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Mitochondria in Lung Diseases

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Summary

Mitochondria are autonomous cellular organelles that oversee a variety of functions such as metabolism, energy production, calcium buffering, and cell fate determination. Regulation of their morphology and diverse activities beyond energy production are being recognized as playing major roles in cellular health and dysfunction. This review is aimed at summarizing what is known regarding mitochondrial contributions to pathogenesis of lung diseases. Emphasis is given to understanding the importance of structural and functional aspects of mitochondria in both normal cellular function (based on knowledge from other cell types) and in development and modulation of lung diseases such as asthma, COPD, cystic fibrosis and cancer. Emerging techniques that allow examination of mitochondria, and potential strategies to target mitochondria in the treatment of lung diseases are also discussed.

Keywords

Mitochondria; fission; fusion; oxidative stress; reactive oxygen species; airway; proliferation; apoptosis; autophagy

1. Introduction

Mitochondria are cellular organelles with distinct features that belie their origins and unique functions. Originally derived from ancient aerobic bacteria [1], mitochondria are critical for meeting cellular energy demands by driving the synthesis of ATP. Possessing their own maternally-inherited genome and transcription/translational machineries has also conferred mitochondria with a certain level of functional autonomy [2]. The mitochondrion differs from the other organelles structurally as well: it is enveloped by a double-membrane assembly, with four distinct parts: outer membrane (OMM), inner membrane (IMM), intermembrane space (IMS), and matrix (Figure 1). The highly folded IMM (called cristae) harbors the oxidative phosphorylation (OXPHOS) enzyme complexes. Within the well-defined confines of the mitochondrion, carbohydrates and long chain fatty acids are metabolized, lipids and steroids are synthesized, ATP is made as a by-product, mitochondrial DNA (mtDNA) is replicated, and gene products are expressed.

As part of their normal functioning, mitochondria generate reactive oxygen and nitrogen species (ROS and RNS) [3,4]. In turn, ROS and RNS are among the key regulators of mitochondrial function. While normal metabolic pathways such as glycolysis and β-oxidation of fatty acids are significant sources of these free radical species [5–7], cells also have defense measures in the form of scavengers: antioxidant enzymes such as superoxide dismutase, peroxidases and catalase [8–10], and small molecules such as melatonin [11] to

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curb the damaging effects of ROS and RNS, thus protecting the cell. The extensive roles that ROS and RNS play in the pathogenesis of several disease conditions, especially in the lung, will be discussed later in this review.

While such ROS and RNS can serve physiological functions [4,12], failure to clear excessive levels of these toxic moieties can be detrimental to the cell. In addition to affecting cell proliferation and apoptosis, ROS can cause mutations in the mitochondrial genome, thereby giving rise to nonfunctional OXPHOS enzymes and impacting cellular metabolism [13,14]. This, in turn, can adversely affect other organelles, and thus disrupt cellular homeostasis. Mitochondria further play a crucial role in cell fate determination by releasing certain molecules into the cytoplasm, triggering a self-destructive enzymatic cascade, leading to apoptotic cell death [15].

While mitochondria are now thought to be multifunctional in nature, emerging discoveries further suggest a dynamic aspect to mitochondrial location and structure [16–28]. In this regard, multiple regulatory mechanisms are thought to be involved in morphological changes of mitochondria, controlling the formation and maintenance of mitochondrial networks (fusion) and disruption of such networks (fission). Such changes result in spatial and temporal regulation of mitochondria. Accordingly, mitochondria and mitochondrially targeted proteins are thought to interact with organelles such as the plasma membrane, endoplasmic reticulum (ER; sarcoplasmic reticulum SR, in muscle cells), peroxisomes, lysosomes, and nucleus, thus enhancing intracellular communication and potentially affecting a wide range of cellular functions [18,29–34]. The relevance of such interactions lies in the broad effects that mitochondria can have on different organelles and processes in the context of disease, and furthermore the effect of mitochondrial dysfunction itself on cellular structure and function.

Overall, mitochondria appear to play an important role in different aspects of cell structure and function, and in the balance between processes that are detrimental vs. protective to the cell. These aspects become important in the cellular responses to a variety of genetic, epigenetic and environmental signals that lead to disease states. This is also important in the context of lung diseases where the idea that perturbations of mitochondrial structure and function are important to disease pathophysiology is gaining momentum. This review is aimed at summarizing and discussing the current state of knowledge regarding mitochondrial structure and function in the lung, how disease processes modulate these aspects, and in turn, how mitochondria can contribute to disease pathophysiology. Here, at the outset, it is important to emphasize that much of this data in terms of normal lung structure/function and lung disease is only emerging. Nonetheless, substantial important information can be gleaned from data in other non-lung cell systems and disease states, setting the stage for exploration of mitochondrial mechanisms in the lung. In this regard, what aspects of mitochondrial structure, function or dysfunction are important is not well-known. In this review, specific emphasis will be given to homeostatic aspects of mitochondrial biology: the fission-fusion balance of the mitochondrial reticulum, synthesis/clearance cycle of oxidants (ROS and RNS), and, proliferation vs. apoptosis or the cell fate determination step, all of which are important in cellular function relevant to the lung. By highlighting the significance of these processes, the review will facilitate better understanding and exploration of emerging concepts of targeting mitochondria to alleviate disease in general, and lung disease in particular.

2. Mitochondrial Fission and Fusion

2.2. Regulation of mitochondrial morphology

Although the origins of mitochondria are common across cell types, mitochondria assume remarkably different shapes, connectivities and morphological parameters in different cells, in different species and under different conditions [35–39]. Furthermore, even within a cell type or within individual cells, mitochondria are not static structures, but show continued changes in their network morphology and intracellular locations. The relevance of altered mitochondrial morphology lies in increasing evidence that regardless of cell types, such changes reflect (or go hand in hand with) mitochondrial function, especially in the setting of apoptotic cell death or oxidative stress [19,35,37,40]. Accordingly, understanding the processes that control mitochondrial morphology become important to determining the role of mitochondria in normal cellular function and mitochondrial dysfunction in the setting of disease. Here, it is important to stress that much of the information is derived from cellular expression systems and a variety of non-lung cell types.

Proteins that control mitochondrial fission vs. fusion are the primary regulators of mitochondrial morphology. Here, the mitofusins 1 and 2 (Mfn1 and Mfn2), and Optic Atrophy protein 1 (Opa1) are essential mediators of mitochondrial fusion [41] (Table 1). Genetic knockout studies have established that consistent with their localization patterns (Mfns on the OMM, and Opa1 on the IMM), these proteins are responsible for the fusion of mitochondrial OMM and IMM, respectively [41], allowing for formation of contiguous chains or networks of mitochondria within the cell. In contrast, fission requires dynamin related protein 1 (Drp1) to be recruited from the cytosol to the mitochondrial surface, a step that involves other proteins such as Fission 1 (Fis1) and Mitochondrial fission factor (Mff) [42]. Additional factors such as MiD49 and MiD51 are also important in the orchestration of fission where these proteins form foci/rings around mitochondria to assist in recruitment of Drp1 from the cytosol when fission is imminent [43].

