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Mitochondria signaling pathways in allergic asthma

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ABSTRACT

Mitochondria, as the powerhouse organelle of cells, are greatly involved in regulating cell signaling pathways, including those related to the innate and acquired immune systems, cellular differentiation, growth, death, apoptosis, and autophagy as well as hypoxic stress responses in various diseases. Asthma is a chronic complicated airway disease characterized by airway hyperresponsiveness, eosinophilic inflammation, mucus hypersecretion, and remodeling of airway. The asthma mortality and morbidity rates have increased worldwide, so understanding the molecular mechanisms underlying asthma progression is necessary for new anti-asthma drug development. The lung is an oxygen-rich organ, and mitochondria, by sensing and processing O_2, contribute to the generation of ROS and activation of pro-inflammatory signaling pathways. Asthma pathophysiology has been tightly associated with mitochondrial dysfunction leading to reduced ATP synthase activity, increased oxidative stress, apoptosis induction, and abnormal calcium homeostasis. Defects of the mitochondrial play an essential role in the pro-remodeling mechanisms of lung fibrosis and airway cells' apoptosis. Identification of mitochondrial therapeutic targets can help repair mitochondrial biogenesis and dysfunction and reverse related pathological changes and lung structural remodeling in asthma. Therefore, we here overviewed the relationship between mitochondrial signaling pathways and asthma pathogenic mechanisms.

INTRODUCTION

Mitochondria, as crucial organelles and the "powerhouse" of the cell, have a key role in biosynthetic reactions and ATP synthesis, as well as other cellular processes, including synthesis of fatty acid, Ca^{2+} homeostasis, and hemoprotein biogenesis. Mitochondria are largely involved in the cell signaling circuitry, and recent evidences demonstrated that mitochondria participate in the regulation of numerous cellular signaling pathways by providing a physical platform for protein–protein interactions and the trafficking of intracellular signaling molecules and adjusting Ca^{2+} hemostasis and ROS production. Mitochondria also regulate the signaling pathways involved in cell death, autophagy, innate and acquired immunity activation, production of growth factors, cellular differentiation, and hypoxic stress responses. On the other hand, dysregulated apoptosis, the main form of programmed cell death, contributes to the pathogenesis of various diseases.[1]

Mitochondria play a key role in modulating innate immune system responses (via regulating RNS generation) and cell death (by releasing apoptotic factors).[2 3] Mitochondrial signaling routes are involved in the release of various metabolites and the balance of mitochondrial dynamics via interactions with other organelles such as the endoplasmic reticulum. Mitochondrial dysfunction induces stress responses that can affect other organelles and disrupt cellular functions. In fact, any dysregulation in mitochondria-dependent signaling pathways would have physiological and pathophysiological outcomes.[4]

Asthma is a chronic airway disease characterized by airways' eosinophilic inflammation, airway hyperresponsiveness (AHR), mucus hypersecretion, goblet cell metaplasia, airway remodeling, increased IgE levels, and also reversible expiratory airflow obstruction.[5] Many environmental stimuli and genetic contributors are known to facilitate asthma development. Asthma, as a prevalent disease, imposes a heavy economic burden on society, patients, and their families. The morbidity and mortality rates of asthma have increased worldwide over the past recent decades. While the currently available anti-asthma drugs effectively control asthma clinical symptoms, they cannot prevent the natural course of the disease.[6] Therefore, it is necessary to scrutinize the molecular mechanisms involved in asthma progression to develop new and curative anti-asthma drugs.

Allergic asthma is mediated by Th2 dominant immune responses. Allergen exposure induces the release of pro-inflammatory cytokines (IL-4, 5, and 13) and mediators (PGs, LT, and histamine), leading to inflammatory cells' recruitment and activation, such as eosinophils. Among other immune cells, eosinophilic inflammation is dominant and a common feature in asthma. Eosinophils release many reactive free radicals and compounds such as superoxide anion, hydrogen peroxide, hydroxyl radicals, peroxynitrite, NO, and so on, mediated by

oxidative pathways' enzymes, such as myeloperoxidase and eosinophil peroxidase. These mediators create an oxidative microenvironment in airways, causing damage and injury to airway cells. In addition, the number of mitochondria has been observed to increase in the airway epithelium of asthmatic children.[7] Oxidative free radicals have been noted to contribute to asthma pathogenesis. In mitochondria, COXETC, as a key oxidative enzyme, catalyzes electron transfer for ATP generation via the coupled process of oxidative phosphorylation and, for this purpose, consumes large amounts of the oxygen content of cells.[8] The inhibition of COXETC causes oxidative stress and triggers mitochondria-mediated apoptosis.[9]

The lung is an oxygen-rich environment, and the physiological activity of this organ depends on oxygen concentration.[10] A fall of O_2 level in the lung leads to HPV secondary to PASMC intracellular Ca^{2+} due to Ca^{2+} release from ryanodine-sensitive stores and voltage-independent/dependent Ca^{2+} entry, coupled with Rho kinase-mediated enhanced Ca^{2+} sensitivity of the contractile apparatus.[11] So, PASMC mitochondria act as O_2 sensors in HPV. Changes in O_2 concentration affect ROS production in mitochondria and subsequently Ca^{2+} and K^+ depolarization pathways. So, mitochondria can contribute to lung pathologic injuries by generating ROS and participating in the activation of pro-inflammatory signaling pathways.[5]

Mitochondria consist of two membranes (the inner and outer), IMS, and mitochondrial matrix. This organelle works with a self-functioning security mechanism, namely. MQC. Mitochondria and the ER have connections with lipid raft-like domains called MAMs, which contain many proteins, which have important roles in Ca^{2+} transfer from the ER to mitochondria and lipid synthesis. Also, MAMs transfer stress signals from the ER to mitochondria and regulate MQC under ER stress.[12]

There is considerable overlap between the pathophysiology of asthma and mitochondrial dysfunction in terms of calcium homeostasis, oxidative stress, and apoptosis. In addition, maternal inheritance is considered the strongest risk factor of asthma.[13 14] Mitochondrial haplogroups have been associated with increased serum IgE levels, and mutations in the mitochondrial genome sequences encoding mitochondrial tRNAs have shown a link with asthma.[14 15] In addition, the gene encoding ATP synthase mitochondrial F1 complex assembly factor 1 has been noted to contribute to asthma progression.[16 17] Mitochondrial metabolism is involved in airways' remodeling and the regulation of immune responses that are critical processes contributing to the pathogenesis of allergy and asthma. Therefore, here, we reviewed the relationship between mitochondrial signaling pathways and asthma pathogenesis.

MITOCHONDRIA DYNAMICS

Overall, mtDNA is much more susceptible to oxidative-induced damage and degradation than nuclear DNA.[18] Mitochondrial dynamics, fission or fusion, maintain their high functional capacities in adverse conditions. The fission leads to mitochondria fragmentation and affects mtDNA integrity and respiration capacity. On the other hand, fusion promotes incorporation and preserves mtDNA function through several distinct mechanisms, including augmenting mtDNA resistance against mutations.[19] When these mechanisms fail, non-functional depolarized mitochondria are selected for degradation, which is attributable to the reduced translocation of PINK1 from the outer to the inner mitochondrial membrane. This actives a series of consecutive reactions, leading to a reduction in mitochondria motility and their capture by phagophore (ie, mitophagy; the selective degradation of mitochondria by autophagy).[20] Finally, the lack of mitochondrial homeostatic function leaves cells susceptible to mtROS-mediated injury.[21]

MITOCHONDRIAL FISSION/FUSION

Fusion in mitochondria is regulated by Mfn1 and 2 that are expressed on the outer membrane, as well as Opa1 on the MIM, which help the two mitochondrial membranes fuse.[22 23] Fission is orchestrated by mitochondrial fission factors, Fis1, MiD49, and MiD51, which facilitate the formation of focal rings around mitochondria, the recruitment of cytosolic Drp1 on the surface of mitochondria, and finally mitochondrial cleavage. The balance between fusion and fission controls cellular metabolism and links mitochondrial structure to its function.[22 24]

Changes in the levels of fission vs fusion proteins play an important role in inflammation. Pink1, a mitochondrial targeted kinase, can interact with Fis1, promote the activity of E3 ubiquitin ligase Parkin, and finally target Mfn to degrade damaged mitochondria. Ubiquitination is in fact a quality-control process that can target Opa1, Drp1, and Fis1 to balance fission/fusion.[22 25]

Mitochondrial biogenesis has been shown to decrease in airways. On the other hand, increased fission and hyperfusion have been noted in COPD.[26–28] In contrast, it was reported that asthma is associated with increased mitochondrial biogenesis, enhanced Drp1, and decreased Mfn2.[26–28] Mitochondria affect Ca^{2+} hemostasis by modulating the function of inositol ryanodine receptor channels and competing with ER Ca^{2+} ATPase for the reuptake of $[Ca^{2+}]_{cyt}$. Moreover, Mfn2, as a physical linker between the ER and mitochondria, controls the mitochondrial buffering of $[Ca^{2+}]_{cyt}$ in ER microdomains, creating for mitochondria a Ca^{2+} reserve. Also, Mfn2 facilitates ER/PM interactions with activator proteins (such as STIM1 that senses ER Ca^{2+} level) and PM Ca^{2+} influx channels (such as Orai1).[22 26 29] Mitochondria also modulate Ca^{2+} influx by altering local gradients.

