Journal Pre-proofs

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Please cite this article as: Kumar Chellappan, D., Raj Paudel, K., Wan Tan, N., Seng Cheong, K., Sert Qi Khoo, S., Min Seow, S., Chellian, J., Candasamy, M., Patel, V.K., Arora, P., Kumar Singh, P., Kumar Singh, S., Gupta, G., Oliver, B.G., Hansbro, P.M., Dua, K., Targeting the mitochondria in chronic respiratory diseases, *Mitochondrion* (2022), doi: https://doi.org/10.1016/j.mito.2022.09.003

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Targeting the mitochondria in chronic respiratory diseases

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ABSTRACT

Mitochondria is one of the basic essential components for eukaryotic life survival. It is also the source of respiratory ATP. Recently published studies have demonstrated that mitochondria may have more roles to play aside from energy production. There is an increasing body of evidence which suggest that mitochondrial activities involved in normal and pathological states contribute to significant impact to the lung airway morphology and epithelial function in respiratory diseases such as asthma, COPD, and lung cancer. This review summarizes the pathophysiological pathways involved in asthma, COPD, lung cancer and highlights potential treatment strategies that target the malfunctioning mitochondria in such ailments. Mitochondria are responsive to environmental stimuli such as infection, tobacco smoke, and inflammation, which are essential in the pathogenesis of respiratory diseases. They may affect mitochondrial

shape, protein production and ultimately cause dysfunction. The impairment of mitochondrial function has downstream impact on the cytosolic components, calcium control, response towards oxidative stress, regulation of genes and proteins and metabolic activities. Several novel compounds and alternative medicines that target mitochondria in asthma and chronic lung diseases have been discussed here. Moreover, mitochondrial enzymes or proteins that may serve as excellent therapeutic targets in COPD are also covered. The role of mitochondria in respiratory diseases is gaining much attention and mitochondria-based treatment strategies and personalized medicine targeting the mitochondria may materialize in the near future. Nevertheless, more in-depth studies are urgently needed to validate the advantages and efficacy of drugs that affect mitochondria in pathological states.

1. **Introduction**

Mitochondria are well-known double-membrane organelles that serve as the powerhouse of cells generating energy in the form of ATP via OXPHOS across the electron transport chain. In contrast to other organelles, mitochondria comprise a maternally inherited DNA (mtDNA) (1). Studies have revealed that mitochondrial biogenesis relies heavily on mtDNA replication and thus, aids in the development and division of existent mitochondria (2, 3). Interestingly, mitochondrial biogenesis regulates a number of essential regulatory mechanisms (mitophagy, cell growth, and the formation of reactive oxygen species (ROS)) (4-6) that aid in selective eradication of defective mitochondria. ER-to-mitochondrion proximity is favourable to intimate communication, generating dynamic platforms known as mitochondria-associated membranes (MAM) that allow calcium flow into the mitochondria as well (4-7) and thus

maintains homeostasis. Any alterations in mitochondrial homeostasis may compromise the OXPHOS mechanism and therefore lead to the build-up and generation of ROS, which then may cause mitochondrial malfunction, establishing a downward spiral to cell damage and death (8, 9).

Importantly, mitochondrial dysfunction is attributed by the long-term exposure of environmental risk factors such as carcinogens, air pollutants, and tobacco smoke, resulting in inflammation and hypoxia. These environmental risk factors increase the mtROS, damage the mtDNA and trigger the release of abnormal intracellular calcium build up and release, thus altering mitochondrial homeostasis. These events will further induce and activate oxidative stress and inflammation resulting in senescence and necrosis (10, 11).

Interestingly, studies have shown that chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and lung cancer are resultant in part by mitochondrial dysfunction (12-14). Despite the available knowledge, however, currently, there is a pressing need for new therapeutic approaches, given that lung diseases are claimed to be one of the leading causes of death worldwide (15-19). In this review, the focus further remains on how mitochondrial dysfunction may lead to aberrant cellular processes and how it might contribute to the development of certain chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and lung cancer. Not only does this help us to better understand how mitochondrial dysfunction contributes to the aetiology of chronic respiratory diseases, but it also points to prospective therapeutic methods that would target malfunctioning mitochondria in the treatment of such ailments.

2. Mitochondria and asthma

2.1 Mitochondria in the pathophysiology of asthma

Asthma, a respiratory disease, is well-characterised by airway hyperresponsiveness due to increased calcium and smooth muscle contractility, and airway remodelling as represented by thickening of the epithelium and smooth muscle cell proliferation (20, 21). Reduced apoptosis is also evident in asthma and the pathogenesis of this condition is strongly associated with mitochondrial roles in ROS generation and processing (22). Furthermore, asthma in humans has been linked to mitochondrial genome polymorphisms or haplotype variations (23). Nevertheless, the function of mitochondria in mediating or regulating the consequences of inflammation in the airways is still yet to be fully elucidated.

As an inflammatory cytokine, tumour necrosis factor-alpha (TNF-α) has a crucial role in airway inflammation. When TNF-α is present, mitochondrial dynamics and outer membrane adhesion are altered, boosting ROS production, leading to cell death in cultured adipocytes and mouse lung vasculature endothelial cells (24, 25). Additionally, the activation of Ca^{2+} buffering function of human airway smooth muscle mitochondria by inflammatory cytokines such as TNF-α and IL-13 has been demonstrated by Delmotte et al. to be protective against Ca2+ overload (26). However, Aravamudan et al. have recently discovered that TNF-α enhances mitochondrial fission, increased Drp1 expression and elevated ROS generation, notably in cells from asthmatic patients, in human airway smooth muscle (27).

Aravamudan et al. describe mitochondria as self-sufficient cellular organelles that perform a wide range of operations, including metabolism, energy generation, calcium buffering, and determining the destiny of the cell. The study reported that the production of energy (that occurs *via* various enzymes, mtGRα, mtERβ, and OXPHOS) plays an important role in cellular health and dysfunction (1). Studies have also discovered that reduction of these enzyme biosynthesis in lung cells mitochondria especially in bronchial epithelial cells causes

allergic airway inflammation (28). Importantly, a decrease in lung mitochondrial mass and induction of receptors in asthmatic human lung epithelial cells was observed by the authors *via* analysis of autopsies from fatal asthma cases. Similarly, Phaniendra et al. also found that ROS and reactive nitrogen species (RNS) from mitochondria can greatly affect nucleic acids, lipids and proteins that may disrupt the normal redox status, ultimately resulting in respiratory diseases such as asthma (29).

On the contrary, experts have shown that the concentration of $[Ca^{2+}]$ in the cytoplasm $([Ca^{(2+)}]_{cytoplasm})$ is increased by several inflammatory cytokines, including TNF-α and IL-13, which may promote airway smooth muscle (ASM) contractility, proliferation and remodelling. For instance, the agonist (e.g. acetylcholine) induced increase in $[Ca^{(2+)}]_{cytoplasm}$ results in a transient rise of $[Ca^{(2+)}]_{mitochondria}$ which is crucial in matching ATP production with ATP consumption. It was also found that TNF-α and IL-13 induce ROS production, endoplasmic/sarcoplasmic reticulum stress, decreased expression of mitofusion2, and mitochondrial fragmentation in human ASM (30).

Furthermore, NO-induced eosinophil apoptosis is mediated by ROS, JNK, and late mPT, as demonstrated by Ilmarinen-Salo et al. Additionally, they reported that NO induces early transient mPT that leads to JNK activation although, it is not significant for apoptosis (31). Moreover, Mabalirajan et al. showed that 12/15-lipoxygenase, an ortholog of human 15-LOX-1, expressed in nonepithelial cells causes epithelial injury resulting in asthma. They also reported that IL-13 induces lipoxygenase, and overexpression of this cytokine causes severe airway epithelial injury. However, the constitutive expression of 15-LOX-1 demonstrated no cause of epithelial injury (32). These studies suggest that 12/15-LOX expressed in non-epithelial cells leads to bronchial epithelial injury. Therefore, IL-13 could be a crucial target against influenza-induced exacerbation of chronic lung diseases (33).

Konga et al. discovered that increased levels of ROS, lipid peroxides and low levels of mitochondrial enzymes also contribute to the development of respiratory diseases (34). Furthermore, airway remodelling in asthma is implicated due to the activation of specific phosphatidylinositol-3-kinase (PI3K) by growth factors and cytokines resulting in a decrease in cell survival in TGFβ-stimulated asthmatic, but not non-asthmatic, ASM cells(35). Nevertheless, asthma exacerbation is also correlated to glycolysis, calcium-binding and mitochondrial activity (36).

2.2 Drugs targeting mitochondria in the treatment of asthma

Currently, the first line of treatment is inhaled corticosteroids (ICS), occasionally combined with short-acting or long-acting beta-agonist (SABA or LABA) and leukotriene inhibitors. However, certain patients still experience poor control despite maximal dosing of oral corticosteroids. On the other hand, moderate to severe refractory asthma can be treated with humanized neutralizing monoclonal antibodies and specific cytokines/ chemokines antagonists in addition to ICS. Due to the disease's heterogeneity, these treatments only have a suboptimal effect on asthma control (37).

Simvastatin, a well-known 3-hydroxy-3-methyl-glutarylcoenzyme A (HMG-CoA) reductase inhibitor used to treat dyslipidaemia, has garnered attention due to its pleiotropic effects and potential application in lung diseases. Simvastatin inhibits mitochondrial fission by inhibiting mitochondrial Drp1 (38).

Wei et al. have revealed that aminophylline, a nonselective phosphodiesterase shows therapeutic effect by promoting the synthesis of mitochondria in cultured human pulmonary bronchial epithelial cells (HPBECs). It was found that aminophylline activates the CREB signalling pathway which results in induced expression of PGC-1α, a transcriptional coactivator, and transcriptional factors of NRF1 and TFAM. At the cellular level, a rise in the

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respiratory rate of mitochondria and a reduction in oxygen content can be observed when HPBECs were treated with aminophylline (39).

Asthma may also be treated with montelukast which it is known as an antagonist of cysteinyl leukotriene receptors. It promotes mitochondrial biogenesis and functional gain in mitochondria by acting on the bronchial epithelial cells of Beas-2b, which stimulate the expressions of PGC-1α, NRF-1 and TFAM. As a result, the expressions of cytochrome B and mtDNA/nDNA were shown to have increased (40). The treatment with antioxidant compounds n-acetyl cysteine (NAC) or diphenyleneiodonium (DPI) in ovalbumin induced allergic asthmatic mice resulted in reduced inflammatory cell infiltration in the lung space and reduced proinflammatory cytokines in the lungs. However, an improvement in the mitochondrial energy metabolism was shown in NAC (but not DPI) (41).

2.3 Novel therapeutic strategies targeting mitochondria in the treatment of asthma

Aside from the conventional asthmatic drugs, many compounds such as vitamin E (42), hydrogen sulfide donors (example HaHS, GYY4137, AP39) (43), esculetin (44), L-arginine (45), novel cinnamate, ethyl 3',4',5'trimethoxythionocinnamate (46), stems cells and peptides such as human induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs) (47, 48), LF-15, T3 and T7 tumstatin-derived peptides (49) that may not be indicated for asthma may affect mitochondria. Several studies have been carried out, investigating the effect of naturally occurring or synthetic drugs on mitochondria mostly in animal models. Table 1 summarizes the research on novel compounds affecting mitochondria in targeting asthma. Most of these compounds attenuate cytokine level and reduce cytosolic cytochrome c levels, reducing airway injury and inflammation.

2.4 Nanoparticles in targeting mitochondria for asthma treatment

Persistent airway inflammation in asthmatic conditions may result in structural changes of the airways leading to airway remodelling (50). With the existing treatment options like the use of steroids, these changes are not fully reversed. Although, steroids remain the mainstay in asthma treatment, their short duration of action can be troublesome.