A number of factors can modulate mitochondrial fission and fusion proteins. Indeed, intracellular Ca^{2+} can itself be a factor where mitochondrial fragmentation is activated during high Ca^{2+} conditions, and suppressed at resting $[Ca^{2+}]_I$. Such effects may serve to modulate mitochondrial localization for maximal energy utilization or other functions, and/or they may be outcomes of other changes induced by altered Ca^{2+}. Changes in mitochondrial morphology are also linked to oxidative stress-mediated signaling and cell fate determination such that these events share several proteins [26,44–49]. For example, the Bcl2-family proteins (Bax and Bak), initially thought to regulate apoptosis by merely inducing cytochrome *c* release into the cytosol, have now been shown to also directly interact with the fission-fusion machinery [44,47,50,51]. By sequestering fusion protein Opa1, the Bcl2-family member Bnip3 promotes mitochondrial fission [47], and Bcl-xL, a related protein, has been shown to regulate both fission and fusion via binding to Drp1 [50]. In addition, data in human skin fibroblasts show that mitochondrial morphology is modulated by mitochondrial membrane potential (MMP) where dissipation of MMP causes fragmentation and inhibition of glycolysis worsens OXPHOS defect-induced morphological changes [45]. Apoptosis follows Bax-mediated Opa1 disassembly, cristae remodeling and CytC release [49].

Interactions between fission and fusion proteins can also regulate mitochondrial morphology. For example, Huang et al. found that the association between two heptad regions in Mfn2 renders this protein incapable of mediating fusion, and when Mfn2 is free to interact with Drp1 under these conditions, fission ensues [26].

Relevant to disease states, one mechanism by which mitochondrial morphology can be altered is via changes in the turnover of the fission and fusion proteins. In other words, in addition to activating signaling pathways that alter the transcription and translation of fission/fusion proteins, stimuli such as inflammatory mediators or environmental triggers also induce post-translational modifications like proteasome-mediated degradation [52]. This is best illustrated by data from familial Parkinson's disease (PD); here, Pink1, a mitochondrially targeted Ser/Thr kinase, interacts directly with the fission protein Fis1, and regulates mitochondrial dynamics [53] by acting as a co-factor for Parkin, an E3 ubiquitin ligase, and targeting Mfn, helping to degrade damaged mitochondria [54]. Defects in Mfn2 protein turnover due to gene mutations are associated with CMT2A neuropathy [55]. The role of post-translational modification by ubiquitination or SUMOylation in controlling the degradation rates of other members of the fission-fusion machinery such as Opa1, Drp1, and Fis1 have also been reported [56–58]. Overall, these studies highlight the evolution of cellular quality control mechanisms directed towards protecting mitochondrial fission-fusion equilibrium, and their potential relevance to disease states. What is not known is the relative importance of fission and fusion machineries in different lung cell types, their roles in normal cell function, and the extent of their involvement in specific lung diseases.

2.2. Mitochondrial morphology and cell function

The relevance of mitochondrial fission/fusion processes is clear during the cell cycle. Mitochondria fragment and rejoin constitutively during the G0 phase (quiescent state) of the cell cycle. However, when the cell enters interphase, mitochondrial fusion is promoted, leading to extensive networking (Figure 2). As the mitotic and cytokinetic events occur during the M phase, mitochondria get fragmented, mixed and distributed equally among the progeny cells. At M phase exit, fusion resumes [27,59–62]. Furthermore, proteins involved in mitochondrial dynamics, such as Fis1 and GDAP1, have been reported to interact with microtubules, which are integral members in cell division [63]. These fission/fusion processes during the cell cycle become relevant to the disease state when factors such as inflammatory or infectious mediators, ROS/RNS and environmental stimuli alter these processes (e.g. in the airway or lung), influencing cell proliferation vs. stasis/apoptosis and overall cell numbers [64,65].

The dynamin GTPase Opa1 (regulator of mitochondrial inner membrane fusion and cristae remodeling) is thought to mediate adrenergic control of lipolysis by functioning as a cytosolic A-kinase anchoring protein (AKAP) [66,67]. Decreased Opa1 expression and increased fragmentation have been observed in failing hearts in both humans and rats [68]. In addition to its role in mitochondrial fusion, Mfn2 has been reported to be involved in oxidative metabolism, vascular cell proliferation, obesity, type 2 diabetes and regulation of the cytokines TNFα and IL-6 [69]. Mutations in Mfn2 gene have been linked to the autosomal dominant neurogenerative disease Charcot-Marie-Tooth 2A [70]; however, Mfn2 can also function as a protector of mtDNA integrity. Loss of Mfn2 results in accumulation of mutations in skeletal mtDNA in humans and mice [71]. It is evident from these emerging data not only that mitochondrial morphology and function are finely balanced domains, but also that their interdependency is an important factor in the role of mitochondria in disease development.

2.5. Mitochondrial fission/fusion and mitochondrial movement and distribution

Aspects other than mitochondrial morphology can also drive cellular functions. Here, mitochondrial localization to and interaction with other organelles are particularly important. In this regard, mitochondrial contacts with ER have been studied more extensively [23,24,29,30,72–75]. Acting as tether sites for specific proteins, mitochondria-ER junctions can aid in lipid synthesis, Ca^{2+} buffering and signaling, intracellular trafficking and

mitochondrial biogenesis [24,75–78]. The fusion protein Mfn2 can physically harness ER to the mitochondrion via its association with Ras, thereby controlling mitochondrial uptake of Ca^{2+} from ER microdomains [23,72,74]. Regulation of cytosolic and ER luminal Ca^{2+} levels via such buffering is particularly important in tissues such as cardiac muscle, where ATP and ROS, as well as Ca^{2+}, are rapidly and consistently exchanged between mitochondrion and SR. This bidirectional regulation of $[Ca^{2+}]_i$ makes the mitochondria a major source of Ca^{2+} for contraction in addition to the SR [79]. The relevance of such mitochondria-ER interactions in the lung lies in the fact that several lung cell types including alveolar epithelium and airway smooth muscle use ER/SR Ca^{2+} release for contraction or other cellular functions, including protein regulation. Accordingly, mitochondrial modulation of plasma membrane vs. intracellular organelle function becomes important.

Mitochondria in proximity to the PM may be important in regulation of Ca^{2+} influx in different cell types. Mitochondrial buffering of the Ca^{2+} that enters may sustain or promote Ca^{2+} entry due to perceived lack of cytosolic Ca^{2+}, or alternatively prevent Ca^{2+} entry through signaling interactions [80–85]. Whether mitochondrial fission or fusion proteins are involved in such modulation of Ca^{2+} influx remains to be determined. The importance of such a mechanism lies in the fact that several cell types, including those in the lung are highly dependent on Ca^{2+} influx.