MITOCHONDRIAL AUTOPHAGY/MITOPHAGY

Autophagy represents a series of cellular processes aiming to recycle components and organelles through channeling them into lysosomes. In lung diseases, autophagy can protect cells against damaged cellular components due to inflammation or ROS. The clearance of damaged mitochondria (ie, mitophagy) is a type of selective autophagy that can activate apoptotic signaling pathways.[22 30 31] In bronchial epithelial cells, mitophagy initiates after exposure to cigarette smoke and progresses by the stabilization of the mitophagy-associated protein, Pink1. During mitophagy, the expression of Pink1 increases in epithelial cells, resulting in a reduction in the number of functional mitochondria, ending up in mitophagy-associated cell death.[22 32] In asthma, mitophagy-associated proteins (such as AMPK)

may act as a regulator and sensor of cellular energy and influence glycolysis and oxidative phosphorylation.[22 30]

Some other signaling pathways that regulate autophagy include TORC1 and cAMP-dependent PKA.[33] During autophagy (as an evolutionarily conserved lysosome-dependent degradation process), cytosolic cargos are engulfed by a double-membraned lipid bilayer that seals to form autophagosome. Autophagosomes then fuse with lysosomes, leading to the destruction and recycling of the cargo engulfed. Under stress conditions, autophagy occurs in all cells and has crucial functions in disease and health.[22 33 34]

Mitochondria can regulate the initiation of autophagy signaling processes and serve as a membrane source for autophagosome formation. Most commonly, the kinases of ULK1 and/or ULK2 must be activated to trigger autophagy.[33 34] The ULK1 kinase forms a complex with FIP200 (RB1CC1) and ATG13, which their activation leads to autophagosome nucleation and elongation. The activity of ULK1 is regulated through phosphorylation by upstream kinases. The activation of the mTORC1 kinase complex inhibits ULK1 and ATG13 phosphorylation, suppressing autophagy.[22 34 35] Mitochondria provide a membrane source for formation of the autophagosome, specifically following starvation.[36] In starvation, a reduction of the ATP content of cells leads to the activation of AMPK that in turn directly activates ULK1 by phosphorylation. On the other hand, suppressing the phosphorylation of mTORC1 indirectly regulates TSC2 and RAPTOR.[22 34 35] So, mitochondria, through regulating ADP and ATP levels, modulate autophagy. In addition, on starvation, the JNK-mediated phosphorylation of Bcl2 disrupts the Bcl2–beclin-1 interaction, triggering autophagy.[22 33 35]

Mitochondria have also an important role in autophagy regulation by affecting intracellular Ca^{2+} levels.[37] Mitochondria take up the Ca^{2+} released by the ER on IP3R activation to warrant efficient oxidative phosphorylation and ATP generation and inhibit autophagy by suppressing AMPK activity. Also, mitochondria regulate autophagy initiation through ammonia generation mediated by mitochondrial-dependent glutaminolysis.[38] Autophagy is upregulated by ammonia in a non-conventional (ie, ULK1 or ULK2 independent) manner. In some conditions, mitochondria initiate autophagy by modulating PKA activity. Mitochondrial respiratory deficiency leads to the upregulation of PKA that inhibits autophagy by suppressing Atg8 expression (microtubule-associated protein LC3), which is required for autophagy.[22 33 39]

Under some circumstances, autophagy selectively degrades organelles. The autophagic degradation of mitochondria is called mitophagy that can remove damaged mitochondria to maintain healthy organelles. Also, mitophagy is an important process during erythropoiesis, depleting mitochondria during reticulocyte-to-erythrocyte maturation.[40–42]

The translocation of Parkin, a cytosolic E3 ubiquitin ligase, into dysfunctional mitochondria initiates mitophagy. The recruitment of Parkin to mitochondria depends on the function of PINK1, a mitochondrial kinase. Under normal conditions, this kinase is imported into the mitochondrial intermembrane space where it is inactivated through being cleaved by the rhomboid protease (PARL). On MOM, when mitochondrial membrane potential is lost (ie, a feature of unhealthy mitochondria), PINK1 accumulates and then recruits Parkin (a process that is dependent on PINK1 kinase activity). Finally, Parkin promotes mitophagy in an AAA +ATPase p97-dependent manner.[43–45]

During erythrocyte maturation, programmed mitophagy effectively removes all mitochondria from reticulocytes. A protein called NIX (BNIP3) is a key player in this process[42] and recruits mitochondria into maturing autophagosomes through binding to a key autophagy protein (LC3). On the outer membrane of mitochondria, NIX displaces beclin-1 from anti-apoptotic Bcl2, promoting formation of autophagosome on mitochondrial membrane. A newly identified mitophagy pathway requires the autophagy proteins of ATG12 and ATG3, during which ATG12 covalently binds to ATG5, causing LC3 conjugation to PE. Also, ATG12 covalently attaches to ATG3, and this complex promotes mitophagy following the dissipation of mitochondrial membrane potential. In addition, the ATG12–ATG3 complex regulates mitochondria homeostasis via inhibiting mitochondrial expansion and promoting mitochondrial fusion.[44 46 47] Therefore, mitochondria are able to control many signaling processes. Another major metabolic function of mitochondria is citrate production, as a major source of acetyl-CoA for protein acetylation, an important post-translational modification of many key signaling molecules. Both autophagy and mitophagy increase mitochondrial damage and boost cells' sensitivity to death and inflammatory cytokines' production. In addition, mitochondria signaling is particularly important in the pathogenesis of some diseases such as allergy and asthma.

MITOCHONDRIA AS A SIGNALING MACHINE

Mitochondrial along with nuclear DNA is necessary for the normal functioning of cells, and miscommunication between these two sets of DNAs can lead to pathological events in cells. Mitochondrial ATP generation is necessary for the normal thermodynamics of biochemical reactions. Also, mitochondria are involved in producing the proteins containing heme and porphyrin moieties; a phenomenon that is dependent on the membrane potential. The metabolites generated during the TCA cycle are precursors for the biosynthesis of lipids, proteins, nucleotides, and carbohydrates. Thus, mitochondria are bioenergetic and biosynthetic organelles with signaling functions beyond their metabolic roles.[4]

Signal transduction to the cytosol from mitochondria is referred to retrograde while that from the cytosol to mitochondria is known as anterograde signaling. Mitochondria-derived signaling encompasses the release of cytochrome C to initiate cell death, ROS production to induce the expression of hypoxic genes, localization of AKAPs to the mitochondrial outer membrane, and allowing cAMP-dependent PKA to phosphorylate substrates on the outer membrane. Dysfunction in these pathways can induce mitochondria-specific HSPs and promote cytosolic calcium-dependent signaling.[4 48 49] Cytochrome C is an essential component of the electron transport chain and a key player for ATP generation. NADPH oxidase activity promotes signaling pathways by oxidizing particular cysteine residues in proteins, which modulates their activity. In fact, mtROS includes signaling molecules that facilitate communications

between mitochondria and other parts of the cell. Hypoxia stimulates ROS release from mitochondria, stabilizing HIFs and inducing the genes responsible for metabolic adaptations in low oxygen conditions.[50–52] Further, mtROS regulates cellular metabolism and TNF-receptor signaling and is necessary for the optimal progression of many immune responses. Eight sites within the MIM and matrix are mainly responsible for ROS generation, which is controlled by mitochondrial oxygen concentration and membrane potential, as well as the redox state of electron transport chain complexes.[53 54] AKAPs could be tethered to the outer mitochondrial membrane, which bind to the cAMP-dependent serine/threonine kinase (ie, PKA), leading to the assembly of PKA with multiple signaling proteins. Survival signaling induces PKA-dependent phosphorylation and inactivation of BAD, a pro-apoptotic Bcl-2 family member that specifically binds to MOM.[55 56] Mitochondria-bound PKA–AKAP complexes regulate oxidative reactions, fusion/fission machinery, and hypoxic responses and allow cellular signaling pathways to converge on the mitochondrial PKA–AKAP axis and control mitochondrial function. AKAPs, as anchor for PKA, act as scaffolds to coordinate many signaling enzymes' activities, such as kinases and phosphatases.[4 57 58] Thus, the mitochondrial outer membrane serves as a scaffold for the complexes that regulate immune responses.

MAVS is a crucial adaptor for RIG-I-like receptor signaling. In response to viral RNAs, RIG-I is induced and then promotes type I interferons' production through MAVS, which is located in the outer mitochondrial membrane. Interferons (type I) can activate ISGs, as well as the NF-κB pathway to upregulate antiviral proteins and several proinflammatory cytokines and chemokines. Other innate immune molecules involved in TLR and NLR signaling are also associated with the MOM.[59–63]

Decreased ATP production, for example, during ischemia, regulates metabolic signaling pathways by increasing AMP production and adenosine breakdown. An increase in AMP level concomitant with decreased ATP level activates the AMPK kinase, which halts multiple anabolic processes and also promotes catabolic events such as autophagy in the cell.[4 64]

MAMs are necessary for the rapid transmission of Ca^{2+} signals between the mitochondria and ER to regulate intracellular Ca^{2+} levels. MAMs also regulate mitochondrial shape and motility, production of ATP and ROS, ER stress, autophagy, and immune cells' signaling pathways.[65 66]

MITOCHONDRIAL ENERGY SIGNALING

The triggers of mitochondria-related transcriptional signals are classified into the anterograde and retrograde categories. While anterograde signaling is the nuclear control of the mitochondrion, retrograde signals originate from the organelle itself and induces nuclear transcriptional reprogramming (a phenomenon that is often referred to as feedback or the backward flow of information).[67 68]

The ABI4 and 15/40/63 WRKY-type transcription factors are downstream regulators in mitochondrial retrograde signaling. Moreover, Ca^{2+} is involved in the mitochondrial retrograde response, and Ca^{2+} concentration is actually linked to ATP production. A uniporter complex using the mitochondrial membrane potential is involved in Ca^{2+} import. Also, Ca^{2+} influx into the mitochondrial matrix occurs in response to various abiotic stresses.[67 69–72]

MITOCHONDRIAL OUTER MEMBRANE AND APOPTOSIS

The MOM's integrity is strictly regulated via interactions between the pro-apoptotic and anti-apoptotic members of the Bcl2 protein family. In the mitochondrial pathway of apoptosis (intrinsic), pro-apoptotic insults such as DNA damage activate two main Bcl2 family proteins, Bax and Bak. Activated Bax and Bak lead to MOMP and the release of cytochrome C and SMAC (DIABLO) into the cytoplasm where they promote caspases' activation. When MOMP occurs, cells undergo caspase-independent cell death, which is most likely a consequence of a progressive decline in mitochondrial function.[1 73]

Although MOMP is associated with apoptosis induction, under some conditions, it can also promote non-lethal signaling functions. For example, mitochondria-mediated caspase-3 activation is required for the effective internalization of the AMPA receptor via postsynaptic membranes, and also, mitochondrial activation of caspase-9 regulates myocyte differentiation. Interestingly, incomplete MOMP is probably essential for its non-cytotoxic signaling functions in the conditions where the cell's fate is to survive. Incomplete MOMP occurs through two mechanisms: (1) via expressing high levels of anti-apoptotic Bcl2 proteins and (2) via regulating mitochondrial dynamics. Mitochondria constantly undergo cycles of fission and fusion with one another, and the inhibition of mitochondrial fusion promotes incomplete MOMP. Another potential mechanism of incomplete MOMP includes the localized activation of BH3-only proteins (ie, a protein family that triggers Bax and Bak activation and MOMP).[1 74–76]

MITOCHONDRIAL PERMEABILITY TRANSITION ROLE IN APOPTOSIS AND NECROSIS

BA is one of ANTs (1, 2, and 3 in humans). CsA and BA inhibit apoptosis induction (eg, glucocorticoid-triggered thymocyte death or TNF-α-induced hepatocyte apoptosis) and protect mitochondria against the MMP-inducing effects of Bax and Bid recombinant proapoptotic proteins (two members of the Bcl-2 family). Moreover, the overexpression of cyclophilin D inhibits apoptosis induced by ANT1 (cyclophilin D is an antagonist for ANT1 and 3). Also, cyclophilin D was shown to inhibit apoptosis induced by caspase-8, but not by Bax or RIP.[77–79]