Interestingly, studies have shown that nanoparticle-combined steroids may provide longer duration of action and may prove to be effective in treating airway inflammation (51, 52). It was also found that budesonide nanoparticle oligomers demonstrated a favourable morphology, allowing rapid absorption of liquid-based drug formulations. Enhanced bronchodilation was also evident by the employment of nanoparticles. Bhavna et al. illustrated that nanoparticles condensed with albuterol engage more effectively with the respiratory epithelium. However, perivascular accumulation and paracellular movement back to the trachea-bronchial region are more intense which may lead to an increased and more prolonged drug content availability in the blast zone (53). STAT4 signalling pathway has been primarily responsible for this effect. Chitosan nanoparticles may also be useful for treating airway inflammation in people due to the general similarity between mice and humans in the T-cell synthesis pathway (54).

Liposomal medication delivery methods such as liposomal budesonide has proven to treat asthma in mice as it is effective in reducing the harmful effects of inhaled steroids, such as albuterol and cortisone (54). The anti-asthmatic effects of free salbutamol sulphate have been improved by liposomal delivery of salbutamol sulphate, as reported by Chen et al. (55). Asthma-related inflammatory respiratory disorders have also been treated using dendrimer treatments. For example, G4-PAMAM conjugated with methylprednisolone may reduce swelling by increasing the amount of time the medication spends residing in the body (56).

2.5 Alternative therapies affecting mitochondria in the treatment of asthma

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Nutraceutical-based therapies including medicinal plant extracts, single/pure compounds isolated from plants, and probiotics are gaining considerable attention in the management of chronic respiratory diseases (57-63). Suhuang, a traditional Chinese antitussive medicine has been found to be effective in the treatment of asthma, as it can effectively reduce ROS overproduction, mTDNA release, MPTP opening and potential mitochondrial membrane collapse. Interestingly, NF-κB signaling is reduced by inhibiting phosphorylation of NF-κB-p65 and IκBα degradation. By blocking the interaction of NLRP3-ASC and promoting NLRP3 ubiquitination degradation, NLRP3 inflammasome activation can be reduced. The inhibitory effects that Suhuang exerts on uncontrolled inflammation was observed through two sepsis mice models (64).

Baicalein, a bioflavone found in the dry roots of *Scutellari baicalensis*, has been known for its antioxidant and anti-inflammatory activities (65). It was found that Baicalein can reduce airway hyperresponsiveness and inflammation in a standard experimental asthma model of male Balb/c mice sensitized with ovalbumin (OVA) and an alternate model administered with IL-4 or IL-13 intranasally. Reduction of sub-epithelial fibrosis, 12/15-LOX activity, TGF-β_1 and goblet cell metaplasia bronchial epithelia apoptosis were reported. Thus, baicalein can reduce airway injury and replenish mitochondria function (66).

The parameters of oxidative stress in the lung of the asthmatic murine model that were treated with Bu-Shen-Yi-Qi formula (BSYQF), a traditional Chinese medicine (TCMs) used in the clinical treatment of asthma in China were analysed by Cui et al. (67). They found out the ATP levels, mitochondrial ultrastructural, and bronchial epithelia in the lung of the mice returned to normal.

Moreover, Wang et al. had documented the potential of *Adhatoda vasica* (AV) aqueous extract to treat severe steroid-resistant asthma (68). The examination of mitochondrial bioenergetic profiles and morphology in mouse models with acute allergic and severe asthma that were treated with AV extract had revealed that the molecular signatures of steroid (dexamethasone) resistance like IL-17A, KC (murine IL-8 homologue), and HIF-1α (hypoxia-inducible factor-1α) were alleviated. The alleviation of HIF-1α (hypoxia-inducible factor-1α) is due to the PHD2 (prolyl hydroxylase domain-2) restoration, where PHD2 is a negative regulator of HIF-1α. All these events led to a reversal in cellular hypoxia-induced mitochondrial dysfunction in human bronchial epithelial cells (68) (Figure 1).

3. MITOCHONDRIA AND COPD

3.1 Mitochondria in the pathophysiology of COPD

COPD is considered as a disease of accelerated lung ageing because they exhibit all the key characteristics of senescence, which include shortening of telomere, cellular senescence, stimulation of PI3 kinase-mTOR signalling, impaired mitophagy, mitochondrial dysfunction, stem cell depletion, epigenetic changes, irregular microRNA statuses, immuno-senescence, and a low-grade chronic inflammation due to senescence-associated secretory phenotype (SASP) (69, 70). These ageing mechanisms are believed to be triggered by extrinsic and intrinsic oxidative stress (69-71).

Cigarette smoke, a major causative factor of COPD, can induce mitochondrial dysfunction in airway epithelia (72-74). Mitochondrial dysfunction is correlated with excessive mitochondrial ROS levels, leading to increased inflammation and hyperproliferation of airway smooth muscle (ASM) cells in COPD patients (75). ASM cells of these patients were found to

have reduced mitochondrial membrane potential ($\Delta\Psi m$), adenosine triphosphate content and mitochondrial complex proteins expression in comparison to healthy subjects (60). This leads to inadequate respiration, lower basal and maximum respiration levels, and reduced respiratory reserve capacity among COPD patients (75). Furthermore, mitochondrial abnormalities may also induce impaired healing and decreased corticosteroid sensitivity in the lung epithelium (76). The mitochondrial dysfunction pathways in COPD and lung epithelial cells along with the potential targets for therapy are summarized in Figure 2.

3.1.1 Role of autophagy and mitophagy in COPD pathogenesis

Autophagy is a membrane-dependent process that enables subcellular components such as proteins and organelles to be sequestered, transferred, and lysosomal-recycled (77). It is a crucial cellular defensive mechanism to prevent oxidative stress and other associated conditions that result in the build-up of abnormal proteins or organelles including mitochondria (78-80).

Mitophagy, a process responsible for removing damaged mitochondria, is mediated by the PINK1 (PTEN induced putative protein kinase 1) and PRKN (parkin RBR E3 ubiquitin protein ligase) pathways with Akt ubiquitin E3 ligase as a key mediator (81). Cigarette smoke can induce oxidative stress which leads to lipofuscin accumulation leading to incomplete mitophagy (82). Decreased PARK 2 expression in COPD patients' lungs may cause insufficient mitophagy and thus has a role in COPD pathogenesis (83). Excessive dysfunctional mitochondria due to impaired Parkin (ubiquitin-related degradation molecule) translocation upon Pink-1 mitophagy pathway activation can account for this increased risk of lung carcinogenesis (84).

The development of chronic obstructive pulmonary disease (COPD) has been related to both increased and reduced mitophagy (85). Mitophagy may be induced by cigarette smoke by stabilizing the mitophagy regulator PINK1. Necroptosis has been associated to increased expression of the mitophagy-promoting PINK1 gene (85). Necroptosis or programmed necrosis is often accompanied by an increase in inflammation (86). This new cell death mechanism precipitates airway inflammation by increasing the secretion of inflammatory chemicals during epithelial death, a process not evident in apoptosis-derived cell death (86). Genetic deficiency of PINK1 was shown to reduce mitochondrial dysfunction and CS-induced cell death *in vitro* via reduction of a substrate for the receptor-interacting protein kinase 3 (RIP3), mixed lineage kinase domain-like protein (MLKL) in the necroptosis pathway (87, 88). Furthermore, upon CS treatment, Pink1(-/-) mice were prevented from mitochondrial damage, airspace expansion, and mucociliary clearance (MCC) dysfunction (88). Inhibition of PINK1 (PTEN-induced putative kinase 1)-PARK2-mediated mitophagy is shown to reduce the removal of cigarette smoke-induced damaged mitochondria, leading to increased mitochondrial ROS production and primary human bronchial epithelial cell (HBEC) senescence (83). Mitochondria then releases undegraded pro-apoptotic proteins such as cytochrome C, activating necroptosis and potentially depleting the functional mitochondria (82, 87).

In contrast, decreased PARK2 levels may lead to insufficient mitophagy in bronchial epithelial cells which causes rapid cellular senescence (85). Furthermore, *in vitro* experiments have showed that PRKN overexpression was able to promote mitophagy during cigarette smoke consumption even when PINK1 protein numbers were low, reducing mitochondrial ROS generation and cellular senescence (81). The study demonstrates that there is an increased

airway wall thickening and emphysematous alterations in PRKN knockout mice compared to wild-type mice (81). PINK1 overexpression, on the other hand, was unable to restore defective mitophagy induced by PRKN knockdown (81). Besides, accelerated cellular senescence due to the build-up of damaged mitochondria and enhanced oxidative alterations can also be observed in PRKN knockout mice (81). All these findings indicate that PRKN protein levels may be the rate-limiting component in PINK1-PRKN-mediated mitophagy (81).

3.1.2 Alterations in mitochondrial morphology

The functionality of the mitochondrion is usually determined by the dynamic balance between mitochondrial biogenesis, fusion and fission, and mitophagy-mediated elimination of damaged mitochondria (89). It was found that the morphology of mitochondria in primary bronchial epithelial cells of COPD patients experience changes that may include swelling, elongation, fragmentation, and cristae depletion (90). The mitochondria defend themselves from oxidative damage under stress by fusing into a network to enhance bioenergetic efficiency (91). Mitochondrial injury causes mitochondria fission by fragmenting away from the mitochondrial network and proteolytic degradation by mitophagy (91). Intriguingly, exposure to CS in humans cause proliferation and remodelling of the airway in these cells, implying that induction of mitochondrial fission promotes glycolysis in cells to seek energy sources (92). After mitochondrial morphologic changes, post-translational mitochondrial proteome alterations and nuclear crosstalk occur to sustain energy generation and redox equilibrium by reconfiguring the metabolic gene expression (91).

3.1.3 Role of mitochondria-related genes in COPD development

The role of mitochondria-related genes in COPD development is under much study via mitochondrial genome sequencing (82). Albeit the genes controlling bioenergetics homeostasis, especially lipid metabolism in COPD is lesser-known, FAM13A (family with sequence similarity 13 member A), a gene family which modulates the fatty acid oxidation (FAO) pathway may contribute to COPD development (93). It was speculated that FAM13A activates sirtuin 1 and increases carnitine palmitoyltransferase 1A (CPT1A) expression in mitochondria, thereby increasing FAO and contributing to COPD (93). Other studies have discovered that FAM13 may cause lung epithelial tissue destruction and reduced restoration capacity, though it was observed that the effect may not be due to β-catenin inhibition (94).

Iron-responsive element-binding protein 2 (IRP2) was found in mitochondria and it plays a major role in regulating iron concentration (95). It was found that upregulation of IRP2 due to a specific genetic risk factor may lead to COPD which may impair the function of cilia to remove pollutants and pathogens from the lungs. In contrast, the study shows that when IRP2-deficient mice were exposed to smoke, the build-up of iron in mitochondria was lesser (95).

3.1.4 Alteration in mitochondrial Ca^{2+} balance

Several studies have linked COPD to an abnormal increase in intracellular Ca^{2+}, which leads to mitochondrial ROS production and thus subsequent stimulation of the induction of apoptosis pathway (96). To maintain the homeostasis state of Ca^{2+} in mitochondria, the equilibrium between mitochondrial Ca^{2+} efflux rate and the up taking rate are important. The entering of Ca^{2+} into the mitochondria causes transient depolarisation of its membrane potential thus, altering the metabolism. The concentration of Ca^{2+} in mitochondria greatly affects the tricarboxylic acid (TCA) cycle, where the activity of the TCA cycle will be promoted, leading to the increase of TCA flux. As a result, the electron transport chain (ETC) function is enhanced

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leading to the generation of the ROS, initiating apoptosis and/or necrosis. Mitochondria regulates its metabolism and ATP generation by buffering Ca^{2+}. When the Ca^{2+} equilibrium is altered, cellular activity including apoptosis and necrosis are compromised (97).

3.1.5 Production and accumulation of reactive oxygen species (ROS)

Reactive oxygen species (ROS) is usually a by-product of cellular metabolism, particularly through peroxisomes and mitochondria (97). The compromised redox homeostasis due to ROS causes inflammation, which further boosts ROS production, causing alterations in transcriptional factors that modulate cellular stress response pathways (98). The surge in ROS can be caused by oxidizing enzymes activity notably NADPH oxidases and manganese-superoxide dismutase (MnSOD) (97). Catalase, on the other hand, acts as an antioxidant enzyme that downregulates ROS. Chung et al. revealed that overexpression of transforming growth factor-β (TGF-β) suppresses the expression of MnSOD and catalase, but stimulates NADPH oxidase 4 (Nox4), leading to an elevated ROS level and IL-6 in COPD patients (97). Subsequent findings reveal that Smad3 and phosphatidyl-inositol kinase-mediated pathways have a crucial role in the induction of oxidant/antioxidant imbalance and IL-6 release (97). The antioxidants NAC and ebselen inhibited Smad3 phosphorylation, reverse the alterations in oxidant/antioxidant enzymes and IL-6 levels, suggesting that TGF-β activates Smad3 in a redox-dependent manner (97).