Mitochondria may also indirectly facilitate ER-plasma membrane interactions. For example, in airway smooth muscle and other cell types, the ER/SR protein STIM1 senses SR Ca^{2+} release, and either translocates to or aggregates at the PM Ca^{2+} channel Orai1 [86–91], facilitating store-operated Ca^{2+} entry. It has recently been shown that STIM1 mobilization to ER-PM junctions also occurs upon mitochondrial release of Ca^{2+}, and is an Mfn2-dependent step [78]. The relevance of these interactions lies in the fact inflammation has been shown to regulate mitochondrial Ca^{2+} in the airway [81], mitochondrial regulation of intracellular Ca^{2+} levels via the STIM1/Mfn2 pathway remains to be determined.

There is now strong evidence that mitochondrial movement and distribution within the cell is also important. Coordinated interactions between cytoskeletal elements (particularly actin and microtubules) and motors (such as kinesin and dynein) are thought to enable proper positioning of mitochondria. The mitochondrially-localized Miro protein seems to be a key regulator of mitochondrial motility, with its activity depending on its Ca^{2+}- sensing and kinesin-binding abilities [92,93]. Interestingly, Miro suppresses Drp1-mediated fission, thus connecting mitochondrial fragmentation (activated during high Ca^{2+} conditions, and suppressed at resting $[Ca^{2+}]_i$), and fusion with mitochondrial movement [84]. While it has been known that mitochondria rely on the motor proteins kinesin and dynein for transport along microtubules [94,95], recent evidence suggest that dynein mediated mitochondrial transport may be essential for mitochondria-ER interactions during inflammation [96]. Similarly, mechanisms that regulate actin polymerization also seem to modulate mitochondrial fission [29]. Actin filaments that connect ER to mitochondria may initiate mitochondrial constriction, which then is completed by the fission protein Drp1. It is easy to envision mitochondrial movement being motivated by energy-demanding areas of a cell, i.e., mitochondria become less mobile and cluster at sites that require additional metabolic energy for function, signaled by factors such as $[Ca^{2+}]_i$ or signaling intermediates. The relevance of mitochondrial movement and its modulation by fission/fusion in lung diseases lies in the potential disruption of these relationships by inflammation, ROS or other factors in different cell types. Such disruption may lead to a mismatch between energy demand and supply (particularly in bronchial or epithelial cells that undergo high turnover), availability of mitochondria for regulating other cellular organelles such as the ER/SR and PM, leading to dysregulating Ca^{2+} and contractility, cell proliferation/apoptosis etc.

3. The importance of ROS

3.1. Production and clearance of mitochondrial ROS

As mentioned above, mitochondria are the sites for metabolic processes and the electron transport chain, both of which give rise to significant amounts of ROS and RNS [97]. When production of these moieties overwhelms the innate scavenging mechanisms of the cell (via enzymes like SOD, catalase or peroxidase, to name a few), the resultant oxidative stress is deleterious to the cell at multiple levels [98]. In the lung, uncontrolled oxidant levels can contribute to pulmonary fibrosis and other diffuse lung diseases [12]. Several studies have shown that mitochondrial ROS subserve several functions, acting as signal transducers that trigger expression and/or release of proinflammatory cytokines and the relevant pathways [99,100], as well as modulators of transcription factors that are important in normal cellular redox homeostasis, cell proliferation/survival, and responses to inflammation [101]. These mediators play vital and multiple roles in the development and exacerbation of asthma and allergy, COPD and fibrosis by altering processes such as cell proliferation, extracellular matrix production, Ca^{2+} regulation [81,87,89,100,102–110]. Importantly, inflammatory mediators and ROS can in turn modulate mitochondrial morphology and function, thus linking mitochondria, ROS and inflammation in a functional cycle of consequence to disease pathophysiology. For example, IL-6 induces Bcl2, which interferes with mitochondrial Bak-Mfn2 interactions and blocks apoptosis [111]. Oxidative stress enhances Drp1 translocation from the cytosol, mitochondrial fragmentation, CytC release and apoptosis, while treatment with an antioxidant or overexpression of Mfn2 nullifies these effects [112].

In addition to oxidative stress occurring as a result of intracellular processes, the lung can be exposed to hyperoxia (e.g. in patients in the ICU, or those needing supplemental oxygen) as well as hypoxia (e.g. as in COPD, pulmonary fibrosis or pulmonary hypertension). We recently demonstrated that fetal airway smooth muscle cells exhibit an O_2 dose-dependent response in terms of Ca^{2+} signaling, proliferation and mitochondrial morphology [113]. Here, we reported that both hypoxia and increasing levels of oxygen induce mitochondrial fragmentation and enhance expression of fission proteins (but blunt fusion protein levels). Hypoxia can also induce ROS and RNS release from IMM [4] which activates transcription factors such as HIF1 (Hypoxia inducible factor 1) [114] that plays a significant role in mediating hypoxia-induced effects on the mitochondria by hyperpolarizing OMM, increasing mitochondrial glycolytic activity and promoting cell proliferation [115,116].

In summary, mitochondria are not only the sites for releasing oxidative free radicals; they respond to the corresponding signals by altering their structural and functional features, and these are conveyed to downstream effectors as regulatory instructions for alterations in cellular behavior.

3.2. Mitochondrial Membrane Potential

Release of the mitochondrial IMS enzyme CytC into the cytoplasm is a marker for the onset of apoptosis since this event can occur only if OMM becomes permeable following mitochondrial depolarization. Mitochondrial membrane potential (MMP; $\Delta\Psi$) is thus an indicator of mitochondrial function, or dysfunction. Thus, in cancers where cell death is the intent, manipulation of MMP towards depolarization can boost sensitivity to chemotherapy agents [117,118]. Here, we recently showed a “protective” role for the mitochondrial protein MICU1 [119]. Conversely, a stable OMM barrier and inhibited apoptosis may be beneficial under other conditions [120]. Regulation of MMP appears to be linked to mitochondrial fission-fusion machinery but not fission/fusion itself. Inhibition of the fission protein Drp1 directly prevents CytC release and apoptosis by blocking mitochondrial depolarization [15,121]. The story is more complex as the fusion protein Mfn2 is also thought to predispose

cells (cardiac myocytes) to apoptosis by expediting mitochondrial depolarization or fusion-triggered membrane destabilization and leakage [122,123]. However, it is likely that Mfn2 plays roles other than just mitochondrial union; in fact, Mfn2 has been shown to mediate autophagosome-lysosome fusion as well, and this process triggers apoptosis in cardiac cells [124]. Thus complex interactions between MMP, fusion/fission proteins and cell fate exist, and may color the overall effects of factors that induce mitochondrial depolarization.

4. Mitochondrial fission/fusion, apoptosis and proliferation

4.1. Mitochondria-dependent cell death

Several studies, including our own, have confirmed that mitochondria fragment following exposure to a wide range of toxic substances, signaling the onset of apoptosis [25,28,113,125,126]. Such a sequence is triggered by Drp1 translocation to mitochondria [109]. However, mitochondrial fission can occur, independent of apoptosis. For example, attenuation of mitochondrial fragmentation by Drp1 inhibition blunts CytC release into cytosol, but other mitochondria-derived cell death-related factors (caspases, adenylate kinase etc.) are not blocked [127,128]. Bax and Bak promote fragmentation and apoptosis [129,130], yet antagonizing these proteins inhibits CytC release but does not necessarily prevent fragmentation [131].

