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Mitochondrial biology in airway pathogenesis and the role of NRF2

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Abstract

A constant improvement in understanding of mitochondrial biology has provided new insights into mitochondrial dysfunction in human disease pathogenesis. Impaired mitochondrial dynamics caused by various stressors are characterized by structural abnormalities and leakage, compromised turnover, reactive oxygen species overproduction in mitochondria as well as increased mitochondrial DNA mutation frequency, which leads to impaired energy production and modified mitochondria-derived cell signaling. The mitochondrial dysfunction in airway epithelial, smooth muscle, and endothelial cells has been implicated in diseases including chronic obstructive lung diseases and acute lung injury. Increasing evidence indicates that the NRF2-antioxidant response element (ARE) pathway not only enhances redox defense but also facilitates mitochondrial homeostasis and bioenergetics. Identification of functional or potential AREs further supports the role for Nrf2 in mitochondrial dysfunction-associated airway disorders. While clinical reports indicate mixed efficacy, NRF2 agonists acting on respiratory mitochondrial dynamics are potentially beneficial. In lung cancer, growth advantage provided by sustained NRF2 activation is suggested to be through increased cellular antioxidant defense as well as mitochondria reinforcement and metabolic reprogramming to the preferred pathways to meet the increased energy demands of uncontrolled cell proliferation. Further studies are warranted to better understand NRF2 regulation of mitochondrial functions as therapeutic targets in airway disorders.

Keywords

lung; antioxidant response element; metabolism; sulforaphane

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Conflict of Interest

All authors declare that they have no conflict of interest.

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Redox-based therapeutic target Nrf2 in human diseases

Introduction

Nuclear factor, erythroid 2 like 2 (NFE2L2) or NF-E2-related factor 2 (NRF2) is a transcriptional activator of antioxidant response element (ARE)-bearing genes encoding antioxidant, drug-metabolizing, glutathione-homeostasis enzymes as well as many other host defense proteins. For this, NRF2 dimerizes with other transcriptional activators including small Maf proteins (MafF, MafG, MafK). NRF2 homeostasis is regulated by a cytoplasmic inhibitor Kelch-like ECH-associated protein 1 (KEAP1) under housekeeping proteolytic demands or by exogenous stimuli such as oxidants, xenobiotics, carcinogens as well as antioxidants and chemopreventive agents (Taguchi et al. 2011). During the last two decades, *in vitro* and transgenic murine model (Cho 2013) studies revealed that NRF2 contributes to a broad spectrum of cellular functions including redox balance, cell cycle and death, immunity, metabolism, selective protein degradation, development, aging, and carcinogenesis.

Mitochondria are dynamic and multifunctional organelles that produce ATP and many biosynthetic intermediates by means of oxidative phosphorylation (OXPHOS) in response to cellular bioenergetic and biosynthetic demands. In this process, electrons liberated from reducing substrates are delivered to oxygen via respiratory proton (H^+) pumps in complexes I-IV to establish a H^+ gradient across the inner mitochondrial membrane, and electrochemical energy of this gradient is used to complete ATP synthesis by complex V (ATP synthases). Importantly, the mitochondrion is the only non-nuclear organelle bearing its own genome which encodes 13 polypeptides of the OXPHOS subunits and respiratory chain as well as two ribosomal RNAs and 22 transfer RNAs necessary for translation of polypeptides inside mitochondria in humans and mice. In addition, mitochondria are the major source of endogenous reactive oxygen species (ROS) production. Mitochondria also provide temporal storage of calcium ion (Ca^{2+}) and are essential in cellular calcium homeostasis. Other important metabolic reactions that occur in mitochondria include steroid hormone and porphyrin synthesis, urea cycle, lipid metabolism, and interconversion of amino acids. Recent research has provided enormous insights into the molecular mechanisms underlying mitochondrial bioenergetics (ATP production) as well as biogenesis (increase in mitochondrial mass by *de novo* generation), fusion (mixing contents within a mitochondria population) and fission (binary division of mitochondria) in relation to mitophagy (lysosome-dependent selective degradation of defective mitochondria) as quality control programs. Maintenance of mitochondrial homeostasis and functions are critical components of mitochondrial health and interruption of these features are key determinants in aging and in the pathogenesis of neurodegenerative disorders, cardiac ischemia, type 2 diabetes, glaucoma, cancer, and other diseases (Ashar et al. 2017; Yue et al. 2018).

Mitochondria are constantly recycled as damaged or aging mitochondria undergo mitophagy for which PTEN-induced kinase 1 (PINK1), a mitochondrial serine/threonine-protein kinase, renders parkin (PARK2, E3 ubiquitin ligase) to bind to depolarized mitochondria to form autophagosome to be self-digested and replaced (Pickles et al. 2018). Mitophagy impacts various cellular process and diseases and may be either protective or deleterious. Biochemical changes in dysfunctional mitochondria include impaired OXPHOS subunits and energy production, diminished membrane potential, altered metabolic pathways such as

glycolysis and fatty acid β-oxidation (FAO), disturbed calcium homeostasis, and increased ROS production. Dysfunctional mitochondria also change their physical morphology or lose their membrane integrity and may allow leakage of mitochondrial components including damage-associated molecular pattern (DAMP) molecules. DAMPs from mitochondria are mitochondrial DNA (mtDNA), ATP, mitochondrial transcription factor A (TFAM or mtTFA), succinate, cardiolipin, and N-formyl peptides [See review (Nakahira et al. 2015)]. Mitochondrial dysfunction can lead to necrotic and/or apoptotic cell death and interrupt cell proliferation (Ni et al. 2015). Dying cells (mostly necrotic) release DAMPs which alarm and activate the innate immune system (Venereau et al. 2015).

Mitochondrial numbers and morphology vary according to cell type and demand, whereby the balance between mitochondrial fusion/fission regulates mitochondrial distribution, morphology, and function. Depending on the energy substrates available, energy demands, or the redox state, the cell may increase or decrease the number and size of mitochondria, and altered copy number by damage can result in mitochondrial dysfunction (Clay Montier et al. 2009; Mishra and Chan 2016). Compared to genomic DNA, mtDNA is more susceptible to mutation and ROS damage possibly in part due to the lack of histones and less efficient DNA repair mechanisms (Alexeyev et al. 2013). mtDNA point mutations [including small insertion/deletion (indel) mutations] and mutations of nuclear genes encoding respiratory chain subunit proteins constitute a significant cause of human diseases such as mitochondrial encephalopathy (Schapira 2012). Currently, more than 250 pathogenic mtDNA mutations have been identified in various diseases (Alston et al. 2017) and a pathogenic mutation (e.g., m.3243A>G, m.8344A>G) is associated with multiple clinical features (phenotype heterogeneity), making disease prognosis extremely difficult to predict (Orsucci et al. 2018). In addition, non-repaired, damaged mtDNA under oxidative environments may be fragmented and released as a DAMP into the cytosol and extracellular space (Venereau et al. 2015) to trigger innate immune responses through toll-like receptor 9, cyclic guanosine monophosphate-adenosine monophosphate (GMP-AMP) synthase, and/or the nod-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome (Banoth and Cassel 2018). As sources of ROS and reservoirs of antioxidant enzymes, mitochondria are critical organelles in ROS-dependent cellular redox pathways, which primarily connect mitochondria with NRF2.

Respiratory airways continuously interface a high oxygen environment for gas exchange and various environmental oxidants. In addition, the airway is a dynamic organ which constantly requires energy for various functions such as bronchial smooth muscle contraction, mucociliary clearance, and surfactant and mucus secretion. Therefore, airway cells place a large demand on mitochondrial bioenergetic functions. Recent studies have characterized the roles of mitochondrial dysfunction in diverse airway disorders. In this review, we profile the updated information on the role of NRF2 pathway in mitochondrial biology and discuss the association of NRF2 in airway pathogenesis.

NRF2 Regulation of Mitochondrial Function

Recent studies indicated that loss or genetic mutation of *NRF2* (or *Nrf2*) leads to oxidative stress-induced mitochondrial dysfunction and metabolic disorder (Mitsuishi et al. 2012;

Ludtmann et al. 2014; Kovac et al. 2015). Accumulating evidence has determined that NRF2-mediated nuclear control of mitochondrial function is directly through ARE-mediated transcriptional activation of mitochondrial antioxidant defense, bioenergetic process, mitophagy, and biogenesis as well as mitochondria-associated intermediary metabolism (Piantadosi et al. 2008; Abdullah et al. 2012; Mitsuishi et al. 2012; Hayes and Dinkova-Kostova 2014; Cho et al. 2019) as summarized in Table 1. In addition, the NRF2-KEAP1 axis is known to interact with various proteins for direct and indirect influence of NRF2 on mitochondrial function (Fig. 1). An oncogenic role for overexpressed NRF2 is thought to be in part through facilitation of mitochondrial function and metabolic reprogramming to meet the increased energy demand of rapidly growing cancer cells.