Furthermore, endogenous chemicals may increase ROS production. It was found that cytokines IL-1, IFNγ, and TNFα, lead to larger quantities of mitochondrial-derived ROS when ASM cells are exposed to inflammatory stress (99). MMP-2 was found to have a significant role in mitochondrial damage. The loop of negative feedback seems to exist between mitochondria and MMP-2 whereby mitochondria-derived ROS activates MMP-2 and MMP-2 drives the generation of ROS and decreases mitochondrial function (99).

Leakage of electrons from the mitochondrial electron transport chain (ETC) and normal physiological formation of reactive oxygen species (ROS) coupled with intra- and extracellular factors may lead to oxidative-antioxidative imbalance (oxidative stress) in the pathobiology of COPD (99). Studies have shown that biopsies on the vastus lateralis and external intercostal muscles of COPD patients revealed the obstruction in ETC and increased generation of ROS in the skeletal muscle mitochondria suggesting the abnormalities in mitochondrial function (100).

Studies have also found that exposure to particulate matter (PM2.5) can lead to cytosolic ROS overproduction in human bronchial epithelial (BEAS-2B) cells, normal human bronchial epithelial (NHBE) cells, and COPD-diseased human bronchial epithelial (COPD DHBE) (101). This further leads to oxidative damage and stimulates the activation of the oxygen-sensitive NRF2 and NF-kB pathways (101). This cell-damaging exposure to PM2.5 induces an oxidative increase that impairs mitochondrial redox equilibrium, leading to mitochondrial dysfunction over a long-term period and also a reduced cell energy supply. This oxidative boost was partly suppressed by the NRF2 signalling pathway (101).

Production of ROS is also known to induce senescence. Cellular senescence is the permanent loss of ability for somatic cells to replicate, a characteristic in the ageing lung and in a variety of age-related respiratory disorders, including COPD (102, 103). mtDNA depletion, downregulation of sirtuin3 (SIRT3) or mitochondrial ETC inhibition may implicate mitochondrial dysfunction-associated senescence (MiDAS) (102). In cellular senescence, telomere instability and disruptions in mitochondrial homeostasis are common and are detrimental in the formation of the senescence-associated secretory phenotype (SASP) (102). Furthermore, senescent lung epithelial cells have an increased activity of the mitochondrial biogenesis pathway, which is controlled by the mTOR/PGC-1/ axis and triggered by the

mammalian target of rapamycin (104). Overactive biogenesis and impaired mitophagy, which would normally eliminate damaged mitochondria, might also explain the build-up of large, dysmorphic mitochondria reported in old mice when the mTORC1/PGC-1 axis is chronically activated (104).

3.1.6 Other pathobiology involving mitochondria dysfunction

Skeletal muscle dysfunction is often evident in patients with COPD. A possible explanation is that the abnormal kinetics of mitochondrion permeability transition pore (MPTP) promotes the leakage of mitochondrial matrix materials, for example, cytochrome C, inducing apoptosis in skeletal muscles (105). Furthermore, reduced mitochondrial oxidative capacity and fibre type alterations may also lead to locomotor muscle impairment (106). On the other hand, a reduction in ATP generation in skeletal muscle mediated by high oxidative mitochondrial enzyme activity was observed in the early phases of COPD as well (107). Greater amounts of superoxide anion in mitochondria and its membrane compartments are also evident in diaphragm specimens of COPD patients (108). The oxidation of diaphragm proteins which are essential for energy synthesis and contractile function is likely to play a role in the reported respiratory muscle failure in severe COPD patients (108).

Phagocytosis was found to increase early mROS and mitochondrial membrane potential in monocyte-derived macrophages of two types, those with granulocyte macrophage-colony stimulating factor or those with macrophage-colony stimulating factor (101). Interestingly, it was found that defective phagocytosis observed in COPD macrophages is associated with altered mitochondrial activity and mROS activity (101).

In COPD, hypoxic pulmonary vasoconstriction (HPV) is reduced (109). Alveolar hypoxia causes mitochondrial sensors to change ROS and redox couples in smooth muscle cells of the pulmonary artery dynamically (109). This causes the inhibition of K^+ channels, depolarization, activation of voltage gated Ca^{2+} channels, a surge in Ca^{2+} concentration and vasoconstriction. If hypoxia is sustained, rho kinase and hypoxia-inducible factor (HIF)-1α will be activated, worsening vasoconstriction, and implicating pulmonary vascular remodelling and pulmonary hypertension (109).

3.2 Potential targets for therapy in mitochondria dysfunction for COPD

Despite advancements in treatment strategies, effective therapies to stop or reverse lung destruction in COPD are yet to be established (110). Nevertheless, many studies have investigated the possibility of targeting mitochondrial enzymes or associated proteins as a revenue for treatment. Thus, enzymes and proteins associated with mitochondrial dysfunction in COPD is summarized in Table 2.

3.2.1 Damage-related molecular patterns (DAMPs)

Furthermore, chronic exposure to cigarette smoke can cause immunogenic cell death in structural airway cells, which stimulates the production of damage-related molecular patterns (DAMPs) (111). In COPD patients' extracellular lung fluids, Pouwels et al. reported that the quantities of certain DAMPs such as S100 proteins, defensins, and high-mobility group box-1 (HMGB1) are elevated (111). Besides, DAMPs and DAMP receptors were upregulated *in vivo,* and galectin-3 release was increased *in vitro* (109). DAMP production may promote neutrophilic airway inflammation because DAMPs can recruit and stimulate immune cells when they attach to pattern recognition receptors PPARs (111). mtDNA, a DAMP, may trigger

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intracellular response cascades involving TGF-β1 upon release and binding to toll-like receptor 9 (TLR9) (112).

3.3 Targeting mitochondria in COPD therapy

3.3.1 NOX inhibitors

There are additional pathogenic processes, such as cell death and scarring, that result from persistent exposure to oxidants in cigarette smoke. Both exogenous (from cigarette smoke and pollution) and endogenous (from NOX, mitochondria, inducible nitric oxide synthase (iNOS) and myeloperoxidase) oxidants contribute to the progression of chronic obstructive pulmonary disease (COPD) (113). Additionally, the pathogenic activation of NLRP3 inflammasomes was shown, especially in emphysema due to inflammation, thus contributes to the development or progression of COPD (114). Barnes et al. had summarised the recent research on the mechanisms of Nrf2-mediated lung protection in COPD. Drugs and techniques that target Nrf2 signalling in the treatment of COPD are being studied, despite the difficulty of developing targeted and effective Nrf2 agonists (115). As a key regulator of Nrf2 signalling, melatonin lowers the status of oxidative stress. This endoplasmic stress mediator has also been reported as an inhibitor of the TXINP/NLRP3 pathway that promotes TRx1 activity (116).

3.3.2 Drugs targeting cellular senescence

It has been well acknowledged that COPD is associated with a dysfunction of the systems that govern mitochondrial activity, and so may be a target for future treatments. One of the most promising new treatments for senescence is the PI3K-mTOR pathway-inhibiting medication metformin, which has already been used to treat type 2 diabetes. *In vitro* studies have showed

that metformin reduces cellular senescence and the SASP response (117). Senescent cells have higher levels of pro-survival networks' expression, which confers apoptotic resistance to the cells, according to Barker et al (117).

In addition, a study was conducted to investigate the beneficial of chronic hemin treatment against senescence and its effect of improving mitochondrial dysfunction (118). Heme oxygenase (HO)-1 plays a key role in protecting the lungs from oxidative damage and inflammation. A senescence phenotype of lung fibroblast is characterised by a reduction of replicative capacity, an accelerated senescence and inflammatory profile (118). These characteristics are shown in the senescent COPD fibroblasts where the mitochondrial activity (respiration, glycolysis, and ATP levels) is lowered, ROS production, mitochondrial biogenesis and impaired mitophagy are increased. Hemin exposures enhance HO-1 gene expression levels in fibroblasts, decrease ROS levels, senescence, inflammation, and reverse mitochondria dysfunction in COPD cells by restoring mitophagy (118).

3.3.3 Antioxidant mimetics

3.3.4 Anti-inflammatory drugs

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation that primarily affects the lung parenchyma and peripheral airways, resulting in irreversible and progressive airflow restriction. The efficacy of Heche Chongcao Capsule (HCC) was tested in the lung tissue of rats with COPD by Dong et al (119) where the model group of rats was exposed to cigarette smoke and were injected with lipopolysaccharide intratracheally. Certain conditions were observed on the lung tissue of model group like, a disordered arrangement of cilium in the trachea, degenerated type I and type II alveolar cells, emptied lamellar body, and observed free fragment within the alveolar space (119). As compared to the model group, a better condition of lesions was found in all medicated groups, especially the group which

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treated with a medium dose (0.5 g/kg). Besides, HCC also helps to reduce the release of TNF-α protein as the reduction of TNF-α protein release is significantly obvious in groups treated with medium dose (0.5 g/kg) and low dose (0.25 g/kg) of HCC (119). Thus, HCC may effectively inhibit the inflammatory response in COPD rats.

Other than HCC, Li et al. have also conducted a study on rats to investigate the anti-inflammatory effect of *Radix platycodon* in combination with heat cleaning and detoxification herbs (120). The other herbs used in this study were *Flos lonicera* and *Fructus forsythia*. The rats in the model group of this study were also exposed to cigarette smoke and were injected with lipopolysaccharides. The observation on the lung tissues of the model group were in comparison to the observations of Dong et.al., whereby the alveolar structure was disrupted, widened/fused alveolar septa, massive infiltration of inflammatory cells, partially shed of branchial epithelial and significant infiltration of inflammatory cells were observed (120). Besides, the expression of TNF- α, TGF-β and IL-1, also the WBC count increases significantly in the bronchoalveolar lavage fluid (BALF) while the expression of TFF3 mRNA in lung tissue was decreased significantly (120). However, after treating with *Radix platycodon*, *Flos lonicera* and *Fructus forsythia*, and the combination of all these three herbs, the pathological morphology of lungs was observed to be less severe (120). The reduction of the TGF-β and IF-1 as well as WBC count were significant. The increased TFF3 mRNA expression was also noted, especially in the group treated with the combination of all the herbs. The study suggested the synergistic anti-inflammatory effect of *Radix platycodon*, *Flos lonicera*, and *Fructus forsythia* may help to improve the pathological changes in COPD (120).

Resveratrol is a naturally occurring molecule that has anti-inflammatory, antioxidant, metabolic, and cardioprotective properties (121). As a result, resveratrol may alleviate both

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pulmonary and extrapulmonary pathology in COPD patients. Research was carried out to assess the impact of resveratrol on lung damage, muscle metabolism, and cardiovascular risk profile, as well as to explore if resveratrol is beneficial to COPD patients (121). Reduction of inflammation and oxidative stress shown in the models of COPD after treated with resveratrol. Hence, it may ameliorate the skeletal and respiratory muscle impairment indirectly (121).

3.3.5 Mesenchymal stromal cells (MSCs) in mitochondrial dysfunction

Mesenchymal stem cells (MSCs) are a type of non-hematopoietic multipotent stromal cell that can develop into tissue from a single germ layer (122). MSCs have a promising potential in the treatment of chronic lung diseases with significant morbidity and death rates, including idiopathic pulmonary fibrosis (IPF), COPD, and obstructive bronchiolitis (OB) (122).