The connection of Drp1/fission to apoptosis and of Mfn/fusion to proliferation is not straightforward. Contrary to the idea that Drp1 is pro-apoptotic, recent studies show that Drp1 can induce a hyperproliferation phenotype in vascular smooth muscle cells relevant to pulmonary hypertension and in lung cancer [132,133]. On the other hand, several studies show that mitofusins (especially Mfn2) promote proliferation [48,134,135], with some reports of suppression [132,136,137]. Mfn2 expression is downregulated in atherosclerosis and other vascular disorders [138] and in hyperinsulinemia [139]. In airway smooth muscle cells, however, we find Drp1 to be anti-proliferative and Mfn2 to be anti-apoptotic (unpublished results, paper currently in review). Perhaps differences in cell type, stimuli, and growth conditions underlie such discrepancies, but the essential point is that mitochondrial morphology itself can drive cell survival vs. death. Accordingly, the role of mitochondrial morphology in the pathogenesis of lung diseases becomes important.

4.2. Autophagy/mitophagy

Autophagy is a cellular homeostatic process where organelles including mitochondria, proteins or bits of cytoplasm get enveloped by double-membraned vesicular structures (autophagosomes) that then fuse with lysosomes to get degraded and recycled [140]. Recycling of digested cellular components helps produce ATP during starvation [141]. Relevant to mitochondria (where the process is termed mitophagy), depolarized and damaged mitochondria, if left unchecked, will activate apoptotic signaling [142,143], and thus mitophagy plays a key role in cytoprotection, stress adaptation and cellular housekeeping [1]. The PINK1-parkin signaling system has been attributed a critical role in the pathogenesis in Parkinson’s disease (PD), as mutations in these genes cause defective mitophagy; lack of clearance or the accumulation of damaged mitochondria initiates apoptosis and thus to selective loss of dopaminergic neurons in substantia nigra [53,54,144]. There is now considerable interest in autophagy processes in lung diseases, particularly relevant to the effects of inflammation, especially because this phenomenon is no exception to the repetitive motif of balance and homeostasis in biological systems: emerging evidence suggests that autophagy can be either protective or deleterious in the lung in disease conditions such as pulmonary arterial hypertension, COPD, cystic fibrosis, and pneumonia [1,142,145–151]. What is less clear is which effect predominates whether autophagy should be inhibited or promoted in the context of therapy. The autophagy protein LC3B has a

protective effect in PAH by regulating hypoxia-induced cell proliferation [149], but is detrimental in COPD and cigarette smoke-induced lung cell death [145,146]. Specific modifications of autophagy/mitophagy-associated proteins are essential for maintaining mitochondrial and cellular homeostasis: failure by AMPK (adenosine monophosphate-activated protein kinase), a cellular energy sensor, to phosphorylate autophagy proteins results in defective clearance of mitochondria [152]. How mitochondria in turn may play a role in modulating autophagy, and the link between autophagy, mitochondrial regulatory proteins and lung diseases are still under investigation.

5. Mitochondrial dysfunction in lung diseases

Most of the current knowledge on mitochondrial morphology and functions related to fission/fusion and other mechanisms is derived from neurons, cardiomyocytes and immune cells (summarized in Figure 3 and Table 2). While the fission-fusion machinery in these cells is fundamentally identical to that in the cells of the lung (smooth muscle, epithelium, fibroblasts, resident immune cells), dysregulation of mitochondrial morphology, localization or function (due to fission-fusion imbalance, for example) is very likely to be cell-type and context (disease)-specific. The airways and lung parenchyma are sensitive and responsive to numerous stimuli such as allergens, toxins, pollutants and microorganisms, all of which can enhance inflammation, oxidative and cellular stress and metabolism-induced signaling to different extents, resulting in different pathophysiological responses. Chronic exposure to such stimuli or the resulting cellular responses are thought to drive processes such as enhanced Ca^{2+} and contractility, greater cell proliferation with reduced apoptosis, mucus secretion, and overall structural and functional changes in the lung. As primary sources of ROS and as crucial modulators of a number of cellular processes relevant to lung disease, the importance of mitochondria is obvious. However, there is considerable paucity in our understanding of mechanisms by which mitochondrial structure and function regulate airway health and disease.

5.1. Airway hyperresponsiveness and asthma

As discussed above, the roles of mitochondria in ROS generation and processing, cell proliferation vs. apoptosis, regulation of Ca^{2+} and of other organelles are all relevant to inflammatory airway diseases such as asthma, which are characterized by hyperresponsiveness of the airway resulting from increased Ca^{2+} and contractility of airway smooth muscle, and by airway remodeling represented by epithelial and smooth muscle thickening, cell proliferation/migration and reduced apoptosis. Furthermore, polymorphisms or haplotype differences in mitochondrial genome have been found to be associated with asthma in humans [153]. However, there is limited but emerging data on how mitochondria may play a role in mediating or modulating the effects of inflammation in the airway.

Tumor necrosis factor-alpha (TNFα) is an early cytokine relevant to airway inflammation. TNFα has been shown to alter mitochondrial dynamics and OMM permeabilization, increasing ROS generation, and causing apoptosis in cultured adipocytes, and in murine lung microvascular endothelium [104,154–156]. Delmotte et al. recently showed that inflammatory cytokines such as TNFα and IL-13 have profound effects on the Ca^{2+} buffering activity of mitochondria of human airway smooth muscle [81], which may play a protective role in preventing Ca^{2+} overload. However, cytokines may also overwhelm the protective mechanisms of mitochondria, as we recently found in human airway smooth muscle where TNFα induced mitochondrial fission, increased Drp1 expression (with a concomitant decrease in Mfn2), and increased ROS production, particularly in cells derived from asthmatic patients [125]. Inhibiting ROS with antioxidants blocked these effects on mitochondrial morphology highlighting the link between ROS and mitochondrial fission/fusion in the airway. Interestingly, inhibition of Mfn2 increased ROS in airway cells, while

inhibition of Drp1 suppressed it, suggesting that the fission/fusion machinery may play an active role in modulating oxidative stress in the inflamed airway.

TNFR1 has been implicated in TRAPS (an autoinflammatory disorder resulting from missense mutations in TNFR1 gene), possibly by causing mitochondrial dysfunction, increasing ROS production and cytokine levels [103]. Similarly, glucocorticoid receptor GRα and estrogen receptor ERβ, critical signaling pathway components in the cell, have been shown to localize to the mitochondria; their expression declines during allergic asthma [157]. It is possible that, at a molecular level, a combination of decreased apoptosis-promoting receptors and increased survival-enhancing receptors influences mitochondrial function and morphology, and eventually leading the airway cells towards proliferation and remodeling. In addition to the usual cytokine players, another group of inflammatory modulators is lipid metabolites and the enzymes involved in their production. For example, 13-S-hydroxyoctadecadienoic acid, a lipid metabolite derivative of linoleic acid, induces features of severe asthma and importantly mitochondrial dysfunction [158], while 12/15-lipoxygenase, an enzyme induced by the Th2 cytokine IL-13in can cause bronchial epithelial injury again involving mitochondria [159].