Redox homeostasis

During consumption of oxygen for cellular ATP production, mitochondrial electron transport chain of the OXPHOS complex transfers single electrons to oxygen to form ROS, mainly superoxide hydrogen peroxide (H_2O_2) by complexes I and III. Mitochondria contain their own antioxidant defense system to detoxify and minimize ROS. This mitochondrial redox buffering capacity is controlled precisely to avoid mitochondrial dysfunction and cell death. However, mitochondrial ROS are increased under various pathophysiological conditions caused by hypoxia, ischemia/reperfusion injury, chemical stress, drug treatment, genetic defects, or metabolic fluctuations. NRF2 directly regulates mitochondrial ROS homeostasis through ARE-mediated induction of antioxidant enzymes localized in mitochondria. These enzymes include superoxide dismutase (SOD2 or MnSOD) neutralizing superoxide, glutathione (GSH) and multiple thiol-homeostasis enzymes such as glutathione reductase (GSR), glutathione peroxidases (e.g., GPX1, GPX4), glutathione-S-transferases (e.g., GST-A1, GST-T1, GST-P1), glutaredoxin 2 (GRX2, also called thioltransferase 2), thioredoxin 2 (TRX2), thioredoxin reductase 2 (TXNRD2), peroxiredoxins (e.g., PRDX3, PRDX5) (Ribas et al. 2014; Ryoo and Kwak 2018). PGAM family member 5, mitochondrial serine/threonine protein phosphatase (PGAM5) in the mitochondrial outer membrane is known to form a ternary complex with KEAP1 and NRF2, and thus an intact PGAM5-KEAP1-NRF2 complex constrains KEAP1 and allows NRF2 to sense and respond to mitochondrial ROS (Lo and Hannink 2008).

NRF2 transcriptionally induces all the enzymes involved in the synthesis of cellular reducing agents, nicotinamide adenine dinucleotide phosphate (NADPH) and GSH, in both normal and oxidant-stimulated tissues including lung (Cho 2005). GSH is transported to mitochondrial matrix by carriers, 2-oxoglutarate carrier (OGC or SLC25A11), dicarboxylate carrier (DIC or SLC25A10), and tricarboxylate carrier (TTC or SLC25A1) located in the mitochondrial inner membrane. Maintaining high mitochondrial GSH concentration (5–10 mM) is critical to prevent or repair oxidative damage generated during normal aerobic metabolism (Mailloux and Willmore 2014). In addition to ROS detoxification, recent studies indicated that mitochondrial GSH undergoes reversable S-glutathionylation by GST-P and GRX2 to prevent oxidation of mitochondrial proteins (Young et al. 2019). Protein S-glutathionylation is also known to be beneficial in mitochondrial metabolism processes, ATP production, ROS release, solute importation, permeability transition, protein uptake, and fission/fusion (Mailloux and Willmore 2014). OXPHOS complex I subunits

(NADH:ubiquinone oxidoreductases or type I NADH dehydrogenases, e.g., MT-ND3, NDUFV1, NDUFS1) and uncoupling protein 3 as well as many other OXPHOS proteins are suggested to be targets of GRX2 for glutathionylation (Mailloux and Willmore 2014). Lack of murine *Grx2* diminished ATP production and increased H_2O_2 and superoxide release from liver and cardiac mitochondria, and complex I and III proteins as well as mitochondrial redox sensor and tricarboxylic acid (TCA) cycle proteins including pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase complex (OGDC) are suggested to be S-glutathionylation targets in mouse mitochondria (Mailloux et al. 2014; O'Brien et al. 2017; Chalker et al. 2018). Overall, these studies support the importance of NRF2 in glutathione supply and usage for mitochondrial redox balance.

Bioenergetic process

Metabolic end-products of carbohydrates, fats, and proteins end up in mitochondrial FAO and TCA cycle and generate NADH (reduced form of NAD) and flavin adenine dinucleotide ($FADH_2$, the reduced form FAD) for electron transport chains of OXPHOS complexes to produce ATP. Functional roles for NRF2 in mitochondrial metabolism and bioenergetics have been found from murine studies of high-fat diet-induced obesity and methionine-choline-deficient diet-induced fatty liver disease. In these studies, NRF2 negatively regulated gluconeogenic (e.g., phosphoenolpyruvate carboxylase, glucose-6-phosphatase) and lipogenic (e.g., acetyl-coA carboxylase 1) genes to protect against the metabolic disorders (Chartoumpekis et al. 2011; Kay et al. 2011; Uruno et al. 2013). The underlying molecular mechanisms are unclear. Furthermore, murine *Nrf2* deficiency enhanced outer membrane permeability, reduced mitochondrial membrane potential, and suppressed oxygen consumption rate, and FAO in various tissues, cells, and isolated mitochondria (Ludtmann et al. 2014; Strom et al. 2016). Inhibition of OXPHOS complex II augmented neuron and astrocyte toxicity in *Nrf2*-deficient ($Nrf2^{-/-}$) cells more than in wild-type cells, and *Nrf2* overexpression reversed the inhibition effects in the cells (Calkins et al. 2004). Consistent with these findings, enhanced mitochondrial functions and upregulated FAO genes under caloric restriction were found in *Keap1-knockdown* mice (Kulkarni et al. 2013). Altered NAD and FAD homeostasis coupled with reduced ATP production were found in neurons from $Nrf2^{-/-}$ mice (Holmstrom et al. 2013).

As *Nrf2* deficiency did not alter OXPHOS enzyme activities in this study, Holmstrom *et al.* (2013) proposed that *Nrf2* deficiency mainly limited respiratory chain substrates availability. Supporting this hypothesis, limited substrates and impaired activity of complex I coincided with enhanced mitochondrial and total ROS production were found in cortical slice and glio-neuronal co-culture from $Nrf2^{-/-}$ mice (Kovac et al. 2015). In addition, functional AREs were determined in many genes generating the OXPHOS substrates. They include aldo-keto reductase family (e.g., AKR1B15) in the FAO process and pentose phosphate pathway (PPP) genes including glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (PGD), malic enzyme 1 (ME1), in oxidative phase (generating a cellular reducing agent NADP), and transketolase (TKT) and transaldolase 1 (TALDO1) in non-oxidative phase (generating ribose 5-phosphate for nucleotide synthesis) (Nishinaka and Yabe-Nishimura 2005; Mitsuishi et al. 2012). However, potential AREs were identified in mitochondrial bioenergetic genes that were Nrf2-dependently activated by NRF2 agonists in

mouse lung and liver (Abdullah et al. 2012; Cho et al. 2019). They included complex I-NADH dehydrogenases (e.g., *Ndufs1*), complex II-succinate dehydrogenases (e.g., *Sdhb*), complex III-ubiquinol-cytochrome c reductases (e.g., *Uqcr11*) and coenzyme Q9 (*Coq9*), complex IV-cytochrome c oxidases (e.g., *Cox7a1*), complex V-ATP synthases (e.g., *Atp5a1, Atp5g1*), acyl-Coenzyme A dehydrogenase, long-chain (*Acadl*), isocitrate dehydrogenase 3 (NAD+) alpha (*Idh3a*), solute carrier family 27 (fatty acid transporter), member 2 (*Slc27a2*), and ornithine aminotransferase (*Oat*) (Table 2). Some of these potential AREs have been functionally verified by chromatin immunoprecipitation followed by DNA sequencing (ChIP-Seq) analyses (Chorley et al. 2012; Mouse et al. 2012; Yue et al. 2014). Therefore, NRF2 may increase mitochondrial biogenesis by acting directly on OXPHOS enzyme transcription in addition to OXPHOS substrate production through induction of intermediary metabolism enzymes.

Biogenesis, mitophagy, and quality control

Mitochondrial biogenesis is known to be cooperatively regulated by several transcription factors. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α, gene is *PPARGC1A*) is a master co-regulator (Austin and St-Pierre 2012; Johri et al. 2013), which forms heteromeric complexes with nuclear respiratory factors, NRF-1 (also called alpha palindromic-binding protein, α-PAL) and NRF-2 (also called GA binding protein transcription factor subunit alpha, GABPA), and the nuclear receptors (e.g., peroxisome proliferator-activated receptors such as PPARα, PPARδ, and PPARγ). These transcription factors, in turn, regulate the expression of OXPHOS complex genes including cytochrome c (*CYCS*) as well as TFAM and families (*TFB1M, TFB2M*) that are essential in transcription of respiratory chain subunits and translational components (Hock and Kralli 2009). PGC-1α also induces the expression of genes involved in heme biosynthesis, ion transport, mitochondrial protein translation, and protein importation and also stimulates respiratory function (Austin and St-Pierre 2012). Importantly, NRF2 is reported to control mitochondrial quality characterized by copy number, mitophagy, and biogenesis. Piantadosi *et. al.* (2008) first demonstrated that NRF2 induces NRF-1 through functional AREs via the heme oxygenase 1 (HO-1)-carbon monoxide (CO) system in mice. Overexpression of HO-1 caused CO production to activate phosphoinositide 3-kinase (PI3K)-serine/threonine-specific protein kinase (Akt) pathway, which in turn liberated NRF2 from Keap1 sequestration to induce NRF-1 and enhanced mitochondria copy number in murine cardiomyocytes (Piantadosi et al. 2008). A similar observation for CO- and NRF2-mediated mitochondrial biogenesis was demonstrated in rodent liver, neuron, and lung (Athale et al. 2012; Hota et al. 2012; MacGarvey et al. 2012). The mouse PGC-1α gene (*Ppargc1a*) also possesses multiple putative AREs (Cho et al. 2019) and a functional ARE was determined in PPARγ-encoding gene (Cho et al. 2010). In mice trained with treadmill exercise, *Nrf2* deficiency reduced whole body energy expenditure accompanying decreased skeletal muscle mitochondria mass and antioxidant activities (Merry and Ristow 2016), supporting further the role for NRF2 in mitochondrial biogenesis.