Asthma, acute respiratory distress syndrome, and COPD are just a few of the respiratory conditions that MSCs have shown promise in treating during preclinical studies. The first animal research studies to examine the benefits of MSC-based cell therapy in COPD revealed promising and encouraging results (123). An increasing number of research studies have further examined the impact of MSCs on mice and other models of COPD, and other lung diseases. Furthermore, it was observed that even a modest MSC dose may have a significant impact in restoring both alveolar and endothelial tissues to mice treated with MSCs (124). In addition, Shigemura et al. found that MSC-based treatment improved lung architecture, decreased apoptosis, and increased cell proliferation in a series of *in vivo* experiments (123).

Lung tissue histology and evaluation of proinflammatory cytokine mRNA expression were two common methods used to assess the effects of MSC therapy on inflammation during studies conducted in animals. These cells were less prevalent in the alveolar, and periphery of the blood vessels in comparison to the control group, while the number of macrophages was high (123, 125). As a result, it may help to reduce inflammation and enhance the body's ability to repair itself. Treatment with MSCs significantly reduced levels of inflammatory cytokines implicated in COPD aetiology, including IL-1beta and TNF-α, according to the results of an analysis. Because of the tissue degradation caused by COPD, various matrix metalloproteinases, such as MMP2, MMPs 9 and 12, have been reported to be abundant and may be reduced by MSC therapy (126-131). Additionally, pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) may help in attenuating oxidative stress-induced mitochondrial dysfunction, airway inflammation and hyperresponsiveness. This again shows the potential of iPSC-MSCs being used as oxidative stress-dependent lung disease therapy (132).

The sources of the MSC also play a crucial role, as it might affect the efficacy as well as the transfer capacity of mitochondria. *In vivo* and *in vitro* studies have been conducted to study the benefit of transferring mitochondrial from the human-induced pluripotent stem cell-derived MSCs (iPSC-MSCs) or adult bone marrow-derived MSCs (BM-MSCs) to the epithelial cells exposed to cigarette smoke (133). The transfer of mitochondria is done by the formation of tunnelling nanotubes. *In vivo* studies showed that mitochondrial transplantation from iPSC-MSCs and BM-MSCs could reduce alveolar destruction and fibrosis severity (132, 133). However, the reduction of alveolar destruction and fibrosis severity are more significant with the mitochondrial transplantation from iPSC-MSCs. *In vitro* studies also show higher transfer capacity and efficacy of mitochondrial transplantation from iPSC-MSCs compared with BM-MSCs (133). Moreover, therapeutic effects of MSCs and MSC-derived exosomes (EXO) has

been reported in animal models of inflammation. The role of MSC and EXO on lung inflammation induced by CS has been determined in a recent study (134). The study shows that intraperitoneal injections of both MSC and EXO have higher protective effect from the CS exposure while comparing with the individual treatments (134). Exposure to CS will result in increase of mitochondrial fission protein DRP1 and DAMPs pathway mediators such as HMGB1, RAGE, AGE, S100A4, and S100A8. However, combination of MSC and EXO helps to increase the fusion gene expression of the mitochondria, which are mfn1, mfn2 and opa1. Apart from that, this combination also helps to increase the rhot1 gene expression (134). In short, the combination treatment of MSC and EXO is protective against the early events of mitochondrial gene while exposed to CS (134) (Figure 3).

4. MITOCHONDRIA AND LUNG CANCER

4.1 Lung cancer and its epidemiology

Among the known cancer types, lung cancer still ranks top as one of the major causes of deaths due to cancer, with more than 1.7 million deaths each year worldwide. Non-small-cell lung cancer (NSCLC) accounts for around 80% of the cases of lung cancer (63). According to the latest data, both small cell and NSCLC are the second most prevalent malignancy across both genders in United States (US) (135). In 2022, it has been projected that new cancer cases will reach 1,918,030 and cancer deaths will reach 609,360 in the US. This constitutes to approximately 350 deaths per day from lung cancer (136). The lack of early-stage diagnostic biomarker for lung cancer is one of the hurdles in its management and therefore, researchers are now focusing on developing the novel early detection markers such as measuring the level of microRNA in body fluids and designing new experimental models for lung cancer detection and therapy (137). On the brighter side, there is an improvement of 10% in term of 5-year relative survival of lung cancer from 1975 to 2022. Moreover, between 1990 and 2015,

mortality of lung cancer in males had decreased by 45%, while deaths due to lung cancer in females have decreased by 19% between the years 2002 and 2015 in the US (138). This is possibly due to the increased awareness of the consequences of smoking and decision to quit smoking. In addition, the advancements in the current treatment and diagnosis technology also contribute to a decrease in lung cancer incidences (139).

4.2 Mitochondria in the pathophysiology of lung cancers

The Complex I subunit of the ETC is particularly important in terms of tumour progression and metastases. Often, the mutation of complex I occurs in patients without any smoking history (140). This leads to malfunction in the formation of super complexes between Complex I and Complex III, as a result, an inappropriate number of electrons are transported, and the overload of ROS is subsequently undetectable by these cells (140). This results in an excessive production of ROS, additional energy loss, and accumulation of oxidative stress. Besides, Zhang et al. also observed formation of superoxide particles, and unregulated growth of lung cancer cells because of complex I mutation (140).

A change in the metabolic state is noticed in mitochondria of cancer cells. Malfunctional mitochondria in cancer cells are found to constantly undergoing a process known as the Warburg effect which involves the excessive use of glycolysis to produce ATP despite the availability of oxygen (141). Although this resulted in 16 times fewer ATP than oxidative phosphorylation system (OXPHOS), it is favourable in the proliferation of cancer cells (142). Johanna et al. proposed that in a hypoxic condition which is commonly found in the microenvironment of lung cancer cells, mitochondria expand themselves to escape apoptosis which causes the proliferation of cancer cells (143). Hypoxia-inducible transcription factor (HIF-1α) that stabilizes by hypoxic environment is a transcription factor that enables cells adapt to harsh conditions (144).

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The carcinogenic ROS that are produced from OXPHOS, and endogenous carcinogens like smoking consistently get exposed to mitochondria, which will cause an overload of oxidative stress (145). This can affect the functionality of the respiratory chain and induce escape of proton and destruct mitochondrial DNA (mtDNA). In contrast to nuclear genomic DNA, mtDNA possesses a greater risk of developing mutations partly due to the absence of histones which act as a protective barrier, and the lack of introns and DNA repair systems. Furthermore, antioxidant defence mechanisms generally do not function in tumour cells, hence the cells cannot be shielded from ROS damage (145). Collectively, these mechanisms favour the growth and metastasis of lung cancer cells.

4.3 Novel techniques that target mitochondria in lung cancer

NAD-dependent class III histone deacetylase Sirtuin-1 (SIRT1) is a member of the sirtuin family in mammals. SIRT1 seems to be a critical player in ageing, DNA damage, metabolic stress, inflammation, and cancer, according to growing evidence (146). Stress defence and DNA repair processes have previously been linked to genomic integrity. Anaerobic glycolysis is clearly recognised as cells undergo the epithelial-mesenchymal transition (EMT) process. Interestingly, downregulation of the silent information regulator 1 (SIRT1), a target of TGF-1, prevents mitochondrial dysregulation and EMT from occurring, indicating a critical role for the integrity of mitochondrial metabolism in EMT regulation. Supplementation with NAD+ causes mitophagy mostly through Sirtuin-dependent pathways. Sirtuins are a family of signalling proteins that play an important role in the regulation of cellular metabolism, including mitochondrial biogenesis and mitophagy. They are dependent on NAD+ levels to do their work (147).

Curcumin encapsulated vesicular drug selectively increases p53 expression at G(2) phase of lung carcinoma cells and releases cytochrome c from mitochondria, which is an essential requirement for apoptosis (148). The internal nanostructure, shape, and structural evolution of diverse protein, peptide, and lipid-protein complexes may be detected and determined using biological synchrotron small angle X ray setting (BioSAXS). It was also found that at the outer mitochondrial membrane, the voltage-dependent anion channel 1 (VDAC 1) plays a critical role in mitochondria-mediated apoptosis. Anti-apoptotic activity is consequently promoted by the interaction of VDAC1 with HK-11 (hexokinase), an enzyme in the glycolytic pathway (149). Thus, a new anti-cancer therapeutic strategy focuses on this complex. Interestingly, Yuan et al. found that lysosomes are the only place where mature cathepsin B may be found (150). As soon as the integrity of the lysosomal membrane is compromised, it is released into the cytoplasm, where it helps to trigger apoptosis. By activating Toll-like receptors like TLR 2, 4, and 9, as well as NF-kB, MAPK, and JNK signalling pathways, the absorption of different nanomaterials, such as carbon black, causes macrophage activation and the release of cytokines, producing inflammation (150). TLR2 and TLR4 are involved in the onset of lung disease and airway inflammatory exacerbations (151). Carbon-based nanoparticles were also found to have an increased anti-cancer impact. Additionally, Tian et al. generated modified liposomes that included modified liposomes that contained hyaluronic acid (HA), a biodegradable and biocompatible molecule that binds with certain tumour cell ligands (152). Non-small cell lung cancer (NSCLC) patients who had never smoked exhibited a decrease of IkB-alpha expression in roughly 16% of the patients in the study. As NFKBIA silencing seems to be a driving factor in the formation of this class of tumours, individuals with an IB deficit were more often found to have it than those with EGFR, K-Ras, or EML4-ALK genetic abnormalities. Erlotinib (EGFR-tyrosine kinase inhibitor) therapy in EGFR-mutant lung cancer cells was observed by IB silencing through interference RNA expression (153). High levels of

IB in patients treated with EGFR inhibitors suggest a therapeutic response, which is in line with this finding (153). However, cisplatin-induced cell death was increased in lung cancer cells when a chemical targeting IB was used instead (153). Interestingly, microRNA such as ZEB1 (Zinc finger E box binding homeobox 1) expression was found to be inhibited by upregulation of miR-199a-3p in NSCLC cells, preventing tumour stem-like features from arising (154).

4.4 Genetic alteration (gene therapy) to target mitochondria in lung cancer

Through ATP supply, metabolic signal transmission, intracellular calcium balance, and apoptosis, mitochondria play a key role in cancer cell biology. Consequently, it is one of the most important targets for cancer chemotherapy. Since extreme ER stress can inhibit the growth of tumour cells hence it is reasonable to assume that ER-mitochondrial crosstalk is critical to the development of NF1-deficient cells, given the intensive molecular crosstalk and coordinated control of Ca2+ homeostasis (155).

Selectively reducing the ATP levels might impede autophagy and lead to the demise of cancer cells via inhibiting ALDH through OXPhos (156). Because cancer cells have a greater OCR than normal cells, they employ mitochondrial oxidative phosphorylation to generate ATP. The cytosolic NADH generated by dehydrogenases such as aldehyde dehydrogenase (ALDH) is a major source of electrons for ATP generation in OxPhos cancer cells, while NADH is produced via the tricarboxylic acid cycle in normal cells (TCA cycle) (156). Furthermore, depletion of ARID1A/BRG-1 causes a rise in ROS and synergizes with inhibitors of OXPHOS, increasing the dependency on that enzyme (157). Mitochondrial inhibitors, as reported by Martin et al., suppress the growth of KRAS mutant tumours *in vivo*, paving the way for the development of new approaches to combat KRAS-driven cancer (158).

ATP-driven multidrug efflux pumps, such as P-gp, increase the activity of the ETC in certain cancer cells due to the high turnover of cancer cells, which in turn increases the production of ATP and ROS, which control proliferation, migration, and the conferral of drug resistance (157). Other than the effects of the tumour's surroundings on its dormancy and reliance on OXPHOS, genetic abnormalities in certain kinds of lung cancer led to an abnormal reliance on OXPHOS. The therapeutic potential of miRNAs has now been reported (159). Nanovesicles can transport MiR-200c to lung carcinoma cells (160). As a result, the expression of tumour suppressors miR-29b and MiR-1247 in metastatic and nonmetastatic lung cancer cells may be increased by nano miR-200c. It can also inhibit the migration and invasion of lung carcinoma cells.

The Nrf2 transcription factor controls the production of genes that protect the lungs and other organs from oxidative damage by regulating the expression of Nrf1 and Nrf2. Antioxidant genes are transcribed via antioxidant response elements (AREs) when fumarate hydratase deficiency is present in a patient (AREs) (161). Thioredoxin 2, peroxiredoxins, and SOD2 are only some of the genes that make up this group of genes, which also includes transporters and enzymes involved in glutathione production and redox recycling.