While exposure to environmental factors such as ozone, diesel exhaust particles, or cigarette smoke has been known to trigger and worsen asthma and allergy symptoms, the role of mitochondria in the airway is emerging. Here, the role of oxidative stress and ROS appears to be important. In general, it appears that levels of different antioxidants in serum, bronchoalveolar lavage (BAL) or tissues are lower in asthmatics (see [160] for review). For example, peripheral cells and tissues from asthmatic patients show lower antioxidant enzymes [161–163] such that serum SOD activity, but not manganese or copper- and zinc-containing superoxide dismutase, is lower, and correlates to the degree of airway constriction. In terms of ROS, exposure to either unfractionated cigarette smoke extract or its components generates excessive mitochondrial ROS in ASM and alveolar epithelial cells [125,164]. Similarly, exposure to allergens such as ragweed pollen enhances ROS generation, which in turn, damages components of the mitochondrial respiratory chain complex [165]. Interestingly, the innate metabolic and respiratory events, which are the primary source of ROS, have been noted to become more robust in some airway diseases; that is, oxygen consumption and mitochondrial biogenesis are elevated in asthmatic airway cells [166], suggesting an almost ‘suicidal’ mechanism of disease pathogenesis.

Overall, these limited but interesting data suggest that factors that contribute to asthma can influence mitochondrial ROS generation or handling in the face of reduced antioxidants, thus sustaining oxidative stress in the airways. Here, mitochondrial fission and fusion machinery may play a more active role in modulating oxidative stress in the inflamed airway. Accordingly, targeting of oxidative stress in general or aspects of the mechanisms that control mitochondrial morphology may be helpful in alleviating the effects of factors that cause asthma.

5. 2. Cystic Fibrosis (CF)

Cystic fibrosis is an autosomal recessive disease, most commonly due to a mutation ΔF508 in the CFTR gene, and characterized by abnormal transport of chloride and sodium ions across the epithelium, leading to viscous airway secretions. There is limited data showing that mitochondrial buffering is compromised in airway epithelial homozygous for ΔF508CFTR [167]. Cells with mutated CFTR also display extensive mitochondrial fragmentation, and OMM depolarization. In addition, the hypoxic pulmonary vasoconstriction (HPV) that occurs in CF is partly attributable to impaired mitochondrial OXPHOS in pulmonary arterial smooth muscle and endothelial cells under hypoxia, which alters ROS levels and AMPK activation [168]. Furthermore, enhanced ROS production and

apoptosis in CF bronchial epithelium appear to be due to aberrant mitochondrial function [169]. These observations suggest that mitochondrial dysfunction can be a substantial contributor in CF.

5. 3. Chronic Obstructive Pulmonary Disease (COPD)

COPD involves a number of mechanisms including protease-antiprotease imbalance, oxidant-antioxidant imbalance, and ineffective repair mechanisms. Exposure to cigarette smoke is well known to be a major risk factor for developing COPD [145,170–177]. Given the importance of ROS in mediating cigarette smoke effects, oxidative stress is now recognized as a major factor in COPD pathogenesis [178], since antioxidant capacity is also reduced in COPD. Here, the role of mitochondria-derived ROS appears to be prominent [178]. Beyond ROS generation, the mechanisms by which mitochondria could contribute to COPD are still under investigation. For example, differential expression of the aryl hydrocarbon receptor in lung epithelial cells and fibroblasts, which renders some smokers more vulnerable to COPD, can induce mitochondrial dysfunction and apoptosis, and downregulate expression of antioxidant enzymes [179]. In COPD, the electron transport chain, mitochondrial membrane permeability and apoptotic signaling are all affected [180–182]. Aberrant apoptotic signaling may also reduce mitochondrial density and biogenesis [175]. A recent study includes sickle cell anemia as a risk factor for cigarette smoke-induced airway obstruction in children [183], bolstering the notion that O_2 respiration, mitochondrial function, and COPD pathogenesis are linked.

5.4. Lung cancer

While a number of mechanisms can contribution to initiation and propagation of lung cancers, mitochondrial dysfunction during the initial stages of neoplastic transformation may result in superfluous ROS resulting in mtDNA damage and mutations [184,185]. Furthermore, mutations in traditional oncogenes and tumor suppressor genes such as Ras, Myc and p53 can induce mitochondrial dysfunction and uncontrolled production of ROS [186]. Anomalies in mitochondrial respiratory chain complexes and ROS generation mechanisms not only impair the normal autophagy-apoptosis stability, and thus initiate tumorigenesis, but can also render cancer cells less susceptible to chemotherapeutic agents [117,148].

The connection between mitochondrial morphology and lung cancer development is complex. Overexpression of Mfn2 may be pro-proliferative, but some studies show fission to be favored in lung cancer cells, where a simultaneous increase in Drp1 is noted (along with reduced Mfn2) such that Drp1 inhibition or Mfn2 overexpression increases apoptosis [133]. How these proteins regulate cell fate is not known and may also be context dependent. Regardless, the limited data suggest a potential novel target for lung cancers that may have pleiotropic effects.

6. Targeting mitochondria in therapy

Pharmacological agents that alter mitochondrial structure and/or function have been considered. Drugs such as glucocorticoids, antioxidants, phosphodiesterase inhibitors, leukotriene receptor antagonists and bronchodilators can regulate mitochondrial structure and function[106,187–190]. For example, lack of L-arginine bioavailability is thought to occur in asthma, leading to peroxynitrite accumulation and mitochondrial dysfunction [191,192]. Mabalirajan et al. found that in the bronchial epithelia of ovalbumin-sensitized/challenged mice, L-arginine administration restores mitochondrial ultrastructure (reduced fragmentation), and function (increased ATP levels and mitochondrial CytC activity), suggesting L-Arg as a promising asthma drug [191]. Potent antioxidants such as MitoE(2)

and MitoQ(10) have been shown to increase mitochondrial matrix [Ca^{2+}], which may favor Ca^{2+} overload, and alter the apoptosis-proliferation balance [193].Other antioxidants that have also shown promising results in preclinical studies are: baicalein [194,195], esculetin [196], and vitamin E [197,198]. For example, esculetin, a plant-derived coumarin has been reported to have potent bronchodilating properties, and to restore mitochondrial function in a mouse model of experimental asthma [196]. In neurons, glucocorticoids have been shown to induce glucocorticoid receptor interaction with Bcl2 and translocation to mitochondria [199], which may be beneficial in asthma. Here, it is interesting to note that steroid receptors (glucocorticoid, estrogen) are localized to mitochondria and participate in induction of apoptosis, but are downregulated in bronchial epithelium of allergic asthma patients [157]. Accordingly mechanisms to enhance such receptor expression may be helpful in inducing apoptosis and thus preventing airway remodeling in asthma. Here, it may be worth considering the idea that inducing mitochondrial dysfunction (rather attempting to normalize it) may actually be beneficial.