Mitochondria constantly undergo fission and fusion, and the rates of fusion and fission are tightly controlled. When cells experience metabolic or environmental stresses, mitochondrial fusion and fission work to maintain the balance. An increase in fusion activity leads to

mitochondrial elongation, whereas an increase in fission activity results in mitochondrial fragmentation (Chan 2012). Mitofusin-1 and −2 (MFN1, MFN2) mediate fusion. Complex of dynamin-1-like protein (DRP1) and mitochondrial fission factor (MFF) promote fission and it is coordinated with DNA replication (biogenesis) as fission requires mtDNA for their function. Mitochondrial fission has significant implications in stress response and apoptosis (Chan 2012). KEAP1 was identified as a negative regulator for this process, and KEAP1 inhibition/NRF2 activation degraded the mitochondrial fission protein DRP1 (Sabouny et al. 2017). ARE motifs were identified in the promoters of mouse *Mfn2* (Cho et al. 2019). When damaged mitochondria are separated from the mitochondrial network by fission, mitophagy subsequently degrades them to maintain a healthy pool of mitochondria. Mitophagy is also accelerated by oxidative stress and monitored and regulated by redox sensors; PINK1 recruits and phosphorylates PARK2 in mitochondria to ubiquitinate MFNs and other proteins (e.g., voltage-dependent anion-selective channel 1, VDAC1) to induce mitophagy. The ubiquitinated mitochondrial proteins are then recognized by autophagy proteins, p62/sequestosome 1 (SQSTM1) and microtubule-associated proteins 1A/1B light chain 3B (LC3B), to form an autophagosome for lysosomal degradation. NRF2 increased PINK1 transcription in damaged mitochondria under various stress conditions (Murata et al. 2015). The p62/SQSTM1 promoter also possesses functional AREs (Jain et al. 2010). Nrf2 activated p62/SQSTM1 following mitophagy induction in neuroblastoma cells, and *Nrf2* knockdown impaired increase in p62 (Ivankovic et al. 2016). In addition, p62 (KEAP1-interacting region) competed with NRF2 (DLG motif) for KEAP1 binding (in the DC pocket) and thus accumulation of p62 caused persistent NRF2 activation (Kageyama et al. 2018) (Fig. 1). NRF2 and p62 are also known to cooperate in suppressing aberrant KEAP1 activity for mitochondrial motility (Bertrand et al. 2015). Mitochondria are anchored to microtubule motor proteins by attachments with the small mitochondrial Rho GTPases (MIRO1, MIRO2) in the mitochondrial outer membrane and they primarily traffic on microtubules in the cell. NRF2 or p62 binding to KEAP1 within the PGAM5 complex on the mitochondrial outer membrane suppressed KEAP1-CUL3 ligase-mediated degradation of MIRO2 and preserved mitochondrial motility (O'Mealey et al. 2017).

Protein deglycase DJ-1 (also called Parkinson disease protein 7, PARK7) is essential in mitochondria quality control. DJ-1 is linked to recessively inherited Parkinson's disease when mutated, i.e. human carriers of E64D mutation (c.926G>A) develop early-onset recessive Parkinsonism accompanying selective dopamine-generating cell death and oxidative stress (Bonifati et al. 2003). Oxidation of the Cys106 residue caused mitochondrial localization of DJ-1 (Canet-Aviles et al. 2004). Loss of DJ-1 caused impaired mitochondrial respiration, increased mitochondrial ROS, mitochondrial membrane depolarization, mitochondrial fragmentation, and alterations of mitochondrial shape in human neuronal cells and mouse embryonic fibroblasts (Krebiehl et al. 2010; Thomas et al. 2011). Defective mitochondrial complex I activity in *PARK7* null dopaminergic neuronal cells was thought to be due to the aberrant formation of the supercomplex, which impaired the flow of electrons through the channels between respiratory chain complexes, resulting in mitochondrial dysfunction (Heo et al. 2012). Importantly, DJ-1 was known as an upstream regulator for NRF2 to prevent its KEAP1 binding and subsequent ubiquitination (Clements et al. 2006) (Fig. 1). Knockout of *Park7* in mice or rodent neuronal cells significantly reduced ARE-

mediated TRX1 and glutamate cysteine ligase catalytic expression and increased susceptibility to oxidants (Zhou and Freed 2005; Im et al. 2012). DJ-1 was also proposed to regulate redox and mitochondrial homeostasis independent of Nrf2 [See review (Ariga 2015)]. In summary, these studies implied that DJ-1-NRF2 axis contributes to mitochondrial dynamics and quality control through maintenance of redox balance.

NRF2 in Mitochondrial Dysfunction of Airway Disorders

Mitochondrial biology has been extensively investigated in airway disorders during the last decade. Mitochondrial energy provision is an important function of the airway cells. For example, alveolar type 2 cells in the parenchyma are enriched with mitochondria to provide ATP for surfactant synthesis, secretion, and recycling as well as differentiation into type 1 pneumocytes (Schumacker et al. 2014). Mitochondrial [Ca^{2+}] and ATP production are also critical for ion transport, maintaining airway surface hydration, ciliary beat, and mucin secretion in bronchial epithelium (Lazarowski and Boucher 2009). In addition, mitochondria have a vital role in bronchial airway smooth muscle (ASM) contraction. The mitochondrial genome has been known as a cellular target under oxidative stresses such as hyperoxia, acute lung injury (ALI), or pneumonia (Schumacker et al. 2014). To compensate for damaged mitochondria in airway disorders, promotion of mitochondrial biogenesis or mitochondria transfer has been suggested (Schumacker et al. 2014). In the case of mitochondrial transfer, donation of healthy mitochondria by mesenchymal stem cell (MSC)-conducted mitochondrial transfer to the mouse airway epithelial or ASM cells increased alveolar ATP concentration and protected against experimental lung inflammation and hyperresponsiveness (Islam et al. 2012; Li et al. 2018). These findings indicated transfer may be a therapeutic strategy for diseases linked to genetic mitochondrial defects. In airway pathogenesis, there is relatively little evidence of a role for NRF2 in mitochondrial functions. However, studies discussed below have implicated NRF2 as an important component to support airway mitochondrial structural and functional integrity against oxidative stress and inflammation.

Chronic obstructive pulmonary disease (COPD)

Cigarette smoke (CS) contains over 500 chemical compounds and is a well-known source of ROS (e.g., H_2O_2, superoxide). COPD and lung cancer are the two major CS-related lung diseases. COPD is a complex, debilitating lung disease that encompasses a variety of phenotypes including chronic bronchitis, oxidative stress, loss of alveolar surface area (emphysema), and remodeling of small airways (Barnes et al. 2003). Diagnosis of COPD is difficult until it is clinically apparent, and the prognosis remains poor. Moreover, current treatment options do not substantially alter the course of the disease. Patients with COPD are at increased risk (2–5 fold) for both the development of lung cancer and poor outcome after lung cancer diagnosis and treatment (de Torres et al. 2007).

Many *in vitro* studies have reported CS-induced mitochondrial defects in primary airway cells from patients or airway epithelial cell lines. CS increased ROS production from mitochondria and decreased mitochondrial membrane potential, released mitochondrial DAMPs, apoptosis, and mitophagy leading to airway injury [See review (Ryter et al. 2018)].

Evidence suggests that mitophagy is in general deleterious in the lungs of COPD patients, and LC3B and other autophagic proteins have a pro-pathogenic role in COPD. Relative to wild-type littermate mice, significantly decreased lung apoptosis and emphysema were found in LC3B gene (*Map1lc3b*)-deficient mice after CS exposure (Chen et al. 2010; Lam et al. 2013). Consistent with this observation, *Pink1*-knockout mice were resistant to mitochondrial dysfunction and COPD phenotypes development by CS exposure (Mizumura et al. 2014). Alveolar macrophages from COPD patients were found to have increased mitochondrial ROS and reduced bactericidal activity against *Streptococcus peumoniae* (Bewley et al. 2017). Necroptosis is a programmed form of necrosis regulated by receptor-interacting protein kinase 1 (RIPK1), RIPK3, and mixed lineage kinase domain-like pseudokinase (MLKL) in chronic lung diseases including COPD (Mizumura et al. 2014). Increased RIPK3 level coincided with increased amount of PINK1 in macrophages of COPD lung cells (Mizumura et al. 2014), indicating mitophagy may induce the necroptosis pathway and contribute to COPD phenotypes. Therefore, mitophagy and necroptosis are potential targets for COPD treatment. Differential susceptibility to CS-induced neutrophilia of inbred mouse strains was related to the release of mtDNA into bronchoalveolar lavage fluids (BALFs) (Pouwels et al. 2017). Inhibition of mitochondrial iron chelation and FAO was also suggested as a therapeutic approach based on a genome-wide association study (GWAS) which determined iron-responsive element-binding protein 2 (IREB2 or IRP2) as a susceptibility gene for COPD (DeMeo et al. 2009). IRP2 increases cellular iron uptake by iron responsive element-mediated transcriptional activation under iron depletion conditions. Excess buildup of iron in mitochondria by IRP2 was thought to lead to mitochondrial dysfunction due to inefficient oxygen consumption and energy metabolism. Consistent with this finding, inhibited iron (non-heme) loading and complex IV (COX) was found in *Irp2*-deficient mice and these mice were resistant to CS-caused experimental COPD (Cloonan et al. 2016). COPD is also a multiorgan systemic disease including locomotor muscle dysfunction (i.e., skeletal muscle weakness, cachexia). COPD patients stratified by cachexia had reduced expression of PPARα/PPARδ, PGC-1α and TFAM in skeletal muscle, relative to healthy subjects (Remels et al. 2007). These findings indicated that disrupted mitochondrial biogenesis may underlie skeletal muscle dysfunction in COPD.