Although the nuclear factor erythroid 2 p45-related factor 2 (NRF2) is an established important regulator of cellular redox homeostasis, it can block the mitochondrial redox response and therefore preserve or restore the crucial cellular redox balance (161). Mutations in the mitochondrial DNA (mtDNA) have been found in NSCLC, and mt protein expression is much greater than in the surrounding stromal cells (162).

Recent studies have found a link between cancer cell activation, metabolic and epigenetic reprogramming in TME. The preservation of stem cell phenotype in BC is linked to epigenetic and metabolic reprogramming, according to experimental findings (163). Henna and

DNA modification enzymes are both involved in cancer development, and their activity is altered when epigenetic reprogramming is disrupted (163). It is not just DNA methylation that alters gene expression, but histone modification and microRNAs as well. To starve tumour cells, Moreno et al. suggested to use the succinate dehydrogenase complex in conjunction with the hypoxia response and other important pathways to promote RNA network interaction (164). Furthermore, the idea of dosage versus time thresholds is fundamental in measuring tumour and normal tissue responses. S-glutathionylated serine protease inhibitor has been employed as a translational biomarker to titrate ROS produced by radiation exposure (165).

Recent studies have also shown that mitochondrial oxidative phosphorylation is induced in cancer cells resistant to the EGFR-TKI inhibitor. As glutamine is mostly processed in mitochondria, it is expected that EGFR-TKI-resistant cancer cells' metabolism would be more active than that of their parental cells (166). Suppressing EGFR promotes the up-regulation of Beclin1 in cancer cells, which in turn promotes autophagy (167).

The acquisition of resistance in NSCLC to epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) is mostly due to the suppression of PI3K/AKT/mTOR signalling pathway. It has recently been discovered that the FASTK gene family has been found commonly mutated in a variety of cancers, pointing to its potential as a therapeutic target. FASTK and FASTKD3 were shown to be amplified in ovarian and lung malignancies, respectively, whereas elevated mRNA levels for all FASTK members were discovered several lung cancers (168). Similarly, PKM2 and PKM1 heterotetrametric hybrids in oesophagus and stomach, as well as in lung and breast cancer cell lines, have recently been discovered (169). Lung cancer has demonstrated mitochondrial energy metabolism pathway changes as an essential characteristic, and efforts to target the protein expression process in mitochondria have been found to be enormously successful. Targeting the mRNA expressions of ALDH1841

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and CPT1B, for example, may have a considerable impact on tumour development progression (170). Nonetheless, it has been shown that chelidonine efficiently inhibits the growth of cells with EGFR L858R/T790M mutations when used in NSCLC therapy (171). Mitochondrial respiratory chain activity was lowered by chelidonine, as shown by proteomic analysis, and apoptosis was reduced by the AMPK inhibitor.

4.5 Drugs that affect mitochondria in lung cancer

There are several therapeutic agents that target NSCLC. However, the effect of most drugs on mitochondria and the relevant mechanism of action are not extensively investigated. On the other hand, several drugs with indications other than lung cancer have gained attention for their anticancer properties involving mitochondria. This may be due to the increase in the need for alternative options to overcome the issue of resistance in NSCLC chemotherapy. Table 3 summarizes the drugs the affect mitochondria in lung and cancer and the findings of the studies.

4.6 Endogenous/Exogeneous chemicals that affect mitochondria in lung cancer

Multidrug resistance (MDR) has been the major cause of inefficient chemotherapy. The ATP produced by mitochondria has been related to the drug efflux pump overexpression and hence leading to MDR. To overcome MDR and actively target the mitochondria, mitochondria-oriented transportation through small liposomes have been studied (152). The preparation of mitochondria-targeting paclitaxel (PTX)-loaded liposomes is through thin-film hydration and hyaluronic acid (HA) coating via the process of electrostatic absorption. It was found the particle size of PTX liposomes was increased by approximately 40nm and the encapsulation efficiency of PTX liposomes has greatly increased to more than 85%. Stability studies also show that the PTX liposomes were physically and chemically stable for more than a week under 4°C (152). The HA-coated PTX liposomes is a dual-functional PTX liposomes system

as it can target both CD44 and mitochondrial and so the PTX is successfully accumulated in mitochondria, triggering the apoptosis of MDR cancer cells. Studies have showed that the mitochondria-targeting PTX loaded liposomes coated with HA significantly inhibit the A549 and A549/Taxol cells (152).

Other studies have also demonstrated the important role of HA in the mitochondrial targeting delivery system. PTX has been loaded into the TPP-Pluronic F127-hyaluronic acid (TPH) to obtain nano micelles (172). The triphenylphosphine (TPP) head group is required for the nano micelles to obtain mitochondrial targeting properties. As the PTX-loaded TPH (TPH/PTX) enter the acidic lysosomes, hyaluronidase (HAase) will digest the HA and so the TP/PTX nano micelles are completely exposed to the acidic lysosome. This is the mechanism of TPH/PTX localizing mitochondria in A549/ADR cells. TPH/PTX nano micelles also inhibit the antiapoptotic Bcl-2 which causes the permeabilization of the mitochondrial outer membrane. This resulted in the release of cytochrome C and the caspase-3 and capase-9 activation. In short, TPH/PTX shows significant antitumor and mitochondrial targeting properties (172). The mechanism of action of TPH/PTX is depicted in Figure 4.

4.7 Other substances that affect mitochondria in lung cancer

Palladium (II) complex [PdCl(terpy)](sac)·$2H_2O$ is known to have anti-cancer properties (173). An inhalable micellar dispersion containing palladium (II) complex (PdNP) was studied for its physicochemical characteristics. A549 and H1299 NSCLC cell types were used in these studies and the increased level of ROS have been found to trigger PdNP, inducing the mitochondria-dependent apoptosis and disruption of mitochondria membrane (173). The PdNP has high encapsulation efficacy of up to 97%. Besides, approximately 35% and 47% of the dispersed

micellar can reach the alveolar region and the bronchial region respectively. These factors make PdNP a potential inhalable novel complex for NSCLC therapy (173).

The dysregulation of calcium homeostasis has shown as a contributing factor in oncogenesis. It was found that the inhibition of calcium channels can help to restore the intracellular calcium level leading to apoptosis induction, suppression of cell division, and tumor growth (174). Dolutegravir (DTG) is a first line drug for Acquired Immune Deficiency Syndrome (AIDs) management, which can increase the ROS levels and intracellular calcium levels. DTHP, or known as 7-methoxy-4-methyl-6,8-dioxo-N-(3-(1-(2-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)phenyl) 3, 4, 6, 8, 12, 12a-hexahydro-2H-pyrido [1',2':4,5] pyrazino [2,1-b] [1,3] oxazine-9-carboxamide is a derivative of DTG, and its anti-tumour properties were studied. DTHP is non-cytotoxic to healthy cells, it can inhibit the colony-forming ability and the proliferation of NSCLC cells. Induction of apoptosis and increased intracellular calcium were associated with DTHP treatment. Hence, the DTHP-induced apoptosis has been correlated to the intracellular calcium levels. Besides, DTHP also promotes ROS production in mitochondria resulting in the dysfunction of mitochondrial and cell death. Thus, DTHP could be a potential drug candidate for anti-cancer treatment (174).

Bak is an integral protein present on the surface of mitochondria and endoplasmic reticulum. It is a Bcl2 family protein and is an important molecule to induce apoptotic cell death. Studies have correlated poor prognosis of non-small cell lung cancer (NSCLC) with the high levels of Bak expressions (175). The BH3 domains on Bak function as death domain, which is necessary for the activation of apoptotic cell death. Thus, screening has been done to search for small molecules which have a high affinity to the BH3 death domain binding pocket (aa75-88). A lead compound, BKA-073 was identified. BKA-073 does have high affinity and

selectivity at the BH3 binding site of Bak (175). The binding of BKA-073 to the BH3 domain helps to promote the priming of mitochondrial and oligomerization of Bak and activates the proapoptotic function of Bak. As a result, BKA-073 inhibits tumor growth in small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) xenografts, patient-derived xenografts, and genetically engineered animal models of mutant KRAS-driven cancer with no significant tissue toxicity. As the accumulation of Bak occurs in the radioresistant lung cancer cells, BKA-073 can also reverse this radio-resistance. Strong synergistic effects were shown in studies when BKA-073 was used in a combination with venetoclax, a Bcl-2 inhibitor (175).

Cisplatin and tyrosine kinase inhibitor (TKI) is used to treat non-small cell lung cancer (NSCLC) but cisplatin and TKI resistance remains an unresolved issue (176). A group of near-infrared heptamethine carbocyanine fluorescent dyes (DZ) possesses tumour-homing property on cancer cells via differently expressed organic anion-transporting polypeptides. Hence, it can transport the therapeutic molecules to the tumour cells precisely in the form of a chemical conjugation. DZ-simvastatin (DZ-SIM) was found to be capable of destroying both cisplatin-sensitive and cisplatin-resistant as well as EGFR-TKI-sensitive and EGFR-TKI-resistant lung cancer cells (176). DZ-SIM is accumulated specifically in the xenograft tumours formed by the first-generation (H1650) and third generation (PC9AR) EGFR TKIs resistant NSCLC cells, the growth of the xenograft tumours is also inhibited effectively by DZ-SIM. DZ-SIM also caused cell death by interfering with mitochondrial structure and function. These make the DZ-SIM a potential therapy for cisplatin and YKI resistance in NSCLC (176).

4.8 Natural products and isolated molecules that interact with mitochondria in lung cancer therapy

Myricetin, the secondary metabolite flavonoid which distribute in a variety of plants was found to have anti-cancer properties against A549 lung cancer cells (177). A study was conducted to test the efficacy of myricetin against A549 cells in different doses, 73 ug/ml has been found to be the effective dose to stop cancer progression (177). The mechanism of myricetin's anticancer activity is through the induction of sub-G1 phase aggregation. The portion of cells entering the S phase following other phases of cell cycle decreases resulting in cell apoptosis. Additionally, myricetin engenders a massive number of free radicals and thus leading to the alteration of the membrane potential of mitochondria in A549 cells (177). Apart from that, myricetin enhances the expression of P53 while suppressing the expression of EGFR in A549 cells. In short, myricetin exerts its cytotoxicity through the interruption of the cell cycle and ROS-dependent mitochondria-mediated mortality in the A549 cancer cells (177).

Aside from treating constipation, aloe vera have other therapeutic properties such as antitumor, anti-inflammatory, and immune regulatory effects. The anticancer activity and safety of *Aloe vera* barbadensis extract C (AVBEC) has been tested (178). The results indicated no deaths nor substance-induced toxicity in both acute and chronic (6 months) toxicity studies. Furthermore, there are relatively higher incidents of cancer cell deaths that are induced by AVBEC compared to cell death incidents caused in healthy cells. It was found that AVBEC promotes cancer cell apoptosis by decreasing the level of ATP and inducing the production of ROS. Thus, AVBEC could be a potential compound for cancer malignancies therapy (178).

Furthermore, berberine has also been found to be effective in treating NSCLC. Chang et al. have synthesized six berberine derivatives and their anti-NSCLC properties which were tested (179). Some derivatives have higher effectiveness towards NSCLC in the inhibition of cell proliferation while compared to berberine itself, especially the 9-O-decylberberrubine bromide (B6) and 9-O-dodecylberberrubine bromide (B7) (179). B6 and B7 derivatives are

incapable to induce cell death in NSCLC. However, it can regulate the cell cycle, inhibit tumorigenesis, and block the autophagic flux. Thus, B6 and B7 berberine derivatives may be used for NSCLC treatment where further studies on their anti-cancer activity are required (179).

5. MITOCHONDRIA AND CYSTIC FIBROSIS

5.1 Mitochondria in pathophysiology of cystic fibrosis

Cystic fibrosis (CF) is a multi-organ disease, primarily affecting the lungs. The most common gene mutation that results in the progression of CF is CF transmembrane conductance regulator (CFTR) (180). Apart from CFTR, the involvement of IL-8 in CF is well characterized. IL-8 is a neutrophil chemoattractant agent that causes massive release of proteases and elastase in the CF patients' bronchoalveolar compartment (181). Although, the detailed mechanism that promotes CF progression is not yet fully understood, recent studies suggest the association of mitochondrial health and CF since mitochondria play crucial role in inflammation and regulating host response (182).