Non-traditional pharmacological approaches to targeting mitochondria have been tested. For example, a cinnamate compound enhances normal mitochondrial function, and reduces ROS production and mitochondrial fission, suggesting this drug as a good candidate for asthma treatment [200]. Similarly, Rd, a major ginsenoside component found in *Panax ginseng*, has been shown to ameliorate mitochondrial dysfunction in brain cells of a rat model for stroke [201], raising the possibility that this compound may have comparably beneficial effects on airway diseases.

In addition to pharmacological targeting of mitochondria, there are some non-traditional approaches that are yet to be applied to lung diseases but may hold potential. For example, CTVT, a cancerous cell line circulating in feral dogs, is thought to have acquired mitochondria from its host [202]. Based on this finding, a ‘mitochondria replacement therapy’ procedure to successfully abrogate symptoms of lipopolysaccharide-induced acute lung injury (ALI) in mice using mitochondria from bone marrow-derived stromal cells (BMSCs) has been recently reported [203]. Furthermore, transmitochondrial cybrid systems (cybrids) by fusing mitochondria from an aggressive tumor cell line with those from benign or less aggressive cancer cells have been shown to exhibit features of the non-cancerous (‘healthy’) mitochondria, and are capable of erasing the oncogenic properties of the aggressive tumor cells. Microarray analysis shows that oncogenic pathways are inhibited in cybrids with non-cancerous mitochondria, leading the idea that crosstalk between mitochondria and nucleus occurs, which helps repair mitochondrial dysfunction [204]. Another approach has been the targeting of microRNAs. For example, miR-210, which is overexpressed in late-stage non-small cell lung cancer, induces loss of OMM permeability and mitochondrial dysfunction, and regulates succinate dehydrogenase [205,206]. In the heart, miRs-499 and 484 are pro-proliferative by suppressing Drp1 and Fis1 [207]. Similar approaches in other lung diseases await investigation.

8. Expert Commentary

The above discussion clearly highlights the emerging recognition that mitochondria do much more than “just provide energy for cellular function”. Indeed, mitochondria are being increasingly seen as critical regulatory elements by virtue of their control of energy, ions such as Ca^{2+} and H^{+}, and cellular processes such as proliferation, migration and apoptosis. In this regard, it appears that mitochondrial structure is a key aspect of mitochondrial function. Although a plethora of studies have provided us with the basics of mitochondrial morphology and function (Figure 4), the significance of this link and its relevance, as applicable to intracellular contacts and communications, are not completely elucidated in the lung. Certainly, a lot can be learned from data in other cell types. However, the emerging

data in other aspects of cellular function clearly demonstrate the vast heterogeneity among cell types (even within an organ) that makes it difficult to simply extrapolate information to cells of the lung, particularly in the context of lung diseases. Accordingly, if we are to develop novel strategies for lung diseases that are based on mitochondrial structure and function, studies on human airways and lungs should focus on building upon what is known in other cell types and species (especially yeast where mitochondria have been studied most extensively), and identifying homologs and paralogs for the molecules that regulate mitochondrial fission, fusion, mobility, and interactions. Dissecting their expression patterns, mechanism(s) of action, both under normal and different disease conditions in cell types of interest will then improve the chances for developing more efficacious therapy regimens.

Five-year view

The authors foresee, based on the robust and multi-faceted advances in the molecular biology and imaging technologies, significant progress in the next few years, in the following: i) identification of various components of the mitochondria fission-fusion machinery (in both yeast and mammalian cells), regulators of mitochondria-ER interactions, and mitophagy. This should give us a clearer picture of the fundamental biology of mitochondria, as it applies to cellular physiology. ii) Evolution of the current imaging methods into better and more sensitive tools that can reliably quantify mitochondrial morphology, motility and interactions. iii) Extrapolation of studies done in neuronal, cardiac and immune cells, with regard to the central role of mitochondria in disease development and progression, to the lung and the airways. Such correlations will become relatively easier to make, as chronic inflammation, vasoconstriction and hyperreactivity are recognized as serious health conditions, and various mechanistic details about them come to light via clinical and basic research investigations. And finally, iv) looking at chronic airway diseases as mitochondrial dysfunctions. This will include recognizing the importance of mtDNA integrity and of mitochondrial ROS production as crucial determinants of lung health, developing novel methods to screening and diagnose detrimental changes in these parameters, and devising efficient therapeutic strategies (both conventional and unconventional) to combat them.

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Key Issues

- Mitochondrial structure, dynamics and interactions with other organelles are tightly linked to their functional role in supplying cells with energy and signaling molecules, and in determining cellular fate.
- Changes in mitochondrial morphology, manifested as fragmented or fused appearance, are not only indicators of mitochondrial stress, but they also correlate well with the metabolic efficiency of the mitochondrion and the resultant ROS production, integrity of mitochondrial membranes, and the subsequent choice of the cell to survive or to go into apoptosis.
- Though considerable progress has been made with regard to the regulation of fission and fusion, mitochondrial movement and interactions, and ROS generation, the relevance of these phenomena to airway function and dysfunction/diseases is not as widely recognized as it is in other paradigms. However, available data suggest that mitochondria contribute significantly to chronic airway ailments (asthma, COPD, CF, PAH etc), and that manipulations that restore mitochondrial function and/or mitochondrially regulated cellular equilibriums ameliorate disease symptoms.
- Integration of data from studies on non-airway tissues will be of paramount importance, as mitochondrial sensitivities, and responses to cellular environments in the airway are comparable to those in other organs. This is likely to not only provide us with mechanistic insights on balanced/normal mitochondrial function in the airway, but also help us improvise methodologies for further analyses, diagnoses and therapeutic interventions.
- Recent emergence of state-of-the-art imaging and quantification techniques, in combination with cell and molecular biological tools, and bioinformatics, has enabled live studies on mitochondria. While a contiguous evolution of this process is to be expected, our current understanding of the genomic complement and the metabolic processes in the mitochondria, and how they relate to cellular and organismal physiology, is taking shape more vividly. Thus, factors like signaling pathway components, regulators of ROS production and scavenging, cell cycle/apoptosis participants are targeted for drug development.

