events in AEC and leads to persistent remodeling of ASM. Although much remains to be understood, targeting mitochondria appears to be a viable strategy for optimizing respiratory health. A greater focus on the role of mitochondria in lung cellular and molecular physiology appears warranted.

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REJUVENATING RESPIRATION: REPAIR, REPROGRAMMING, REPLACEMENT