MITOCHONDRIA AND APOPTOSIS CROSSTALK

Mitochondria have essential roles in apoptosis. Extensive cellular stress can lead to the release of pro-apoptotic molecules, caspase activation, and apoptotic cell death. Induction of apoptosis is partially regulated by the same proteins involved in autophagy. In fact, Bcl-2, as an antiapoptotic protein, regulates both autophagy and apoptosis through binding to Beclin1 (a pro-autophagic protein) and Bax (a pro-apoptotic protein). In stressed cells, the release of Beclin1 activates PI3K and induces autophagy, accompanied by the release of Bax from Bcl-2, simultaneously triggering apoptosis. Thus, mitochondria health is a critical determinant of autophagy or apoptosis and cell fate.[80–82]

Class III PI3K, ATG4D, and Beclin1 can be cleaved by caspases on being translocated into mitochondria where they can amplify mitochondria-mediated apoptosis. In addition, the caspase-dependent cleavage of Beclin1 and PI3K destroys their function as pro-autophagic factors. Under cellular stress, ATG5 is also cleaved by calpains and translocated to mitochondria where it binds Bcl-XL.[80 83 84]

MITOCHONDRIA AND AUTOPHAGY CROSSTALK

One of the cellular survival pathways, autophagy, recycles intracellular components to compensate for the physiological degradation of organelles. Likewise, mitophagy regulates mitochondria number and health and is a key process for guaranteeing mitochondrial health and proper cell function. On the other hand, mitochondria can substantially influence autophagy. The pro-autophagy Beclin1/PI3K complex and the recruitment of activated ATG proteins induce autophagosome formation. Mfn1, Mfn2, and Opa1 are responsible for mitochondria fusion while fission is regulated by Drp1 and Mff. In addition, E3 ligase and MARCH5 regulate fusion through targeting Mfn1 and/or Mfn2. Overall, fusion and fission influence all aspects of mitochondrial function. Fusion is a way for the rapid exchange of metabolites, membrane components, and mtDNA, and fission facilitates mtDNA segregation and mitochondria isolation from the rest of the cell to prepare them for degradation.[80 85–88]

Damaged mitochondria are recognized by a voltage-sensitive kinase, Pink1, which on the loss of the membrane potential ($\Delta\psi m$) is stabilized on the MOM. The Pink1 accumulation on the mitochondrial membrane facilitates the recruitment of Parkin, an E3 ligase, to mitochondria, allowing for the ubiquitination of Mfn1/2 fusion proteins and VDAC1.[89–92] The accumulation of ubiquitinated proteins facilitates the recruitment of the autophagy adaptor, p62, leading to the autophagosomal degradation of the damaged mitochondrion. NIX is another mitophagy-related protein that its role in this process is yet to be further discussed.[80 93 94]

During starvation, a component of the mitochondrial outer membrane (ie, GFPcb5MitoTM) is transferred into autophagosomes, and endogenous Ambra1 is dissociated from Bcl-2, enhancing the interaction between Ambra1 and Beclin1 on the mitochondria and ER membranes. The mitochondrial Ambra1/Beclin1 complex drives autophagosome biogenesis from mitochondrial and ER membranes. Under nutrient-rich conditions, Bcl-2 inhibits autophagy via interacting with both Beclin1 (on the ER) and AMBRA1 (on mitochondria surface). A membrane-shaping protein with pro-autophagic activity (ie, endophilin B1) links mitochondria to autophagosome biogenesis. Endophilin B1 activates the Beclin1–PI3K complex through binding to Beclin1 adaptor (ie, UVRAG82) and drives autophagosome formation using mitochondrial membranes.[80 95–97] Therefore, decreased ATP production induces autophagy in a mTOR/AMPK-dependent manner.

MITOCHONDRIA ROLE IN NON-APOPTOTIC CELL DEATH

Mitochondria have roles in other forms of programmed cell death as well. For example, necrosis-like cell death is activated by several triggers, including death-receptor ligation that requires the RIPK3 kinase. RIPK3-dependent necrosis has important roles in boosting the host's antiviral immunity.[98 99] Mitochondria are a major cellular source of ROS, and during RIPK3-dependent necrosis, they contribute to an increase in cellular ROS.[99 100] Thus, RIPK3-dependent necrosis may be actually initiated by mitochondria. The alternative role of mitochondria in RIPK3-dependent necrosis may be mediated by the rapid depletion of cellular ATP, triggering the process. The interaction between ANT and CYPD is inhibited during RIPK3-dependent necrosis, leading to a reduction in ADP and ATP transport across mitochondria and diminishing cellular ATP levels. However, RIPK3-dependent necrosis was not affected by the absence of CYPD, negating a crucial role for the ANT–CYPD interaction in this pathway. Nevertheless, CYPD is required for necrosis, which is triggered by Ca^{2+} overload and ROS excessive production, implying a key role for mitochondria in necrosis triggered by these stimuli.[98–101] So, mitochondria can have essential roles in non-apoptotic cell death and various types of necrosis.

MITOCHONDRIA AND NECROSIS

Necrosis, as a random and uncontrolled process, leads to accidental cell death and probably plays a main role in diseases' pathogenesis, similar to that of apoptosis and autophagy. "Programmed necrosis" is sometimes inappropriately referred to as necroptosis. In this pathway, PARP1, ADPH oxidases, RIP kinases, and calpains are potential signaling components.[80 102]

Pan-caspase inhibitors such as zVAD-FMK inhibit TNFα-induced apoptosis, which can direct some cells towards necrosis instead[102 103]; a turn that is blocked in the cells lacking RIP1serine/threonine kinase. Under normal conditions, RIP1 mediates the MAPK and TNF-receptor-induced NF-κB signaling pathways and is normally associated with cell survival. Moreover, under certain stress conditions, RIP1 can induce necrotic death by phosphorylating and activating RIP3, which is a key mediator for necrosis progression. RIP3-deficient cells have been demonstrated to be less sensitive to TNF-α- and Smac-mimetic-induced necrosis.[104–106]

Genotoxic stressors (eg, oxidants and alkylating agents) trigger necrotic cell death accompanied by the overstimulation of PARP1, a DNA repair enzyme. Ischemia-induced necrosis was reported to be attenuated by inhibiting PARP1. A strong cellular signaling network is responsible for PARP1-mediated cell death, during which the actions of calpains or CypD may be indispensable as their inhibition would block PARP1-induced cell death.[107 108]

Several studies reported that ROS scavengers could abrogate TNFα-induced necrosis in cells. During TNFα-induced necrosis, the NOX1 and NOXO1 subunits of NADPH oxidase are recruited to form a receptor complex via a RIP1-dependent manner. Moreover, the inhibition of NOX1 blocks the necrotic actions of TNF-α. In contrast, the NOX4 isoform role has been implicated in oxidized-LDL-induced necrosis. Particularly, necrosis in macrophages can happen in a ROS-independent manner.[68 102 109 110]

Proteases, as proteolytic enzymes (eg, caspases, calpains), can also mediate programmed necrosis. Calpains, as cysteine proteases, are activated by Ca^{2+}, and their inhibition results

in anti-necrotic effects. Indeed, PARP1-induced and also TNF-α-induced necrosis is dependent on the activation of calpains, followed by the cleavage and activation of pro-apoptotic Bcl2 family proteins (such as Bax), cytoskeletal degradation, and lysosomal rupture.[111–114]

In the intrinsic apoptotic pathway, pro-death Bcl2 proteins contribute to necrotic death. Also, in particular, Bax, Bmf, BNIP3, and Nix are parts of necrotic cell death.[115 116] PARP1-mediated programmed necrosis is dependent on the mitochondrial translocation of Bax, but not Bak. Bax, in turn, induces necrosis by facilitating the release of AIF from mitochondria. Bmf is one of the important mediators of TNFα-induced necrosis, and its knockdown prevents TNFα-induced and zVAD-induced necrotic death. One of BH3-related proteins, BNIP3, can also induce necrosis. BNIP3 overexpression induces MPT and subsequently necrosis, and BNIP3-induced necrosis is blocked by inhibiting MPT via cyclosporine-A. Another BNIP3-related protein, Nix, can also induce both apoptosis and necrosis. Nix translocation to mitochondria induces apoptosis through activating the canonical intrinsic pathway. In contrast, ER-targeted Nix can induce necrosis through calcium-dependent MPT pore activation.[19 102 117–119]

MITOCHONDRIA REGULATE CASPASE-8 ACTIVITY

Mitochondria can regulate apoptosis through mechanisms other than MOMP, for example, by regulating caspase-8 activation. In the extrinsic pathway, following ligand binding, apoptosis is initiated by death receptors, which requires caspase-8 activation that occurs at the intracellular tail of the receptor–ligand complex. Active caspase-8 either directly induces executioner caspases and promotes apoptosis, or alternatively recruits MOMP for the effective activation of executioner caspases and induction of apoptosis. Caspase-8 induces MOMP through cleaving and activating a pro-apoptotic Bcl2 family protein (ie, Bid) that in turn activates Bak and Bax.[1 120]

MOMP promotes caspase activation by releasing mitochondrial proteins, including SMAC, which blocks the ability of XIAP to inhibit caspase function. Interestingly, mitochondria are required for the effective function of the initiator caspase-8 following death receptor ligation. Caspase-8 is activated at the MOM in a process that is dependent on cardiolipin, a mitochondrial membrane phospholipid. Mitochondria-localized caspase-8 complexes cleave Bid and lead to MOMP. Disruption of the mitochondrial membrane association with caspase-8 inhibits its activity and disrupts MOMP, suppressing apoptosis.[1 121 122] Therefore, MOM as a signaling platform, is important to facilitate and direct caspase-8 activity where it is required.