Supporting a role for NRF2 in COPD, genetic disruption of *Nrf2* caused early onset and severe emphysema in mice (Rangasamy et al. 2004). In cultured bronchial epithelial cells or primary lung cells from COPD patients, air pollution-derived particulate matter ($PM_{2.5}$) exposure partially inactivated the NRF2 pathway and critically impaired mitochondrial redox homeostasis and functions (Leclercq et al. 2018). Most COPD patients are elderly, and many features of COPD including oxidant/antioxidant, protease/antiprotease, and proliferative/antiproliferative balance as well as control of inflammatory response are shared with those in aged lung (the aging hypothesis for COPD) (Ito and Barnes 2009). Silent mating type information regulation 2 homolog 1 (sirtuin 1, SIRT1) is a NAD-dependent deacetylase that modulates oxidative stress response and premature cellular senescence and aging. Recent studies have associated onset/progression of COPD with deacetylation of many transcription factors by SIRT1 [see review (Conti et al. 2015)]. SIRT1 was decreased in the lungs of smokers and COPD patients compared with nonsmokers or healthy subjects, and knockdown of *SIRT1* increased NF-κB acetylation and interleukin (IL)-8 release

(Rajendrasozhan et al. 2008). Consistent with this finding, *Sirt1*-heterozygous knockout mice developed an emphysematous phenotype at 1 year of age and were susceptible to CS-induced COPD symptoms (Yao et al. 2012). SIRT1 has a number of beneficial roles in mitochondria including PGC-1α deacetylation for nuclear translocation to induce mitochondrial biogenesis (Canto et al. 2009). Sirt1 significantly enhanced the NRF2 pathway by KEAP1 depression and/or NRF2 stabilization (deacetylation, reduction of ubiquitination) in rat renal cells (Huang et al. 2017) (Fig. 1). NRF2 also positively regulated protein expression and deacetylation activity of SIRT1 (Huang et al. 2017). In addition, NRF2 and SIRT1 were recruited to AREs to induce multidrug resistance-associated protein 2 (*MRP2* or *ABCC2*) (Kulkarni et al. 2014) which has a role in glutathione excretion and xenobiotic conjugate formation (Vollrath et al. 2006). Taken together, a positive feedback loop by crosstalk between SIRT1 and the NRF2 is potentially beneficial against COPD pathogenesis. Well-known COPD susceptibility genes identified from multiple GWAS includes family with sequence similarity 13 member (FAM13A) in the FAO pathway (Hancock et al. 2012). CS-induced experimental COPD indicated that FAM13A shapes the cellular metabolic response to CS by promoting the FAO, and *Fam13a* deletion attenuated CS-induced mitochondrial respiration interruption and provided resistance to emphysema in mice (Jiang et al. 2017). A recent transcriptome analysis found increased lung ARE-responsive genes (e.g., *Akr1b8, Nqo1, Gpx2*) in CS-resistant *Fam13a* knockout mice relative to wild-type mice (Yun et al. 2017), which linked FAM13A and NRF2 in model COPD.

Idiopathic pulmonary fibrosis (IPF)

IPF is a fatal disease of the lower respiratory tract characterized by inflammation and fibrosis of the interstitium, leading to destruction of alveolar structure. Development of IPF is significantly associated with age, and people older than 75 years have 50 times higher prevalence than people 35 years old or younger (Selman et al. 2010). Mitochondria are particularly susceptible to aging. Structural and functional abnormality of mitochondria have been found with aging and aging-associated mtDNA mutations, likely related to mtDNA replication errors (Bratic and Larsson 2013).

Elevated mtDNA levels in the lung tissue, BALF, and plasma were found in IPF patients, and plasma mtDNA was strongly associated with disease progression and mortality (Ryu et al. 2017). Accumulation of mtDNA and endoplasmic reticulum (ER) stress markers was found in the lung and in type 2 pneumocytes in severe fibrotic areas from IPF patients compared to controls (Bueno et al. 2015; Patel et al. 2015). The IPF type 2 cell mitochondria were dysmorphic, dysfunctional, and defective in LC3-mediated autophagy (Bueno et al. 2015). Old mice (> 18 months) with diminished PINK1 levels were more susceptible to ER stress-induced fibrosis than young mice (2–3 months), and *Pink1*-deficient mouse lungs displayed more swollen mitochondria, lower mtDNA copy number, and higher collagen accumulation than wild-type mouse lungs (Bueno et al. 2015; Patel et al. 2015). *Pink1*$^{-/-}$ and *Park2*$^{-/-}$ mice were susceptible to the fibrogens bleomycin, murine gammaherpesvirus 68 than their wild-type controls (Bueno et al. 2015; Patel et al. 2015; Kobayashi et al. 2016). ATP release from healthy cells is tightly controlled by extracellular ATP/ADPases, and ATP released from injured cells is also a DAMP that initiates NLRP3-mediated immune responses through the purinergic receptor ($P2X_7$, $P2RX_7$) signaling (Mariathasan et al.

2006). Increased ATP levels were found in BALF from IPF patients compared to controls (Riteau et al. 2010). Extracellular ATP level was also associated with bleomycin-induced lung fibrosis, and reduced bleomycin-induced lung inflammation and fibrosis markers were found in *P2rx7*-deficient mice compared to wild-type controls (Riteau et al. 2010).

NRF2 has a protective role in pulmonary fibrosis in mice (Cho et al. 2004; Traver et al. 2017). In fibrotic airways, mitochondrial levels of SOD2 were markedly heightened in $Nrf2^{-/-}$ mice compared to $Nrf2^{+/+}$ controls (Carraway et al. 2008). NRF2 also improved mitochondrial functions by inhibition of NADP oxidase 4 (NOX4) which generates ROS (superoxide) in the inner mitochondrial membrane (Bernard et al. 2017); lung fibroblasts from *Nox4*-deficient mice had improved mitochondrial respiration and biogenesis (determined by mtDNA/nDNA ratio, TFAM, NRF-1) compared to normal mouse cells. In addition, silencing *NRF2* in human lung fibroblast cells abrogated the *NOX4*-knockout effect on mitochondrial bioenergetics and biogenesis (Bernard et al. 2017). These data imply that the protective role of NRF2 in lung fibrogenesis is likely through control of mitochondrial functions.

Asthma

Asthma affects more than 300 million people worldwide and the incidence and severity of the disease increased between 2001 and 2010 (Moorman et al. 2012). Asthma is characterized by reversible airflow obstruction due to ASM hyperresponsiveness and mucus overproduction. ASM resides in respiratory airways from trachea to terminal bronchioles, and ASM plasticity is critical to lung development as well as the pathogenesis of asthma, chronic bronchitis, and emphysema.

Mitochondria sense intracellular [Ca^{2+}] for calcium homeostasis and energy production, which is particularly critical in metabolically active, energy-consuming contractile responses in myocardium, ASM, and vascular smooth muscle for stable and persistent force generation. Increased energy demand of the contractile response must be matched by equivalent increase in energy supply and [Ca^{2+}] in ASM. Increased cytosolic [Ca^{2+}] signals the contractile response through excitation-contraction coupling and elevated mitochondrial [Ca^{2+}] stimulates ATP production. While the major calcium storage in ASM is sarcoplasmic reticulum (SR) or ER, mitochondria have temporal calcium buffering storage. The mitochondrial outer membrane is relatively permeable to most ions and small molecules although Ca^{2+} transport is facilitated by a nonselective VDAC1 (Rapizzi et al. 2002). However, the inner membrane strictly regulates Ca^{2+} transport through highly selective mitochondrial calcium uniport (MCU) and sodium-calcium exchanger (NCX or SLC8A1) for influx and release, respectively (Kirichok et al. 2004; Palty et al. 2010). Mitochondria-ER/SR coupling is therefore essential for activation of MCU for ASM contraction and relaxation (Zhao et al. 2018). In addition to calcium homeostasis, excess ROS production is also a key determinant of ASM hypercontractility (Sutcliffe et al. 2012).

Asthmatics have increased oxygen consumption and impaired calcium homeostasis during inflammation which leads to ROS production and ER/SR stress as well as enhanced mitochondrial biogenesis and cell proliferation (Trian et al. 2007; Delmotte and Sieck 2015). Increased release of extracellular ATP found in BALF from asthmatics indicated

mitochondrial damage in an experimental asthma model (Idzko et al. 2007). Dendritic cell-driven T helper 2 (Th2) cytokine production and bronchial hyperreactivity are suppressed by ATP neutralization (by ATPase or P2 receptor antagonist) (Idzko et al. 2007). Similarly, airborne allergen-treated mouse bronchial cells induced extracellular ATP release accompanying increased intracellular [Ca^{2+}] and IL-33-mediated Th2 cell responses (Kouzaki et al. 2011).