Various environmental stimuli or endogenous factors may impair mitochondrial homeostasis and impede its function resulting in mitochondrial oxidative stress and alteration in mitochondrial Ca^{2+} signalling. Such abnormal state of mitochondria in the airway epithelial and immune cells can promote the progression of CF. Earlier studies in the 1980s suggest that mitochondrial dysfunction in the CF is associated with CFTR deficiency where it was revealed that oxygen consumption rate of mitochondria obtained from CF patients was affected due changes in complex-I and Na^+/K^+ ATPase (183, 184). Furthermore, the expression of MT-ND4 and CISD1 gene that is essential for the mitochondrial electron transport change is downregulated in tracheal cells obtained from CF patients (185). In CF, other mitochondrial dysfunction, for example mitochondrial protein pattern, Ca^{2+}-dependent NLRP3

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inflammasomes activation, and intracellular pH also promotes the generation of reactive oxygen species and membrane lipid peroxidation (186).

5.2 Targeting mitochondria in cystic fibrosis therapy

The ideal therapeutics for CF should be able to rescue the functionality of defective CFTR, repair the mitochondrial dysfunction, and provide the vicious CF cycle to overcome lung inflammation. As such, various mitochondrial specific antioxidants, modulators of Ca^{2+}-exchanges, inhibitors of inflammasome and IL-1β could be a promising compound for the management of CF through their anti-inflammatory and antioxidant activities (182).

Atlante et al., 2016 treated the CF cells with VX-809 and 4,6,4'-trimethylangelicin as "CFTR correctors" and observed that both small molecules improved mitochondrial parameters including oxygen consumption, mitochondrial membrane potential generation, adenine nucleotide translocator-dependent ADP/ATP exchange and both mitochondrial Complex I and IV activities, while decreasing the mitochondrial ROS production and membrane lipid peroxidation suggesting CFTR rescue is associated with the recovery of mitochondrial function (187). Natural product-based single compounds such as resveratrol are potent antioxidants that can preserve mitochondrial membrane potential and mitochondrial DNA from oxidative damage (188, 189). In human primary lung epithelial cells and mouse model of CF, it was observed that resveratrol increased F508del-CFTR dependent salivary secretion, the chloride secretion and cAMP-dependent anionic transport (190, 191). Similarly, the synergistic activity of ivacaftor (approved drug for CF treatment) and resveratrol potentiates the G155D-CFTR channel in HSNE (human primary sinonasal cells) (192). Other mitochondrial specific antioxidants such as, mitovitamin E, mitoquinone, and compounds containing triphenylphosphonium cation moiety are important for repairing mitochondrial dysfunction in CF (193). Mitovitamin E inhibits the mitochordiral oxidative stress and the production of IL-

1β to improve the mitochondrial function (194), while mitoquinone restores the mitochondrial dysfunction (targeting the parameter such as mitochondrial membrane potential, adenosine triphosphate content, complex expression, basal and maximum respiration levels, and respiratory reserve capacity) and airway hyperresponsiveness (75). The decreased level of serum vitamin E in CF patients can be normalized with dietary supplementation of mitovitamin E to preserve the mitochondrial function (195). Taken together, these studies clearly suggest the potential of various mitochondria specific therapeutics could be a future lead drug for the management of CF.

5. Conclusion

In conclusion, the role of mitochondria and the associated chemical molecules are increasingly acknowledged in respiratory diseases. We envisage that the idea of mitochondrial-based treatment will gain traction in the future as mitochondria are potential targets for new treatment modalities. Mitochondria-based approaches can also be an avenue in developing mitochondrially-personalized medicine as mitochondrial gene therapies have shown to have positive treatment effects. Aside from novel synthetic strategies, several naturally existing compounds were found to influence mitochondria. Thus, they may be investigated further to engender newer novel treatment modalities. However, despite the abundance of studies validating the beneficial response of compounds affecting mitochondria, there are limited number of studies that validate the advantages of mitochondria-based approaches over traditional or conventional strategies in alleviating or preventing respiratory diseases. Moreover, most studies investigating the properties of drugs affecting mitochondria stop short at pre-clinical phase. Thus, more in-depth studies investigating the benefits of potential drugs affecting mitochondria must be carried out to draw a solid conclusion regarding their efficacy.

Declaration of competing interest

The authors declare that they have no competing interests.

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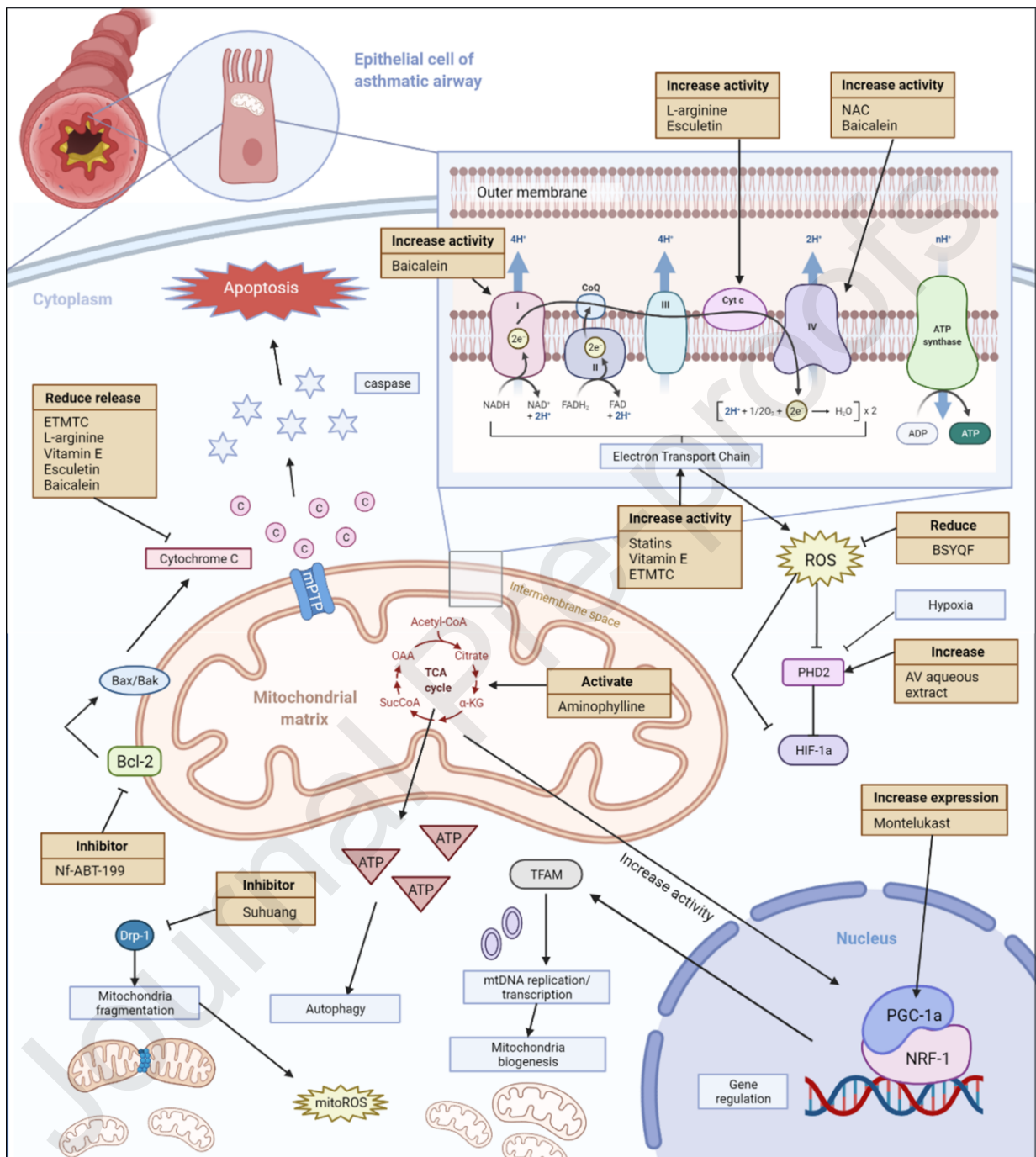
Fig 1. Drugs, novel compounds, and alternative medicinal compounds acting on mitochondria in asthmatic airway epithelia.