MAMS, THE CONNECTORS OF THE ER AND MITOCHONDRIA

MAMs, as membrane structures, connect MOM to the ER. MAMs do not fully fuse the ER and MOM and keep a 10–25 nm distance between them. MAMs, as physical and functional connectors, contain a variety of proteins such as IP3R Ca^{2+} channel (in the ER), VDAC1 (in the MOM), mitochondria dynamic-related proteins (eg, MFN1/2), chaperones (eg, calnexin, Grp75), lipid synthesis-related enzymes and transporters (eg, oxysterol-binding protein, cholesterol acyltransferase), and ER redox regulation enzymes (eg, Ero1α). MAMs connect mitochondria to the ER structurally and functionally and have important roles as signaling molecules and transporters. The IP3R Ca^{2+} channel (in the ER) is linked with VDAC1 (in the MOM) by Grp75 to form an ER-mitochondrial bridge to transport Ca^{2+} between the two organelles.[123–126] Furthermore, vesicle-associated protein (VAPB) in the ER and PTPIP51 in the MOM regulate ER–mitochondria connections. MFN2 can also link the ER with mitochondria through MFN2–MFN2 or MFN2–MFN1 interactions. Also, MFN2 can interact with PERK, an ER transmembrane protein, to link mitochondria with the ER. In addition, Bap31 can be linked to Fis1, which can also act as a tether for MAMs.[12 127–130]

MAMs play a main role in intracellular signal transduction and processes, including Ca^{2+}-mediated signaling pathways, energy production in mitochondria, lipid transport, mitochondrial dynamics, and apoptosis. Ca^{2+}, after being released from the ER, enters the IMS through IP3R-Grp75-VDAC1 channels in MAMs. Then it can enter into the mitochondrial matrix and transmit stress signals through MCU in the MIM.[131 132]

The Ca^{2+} entering mitochondria has an important role in ATP production and determining cell fate. In normal circumstances, Ca^{2+} uptake by mitochondria can increase the TCA cycle activity and ATP production, but Ca^{2+} excess, on the other hand, can lead to mPTP opening and apoptosis. The Miro GTPase 1/2 (miro1/2) present in the MOM has a Ca^{2+}-sensing domain, which regulates mitochondrial movement and maintains mitochondrial Ca^{2+} homeostasis.[133–135]

During ER stress, the number of MAMs increases, promoting Ca^{2+} transport between the ER and mitochondria and boosting mitochondrial energy production. Under stress conditions, IRE1 is enriched in MAMs and promotes cell survival by inhibiting IP3R that stabilizes Ca^{2+} concentration of the mitochondria. In MAMs, PERK can be linked to MFN2 on the MOM, forming MAMs' scaffolds. The elimination of MFN2 leads to ER stress while PERK deletion reduces ROS production and stabilizes mitochondrial Ca^{2+} level. MFN2 in MAMs inhibits PERK activation. MAMs are also rich in chaperones (such as S1R, CNX, and CRT) that significantly contribute to ER stress signaling.[136–140] The transcription of S1R is increased by the PERK/EIF2α/ATF4 pathway, and this molecule inhibits caspase-4 activity and plays a protective role under ER stress. Also, S1R stabilizes IP3R and reduces ER Ca^{2+} release, stabilizing the concentration of this ion in MAMs. CNX and CRT, with a high affinity for Ca^{2+}, buffer its concentration in MAMs and stabilize mitochondrial Ca^{2+} balance under ER stress. Also, CNX directly interacts with SERCA and regulates its activity.[12 139 141 142] Therefore, ER stress is closely related to MAMs and affects their structure by regulating Ca^{2+} channels' activity and chaperone expression. In turn, changes in MAMs affect the transmission of ER stress to mitochondria.

PERK can inhibit protein translation by phosphorylating elf2α and reducing Tim23-dependent protein import. This event reduces mitochondrial protein content and helps maintain protein homeostasis in this organelle. PERK can also increase the expression of LON that strictly regulates mitochondrial protein homeostasis and degrades stress-damaged mitochondrial proteins. In addition, PERK

upregulates Grp75 expression via inducing ATF4. Grp75 helps protein folding in the mitochondrial matrix. These chaperones, however, require energy (ie, ATP) to function properly. Moderately increased Ca^{2+} in mitochondria boosts mitochondrial metabolism via modulating Ca^{2+}-dependent dehydrogenase activity in the Krebs cycle to promote the activity of the respiratory chain complex, ending up in ATP production.[136 143–145] ATP-dependent chaperones and proteases prevent the accumulation of misfolded or unfolded proteins, maintaining mitochondrial protein homeostasis and warranting MQC at the molecular level.

Cytosolic Ca^{2+} overload upregulates XO activity in cytoplasm, leading to ROS production. Then ROS phosphorylates serine 616 of Drp1, resulting in the accumulation of Drp1 on the MOM, which promotes its division. When the membrane potential of mitochondria decreases, PINK1 is transported to the MOM where it recruits Parkin to initiate mitophagy. PINK1 then returns to MAMs when mitophagy promotes the binding of the ER to mitochondria and triggers autophagosome formation.[12 146 147] Therefore, MAM-mediated Ca^{2+} signaling is an important trigger of mitochondria fusion and division under ER stress.

When ER stress is severe, MAMs transmit stress signals to mitochondria to initiate apoptosis. The release of Ca^{2+} from the ER causes mitochondrial Ca^{2+} overload via IP3R-VDAC1 channels, leading to mitochondrial depolarization, Bak and Bax oligomerization on the MOM, mPTP opening, the release of pro-apoptotic factors, and finally the activation of the mitochondrial apoptotic pathway. In contrast to Bax and Bak (pro-apoptotic Bcl-2 family proteins), Bcl-2 and Bcl-xL (anti-apoptotic Bcl-2 family proteins) can translocate to MAMs and promote cell survival by suppressing IP3R. Furthermore, the BH4 domain of Bcl-xL targets VDAC1 and reduces mitochondrial Ca^{2+} influx through these channels, suppressing apoptosis.[148–150] On the other hand, Bax interacts with VDAC and increases mPTP opening, promoting apoptosis. Severe ER stress inhibits the expression of anti-apoptotic proteins and increases that of pro-apoptotic Bcl-2 family proteins, accelerating ER Ca^{2+} release and leading to cell death. During ER stress, truncated SERCA1 (S1T) can be localized on MAMs, a phenomenon that is induced by the activity of the PERK–EIF2α–ATF4 axis of the UPR. Overexpressed S1T amplifies Ca^{2+}-induced apoptotic signals during ER stress.[12 151 152] Therefore, MAMs can transfer death signals to mitochondria, and play main roles in MQC under ER stress, and also promote mitochondrial dynamics and homeostasis. A variety of diseases are associated with ER stress and mitochondria dysfunction, including allergic asthma. Therefore, further studies on the mechanisms regulating MAMs' function under ER stress may help identify new therapeutic targets and develop novel treatment strategies for these diseases, including asthma.

CYTOCHROME C AND HEAT SHOCK PROTEIN-60

Cytochrome C and Hsp60 are two important mitochondrial markers. Cytochrome C is a part of the electron transport chain in mitochondria. The heme group of cytochrome C receives electrons from the B-C1 complex and transfers them to the cytochrome oxidase complex. Extracellular succinate elevates when the integrity of cytochromes C1 and B of the electron transport chain is compromised by either inhibitors or metabolic reprogramming.[153 154]

Hsp60 is a mitochondrial stress protein and a member of the chaperon family, which is induced under cellular stress and injury. Elevated levels of circulating Hsp60 have been detected in patients with asthma. Hsp60 is located in both mitochondria and cytosol, and its function is essential for the folding and assembly of the proteins newly imported into mitochondria; however, this function is compromised when mitochondrial activity is impaired. Hsp60 has been suggested to contribute to the severity of symptoms in asthma. According to studies, cytochrome C and Hsp60 are constitutively expressed in asthmatic fibroblasts, correlating with mitochondrial mass. The inhibition of PRMT1 enzymatic activity or the SMAD2/3 pathway decreased the expression of cytochrome C and Hsp60.[154–157]

PGC-1a is a metabolic regulator and transcriptional coactivator of cellular energy metabolism (ie, fatty acid oxidation, cholesterol catabolism, and gluconeogenesis) and activates mitochondria biogenesis and oxidative phosphorylation. PGC-1a is increased in the fibroblasts of asthmatic airways, as well as TGF-β-stimulated fibroblasts. In addition, PGC-1a methylation by PRMT1 was shown to enhance mitochondrial biogenesis.[154 158–160]

Increased PGC-1a expression in asthmatic fibroblasts expands mitochondria mass. PGC-1a expression is induced by TGF-β1 or PRMT1. On the other hand, TGF-β1 activates myo-fibroblasts to produce extracellular matrix components such as collagen and fibronectin, increases ASM migration, decreases anti-apoptotic signaling, and stimulates cellular proliferation. Therefore, TGF-β can activate SMAD2/3 signaling and induce PGC-1a expression and fibroblast proliferation. Suppressing TGF-β1 by either blocking the SMAD3 pathway or the administration of anti–activin A reduces peri-bronchial fibrosis, ASM proliferation, and mucus hyper-secretion.[154 161 162]

C/EBPs control the promoter of mito-ribosomal protein S12 and mitochondrial seryl-tRNA ligase that regulate Hsp60 mRNA translation. C/EBPb, as a mediator of TGF-β1-induced gene activity, is expressed during cell differentiation and induces the expression of C/EBPa and PPAR-g.[154 163 164] Changes in C/EBPb expression affect the levels of PRMT1 and PGC-1a. All these factors are constitutively upregulated in the fibroblasts of patients with asthma. Thus, C/EBPb is an important mitochondria mass regulator in fibroblasts. Therefore, TGF-β1-dependent remodeling of fibroblasts may help control asthma by suppressing PRMT1 or C/EBPb,[154] both of which present novel diagnostic and therapeutic targets in asthma.