In mouse models of allergic asthma, mitochondrial dysfunction and oxidative stress were key factors in the pathogenesis. Compared to wild-type controls, mice deficient in *Nrf2* or its downstream genes *Gpx2* and *Gsto1* are more susceptible to asthma-like symptoms including elevated oxidative stress, inflammation, mucus, and airway hyperresponsiveness (Rangasamy et al. 2005; Dittrich et al. 2010). Moreover, reduced NRF2 activity was found in ASM cells from severe asthmatic patients (Michaeloudes et al. 2011). NRF2 activation by an agonist (sulforaphane) increased ARE-responsive SOD2 and HO-1 expression and reduced ASM proliferation in cultured ASM cells (Michaeloudes et al. 2011). In peripheral blood monocytes from asthmatics, hyperoxidation rate of NRF2-dependent peroxiredoxins including mitochondrial PRDX3 were related to the severity of asthma (Kwon et al. 2012). These investigations warrant further research on NRF2 in the pathogenesis of asthma.

Pulmonary arterial hypertension (PAH)

PAH is a vascular disease caused by hyperproliferation of vascular cells leading to ventricular failure and premature death. Mitochondrial compromise has been found to be a characteristic of PAH. Mitochondrial ROS and diffusible metabolites and DAMPs activate NLRP3-inflammasome and TLR9, which lead to vascular remodeling and PAH (Sutendra and Michelakis 2014). In a persistent pulmonary hypertension model using newborn lambs, reduced SOD2 expression and activity caused an increase of mitochondrial superoxide, and it depleted endothelial nitric oxide synthase (eNOS) leading to pulmonary artery endothelial dysfunction (Afolayan et al. 2012). Exogenous administration of SOD2 improved eNOS function and alleviated PAH of newborn lambs (Afolayan et al. 2012). In a rat model of PAH and in pulmonary artery smooth muscle cells from PAH patients, mitochondrial fragmentation was associated with decreased MFN2 and PGC1-α levels (Ryan et al. 2013), suggesting a potential MFN2 therapy for PAH in combination with inhibition of mitochondrial fission. Mitochondrial FAO generating substrates for OXPHOS were found to be decreased in association with intracellular lipid accumulation in PAH patients (Talati and Hemnes 2015). In addition, 3-fold greater glycolytic rate was found in pulmonary artery endothelial cells from PAH patients compared to healthy controls, indicating a metabolic switch to anaerobic glycolysis (Xu et al. 2007). In summary, vascular mitochondrial dysfunction and metabolic disturbance may at least partly underlie the PAH pathogenesis and a beneficial role for NRF2 in PAH through modulation of SOD2, PGC-1α, and intermediary metabolism is postulated.

Pneumonia, acute lung injury and other airway disorders

In a murine model of *Staphylococcus aureus* sepsis, mitochondrial biogenesis was enhanced to rescue damaged type 2 pneumocytes and lung function (Athale et al. 2012; Suliman et al. 2017). *Nrf2*$^{-/-}$ mice are highly susceptible to bacterial sepsis and lung inflammation

(MacGarvey et al. 2012). NRF-1 and TFAM mRNA induction was evident in wild-type mouse lungs, but not in *Nrf2*$^{-/-}$ mouse lungs with pneumonia caused by *S. aureus* (Athale et al. 2012). Inhaled CO increased survival after sepsis, which was accompanied by activation of Akt1-NRF2 axis-induced mitochondrial biogenesis factors including NRF-1, TFAM, and PGC-1α. Sepsis-induced loss of hepatic mtDNA copy number was also greater in *Nrf2*$^{-/-}$ and *Akt*$^{-/-}$ mice than in corresponding wild-type mice (MacGarvey et al. 2012). Nrf2-mediated mitochondrial quality control also affected lung resolution from septic pneumonia as reduced mitophagy determined by lowered LC3 levels and elevation of p62 expression was detected in *Nrf2*$^{-/-}$ type 2 pneumocytes and alveolar macrophages compared to the cells from wild-type mice (Chang et al. 2015).

Circulating mtDNA has been associated with severe trauma and sepsis patients, indicating that this DAMP could be a potential biomarker or proinflammatory alarm signal for acute respiratory distress syndrome (ARDS) or sepsis (Kung et al. 2012; Simmons et al. 2013). Another mitochondrial DAMP, cardiolipin, is a rare mitochondrial-specific phospholipid in the inner membrane and is an apoptotic cell surface marker vital for overall mitochondrial function and membrane integrity. Increased lung injury and disrupted surfactant activity and lung function were found in mice treated with cardiolipin (Ray et al. 2010). Markedly elevated cardiolipin was also evident in tracheal aspirates from pneumonia patients and BALF from bacteria-infected mice (Ray et al. 2010). Increased airway cardiolipin secretion and development of pneumonia were detected in type 2 cells of mice with a functional mutation (G308V) in a cardiolipin transporter (ATPase, aminophospholipid transporter, class I, type 8B, member 1, ATP8B1) (Ray et al. 2010). In addition, decreased lung NRF2-ARE responses were found in the aged *Atp8b1* mutant mice (14 month) compared to aged wild-type mice (Soundararajan et al. 2016). Overall, these studies indicated that cardiolipin homeostasis may be protective in age-related lung disorders.

Experimental hyperoxia exposure is a model of supplemental oxygen therapy in ALI/ARDS of adults and bronchopulmonary dysplasia (BPD) of preterm infants. Although supplemental oxygen is clinically important in these clinical settings, oxygen paradoxically causes significant toxicity in the lung. While multiple factors contribute to the risk of the disease, the strongest predictor is lower gestational age which places the most premature infants at greatest risk to BPD. Supporting this notion, hyperoxia exposure exaggerated mitochondrial oxidation in late saccular/early alveolar stage of rodent lung pneumocytes (postnatal days P5-P7) than in matured rodent lung cells (Berkelhamer et al. 2013). The magnitude of hyperoxia-caused arrest in alveolar development was associated with declined mitochondrial respiration and complex I activity in a murine model of BPD (Ratner et al. 2009). Exposure of immature (canalicular/saccular stage) rat fetal lung explants to hyperoxia caused mtDNA damage, impaired branching morphogenesis, and diminished surfactant expression, indicating modulation of mtDNA repair as a potential novel strategy for treatment of oxidant-induced lung disease in the preterm infant (Gebb et al. 2013). Adult type 2 cells and mitochondria isolated from mice exposed to hyperoxia also had significant decrease in mitochondrial respiration and oxygen consumption rate (via complexes I and II) as well as glycolysis, indicating hyperoxia also impairs mitochondrial energy metabolism (Das 2013). NRF2 protects lungs against ALI caused by LPS, hyperoxia, and other insults (Cho et al. 2002; Thimmulappa et al. 2006; Marzec et al. 2007; Cho et al. 2012). In models of ALI

induced by LPS and hyperoxia, mitochondrial localization of SOD2 was increased, supporting the NRF2-mediated increase of mitochondrial redox processes (Carraway et al. 2008). In experimental BPD, mitochondria-associated genes such as *Akr1b8, Sod2,* and GSH synthesis genes (GSH synthase, *Gss;* cystine/glutamate transporter, *Slc7a11*) as well as complex V (*Atp6v1d*) were significantly suppressed in hyperoxia-susceptible $Nrf2^{-/-}$ neonate lungs relative to $Nrf2^{+/+}$ neonate lungs, which indicated significant arrest in alveolarization by *Nrf2* deficiency (Cho et al. 2012). In addition, mtDNA damage was significantly greater in $Nrf2^{-/-}$ neonate lungs than in $Nrf2^{+/+}$ neonate lungs under hyperoxic and normoxic conditions (Cho et al. 2012). These results supported the potential role for NRF2 in pathogenesis and mitochondrial dysfunction of BPD.

Cystic fibrosis, a lethal inflammatory disease accompanied by abnormal mucus secretion, is caused by mutations in cystic fibrosis transmembrane conductance regulator (*CFTR*). Many studies have demonstrated decreased function or expression of OXPHOS subunits (e.g., complex I) in cystic fibrosis patients [see review (Sureshbabu and Bhandari 2013)]. Bacterial flagellin (a TLR5 ligand) exacerbated pro-inflammatory responses of cystic fibrosis airway epithelial cells driven by *Pseudomonas aeruginosa* and MCU had a role in TLR-NLRP3 inflammasome signaling (Rimessi et al. 2015). These findings indicated that mitochondrial [Ca^{2+}] contributes to the regulation of inflammatory cystic fibrosis (Rimessi et al. 2015). A functional ARE was identified in the far upstream (−44kb) enhancer region of *CFTR* (Zhang et al. 2015), which is consistent with a role for NRF2 in mitochondrial dysfunction in this disease.

Lung Cancer

Lung cancer is the leading cause of cancer-related deaths worldwide. In particular, non-small cell lung cancer (NSCLC) including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma accounts for 85% of the cases and is the major cigarette smoking-related lung disease (Tan et al. 2009). Although environmental (e.g., cigarette smoking, COPD with airflow obstruction) and genetic (e.g., epidermal growth factor receptor, EGFR) factors are important contributors to predisposition and progress, lung cancer is also characterized by anti-apoptosis and uncontrolled cell proliferation and angiogenesis processes. While two EGFR tyrosine kinase inhibitors (TKIs, gefitinib and erlotinib) are representative chemotherapeutics for NSCLC, many patients eventually experience resistance to EGFR-TKI due to EGFR somatic mutation development in cancer cells. Overexpression of mitochondria-localized gene clusters was evident in squamous cell carcinoma regardless of presence or absence of COPD (Boelens et al. 2011). TFAM levels were also correlated with lung function indices in NSCLC (Peng et al. 2013).