Note: ATP: adenosine 5′-triphosphate, AV: adhatoda vasica, Bax: Bcl-2 associated X, apoptosis regulator, Bak: (another Bcl-2 family protein), BSYQF: Bu-Shen-Yi-Qi formula, Drp-1: dynamin-related protein 1 (major mitochondrial fission GTPase), ETMTC: novel cinnamate, ethyl 3',4',5'trimethoxythionocinnamate, HIF-1α: hypoxia-inducible factor-1α, MPTP: mitochondrial permeability transition pore, mitoROS: mitochondria ROS, NAC: N-acetylcysteine, NRF-1: Nuclear Respiratory Factor 1, PGC-1a: peroxisome proliferator-activated receptor-gamma coactivator, PHD2: prolyl hydroxylase domain-2, ROS: reactive oxygen species, TFAM: mitochondrial transcription factor A
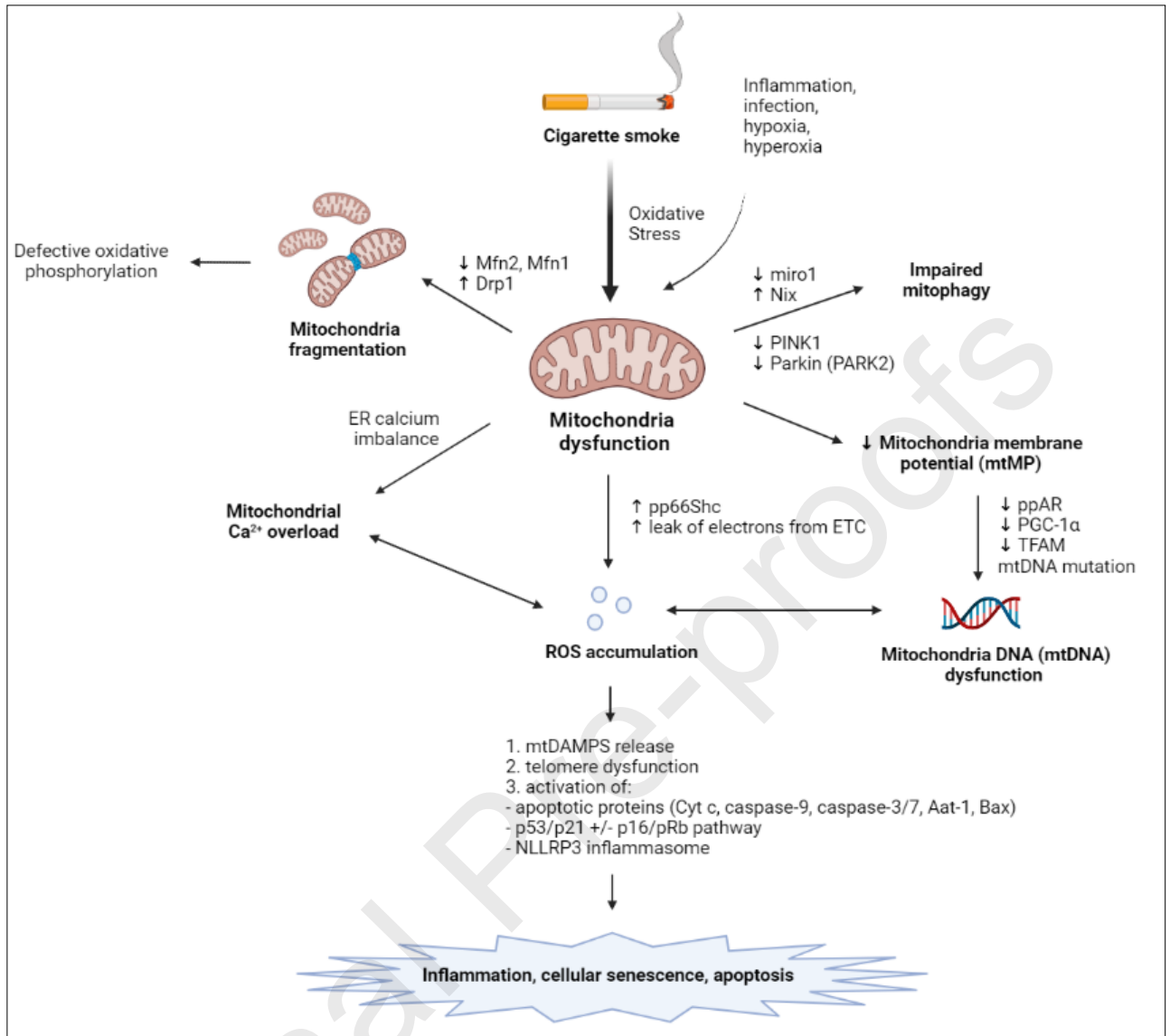
Fig 2. Mitochondria dysfunction pathways in COPD lung epithelial cells involving potential targets for therapy
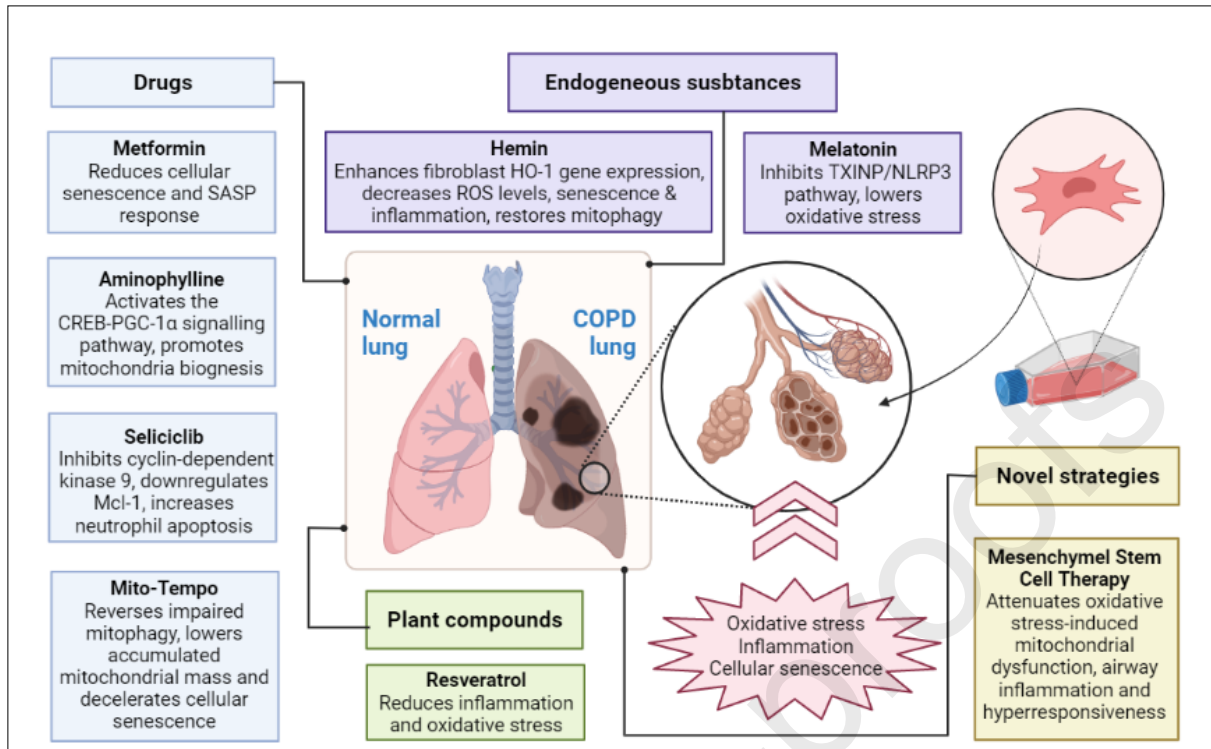
Fig 3. Drugs, plant compounds, endogenous substances and novel strategies affecting mitochondria in COPD.

CREB: cAMP response element-binding protein, HO-1: Heme oxygenase-1, Mcl-1: Induced myeloid leukaemia cell differentiation protein, NLRP3: NOD-, LRR- and pyrin domain-containing protein 3, PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha, ROS: Reactive oxygen species, TXINP: Thioredoxin-Interacting Protein.
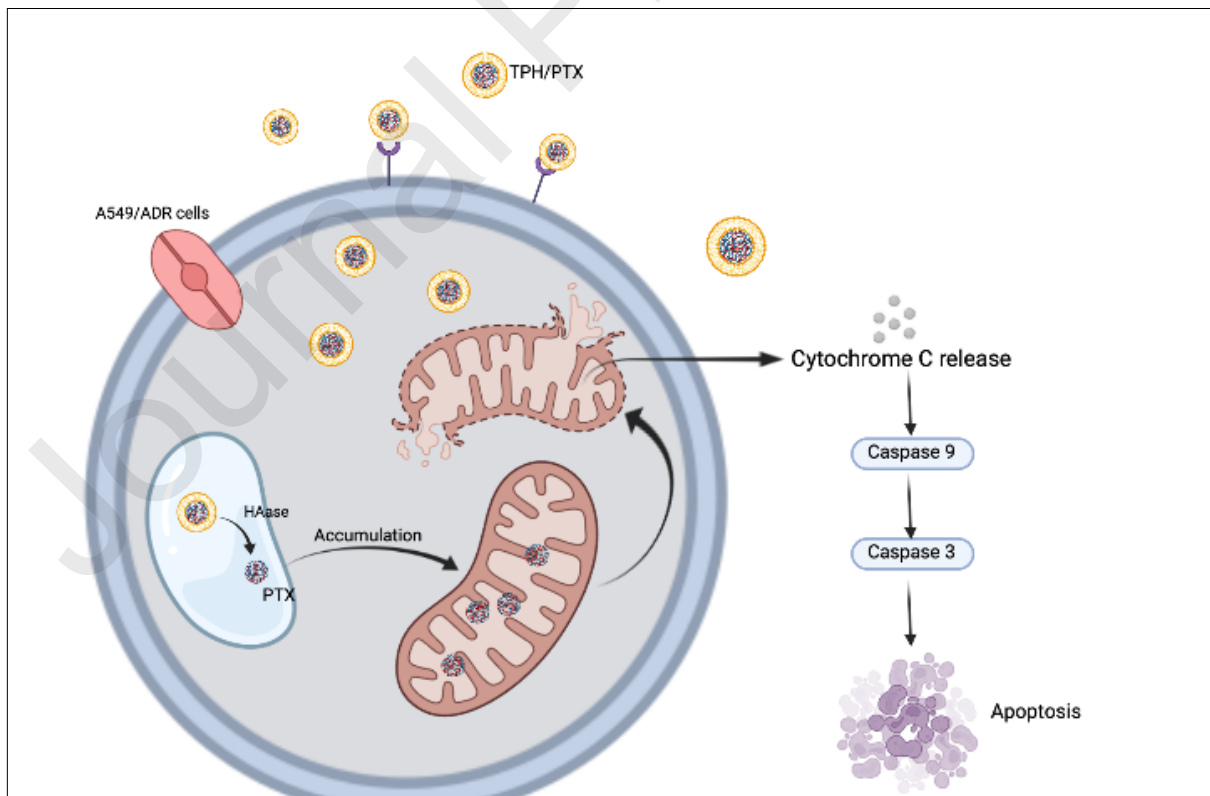
Fig 4. TPH/PTX induced apoptosis in A549/ADR cells.

HAase: Hyaluronidase, PTX: Paclitaxel, TPH/PTX: Paclitaxel-loaded triphenylphosphine-Pluronic F127-hyaluronic acid.

Table 1. Novel therapeutic strategies targeting mitochondria in asthmatic treatment, as elucidated by several authors.

No.	Intervention of study	Route of Administration	Study subject	MOA	Biological effects	Study outcomes	Reference
1	Nf-ABT-199	Intratracheal	Mice	Nanoformulated Bcl-2 inhibitor	● Induces release of Cytochrome C upon mitochondrial fragmentation which activates caspase-9 and caspase-3, causing inflammatory cell apoptosis ● Reduces IL-4 and IL-5 levels	Significantly reduced inflammatory cell recruitment in peri-bronchial areas and excessive mucus production.	(37)
2	N-acetylcysteine (NAC) and Diphenyleneiodonium (DPI)	Intranasal	Mice	NAC: antioxidant thiol ● Scavenger of ROS, converted to glutathione precursor (essential for antioxidant activity) DPI: NADPH oxidase inhibitor ● Reduce superoxide production	NAC: improves mitochondrial energy metabolism Combination of NAC & DPI can reduce: ● Extracellular traps (EETS), ● Goblet cells hyperplasia, ● Eosinophil peroxidase (EPO), ● Nfkb p65 immunocontent proinflammatory cytokines, oxidative stress in airway of asthmatic mice.	● Improved lung function reduces airway inflammation, ● Oxidative stress and EETS formation reduced in asthmatic mice	(41)
3	Vitamin E	Oral	Mice	Antioxidant	● Significantly reduces Th2 cytokines (IL-4, IL-5, IL-13, eotaxin, and OVA-specific ige) in asthmatic mice ● Inhibits expression of 12/15-LOX ● Reduce oxidative and nitrative stress in lung at higher doses ● Increases activity of COX_{ETC} and expression of COX_{ETC} subunit III ● Reduces cytosolic cytochrome C levels	Reduced: ● Asthmatic airway inflammation ● Airway hyperresponsiveness airway remodeling changes	(42)
4	Hydrogen sulfide (H_2S)	(Incubated in male	Mice	● Rapid: NAHS	Inhibit tracheal hyperreactivity to 5-HT at concentrations of:	Decreased airway inflammation	(43)

	releasing donors	BALM/c mice lung tissue with LPS-induced tracheal hyperreactivity)		• Slow: GYY4137 and AP39)	●NAHS: 300 & 1000 µmol/L ●GYY4137: 30 & 100 µmol/L ●AP39: 30 nmol/L <u>Nahs prevent elevation of:</u> ●IL-1β level at 1000 µmol/L ●IL-6 & TNF-α levels at 100 µmol/L <u>Inhibition of increase in IL-6 and TNF-α levels:</u> ●GYY4137 (100 µmol/L) AP39 (30 & 300 nmol/L)	and prevent airway hyperreactivity	
5	Esculetin (6,7-dihydroxy-2H-1-benzopyran-2-one)	Oral	Mice	Coumarin-derived antioxidant	●Reduces Th2 response, lung eotaxin, eosinophilia, airway inflammation, and ova-specific ige. ●Inhibits 15-lipoxygenase and lipid peroxidation ●Reduces linoleic acid-induced oxidative stress ●Restores electron transport chain cytochrome C oxidase activity ●Reduces caspase 9 activity and lung cytosolic cytochrome c level ●Reverses mitochondrial structural changes ●Restores lung ATP levels	●Restored mitochondrial dysfunction ●Reversed structural changes ●Reduced asthmatic features and bronchoalveolar lavage fluid	(44)
6	L-arginine	Oral	mice	Metabolizes into nitric oxide	●Reduces cytosolic cytochrome c ●Increase mitochondrial cytochrome c oxidase activity ●Increases lung ATP levels ●Reduces DNA fragmentation in bronchial epithelia ●Restores mitochondrial ultrastructural changes of bronchial epithelia ●Reduces expansion of intercellular spaces between bronchial epithelial cells	●Reduced airway injury ●Restored mitochondrial dysfunction	(45)
7	Novel cinnamate, ethyl 3',4',5'trimethoxythionocin	Oral	Mice	-	Reduces: ● CAMS expression, NF-κB activation, th2 cytokines, eotaxin and 8-isoprostane	●Reduced lung epithelial injury ●Improved asthma features	(46)

	namate (ETMTC)				● Lung cytosolic cytochrome c and caspase 9 activities ● Oxidative DNA damage marker levels in bronchoalveolar lavage fluid ● Bronchial epithelia DNA fragmentation ● Lung goblet cell metaplasia and sub-epithelial fibrosis. Increases: ● Lung mitochondrial complexes I and IV activities ● 15-(s)-hydroxyeicosatetraenoic acid levels		
8	Human induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs)	Intratra cheal	Mice	Connexin 43 (CX43) mediated mitochondri al transfer to lung epithelial cells	● Reduces Th2 cytokines, IL-33, TSLP ● Inhibits BEAS-2B cell apoptosis under $cocl_2$ challenge	● Reduced lung injury	(47, 48)
9	LF-15, T3 and T7 tumstatin-derived peptides	Human model: endoth elial cell culture Murine model: intrana sal	Huma n, mice	Anti-angiogenic	● LF-15 and T7 attenuates lung endothelial viability and cell tube formation ● T3 peptides only reduce cell viability	● LF-15 and T7 reduces total lung vascularity and attenuates AHR	(49)

Table 2. Summary of potential targets related to mitochondrial dysfunction in COPD.