MITOCHONDRIA MUTATIONS

The ADAM33 gene (on chromosome 20) is strongly associated with asthma.[6] In addition, there are some pieces of evidence indicating the possible involvement of mtDNA defects in asthma etiology. Over 25 genetic loci are associated with asthma, many of which being related to the immune system, including the genetic loci of ORMDL3, 2PBP2/GSDMB/ORMDL3, PDE4D, VEGF, Wnt, MMP-12, importin 13, PRKCA, JAG1, ANKRD5, 12q24, TGF-β1, IL-12 beta, 10, 13, 17, 25, and beta2-adrenergic receptor.[165–167]

Recently, it was shown that the "U" haplogroup (harboring common mitochondrial polymorphisms) was associated with total IgE level in asthmatic patients, highlighting the importance of mitochondrial genes' mutations in allergic asthma.[14 168] Many mutations in the mitochondrial genome have been associated with diseases with an inflammatory component. The A930G polymorphism in the gene encoding cytochrome b has been related to predisposition to bronchial asthma. The rare A3243G-tRNA Leu (UUR) MELAS mutation was also found in asthmatic patients. Interestingly, mitochondrial tRNA and rRNA mutations are more frequent in patients with asthma.[15 169–171] Some mitochondrial mutations have been linked with inflammatory diseases. Fukuda *et al* identified that 9 out of 13 of the genes differentially expressed in allergic patients were mitochondrial (Cytochrome oxidase II, III, NADH dehydrogenase, etc.). Also, the cytochrome b gene's polymorphisms were found in relation with asthma predisposition.[15 170 172]

MITOCHONDRIAL DYSFUNCTION AND LUNG DISEASE

Cellular bioenergy dysfunction leads to epithelial fragility, dysfunctional barriers, impaired secretory activities, and inflammation. Therefore, asthma and also bronchopulmonary dysplasia, pulmonary hypertension, and COPD are strongly associated with mitochondrial dysfunction.[173–175]

The haplogroup "U" of the mitochondrial genome is associated with elevated IgE levels and allergic asthma. In maternal asthma, altered mitochondrial gene expression was observed in the placenta of asthmatic mothers. The deficiency of UQCRC2 in airway epithelial cells was reported to induce mitochondrial dysfunction, allergic airway inflammation, AHR, and mucus secretion. Airway epithelial cells of asthmatic patients show mitochondrial fragmentation and swelling, activated apoptotic pathways, and increased oxidative damage.[15 17 21 157 176]

ROS AND DAMP IN THE AIRWAY

In asthma, the excessive production of oxidant agents triggers the expression of pro-inflammatory cytokines and activates the signaling cascades related to inflammation, extracellular matrix production, and Ca^{2+} trafficking. Inflammation and ROS can modify mitochondrial morphologic characteristics and functions. Damaged mitochondria (due to inflammation and ROS) release multiple mitochondrial components into the cytosol or even extracellular mitochondrial DAMPs in the lung. DAMPs are known to activate PRRs (pathogen recognition receptors) such as TLRs, further exaggerating inflammation. ATP can act as a DAMP to mobilize Ca^{2+}, activate inflammasomes, induce the release of mtDNA into the cytosol, and even increase mitochondrial ROS production.[22 23 177–180]

There is increasing recognition for the role of the autocrine effect of mitochondrial DAMPs in asthma pathophysiology. DAMPs activate PRR such as TLRs and induce inflammation. In airways, ATP is released by multiple cell types and acts as a DAMP when it is released in excess, inducing Ca^{2+} mobilization, inflammasome activation, release of mtDNA into the cytosol, an increase in mitochondrial ROS production, and mitochondria dysfunction.[22 181 182] Also, mitochondria regulate the innate immunity, which has main roles in the eradication of microorganisms or damaged cells through PRRs (PAMPs) that recognize conserved molecular patterns in different microorganisms or the proteins released from damaged cells (DAMPs). This process actives pro-inflammatory cytokines' secretion and signaling, subsequently inducing the adaptive immune response and ROS generation.[22 182 183]

ASTHMA AND MITOCHONDRIAL

Asthma, as a common complex disorder, places heavy economic burdens on individuals and societies. The main pathogenic mechanism of asthma includes allergic and immunologic reactions due to the inhalation of allergens by patients, resulting in the early asthmatic response (EAR).[6 184] Airway remodeling can occur in asthmatic patients. The predominant features of remodeled airways are fibrosis, basement membrane thickening, metaplasia of goblet cells, enhanced smooth muscle mass, and chronic inflammation. Dysmorphic mitochondria are also evident in the smooth muscle cells and epithelium of the airways of asthmatic lungs.[10 26 185 186] In addition, mitochondria can be transferred to remodeled lungs via cell therapy.

In the signaling pathways involved in remodeling, Ca^{2+} has a central role in mediating an increase in mitochondrial mass, which enhances the proliferation of smooth muscle cells and induces lung remodeling. In these pathways, mtROS generation boosts TGF-β expression in airway epithelium in response to allergen challenge.[10 26] TGF-β, as a major growth factor, is implicated in lung fibrosis and remodeling (figure 1).[10 26]

EOSINOPHILS AND ASTHMA

Eosinophils, as innate immune system cells, are involved in the pathogenesis of allergic asthma. Eosinophil account (forming approximately 3% of white blood cells in healthy individuals) is elevated in allergic asthma. Eosinophils, as important cells in asthma progression and airway remodeling, can be activated by IL-5 and then migrate to allergic airways where activated eosinophils release proinflammatory and lipid mediators and toxic proteins (from their granules), which are able to induce bronchoconstriction, mucus hyper-secretion, airway thickening, and airway epithelium damage. The longevity of eosinophils may be enhanced by IL-5, IL-3, and GM-CSF.[187–190] Indeed, IL-5 prevents Bax translocation and cytochrome C release and extends eosinophil survival by inhibiting mitochondrial membrane perturbation and caspase activation.[191] Understanding the pathways involved in eosinophils' survival and apoptosis is extremely important for recognizing the pathogenesis of eosinophilic inflammation in allergic asthma and for developing novel drugs to treat asthma.

Cellular apoptosis is executed via two different pathways, extrinsic (receptor-mediated) and intrinsic (mitochondrion-centered). The extrinsic pathway is actually an immunological process initiated by the ligation of death receptors (Fas/CD95). These receptors lead to the formation of DISC protein that governs the activation of initiator Caspase-8 that then directly activates effector caspases, executing apoptosis or cleaving Bid to create an additional mitochondrial loop. Bcl-2 family proteins have critical roles in monitoring intracellular damage and stress (such as oxidative stress, overload of cytosolic Ca^{2+}, DNA damage, etc.) and

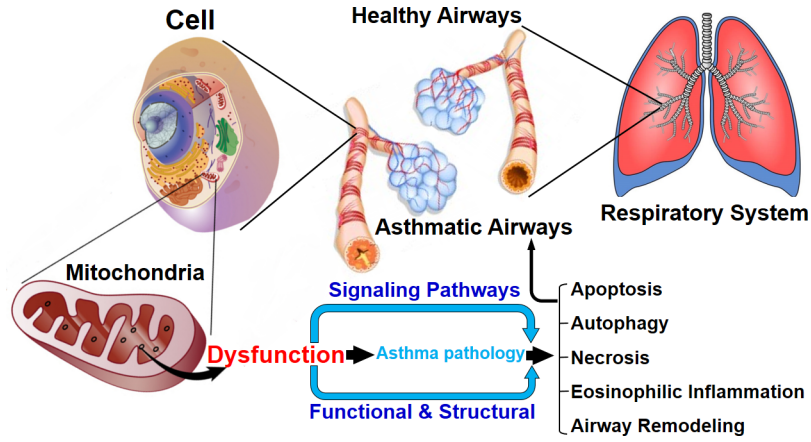

Figure 1 Mitochondria and asthma. Asthma is a major respiratory system disease that involves airways in the lung. Epithelial, goblet, smooth muscle, and immune cells are influenced in asthma. Mitochondria of airway cells are involved in asthma pathophysiology and have main roles in the health of the respiratory system, especially in bronchia, by influencing airway remodeling, fibrosis, eosinophilic inflammation, and cell apoptosis, necrosis, and autophagy, which can be regulated by both cellular and mitochondrial signaling pathways.

activate pore-forming Bax and MMP, as the main triggers of apoptosis. Also, MMP can be mediated through mitochondrial permeability transition (mPT), loss of mitochondrial membrane potential ($\Delta\Psi m$), ATP synthesis suppression, and the release of pro-apoptotic factors such as cytochrome C to the cytosol. Cytochrome C leads to apoptosome formation, which activates initiator Caspase-9 that itself then induces effector caspases 3, 6, and 7 and finally apoptosis (figure 2).[190 192 193]

Eosinophils have a pivotal role in asthma pathogenesis, and delayed eosinophil apoptosis has been reported to participate in asthma pathogenesis. Eosinophil accumulation and persistence at inflammation sites are mediated by IL-5. In fact, eosinophils rapidly undergo apoptosis unless they are exposed to IL-5.[191 194 195] As noted before, interactions between pro-apoptotic (Bak, Bax, Bim, Bik) and anti-apoptotic (Bcl-xL, Bcl-2) proteins control apoptotic pathways, mitochondria-centered reactions, and activation of caspases (as conserved death proteases of cells). Bcl-2 expression in the eosinophils of asthmatic patients is increased, and IL-5 has been reported to upregulate Bcl-2 expression, which is responsible for the pro-survival role of IL-5 and its possible regulatory impacts on other anti-apoptotic molecules such as Bax and Bcl-xL. Pro-apoptotic Bcl-2 homologue, Bid, is a target of Caspase-8 via a mechanism involving an interaction with Bax, leading to the release of cytochrome C, as an apoptosis initiator, and other stress mediators such as AIF and Smac/Diablo from mitochondria. Also, COXETC is a main oxidative enzyme in mitochondria and catalyzes electron transfer for ATP

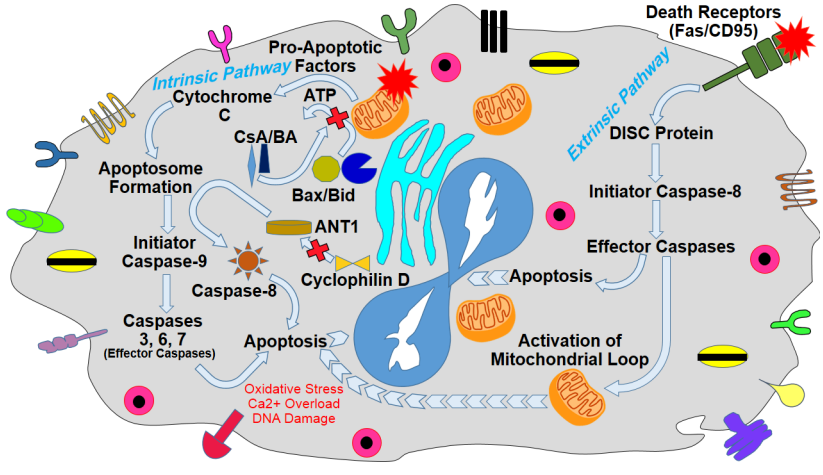

Figure 2 Mitochondrion and eosinophil apoptosis. Cell apoptosis is executed via the extrinsic (ie, receptor-mediated) and intrinsic (ie, mitochondrion-centered) pathways. The extrinsic pathway is activated by the ligation of the Fas/CD95, which leads to the formation of DISC protein and that regulates the activation of the initiator Caspase-8, as well as effector caspases, that to either execute apoptosis or activate the mitochondrial loop. In the intrinsic pathway, changes in the mPT changing forces mitochondria to release pro-apoptotic factors such as cytochrome C to the cytosol. Cytochrome C leads to apoptosome formation, which activates the initiator Caspase-9 and also caspases 3, 6, and 7, that stimulate apoptosis. Eosinophils, as important cells in asthma exacerbations and airway remodeling, can be activated and migrated directed by IL-5. This cytokine prevents cytochrome C release and apoptosis in eosinophil apoptosis. Thus, mitochondrial dysfunction is associated with asthma severity.

generation via oxidative phosphorylation.[9 196–198] Cytochrome *c* release from mitochondria leads to "apoptosome" formation, a caspase-9–activating complex comprising cytochrome C, Apaf-1, procaspase-9, and dATP. The activation of "initiator" caspases (ie, 8 and 9) results in the direct or indirect activation of "effector" caspases (ie, 3, 6, and 7).[196 199] It was shown that IL-5 inhibited eosinophil spontaneous apoptosis via preventing Bax translocation, cytochrome C release, mitochondrial membrane perturbation (by enhancing mitochondrial membrane permeability), and finally the activation of the caspase cascade,[191 194] highlighting an important role for this cytokine in promoting eosinophil survival.