Cancer cells generate elevated levels of ROS and become highly vulnerable to death by oxidative stress. Different from non-neoplastic disorders, the paradoxically pathogenic role of enhanced NRF2 expression and activity in carcinogenesis is well documented and thus inhibition of NRF2 has been developed as a strategy for tumor suppression. Somatic mutations in *NRF2* or in the repressor *KEAP1* (Cho et al. 2015), likely due to mis-incorporation during DNA replication or by exposure to mutagens, are the ‘driver’ mutations causing persistent activation of NRF2-ARE to provide growth advantage and are causally

implicated in cancer development. A recent study indicated that disrupted KEAP1-NRF2 pathway in NCSLC also contributes to the EGFR-TKI resistance, indicating crosstalk between the pathways (Park et al. 2018).

Increased mtDNA copy number is correlated with increased risk of lung cancer and mtDNA instability has been associated with lung cancer. Chronic inflammation during carcinogenesis causes ROS overproduction and implicates mtDNA damage. In NSCLC, there are frequent alterations in mtDNA D-loop which regulates mtDNA replication and expression and the D-loop SNPs (e.g., 16390A) are prognostic markers for NSCLC outcome (Ding et al. 2012). The majority of the coding mtDNA mutations in lung cancers targeted complex I, and a mutation in the *ND5* (G13289A) of the complex I forced its overexpression and led to lung cancer cell proliferation (Dasgupta et al. 2012). Reduced mitochondrial fusion (MFN2) and increased fission (DRP1 and mitochondrial dynamin like GTPase or optic atrophy 1, OPA1) proteins were evident in lung adenocarcinoma tissues or cell lines and increased DRP1 was related to poor prognosis (Fang et al. 2012; Rehman et al. 2012) [Also see review (Nam et al. 2017)].

While normal cells and cancer cells utilize the same metabolic pathways, cancer cells have different preference for metabolic pathways (Chartoumpekis et al. 2015). Cancer cells require more energy and nutrients than normal cells to meet the increased demands of uncontrolled cell growth and proliferation. The most prominent adaptive changes for cancer cells to accomplish this demand is to increase glucose uptake and favor aerobic glycolysis (fermentation). This ‘Warburg Effect’ is used rather than the efficient OXPHOS pathway (Warburg 1956). Other anabolic pathways which provide cancer cells the necessary energy and substrates to synthesize lipids, proteins, and nucleic acids are the glutamate pathway, the PPP, and lipid biosynthesis (Chartoumpekis et al. 2015).

Studies in A549 cells, a human lung adenocarcinoma cell line bearing a *KEAP1* somatic mutation and hypermethylation which drives constitutive NRF2 activation, indicated that NRF2 induces genes encoding PPP enzymes (G6PD, PGD, TKT, TALDO1) as well as NADPH generating ME1 and isocitrate dehydrogenase 1 (Mitsuishi et al. 2012; Hayes and Dinkova-Kostova 2014). These genes are either direct NRF2 effectors or indirectly regulated by NRF2 (Mitsuishi et al. 2012). This reprogramming of glucose metabolism by NRF2 from the aerobic pyruvate/TCA cycle into anabolic PPP was also found in animals with high glucose availability (Chartoumpekis et al. 2015). PI3K-Akt signaling is involved in nuclear accumulation of NRF2 to redirect glucose and glutamate into the adaptive, anabolic pathways (Mitsuishi et al. 2012). Fumarate, an oncogenic metabolite, was also connected with KEAP1-NRF2 pathway in cancer. In the TCA cycle, fumarate hydratase (FH) catalyzes the hydration of fumarate into malate, and germline heterozygous *FH* mutations have been associated with renal cancer (Tomlinson et al. 2002). In *FH*-deficient cells and mouse tumors, ARE-responsive antioxidant enzymes were markedly increased due to the stabilization of NRF2 via succination of critical cysteine residues in KEAP1 with accumulated fumarate (Adam et al. 2011). Other mitochondrial events linked with oncogenic NRF2 is dysregulation of the p62-KEAP1-NRF2 axis (Inami et al. 2011). Aberrant accumulation of p62 (which competes with NRF2 for KEAP1 binding) elevated NRF2 translocation and GSH synthesis leading to chemoprevention tolerance of hepatic cancer

cells (Saito et al. 2016). Increased NRF2 levels in NCSLC tissues with the poor prognosis was also linked with enhanced autophagy proteins (e.g., LC3, Beclin 1) and autophagosome formation was dependent on NRF2 levels in NCSLC cells (Wang et al. 2017b). NRF2 showed oncogenic effects in colon and breast cancer cells by inhibition of a microRNA miR-181c which reduces abundance of mitochondria-encoded COX (e.g., MT-CO1) in complex IV (Jung et al. 2017).

NRF2-dependent Modulation of Lung Mitochondria Transcriptome by Sulforaphane

There is great interest to develop new therapeutic solutions for human diseases related to mitochondrial dysfunction. Pharmacological agents that act as NRF2 agonists, including sulforaphane, resveratrol, 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole (CDDO-Im), and curcumin (Cho and Kleeberger 2015), have been highlighted in studies on mitochondrial biology (Negrette-Guzman et al. 2013; Dinkova-Kostova and Abramov 2015; Denzer et al. 2016). Sulforaphane is a special form of the phytochemical isothiocyanates, and Talalay and Fahey initially recognized its efficacy in induction of ARE-bearing phase 2 enzymes for cytoprotection and cancer prevention (Fahey et al. 1997). Sulforaphane was then found to act mainly through NRF2 stabilization from KEAP1 inhibition (Kensler et al. 2013). Additional investigations found that sulforaphane has an anti-proliferative property (cell cycle arrest) and induces intrinsic apoptosis in cancer cells (Gamet-Payrastre et al. 2000), which provided potential for sulforaphane as a tumor suppressing agent, not a blocking agent, possibly effective in post-initiation stage (Myzak and Dashwood 2006). Further studies identified a role for sulforaphane in mitochondria protection and preservation against xenobiotics while it drove mitochondrial dysfunction to bring the tumor cell to an apoptotic death as an anti-carcinogenetic mechanism (Negrette-Guzman et al. 2013). Through either mechanism, sulforaphane has had a beneficial agent in human disorders.

In airways, NRF2-dependent effects of sulforaphane include attenuation of lung inflammation and phagocytosis against bacterial infection following emphysema, RSV infection, and inhaled arsenic (Cho and Kleeberger 2015). Sulforaphane pre-treatment markedly inhibited $PM_{2.5}$-induced type 2 cell damage by inhibition of the mitochondrial apoptotic pathway (Wang et al. 2017a). Sulforaphane administration also restored benzo(a)pyrene-induced decline of mitochondrial bioenergetic enzyme activities and increased BCL2 levels during lung cancer initiation in mice (Priya et al. 2011). In NCSLC, an active sulforaphane metabolite (sulforaphane-cysteine) inhibited cell viability and activated the ERK1/2-mediated mitochondrial signaling pathway leading to apoptosis (Lin et al. 2017). However, clinical reports have indicated mixed or negative efficacy of sulforaphane (Egner et al. 2014; Wise et al. 2016).

In a murine ALI model, we determined that orally dosing with sulforaphane significantly prevented hyperoxia-induced lung inflammation and injury in a NRF2-dependent manner (Cho et al. 2019). Importantly, lung cDNA microarray analysis elucidated that sulforaphane markedly induced the transcriptome of mitochondrial energy metabolism in wild-type mice, but not in *Nrf2*$^{-/-}$ mice (Cho et al. 2019), consistent with Nrf2-mediated increase of

mitochondrial functions. Expression of many of these mitochondria-related genes were consistently higher in *Nrf2*$^{+/+}$ lungs than in *Nrf2*$^{-/-}$ lungs after hyperoxia exposure. Genes encoding OXPHOS complex subunits and assembly factors/ancillary proteins, TCA cycle enzymes, mitochondrial transporters/symporters, and mitochondrial antioxidants as well as biogenesis and fusion/fission proteins were coordinately increased with those encoding enzymes in FAO, glycolysis, and amino acid degradation pathways (Fig. 2). These results suggested that sulforaphane-NRF2 pathways supply metabolic substrates and electrons for mitochondrial functions and enhance mitochondrial mass to enable the lung to defend against subsequent oxidant attack while *Nrf2* deficiency limits intermediary metabolic substrates and mitochondrial function to impair the mitochondria-mediated cellular processes.

Using a position weight matrix (PWM) statistical model (Wang et al. 2007), we found potential ARE sequences in the NRF2-dependent genes associated to the mitochondrial functions (Cho et al. 2019). The multiple ARE-like motifs (PWM score of ≥ 6.4) were enriched in these genes (Table 2). A similar approach was used to define potential AREs from NRF2-dependently regulated mitochondrial genes by butylated hydroxyanisole treatment in mouse liver (Abdullah et al. 2012). Some potential AREs have been functionally verified by ChIP assays (Mouse et al. 2012; Yue et al. 2014) and further supported the role for the NRF2 pathway in facilitating mitochondrial biology to protect the airway against oxidant disorders.