Potential target	Function	Biological effect upon CSE stimulation/ COPD manifestation	Potential treatment strategy	Refere nces
Enzymes				
PINK (PTEN-Induced Kinase 1)	Mitochondrial serine/threonine-protein kinase ● Recruits E3 ubiquitin ligase Parkin to initiate mitophagy	Increased in PINK 1 expression ● Insufficient mitophagic degradation	● Increase mitophagy ● Reduce mtDAMPs	(83, 196, 197)

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AMP-activated protein kinase (AMPK)	Mitochondrial serine threonine kinase: • Maintain bioenergetics homeostasis, regulate senescence, metabolic activity, and immunological responses	-	AMPK activators	(110)
Protein arginine methyltransferase-1 (PRMT1)	Regulates mitochondrial mass in human airway epithelial fibroblasts • Contribute to tissue remodelling	Increased in expression	Inhibition of the SAMD2/3 pathway or PRMT1 • Reduce TGF-β-induced mitochondrial mass	(198)
carnitine palmitoyltransferase 1A (CPT1A)	Mitochondrial enzyme for the fatty acid oxidation (FAO) pathway	Increased in expression • Enhanced FAO	Inhibition of FAO by etomoxir • Reduce ROS accumulation • Reduce lung epithelial cell death	(93)
NADPH oxidase 4 (Nox-4)	Intracellular mitochondrial enzyme • Catalyze the conversion of O_2 into O_2 • Generate ROS	Increased in expression	Nox inhibitors • Increase mitochondria TFAM level • Stimulate mitochondrial biogenesis	(197, 199, 200)
Prot				
Parkin	E3 ubiquitin ligase	Decreased in PARK2 expression • Insufficient mitophagic degradation • Decreased in FEV1/FVC percentage	• Increase mitophagy • Reduce mtDAMPs	(197, 201)
miro1	GTPase that modulates mitochondrial morphology in trafficking	Decreased in expression [b43] • Impairs mitophagy	-	(202, 203)
Nix	Selective autophagy receptor associated with the proapoptotic BH3-only proteins. • Involved in mitochondrial clearance	Increased in expression • Causes mitophagy which aggravated lung epithelial cell and mitochondria injury	-	(203)
Sirtuin 3 (Sirt3)	Mitochondrial deacetylase that maintains	Decreased in expression of sirt3, manganese superoxide dismutase (MnSOD), mRNA	-	(204)

	mitochondrial function	• Worsen mitochondrial oxidative stress • Implicates cell injury		
Bcl-2 family protein	• Regulate apoptosis: pro-apoptotic proteins - lead to COPD • Anti-apoptotic proteins - protective effect against COPD	Increased in pro-apoptotic protein expression • P53, Bax Decreased in anti-apoptotic protein expression • Bcl-2, Bcl-XL	-	(205)
Connexin 43	Gap junction protein	Downregulation of Cx43 expression in human endothelial cells • May impair alveolar barrier function	Increase Cx43 expression • Promote the transfer of functional mitochondria to damaged cells Administer connexin-mimetic peptides • Suppresses inflammation	(205)
PPARs	Key regulators of mitochondrial biogenesis	Increased in expression in COPD patients' muscle cells	-	(206)
PGC-1α	Key regulators of mitochondrial biogenesis	-	-	(206)
OPA1 isoforms	Mitochondria fusion regulatory protein	• Transformation of OPA1 from long to short isoforms and • Drastic increase in mitochondrial stress-related protein SLP2 levels • Alteration in prohibitins (PHB1 and 2)	Mitochondrial fusion activators: BGP-15 and leflunomide treatment • Preserved the long OPA1 isoform	(201)
Mfn1, Mfn 2	Mitochondria fusion regulatory protein	Decreased in expression	-	(202)
Drp1	Cytosolic mitochondria fission regulatory protein	Increased in expression	-	(202)
TFAM	Maintenance of mtDNA copy number	-	-	(206)
Prohibin	Essential in the normal functioning of mitochondria	Decreased in expression in COPD lung	-	(206)
Iron-regulatory protein (IRP)	Regulate Fe2+ ion homeostasis in cells	Increased in expression in COPD lung	-	(206)

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pp66Shc	Adaptor protein translocated in mitochondria • Generate ROS upon phosphorylation	Increased in expression • Increased ROS production	Silence pp66shc • Reduced ROS generation • Improved other mitochondrial processes	(207)

TRPA1, TRPV1	Transient receptor potential protein (TRP) ion channel
	 • Regulate respiratory tissue damage and inflammation
		Increased the following in in alveolar epithelial cells and bronchial epithelial cells:
		 • Oxidative stress,
		 • Ca2+ influx
		 • Mitochondrial fission proteins (MFF and DRP1) expression
		 • NLRP3 & Caspase-1 protein expression
			1. Inhibition of protein
			 2. Knockout of encoding genes
			 3. Reduce:
			    • Oxidative stress
			    • Influx of Ca2+
			    • Inflammation
			    • CSE-induced alterations in mitochondrial fission & fusion proteins
			    • MRC complex activity
				(208)

Table 3. Drugs that affect mitochondria in lung cancer and outcome of studies

N o	Drug	Indication	MOA	Biological effects	Study/ Clinical outcomes	Refere nce (s)
1	Cisplatin	Cancers	Generate nuclear DNA (nDNA) and mitochondrial DNA (MtDNA)adducts	• Halted DNA replication and transcription • Induced mitochondrial-dependent ROS response • Inhibit mitochondrial ETC enzymes • Elevated electron leakage from the ETC • Increased cytotoxic effect causing cell death	Mitochondrial-ROS response contributed to cisplatin cytotoxicity	(209, 210)
2	Gefitinib	NSCLC with EGFR mutations	Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor	• Significantly enhanced biological activity of mitochondrial succinate-tetrazolium reductase (STR) • Increased mitochondrial membrane potential	Inhibited additive antitumour activity in combination with doxorubicin	(211)
3	Imatinib	NSCLC	Bcr-abl tyrosine kinase inhibitor	• Upregulated mitochondrial enzymes o Electron transport enzymes (cox i, ii and iv) • Improved mitochondrial respiratory capacity	Activated mitochondrial apoptosis in cell lines	(212, 213)
4	Paclitaxel	Cancers • NSCLC ,	Antimicrotubular agent	• Impaired microtubule function • Induced intrinsic apoptotic pathway	Induced p53-independent	(214)

	prostate, and breast cancer			• Causes g2/m cell cycle blockade • Induced mitochondria damage	apoptosis in NSCLC cell lines	
5	Mito-lonidamine (Mito-LND)	Cancers	Antiglycolytic drug • Selectively inhibit aerobic glycolysis and energy metabolism	• Inhibited succinate-ubiquinone reductase activity of respiratory complex II • Inhibited mitochondrial complexes I and II • Increased ROS formation • Stimulated autophagic cell death	Inhibited primary lung tumors and brain metastases in an orthotopic mouse model at low concentrations	(215)
6	Organometallic Gold complex • Cyc-Au-1 • Cyc-Au-2	Novel	-	• Accumulated in mitochondria and induce mitochondrial dysfunction by increasing ROS and ER stress response • Induced simultaneous autophagy and apoptosis	Cyc-Au-2 exhibits lower toxicity and more potent antitumour activity than cisplatin in a murine tumor model	(216)
7	TMU-35435	Novel	Histone deacetylase (HDAC) inhibitor	• Arrested cell cycle at the G2/M phase • Induced mitochondria-mediated apoptosis • Activated unique networks comprising many tumour suppressor gene to suppress tumour • Inhibited Wnt signalling pathway to suppress tumour	• Inhibited cancer growth in lung orthotopic and subcutaneously implanted xenograft models, with good pharmacokinetic properties and safety features • Combination treatment with DNA demethylation reagent, 5-aza-dC have synergistic effects	(217)
8	Pt8(II) saccharinate complexes • Trans-configured complexes 1, 3 and 5	Novel	Dual action in targeting genomic DNA and mitochondria • Interacted with DNA (groove binding) & HAS (hydrophobic IIA subdomain)	• Induced apoptosis • Excessive generation of reactive oxygen species (ROS)	No significant effect observed in lung cancer cells	(218)
Drugs with indications other than NSCLC						

9	Acyclovir	Herpes	Antiviral drug	Caused mitochondrial toxicity • Reduced mitochondrial membrane potential, • altered mitochondrial size and shape • mtDNA damage	• Inhibited colony formation capacity and cancer cell growth in NCI-H1975 cells • Induced apoptosis	(219)
10	Auranofin • Gold (I)-containing phosphine compound	• Rheumatic arthritis (FDA-approved) • Lung and ovarian carcinoma	Selenoprotein thioredoxin reductase (cytosolic and mitochondrial)	• Inhibited mitochondrial activity at IC50 doses, • Increased oxidative stress response	Cancer cell death • Associated with excessive oxidative stress and impaired cytosolic and mitochondrial reductive pathways	(220)
11	Artesunate	Malaria	Inhibits cell cycle progression	• Induced cell apoptosis and cell cycle arrest • Decreased Bcl-2 protein expression • Decreased mitochondrial membrane potential	Significantly Inhibited lung cancer cell growth	(221)
12	Chloroquine	• Malaria • Autoimmune disorders • Viral infections	Target PI3K/AKT pathway • Decrease Bcl-2 expression, increase Bax expression • Decrease mitochondrial membrane potential, • Release mitochondrial cytochrome c into the cytosol • Activating caspase-3 and cleaving PARP	• Induced mitochondria-mediated apoptosis • Blocked autophagy	Suppressed cancer growth	(222)
13	Digoxin	Heart Failure	Inhibit Akt, mTOR and p70S6K phosphorylation	• Arrested the G0/G1 phase and G2/M phase of cell cycle • Induced mitochondria-mediated apoptosis	• Induced autophagy • Inhibited NSCLC cells proliferation	(223)

14	Metformin	Diabetes Mellitus	Inhibit mitochondrial oxidative phosphorylation (OXPHOS) pathway	**Direct anticancer effects:** • Disrupted mitochondrial ETC I and activates AMPK, this inhibiting mTORC1, causing apoptosis • Decreased the mitochondrial carrier SLC25A10 gene expression in a glucose dependent manner **indirect anticancer effect:** • Increased insulin sensitivity • Decreased circulation insulin levels	• Blocked protein synthesis and cancer cell proliferation • Affected nutrient supply and cancer cells' metabolic state	(224, 225)
15	Niclosami de	Helminth infections	Activated intrinsic and caspase-independent pathway	• Translocation of apoptosis-inducible factor (AIF) to nucleus upon mitochondria damage • AMPK/AKT/mTOR pathway involved in response to decrease in ATP	• Suppressed tumour growth • Induced autophagy	(226)
16	Penflurido l	Schizophren ia	Antipsychotic	• Arrested cell cycle at G0/G1 phase • Decreased AKT and NF-kB protein expression • Down-regulated FAK-MMP signalling	• Suppressed growth of A549 cells in xenograft mouse model • Potential drug candidate for NSCLC	(227)

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Highlights

Mitochondria is one of the basic essential components for eukaryotic life survival

This review summarizes the potential treatment strategies that target the malfunctioning mitochondria

The role of mitochondria in respiratory diseases is gaining much attention