The IL-5 receptor induces rapid tyrosine phosphorylation and juxta membranous tyrosine kinase activation. The signal is initiated via the Jak2/STAT and Ras-Raf-1-MAPK cascades. The activation of the Jak2, Syk, and Lyn tyrosine kinases and SHPTP-2 phosphatase is critical for IL-5–induced eosinophil survival. SHPTP-2 activation and its association with Grb2, as an adaptor protein, couple the IL-5 receptor to the Ras signaling pathway, followed by the requirement of Raf-1 serine/threonine kinase to promote IL-5–mediated anti-apoptosis actions.[189 191] This pathway has important therapeutic implications for asthma and the diseases characterized by eosinophil-mediated inflammation. Apoptosis in eosinophils can be accelerated by Fas activation. Fas ligand is a significant pro-apoptotic factor, and the neutralization of the Fas–Fas ligand complex enhances airway eosinophilia in allergic asthma.[189 190 200] Therefore, this pathway can also be another therapeutic target to suppress eosinophilic inflammation in asthma.

Eosinophils, by releasing their granules' proteins, lipid mediators, and pro-inflammatory components, contribute to asthma exacerbation and airway remodeling. The eosinophils derived from the blood of asthmatic patients showed delayed apoptosis when compared with those of healthy people.[201–203] Apoptosis is characterized by nuclear coalescence, mitochondrial changes, cell shrinkage, and DNA fragmentation. Interestingly, NO induces eosinophil apoptosis and regulates eosinophilic inflammation via promoting a pro-apoptotic effect through regulating JNK and caspases 6 and 3. Also, NO stimulates mPT which is a Ca^{2+}-dependent and voltage-dependent channel in MIM, leading to changes in mitochondrial membrane potential ($\Delta\Psi m$), mitochondrial release of cytochrome C and other pro-apoptotic factors to cytosol, and finally apoptosis initiation. In asthma, however, these mechanisms have insignificant roles in inducing eosinophil apoptosis. Nevertheless, the manipulation of these apoptotic pathways can reduce eosinophil survival and lung inflammation. Furthermore, NO-induced apoptosis in eosinophils via activating the JNK route may have therapeutic implications in asthma.[201 204 205]

Eosinophils play an important role in the pathogenesis of asthma, and delayed eosinophil apoptosis has been associated with asthma progression. IL-5 leads to the accumulation and persistence of eosinophils in asthmatic airways and helps eosinophils evade apoptosis.[191 206] Thus, mitochondria in asthmatic patients' immune cells, such as eosinophils, can play important roles in modulating asthma course, urging the development of mitochondrial-directed therapeutics for this disease. Investigating novel immune cell regulatory mechanisms that underlie asthma pathophysiology can lead to the development of effective target-based asthma therapies.

BCL-2 HOMOLOGUES OF EOSINOPHILS

Susceptibility to programmed cell death is influenced by the ratio of death agonists to antagonists and subsequent heterodimerization and homodimerization of death-related proteins via conserved BH3 domains. Bcl-2 homologues act as the critical regulators of the apoptotic pathway and via interactions between pro-apoptotic (Bax, Bak, Bik, Bim) and anti-apoptotic (Bcl-2, Bcl-xL) proteins control the apoptogenic factors released from mitochondria and subsequent activation of caspases (ie, conserved cell death proteases).[191 192]

Eosinophils endogenously express pro-apoptotic Bax and anti-apoptotic Bcl-xL at high levels while the level of anti-apoptotic Bcl-2 is low in these cells. Evidence shows a higher Bcl-2 expression in the eosinophils derived from asthmatic patients. In IL-5–stimulated eosinophils, Bcl-2 is increased, extending the survival of these immune cells.[191 198]

During apoptosis, Bax, as a monomeric cytosolic protein, translocates from the cytosol to the outer mitochondrial membrane where it facilitates cytochrome C releasing and subsequent caspases' activation. Caspases, as aspartate-specific cysteine proteases, govern the final apoptotic phase.[191 198]

The receptor-mediated apoptotic pathway involves the ligation of death receptors (CD95 (Fas/Apo-1) and tumor necrosis factor receptor-1) on the plasma membrane, followed by Caspase-8 recruitment to the receptor complex. The proapoptotic Bcl-2 homologue, Bid, is the target of Caspase-8 in this pathway, which after proteolysis is translocated from the cytosol to mitochondria to interact with Bax and trigger cytochrome C release.[191 198 207] In this apoptotic route, cellular signaling mediators induce perturbation in mitochondrial membrane potential to allow for the release of apoptosis adaptors (cytochrome C, AIF, and Smac/Diablo) and apoptosome formation. Finally, the activation of caspases 8 and 9 induces the effector caspases of 3, 6, and 7.[191 196 198 199 207]

MITOCHONDRIA, OBESITY, AND ASTHMA

Body mass index has a positive correlation with severity of asthma. Therefore, mitochondria can have roles in the pathogenesis of obesity-associated asthma. Mitochondrial dysfunction causes metabolic syndrome and obesity, and the role of mitochondria in the pathogenesis of asthma in obese individuals would be a relatively new concept.[17 167 208]

Metformin has been noted to show anti-asthma potentials and attenuate inflammatory reactions in obese individuals. In patients with COPD, metformin reduces dyspnea and modulates the let-7/lin28 axis, which is a critical contributor to the determination of cellular OxPhos capacity, tissue repair, and aging. Let-7 miRNA was implicated in asthma pathogenesis.[21 209–212] In this regard, miRNAs, as mitochondrial function modulators, appear in the two classes of hypoxamirs and mitomirs. Hypoxamirs are induced in hypoxic conditions and are relevant to asthma. MiR-210, as the best known hypoxamir, is inducible by nuclear NF-κB and is increased in severe asthma. It is noteworthy that ISCU 1/2, which activates redox reactions in mitochondria, and

Foxp3, a transcription factor of T-reg cells, are among the direct targets of miR-210, supporting a pro-inflammatory state during chronic hypoxia.[21 213–215]

Mitomirs are specific miRNAs present in mitochondria and are linked to mitochondrial function and dynamics. Accordingly, miR-149 was reported to enhance mitochondrial biogenesis via inducing poly (ADP-ribose) polymerase-2, peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), and Sirt-1. In addition, MiR-761 and miR-30 are involved in mitochondrial fission, and MiR-183 inhibits OxPhos by targeting IDH2, which may be a relevant therapeutic target. Also, miR-183 downregulates the expression of Ca^{2+}-activated K^+ channels (BKCaβ1); a phenomenon that possibly increases the risk of pulmonary hypertension. The aerosol-based lung delivery of the antagonists of miR-210 and miR-183 has been promising for treating pulmonary hypertension and related diseases.[21 216–218]

Mitochondria transfer may be a beneficial mechanism to replace defective organelles. This can be accessible using methods such as microinjection, gap junctional channel-mediated cell attachment, intact purified mitochondria injection, incubation with mitocytoplasts, and direct transfer from donor to recipient cells via cytoplasmic bridges (called TNT). For efficient penetration of mitochondria into cells, some cell-penetrating peptide-based tags have been effective in next-generation naked mitochondrial transfer techniques. Damage to mitochondria can enhance inflammation and trigger a potent innate immune response; therefore, these organelles should be carefully isolated.[21 219–221] It was found that Miro1, as a calcium-sensitive GTPase, regulates intracellular mitochondrial transport via TNT, suggesting a potential therapeutic target for effective mitochondria transport to treat asthma. Pluripotent stem cell-derived MSCs may be used as rich sources of mitochondria.[21 222 223]

ASTHMA, NO, AND ARG

High levels of FENO are generated by iNOS, and arginine is also catalyzed to NO and citrulline in the epithelium of asthmatic airways. Although iNOS expression and FENO elevation in asthma promote inflammatory injury and AHR, arginine-analog iNOS inhibitors have shown low efficacy in treating asthma. High activity of iNOS and elevated levels of arginine induce ARG to catabolize arginine to ornithine and urea, which both are increased in the plasma of patients with asthma. ARG1 is exclusively present in the cytosol of hepatic cells (participating in the urea cycle), but ARG2 is found in the mitochondria of many tissues. Surprisingly, ARG2 gene variants lie within an asthma-linked region on chromosome 14q24 and are strongly associated with asthma severity. The function of ARG2 in mitochondria is unknown; however, it may be involved in arginine metabolism to support the bio-energetic state. In asthma, ARG2 seems to provide endogenous arginine for NO synthesis.[224–228]

The provision of oxidative intermediates by the arginine/ornithine metabolic pathway in mitochondria may have important consequences for inflammation signal transducers in bronchi. Adaptive transcriptional responses to the TCA cycle are mediated by HIFs. Prolyl hydroxylases use oxygen and αKG for the catalytic hydroxylation of HIFs, targeting these factors for degradation. HIF-1α is expressed ubiquitously while HIF-2α expression is restricted to certain tissues. In inflamed allergic airways, HIFs are in part regulated by IL-13 production. IL-13 induces the typical features of asthma (ie, mucin hyper-secretion and eosinophil recruitment to airways). The majority of IL-13–mediated downstream inflammatory effects are promoted through STAT6. Elevated arginine metabolism through ARG2 increases oxidative metabolism and contributes to signal transduction via HIF and STAT6, suppressing bronchial inflammation and reducing asthma severity.[224 229–232]

In particular, arginine metabolism is interconnected with allergy, metabolic processes, and mitochondrial function. ADMA is an endogenous uncoupler of nitric oxide synthase and is strongly associated with metabolic processes in obesity and asthma. On the other hand, IL-4 modulates ADMA metabolism. The synergistic action of IL-4 and ADMA reduces the number of mitochondria in cells and increases mtROS via prompting cellular hypoxic responses and consequently downregulating TFAM and PGC 1α. Also, the mitochondrial haplogroup U and mitochondria-encoded genes' polymorphisms have been associated with increased IgE production and asthma.[153 224 233 234] Altogether, the transport of ornithine into the mitochondrion contributes to the de novo synthesis of arginine for NO production, modulates the redox state, and suppresses the pathological HIF signaling events, which lead to IL-13 and STAT6 activation. Allergens and air pollutants cause lung inflammation in part through mtROS, and mitochondrial antioxidant coenzymes, Q10 and Mito Q, can be effective to reverse this pathogenic event during mitochondrial transfer therapy.[224] To correct mitochondrial dysfunction in asthma, three main clinical interventions could be applied, including the repair, reprogramming, or replacement of the defected organelle. Therefore, understanding the inflammation pathways affecting mitochondria can provide new potential targets to cure asthma. Induced pluripotent stem cells and MSCs may be applicable to introduce healthy mitochondria to replace dysfunctional ones.