Conclusions

Healthy maintenance of mitochondrial function is essential to prevent various human diseases. It is becoming clear that regulation of mitochondrial function constitutes an important part of cytoprotection by NRF2 under normal physiology as well as in pathological conditions. Many recent investigations have also investigated the novel transcriptional targets of NRF2 in mitochondrial dynamics as well as the crosstalk between mitochondrial proteins/metabolites and the KEAP1-NRF2 axis. NRF2 activates intermediary metabolism pathways which facilitate the flow of carbon into the FAO and TCA cycle for mitochondrial respiration and ATP production. Emerging studies have also linked mitochondrial dysfunction with ROS- and inflammation-associated lung diseases and studies have underscored the role of airway NRF2 in management of mitochondrial dysfunctions in COPD, asthma, IPF, PAH, bacterial pneumonia, and ALI. In lung cancer which has heightened NRF2 activity, an oncogenic role of NRF2 may be attributed to the improvement of mitochondrial quality and function which provides adaptive behaviors and growth advantage of cancer cells. Overall, further accumulation of the insights into the novel roles for NRF2 in modulation of mitochondrial homeostasis and function should lead to new therapeutic or preventive strategies in both neoplastic and non-neoplastic airway disorders.

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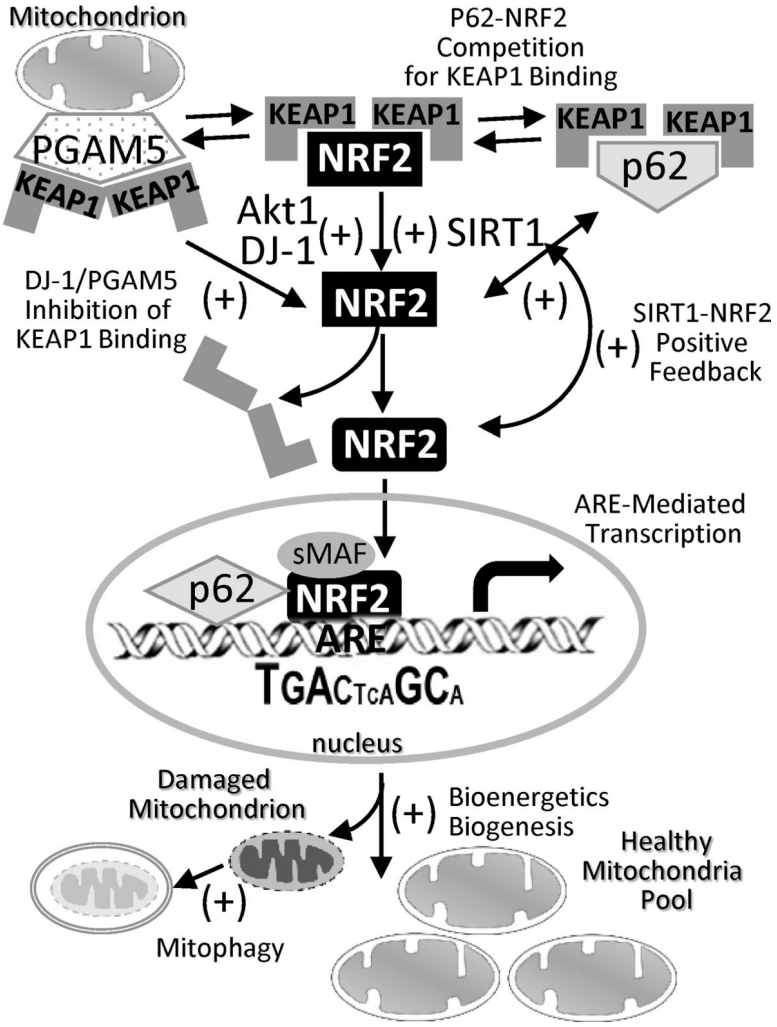

Fig 1. Interactions of NRF2-KEAP1 axis with other proteins in regulation of mitochondrial biology and functions.

Studies with transgenic animal and cell models or human samples demonstrated various proteins that may interact with NRF2-KEAP1 axis under normal physiologic and pathologic conditions to promote NRF2-mediated mitochondrial bioenergetics. NRF2-KEAP1 axis may communication or compete with SIRT1, PI3K/Akt, DJ-1, and/or p62 to liberate NRF2 from KEAP1 for antioxidant response element (ARE)-mediated transcriptional activation of genes involved in mitochondrial energy metabolism, biogenesis, and quality control as described in Table 1. Aktl, serine/threonine-specific protein kinase; DJ-1, protein deglycase (or Parkinson disease protein 7, PARK7); KEAP1, Kelch-like ECH-associated protein 1; p62, ubiquitin-binding protein (or sequestosome 1, SQSTM1); PGAM5, PGAM family member 5, mitochondrial serine/threonine protein phosphatase; PI3K, phosphoinositide 3-kinase; SIRT1, sirtuin 1; sMaf, small musculoaponeurotic fibrosarcoma (MafF, MafG, MafK).

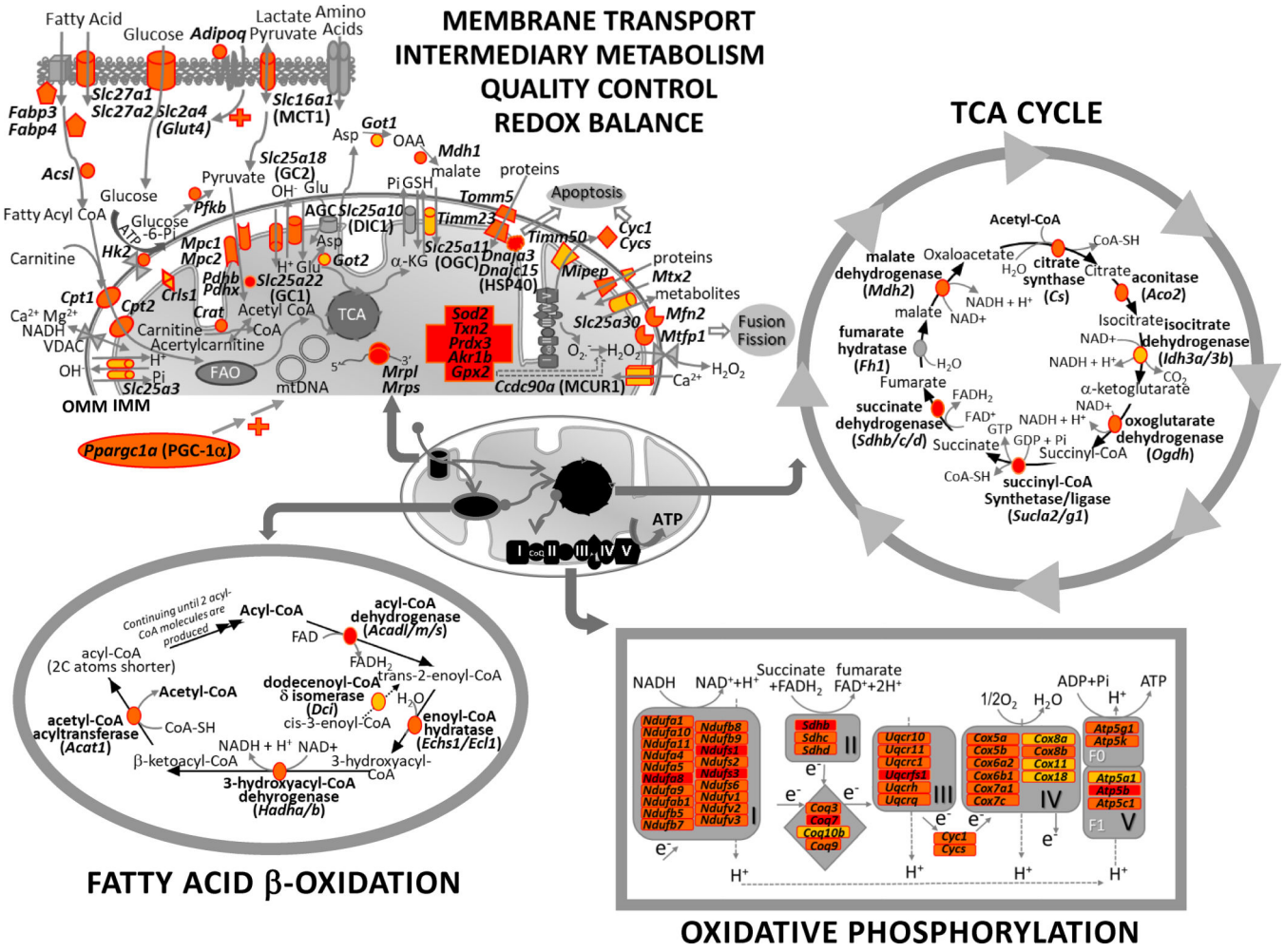

Fig 2. Pulmonary NRF2 effectors known to be involved in mitochondrial bioenergetics and biogenesis.