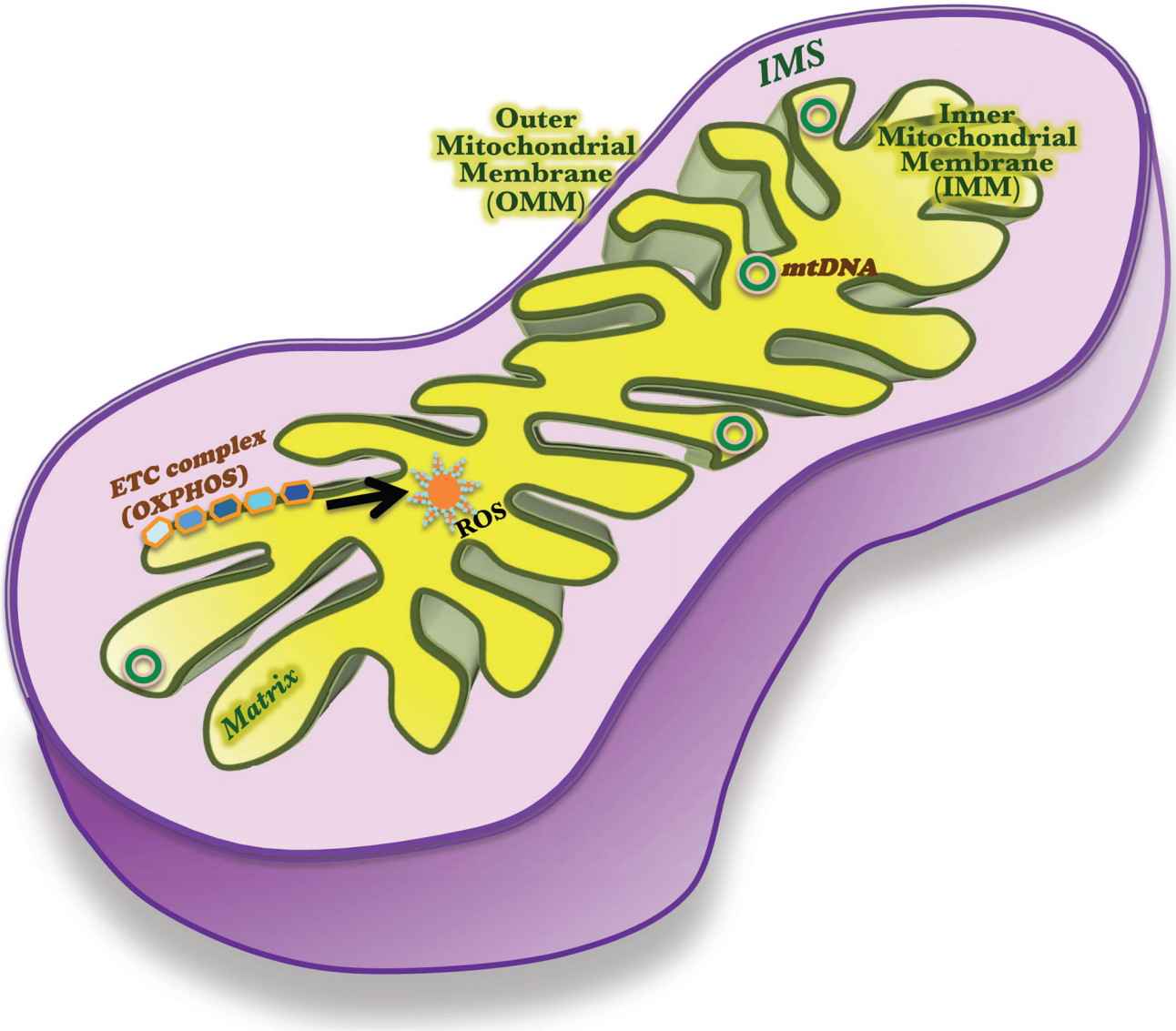

Figure 1. The mitochondrion

This semi-autonomous organelle is bounded by the outer mitochondrial membrane (OMM), which is separated from the second, inner mitochondrial membrane (IMM) by the intermembrane space (IMS). The mitochondrial DNA (mtDNA) codes mainly for electron transport chain (ETC) proteins, which via a series of oxidative phosphorylation reactions (OXPHOS), produce ATP (energy) and reactive oxygen species (ROS).

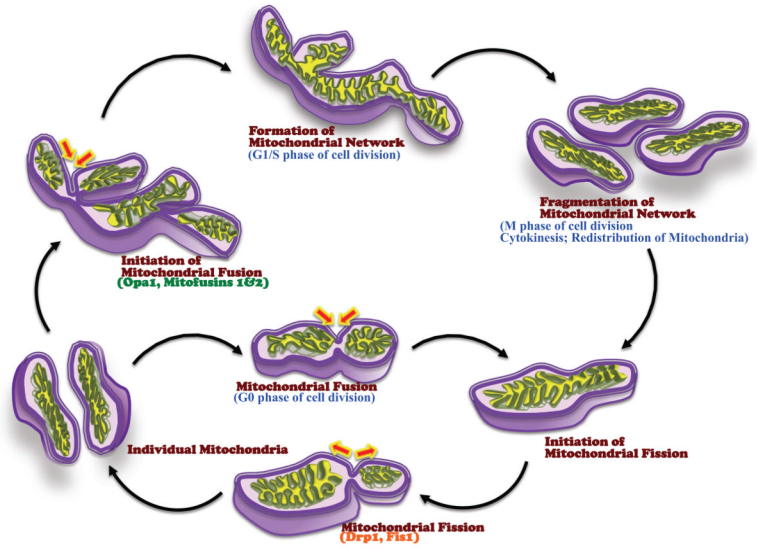

Figure 2. Mitochondrial fission-fusion cycle vs. the cell cycle

During the quiescent (G0) phase, mitochondria fragment and rejoin, maintaining a constant mitochondrial number in the cell. When the cell enters mitosis (M phase), fusion is favored, and is driven by Opa1 and the mitofusins Mfn1 and Mfn2. As cell division comes to a close, the mitochondrial networks break apart and the mitochondria are redistributed between the daughter cells.

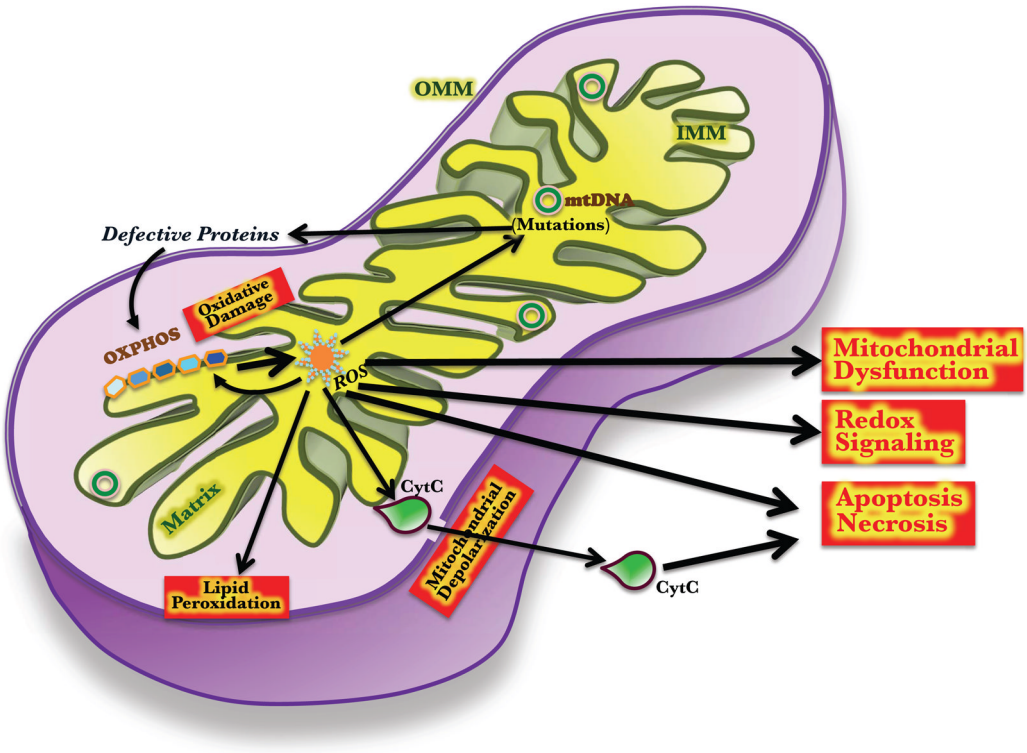

Figure 3. Mitochondrial regulation of cellular functions

ROS produced by normal (and abnormal) glucose and fatty acid metabolism can induce mutations in mtDNA, which in turn can give rise to aberrant ETC proteins, and thus decrease the primary function of the mitochondria: energy production. Lipid peroxidation, and activation of cytoplasmic signals by ROS can lead the cell to apoptosis or senescence. Leakage of apoptosis mediators such as Cytochrome C (CytC), due to OMM depolarization can also initiate apoptosis.