ASTHMA AND GLYCOLYSIS

Evidence indicates that EAR (including hypoxia and ASM contraction) and glycolysis are interrelated with each other.[6 235–237] Glycolysis is a key regulatory process in EAR pathogenesis. Phosphoglycerate kinase 1, as a glycolytic pathway enzyme, catalyzes the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglyceric acid, yielding a single ATP molecule. Phosphoglycerate kinase 1 also enhances glycolytic capacity and helps preserve cellular energetics during hypoxia. Pyruvate kinase, another enzyme in this pathway, transfers a phosphate group from phosphoenolpyruvate to ADP, producing ATP and pyruvate. This energy regeneration pathway is independent of oxygen supply and allows cells to survive under hypoxic conditions.[238 239] Glyceraldehyde-3-phosphate dehydrogenase is another essential glycolytic enzyme that converts glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate during glycolysis. The enhanced expression of all the mentioned proteins in asthma suggests the upregulation of glycolysis during EAR. Beta-enolase and phosphoglycerate mutase are two other glycolytic enzymes found in lung

proteome and are involved in the regulation of glycolysis rate. Decreased expression of these enzymes can modulate glycolysis rate and the ATP-dependent reactions involved in EAR. These glycolytic enzymes facilitate the anaerobic production of ATP and increase cellular energy demands on ASM contraction induced by airway obstruction during hypoxia. It should be noted that glycolysis only provides two ATP molecules while lactic acid and oxidative phosphorylation, under hypoxic conditions, are more efficient energy-supplying processes.[6 240]

Glycolytic enzymes may share a role in the hyperplasia or hypertrophy of ASM in asthma. During EAR, glycolytic proteins may contribute to airway remodeling, and the formation of $Ca2^{+}$/calmodulin complex can activate the myosin light-chain kinase that, in turn, mediates ASM contraction. Cells contain a variety of Ca^{2+}-binding proteins (CaBPs) that regulate cytosolic Ca^{2+} level and Ca^{2+}-mediated intracellular signal transduction. S100 proteins are highly homologous low-molecular weight proteins constituting a CaBP family. Two members of this family, S100A8 and A9, also known as calgranulins, play a role in the initiation and progression of asthma through provoking a number of pro-inflammatory signaling pathways, including p38 or p44/42 MAPK and NF-κB. Consistently, S100A8 and S100A9 inhibition reduces inflammatory cells' migration to the lung in asthma. On the other hand, ERp29, as an ER lumen-resident protein, interacts with Ca^{2+}-binding chaperones on the mitochondrial membrane and indirectly influences Ca^{2+} transport. It has been established that a change in Ca^{2+} level can manipulate ASM contractility and mucous glands' and mast cells' secretory activity in asthma. Therefore, the agonist/antagonist-induced manipulation of Ca^{2+} signaling and homeostasis can be regarded as a possible therapeutic strategy to manage asthma. These findings suggest key roles for energy adaptation and Ca^{2+} signaling in the development and progression of asthma.[6 241–243]

AIRWAY REMODELING AND THE ROLE OF MITOCHONDRIA

Increased ASM mass and fibroblast dysfunction are two main characteristics of airway remodeling. In addition, the accumulation of dysmorphic mitochondria may be relevant to airway remodeling in asthma.[22 154 173 244] TGF-β is also involved in subepithelial fibrosis, ASM remodeling, and mucus production. This factor induces remodeling through inducing PRMT1 expression in pulmonary fibroblasts and may contribute to glucocorticoid resistance in asthmatic patients. In turn, PRMT1 can regulate post-translational arginine methylation, which plays important roles in intracellular signal transduction and extracellular biological interactions. Arginine methylation in histones and other proteins, catalyzed by PRMT1, is a relatively new identified protein modification and is implicated in intracellular signaling, DNA repair and integrity, protein–protein interactions, and gene expression regulation.[245–249] PRMT1 augments extracellular matrix deposition through ERK1/2-STAT1 signaling in ASM and fibroblasts and therefore contributes to mitochondria function. The TGF-β-activated signaling pathway boosts PRMT1 expression and leads to mitochondria dysfunction through the SMAD2/3, C/EBPb, PRMT1, PGC-1a signaling cascade. Both TGF-β1 and C/EBPb are upstream to PGC-1a and PRMT1 expression (figure 3).[154 250]

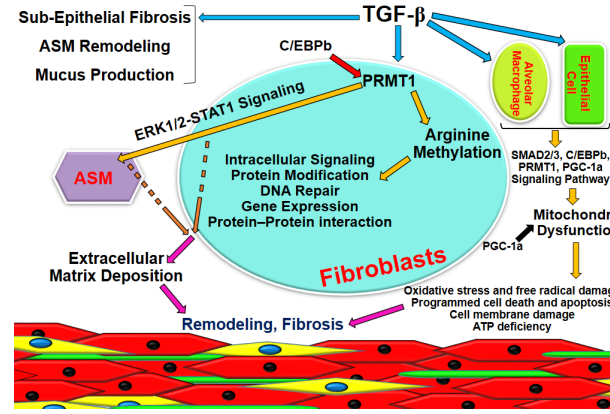

Figure 3 Airway remodeling and mitochondria. The accumulation of dysmorphic mitochondria is related with asthma and airway remodeling and will be done with increased ASM mass and fibroblast dysfunction. TGF-β induces PRMT1 expression in lung fibroblasts and can regulate post-translational arginine methylation which play important roles in signal transduction and events in the extracellular milieu. Arginine methylation displays a novel protein modification which is implicated in the intracellular signaling, DNA repair and processing, protein–protein interaction, and gene expression regulation. PRMT1 upregulates extracellular matrix deposition through ERK1/2-STAT1 signaling. The TGF-β-activated signal pathway augments PRMT1 expression and caused mitochondria dysfunction through the SMAD2/3, C/EBPb, PRMT1, PGC-1a signaling sequence. The TGF-β1 and C/EBPb are upstream of PGC-1a and PRMT1 expression. In the alveolar macrophages and epithelial cells, TGF-β1 overexpression induces mitochondria dysfunction that leads to remodeling of bronchi.

Mitochondria act as the powerhouse of cells by ATP generation, so their dysfunction is linked to the pathogenesis of chronic lung remodeling diseases, including asthma and COPD. Mitochondria modulate intracellular signaling pathways and are a source of intracellular ROS production, through which they can promote apoptosis. In the dust cells (ie, alveolar macrophages) and pulmonary epithelium of asthmatic patients, the overexpression of TGF-β1 induces mitochondrial dysfunction. As a matter of fact, TGF-β1 is an important mediator of sub-epithelial fibroblasts' differentiation and activation.[154 251–253] Therefore, TGF-β therapy to inhibit the production of Th2 cytokines may not be applicable for treating asthma, and this cytokine may be a powerful trigger of lung remodeling and other related problems in asthma.

THREE LEVELS OF MQC

Mitochondria are important organelles for energy generation and cellular apoptosis, signaling, and differentiation. MQC maintains the number of mitochondria, as well as their structure and functional integrity and contains three molecular, organellar, and cellular levels.[12 254–256]

Mitochondria maintain MQC at the molecular level by improving protein folding ability, reducing protein import, and clearing damaged and non-functional proteins. Nuclear DNA encodes most mitochondrial proteins. After synthesis in cytoplasm, these proteins are imported into mitochondria

Table 1 Three levels of mitochondria quality control

MQC	
Molecular level	Improving protein folding
	Reducing protein import
	Clearing non-functional proteins
Organelle level	Fusion
	Fission
	Mitophagy
	Motility
Cellular level	Apoptosis
	Cell death

after correct folding. In the cells that are under stress, the proteins entering mitochondria are not properly folded, which causes unfolded/misfolded protein accumulation in the matrix, triggering mitochondrial UPR. In dysfunctional mitochondria, GCN2 (an EIF2α kinase) is activated, which decreases protein translation, including TiM17A and TiM23, the main subunits of MIM channels, reducing protein import into mitochondria. When mitochondrial protein import efficiency is declined, ATFS-1 is translocated into the nucleus where it upregulates the expression of mitochondrial matrix chaperones and proteases, such as ClpP and HSP60/10. Hsp60/10 helps newly imported proteins to fold properly, and ClpP is responsible for the degradation of misfolded, unfolded, and damaged proteins (table 1).[257–259]

At the organellar level, MQC regulates mitochondrial dynamics to ensure intact mitochondrial fusion and fission, motility, and mitophagy. Mitochondria maintain a dynamic balance between fusion and fission. Under mild stress, mitochondria undergo fusion with the help of MFN1/2 and OPA1 proteins to form a more functional organelle. MFN1/2 on the MOM is responsible for the fusion of outer membranes, and OPA1 on the IMS is responsible for the fusion of MIMs. Mitochondria division increases the number of these organelles under normal conditions and on exposure to severe stress. For this purpose, phosphorylated Drp1 is recruited to the MOM of damaged mitochondria, directing the division process, followed by the removal of these organelle remnants by mitophagy.[260–262] In addition, under abnormal environmental circumstances, mitochondria move to a suitable location, mediated by a MOM receptor, Miro, which is a Rho-GTPase molecule (containing two Ca^{2+}-binding EF-hand motifs and two GTPase domains) and facilitates mitochondria movement.[12 263]

Under severe stress, mitochondria initiate the apoptotic pathway to ensure MQC at the cellular level. Bax and Bak, two pro-apoptotic proteins of the Bcl-2 family, are oligomerized in the MOM and contribute to mPTP opening to maintain MQC under lethal stress. The mPTP is a multi-protein channel consisting of VDAC, cyclophilin-D, and ANT. When mPTP opens, cytochrome C and AIF, as pro-apoptotic factors, are released into cytoplasm where cytochrome C forms apoptosomes with the participation of Apaf-1 to activate Caspase-9 and subsequently other downstream apoptotic factors (caspase 3/7) and mediate programmed cell death. On the other hand, AIF migrates to the nucleus, leading to chromatin fragmentation and DNA degradation, and resulting in cell death.[264–266]

MITOCHONDRIA TARGETING

Mitochondrial dysfunction plays important roles in the genesis of lung diseases, and the impacts of these dysfunctional organelles can be addressed mainly through three strategies: scavenging ROS, reprogramming regulatory pathways, and replacing damaged mitochondria by healthy exogenous substitutes.[21]

For reversing the function of defected mitochondria by scavenging ROS, universal antioxidants such as vitamin E are beneficial. CoQ10, as a strong mitochondrial antioxidant, is conjugated with a triphenylphosphonium cation group, which leads to mitochondrial accumulation and prevents oxidative damage to this cellular component.[21 267] Mito-TEMPO is a triphenylphosphonium-like mitochondria-targeted antioxidant and can protect mitochondria during CS exposure and preserve their function. Tiron is another mitochondria-targeted antioxidant and effective in repairing dysfunctional mitochondria in COPD and after exposure to ozone. Mito-TEMPO attenuates mitochondrial production of ROS and collagen deposition in airway cells during allergic inflammation. Antioxidants like vitamins E and C positively interfere with mitochondrial signaling and biogenesis. Nrf2, as an antioxidant defense transcription factor, exhibits a cytoprotective role in the lung exposed to oxidative stress. Sulforaphane, as a prototypical Nrf2-stimulated molecule, enhances mitochondrial antioxidant defense against a variety of oxidant stressors in respiratory diseases. Nevertheless, Nrf2-inducing agents, when used as potential therapeutics, have been associated with increased risk of cancer.[21 24 268–273] PQQ, a cofactor in the bacterial respiratory pathway, stimulates mammalian mitochondrial biogenesis through a PGC1-α-dependent manner and acts as a powerful mitochondrial antioxidant (1000-fold more powerful than vitamin C). Therefore, PQQ may be beneficial to treat the diseases associated with oxidative stress (eg, asthma), at least partly through repairing mitochondrial function.[21 274 275]

THERAPEUTIC POTENTIAL OF MITOCHONDRIAL TARGETING

The antioxidants specifically targeting mitochondria, such as MitoQ, accumulate in negatively charged mitochondria and suppress ROS production, protecting the organelle against oxidative damage. Another mitochondria-specific antioxidant, mito-TEMPO, are also effective in blunting fibrosis in asthma. MitoQ and Mito-TEMPO help restore mitochondrial function. In association with yet another mitochondria-targeted antioxidant, Tiron, MitoQ reverses mitochondrial dysfunction in the ASM of patients with asthma. Therefore, modulating mitochondrial fission/fusion balance and biogenesis is applicable to control inflammation in allergic asthma. A potential limitation of this approach is that fission and fusion are dynamic processes and even under normal conditions are intricately connected to mitochondrial motility and metabolism, as well as downstream cellular signaling pathways. So, any deviation in the fission/fusion balance can result in unpredicted side effects. It is also noteworthy that mitochondrial damage may not always be evident or result in cell death in vivo, and some levels of damage can stimulate mitochondrial biogenesis.[21 22 273 276 277]

ASTHMA AND MITOCHONDRIAL-DIRECTED THERAPIES

Mitochondrial-directed therapeutics are relatively new, and understanding their role in asthma treatment needs more studies. Mitochondria are sources of ROS and susceptible to oxidative damage (ie, the oxidation of mtDNA, proteins, Coenzyme Q reductase (complex I), and other respiratory complexes), resulting in impaired electron transport chain and increased production of mtROS. Antioxidants can particularly protect mitochondria against oxidative damage. As mentioned, MitoQ, as a mitochondria-targeted antioxidant composed of ubiquinone covalently linked to a cationic moiety, has promised a highly effective therapeutic agent and is able to selectively accumulate within the MIM of negatively charged mitochondria. MitoQ quenches ROS, including superoxide and peroxynitrite, and protects mitochondria against oxidative damage.[10 278 279] Studies suggest that mitochondrial-directed therapies can alleviate lung remodeling. In line, mito-TEMPO, as a mitochondrial-directed antioxidant, was shown to decrease TGF-β activity, a key member of the pulmonary pro-fibrotic signaling pathway.[10 26]

NATURAL ANTIOXIDANTS

Various natural compounds have been reported to protect mitochondria against oxidative damage, such as coenzyme Q, glutathione, α-lipoic acid, α-tocopherol, pyruvate, creatine, acetyl L-carnitine, and choline, which have demonstrated beneficial effects in asthma. Coenzyme Q10 supplementation, as a mitochondria-targeted antioxidant, can reduce the required dose of corticosteroids in asthma. Further, coenzyme Q10 restores eNOS activity and reduces free-radical formation. Also, α-tocopherol reduces mitochondrial dysfunction in asthma, and α-lipoic acid was noted to promote its anti-asthmatic effects by inducing PPAR-δ expression. The PPAR-α/PGC-1α pathway can improve mitochondrial bioenergetics.[280–283] Resveratrol has also been described to improve mitochondrial function in asthma by activating SIRT-1 and SRT1720; while SIRT-1 induces mitochondrial biogenesis via a PGC-1α-dependent route, SRT1720 mitigates the symptoms of allergen-induced inflammation in airways. Resveratrol also increases INPP4A expression, whose role has been documented in asthma.[17 284–290] Although various antioxidants have shown beneficial effects on mitochondrial function and asthma symptoms, their applicability as therapeutic agents needs to be established in future clinical trials.

TARGETING MITOCHONDRIAL REGULATING FACTORS

As two critical phospholipids, CL and PA participate in mitochondrial dynamics. PA is synthesized in the ER and then is transported to the OMM. A portion of PA is converted to CL, which is the major lipid of the IMM. The accumulation of PA augments Mfn-1/2-dependent OMM fusion via Opa-1 conjunction. GTP hydrolysis and Drp-1 oligomerization rearrange the liposome membranes containing CL to form a CL-enriched membrane region that facilitates scission. Also, CL triggers GTPase activity and Opa-1 assembly, resulting in the tubulation and constriction of the liposomal membrane. During fission, actin reorganization and myosin II activity are regulated by the balance between the production and catabolism of PA. Thus, a potential link between Mito-PLD, phosphatide phosphatase-1 action, phosphatidic acid-preferring phospholipase A1 activity, and actin/myosin II-regulated mitochondrial constriction has been proposed to be important in ASM and asthma.[291 292] Dysmorphic mitochondria have been observed in the ASM and epithelium of patients with asthma. Increased baseline respiration and mitochondrial mass are associated with elevated cytosolic Ca^{2+} levels, contributing to AHR and cellular proliferation in asthma. Also, the morphology of fused mitochondria is influenced by the molecular mechanisms governing steroid-resistant allergic airway inflammation. The accumulation of fragmented mitochondria induces the cleavage of Caspase-9 and Caspase-3 and potentiates the mitochondrial apoptotic pathway. So, mitochondria-targeted antioxidants reduce ROS-induced toxicity against this organelle and are emerging therapeutics to treat asthma.[291 293]

Microtubule-dependent movement is a major mechanism of mitochondrial transport. In addition, half of motile mitochondria move anterograde. Several proteins are involved in anchoring mitochondria to microtubules, such as Rho GTPases, Miro 1/2, TRAK1, and TRAK2. During this process, Milton1/2 binds to Miro-proteins and motor-proteins, and TRAK1 attaches to dynein and kinesin (two motor proteins). On the other hand, TRAK2 binds to dynein only for retrograde movements.[294 295] KIF5 is a key motor regulator of mitochondrial trafficking in neurons. Mammals have three KIF5 isoforms. Other proteins, such as synaptabulin, RAN binding protein 2, and fasciculation and elongation protein zeta 1 (FEZ1), interact with KIF5 and mediate anterograde mitochondrial trafficking. The Miro-TRAK1/2-dynein complex is critical to avoid mitochondria accumulation. Moreover, Arp11/Arp10p complex acts as an important mediator for the retrograde movements of mitochondria. Loss of either Miro or Mfn2 halts the spread of dysfunctional mitochondria. Also, disturbed Ca^{2+} homeostasis decreases mitochondrial motility. Abnormal turnover is an important aspect of cell dysfunction, and the modulation of mitochondria transport in the lung is necessary for designing effective therapeutic strategies against asthma.[294 295]

PGAM is an important enzyme in gluconeogenesis and glycolysis that converts 3-phosphoglycate to 2-phosphoglycate. This enzyme also participates in regulating programmed cell death and mitochondrial dynamics through its Ser/Thr/His phosphatase activity. In addition, PGAM5 modulates immune responses such as NKT-mediated inflammation and promotes IL-1β-induced reactions via caspase-1-related inflammasomes. At the cellular level, PGAM5 silencing inhibits inflammation and necrosis. As a phosphohistidine phosphatase, PGAM5 can specifically bind to and dephosphorylate NDPK-B (at H118), inhibiting NDPK-B-mediated histidine phosphorylation. This phosphatase further activates K^+ channels and negatively regulates $CD4^+$ T cells via NDPK-B dephosphorylation. It has been noted that PGAM5 inhibition reduces necroptosis by suppressing Drp1. Exploring the role of PGAM5 in asthma and airway injury may offer a new anti-asthma treatment strategy.[296 297]

Changes in the function and morphology mitochondria result in decreased ATP production, increased ROS levels, and reduced electron transport chain activity. Mitophagy, as a selective autophagy to eliminate impaired mitochondria,

plays an important protective role against inflammation and necroptosis that are fundamental pathological changes in asthma and lung injury. Overall, mitophagy can influence several pathological features of lung diseases such as asthma, and recognizing the molecules and genes involved in this process can provide novel therapeutic targets for treating asthma.[298–300]

CONCLUDING REMARKS

Normally, mitochondria contribute to cellular metabolism beyond production of energy. Functionally and structurally disturbed mitochondria have important roles in asthma pathophysiology, suggesting them as interesting therapeutic targets that have already been embraced in pulmonary medicine. Mitochondrial defects have been recognized in different cells and a variety of chronic respiratory diseases. Therefore, modulating mitochondrial function via agents that selectively regulate mitochondrial biogenesis and repair mitochondrial dysfunction can prevent lung remodeling in asthmatic patients. Mitochondrial defects may facilitate lung remodeling and fibrosis and promote airway cells; apoptosis. Further studies are required to understand the potential therapeutic role of modulating mitochondrial signaling pathways via mitochondria-targeting agents in asthma.

Asthma is an extremely heterogeneous syndrome engaging a variety of cellular mechanisms and molecular endotypes. However, we should focus on a number of specific and most important mechanisms to design effective therapeutics for the successful management and control of pathobiological pathways in asthmatic patients. Studies suggest a novel mitochondria-based framework (intercalated with immune/inflammatory processes) for developing specific anti-asthma medications to target the molecular signaling pathways of this organelle. The present review sheds light on the important role of mitochondria in asthma pathophysiology and presents novel insights into the current knowledge in this field.

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