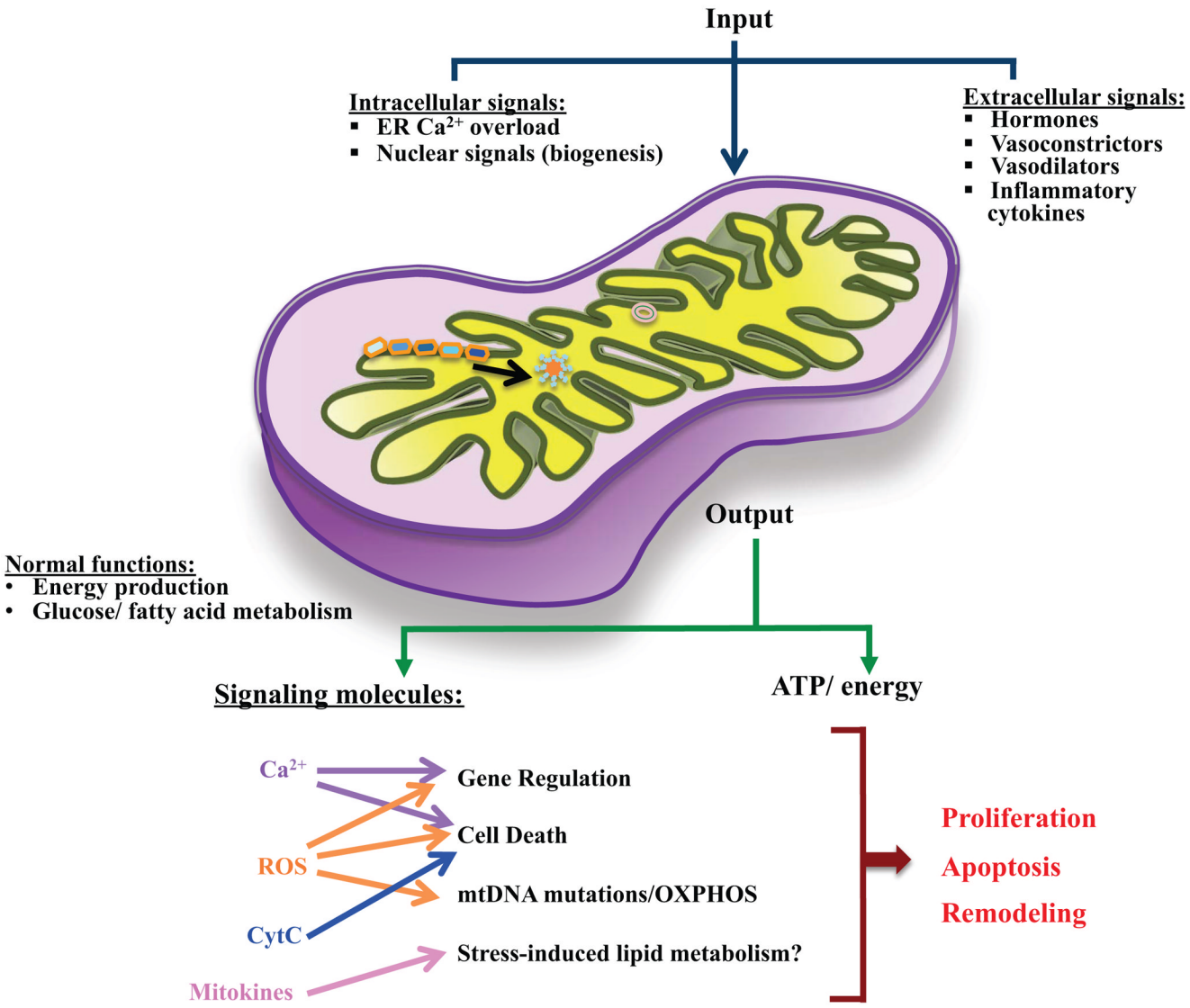

Figure 4. Mitochondrial contribution to lung diseases

By receiving and processing intra- and extracellular signals (especially in chronic inflammation), mitochondria can be a major source for signaling and secondary messengers (ROS, Ca^{2+} etc). Unregulated production of these factors perturbs the proliferation-apoptosis balance of lung cells, and makes the environment conducive for altered cellular structure (proliferation greater than apoptosis) and other aspects of lung disease such as altered cellular protein production.

Table 1

Proteins involved in mitochondrial fission and fusion.

Mammalian Protein	Synonyms/homologs	Function/specific step
Drp1 (Dynamin-related protein 1) Fis1 (Fission 1) Mff (Mitochondrial Fission Factor) Unknown	Dnm1p (*S.c*) Fis1p (*S.c*) Unknown Mdv1 and Caf4 (*S.c*)	*OMM Fission* *Receptor for Drp1 on the OMM* *Alternate receptor for Drp1?* *Adaptor for Drp1-Fis1*
Mfn1/2 (Mitofusins 1 & 2) Opa1 (Optical Atrophy 1) GDAP1 Unknown	Fzo1p (*S.c*) Mgm1p1 (*S.c*) Unknown Ugo1 (*S.c*)	*OMM Fusion* *IMM Fusion* *Linker for Fzo1 and Mgm1*
MARCH5 MAPL SENP3 SENP5 ULK1 & 2	Unknown Unknown Unknown Unknown Atg1 (*S.c*)	*Ubiquitin ligase for Mfn1/2, Drp1 and Fis1* *SUMO ligase for Drp1* *DeSUMOylating protease for Drp1* *DeSUMOylating protease for Drp1* *Autophagy*

Table 2

Mitochondrial proteins that have been implicated in diseases

Disease	Protein (specific function)
Cardiomyopathy Microencephaly, optic atrophy and hypoplasia	Drp1 (Mitochondrial fission)
Charcot-Marie-Tooth (CMT2A) disease Optic Atrophy (ADOA)	Mfn1 (Mitochondrial fusion) Mfn2 (Mitochondrial fusion) Opa1 (Mitochondrial fusion)
Pulmonary Hypertension (PAH)	MnSOD (ROS production)