NRF2-dependently regulated lung genes involved in mitochondrial biogenesis and bioenergetics were determined in a murine study with a NRF2 agonist sulforaphane (Cho et al. 2019). Highlighted proteins in color are encoded from sulforaphane-induced genes in *Nrf2*-deficient mice but not in *Nrf2*-sufficient mice at normal physiological condition (in orange), after hyperoxia exposure (an experimental model of acute lung injury, in yellow), or under both conditions (in red). Most of these protein-encoding genes are found to possess potential antioxidant response elements in their upstream region (Selected list in Table 2). In addition to mitochondrial redox balance through reactive oxygen species quenching and protein S-glutathionylation, NRF2 may enhance cellular entry of nutrients and mitochondrial entry of intermediary metabolites by transcriptional activation of plasma and mitochondrial membrane transporters to increase substrates for oxidative phosphorylation and ATP production. NRF2 may also maintain the healthy pool of mitochondria through quality control by induction of genes involved in biogenesis, fusion, fission, mitophagy, and apoptosis. Most importantly, NRF2 may directly induce expression of the genes encoding enzymes for fatty acid β-oxidation (FAO), tricarboxylic acid (TCA) cycle, and oxidative phosphorylation. Overall, NRF2 may exert its action in various cells such as type 2

pneumocytes, bronchial epithelial cells, smooth muscle cells, and endothelial cells to defend airway from pathogenesis. IMM=inner mitochondrial membrane. OMM=outer mitochondrial membrane. *Acsl,* acyl-CoA synthetase long-chain family member; *adipoq,* adiponectin, C1Q and collagen domain containing; ADIPOR, adiponectin receptor; AGC, aspartate-glutamate carrier; *Aldh,* aldehyde dehydrogenase; Asp, aspartic acid; *Atp,* ATP synthase, H+ transporting, mitochondrial; *Ccdc90a,* coiled-coil domain containing 90A or mitochondrial calcium uniporter regulator 1 *(Mcur1);* CoA, coenzyme A; *Coq,* coenzyme Q; *Cox,* cytochrome c oxidase subunit; *Cpt*, carnitine palmitoyltransferase; *Crat*, carnitine O-acetyltransferase; *Crd1*, cardiolipin synthase; CYC1, cytochrome cl; CYCS, cytochrome c, somatic; Dic1, mitochondrial dicarboxylate transporter; *Dnaj,* DnaJ heat shock protein family; *Fabp,* fatty acid binding protein; GC, glutamate carrier; Glu, glutamic acid; *Glut4,* glucose transporter type 4; *Got,* glutamic-oxaloacetic transaminase; GSH, glutathione; *Gpx2,* glutathione peroxidase 2; *Hk2,* hexokinase 2; HSP40, heat shock protein 40; α-KG, α-ketoglutarate; MCT1, monocarboxylate transporter 1; MCUR1, mitochondrial calcium uniporter regulator 1; *Mdh1,* malate dehydrogenase 1; *Mfn2,* mitofusin 2; *Mipep,* mitochondrial intermediate peptidase; *Mpc,* mitochondrial pyruvate carrier; *Mrpl,* mitochondrial ribosomal protein L; *Mrps,* mitochondrial ribosomal protein S; mtDNA, mitochondrial DNA; *Mtfp1,* mitochondrial fission process 1; *Mtx2,* Metaxin 2; *Nduf,* NADH dehydrogenase (ubiquinone); OGC, oxoglutarate carrier; OAA, oxaloacetate; *Pdh,* pyruvate dehydrogenase; *Pfkb,* 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase; *Ppargc1a,* peroxisome proliferative activated receptor, gamma, coactivator 1 α; *Prdx3,* peroxiredoxin 3; *Sdh,* succinate dehydrogenase complex; *Slc,* solute carrier family; *Sod2,* superoxide dismutase 2; *Timm,* translocase of IMM; *Tomm5,* translocase of outer mitochondrial membrane 5; *Txn2,* thioredoxin 2; *Uqcr,* ubiquinol-cytochrome c reductase, complex. VDAC, voltage dependent anion channel.

Table 1.

NRF2 target proteins involved in mitochondrial biology and function

Categories	Functions	Involved gene products
Nutrient Uptake/Transport	↑ Glucose uptake	GLUT, ADIPO, ADIPOR
	↑ Fatty acid uptake	CD36, SLC27AI/2, ACSL, CPTI/2, FABP 3/4
	↑ Amino acid uptake	GOTI/2. GCI/2
	↑ Metabolites uptake	PDH. ACSL
Intermediary Metabolism	↑ Glycolysis	HK, PFK, PK, ME1, GSR
	↑ Amino acid degradation/Ketolysis	HIBCH. BCKDHA/B, BDHI, OAT
	↓ Gluconeogenesis	G6PC,PEPC
	↓ Lipogenesis	ACAC, ACLY, SCDI, FABP5, ELOVL
Oxidative Phosphorylation	↑ Complex I/II/III/IV/V subunits	
	↑ Electron transfer proteins/donors	CYCS, CYCI, CoQ, NADH, FADH2
TCA Cycle	↑ Entry of metabolic substrates	Acetyl CoA, Malate, α-KG
	↑ NADH/FADH2 generation	MEI, G6PD, IDHI, ACAD
	↑ Enzyme transcription	CS, ACO2, IDH3A/B, OGDH, SUCL, SDHA/B/C, MDH1/2.
Lipid Metabolism	↑ FAO substrates	FAD+/NAD+, FABP 3/4
	↑ FAO enzyme transcription	ACSL, ACADL/M/S, ECHSI/ECHI, HADHA/B, ACATI
	↑ Steroid hormone metabolism	AKRIBI5
Redox Homeostasis	↓ ROS	SOD2, TRX2, TXNRD2, PRDXI/5, ALDH2/6/9/IBI, UCP3
	↑ NADP/NAIY synthesis	MEI, G6PD, IDHI, PGD
	↑ GSH synthesis	xCT, GG T, GST-AI/TI/PI, GS, GSR, GPXI/2/4, GCLC, GCLM
	↑ GSH uptake	OGC, DIC, TTC
	↑ Protein S-glutathionylation	GRX2, GST-P
Quality Control	↑ Biogenesis	PGC-Iα, NRF-I, NRF-2, T FAM, HO-I
	↑ Mitophagy	PINKI, PARK2, p62/SQSTRMI, LC3
	↑ Fusion/Fission	MFNI/2, DRPI
	↑ Apoptosis	CYC, HSP40
	↑ Mobility	MIRO2
	↑ mtDNA repair	OGGI
	↑ Calcium homeostasis	MCUI
	↑ Membrane integrity	CRDI
Cancer & Rapid Proliferating Cells	↑ Pentose phosphate pathway	G6PD, PGD, MEI, TKT, TALDOI, PPAT, MTHFD2
	↑ Lipid biosynthesis	ACACA, FASN, SCDI, ACLY
	↑ Glutamate pathway	GLSI, GLUD

↑, increase in function. ↓, decrease in function. ACAC, acetyl-CoA carboxylase; ACAD, acyl-CoA dehydrogenase; ACAT1, acetyl-CoA acetyltransferase, mitochondrial; ACLY, ATP-citrate lyase; ACSL, acyl-CoA synthetase long-chain family member; ACO2, aconitase 2; ADIPO, adiponectin, C1Q and collagen collagen domain containing; ADIPOR, adiponectin receptor; ALDH, aldehyde dehydrogenase; BCKDH, branched chain ketoacid dehydrogenase E1; BDH1, 3-hydroxybutyrate dehydrogenase, type 1 CoQ, coenzyme Q; CPT, carnitine O-palmitoyltransferase; CRD1, cardiolipin synthase; CS, citrate synthase; CYC1, cytochrome c1; CYCS, cytochrome c, somatic; DIC1, mitochondrial dicarboxylate transporter; DRP1, dystrophin related protein 1; ECH, enoyl Coenzyme A hydratase; ELOVL, ELOVL fatty acid elongase; FABP, fatty acid binding protein; FAO, fatty acid-β-oxidation; FASN, fatty acid synthase; FH, fumarate hydratase; G6PC, glucose-6-phosphate dehydrogenase; GC,

mitochondrial glutamate carrier; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM GCL modifier subunit; GGT, γ-glutamyltransferase; GLS, glutaminase; GLUD, glutamate dehydrogenase; GLUT, glucose transporter; GOT, glutamic-oxaloacetic transaminase; GPX, glutathione peroxidase; GRX2, glutaredoxin 2; GS, glutathione synthase; GSH, glutathione; GSR, glutathione reductase; GST, glutathione-S-transferase; HADH, hydroxyacyl-CoA dehydrogenase; HIBCH, 3-hydroxyisobutyryl-CoA hydrolase; HK, hexokinase; HO-1, heme oxygenase 1; HSP40, heat shock protein 40; IDH1, isocitrate dehydrogenase (NADP(+)) 1, cytosolic; KEAP1, Kelch-like ECH-associated protein 1; α-KG, α-ketoglutarate; LC3 (MAP1LC3), microtubule Associated Protein 1 Light Chain 3; MCU1, mitochondrial calcium uniporter; MDH, malate dehydrogenase; ME, malic enzyme; MFN, mitofusin; MIRO2, mitochondrial rho GTPase 2; MTHFD2, methylenetetrahydrofolate dehydrogenase (NADP+ Dependent) 2; NRF-1, nuclear respiratory factor-1; OAT, ornithine aminotransferase; OGC, oxoglutarate carrier; OGDH, oxoglutarate dehydrogenase; OGG1, 8-oxoguanine DNA glycosylase; PARK2, parkin; PDH, pyruvate dehydrogenase; PEPC, peptidase C; PFK, Phosphofructokinase; PGC-1α, peroxisome proliferative activated receptor, gamma, coactivator 1 α; PGD, phosphogluconate dehydrogenase; PINK1, PTEN-induced kinase 1; PK, pyruvate kinase; PPAT, phosphoribosyl pyrophosphate amidotransferase; PRDX, peroxiredoxin; SCD1, stearoyl-CoA desaturase; SDH, succinate dehydrogenase complex; SLC, solute carrier family; SOD2, superoxide dismutase 2; SQSTM1, sequestosome 1; SUCL, succinate-CoA ligase; TALDO1, transladolase 1; TCA, tricarboxylic acid; TFAM, transcription factor A, mitochondrial; TKT, transketolase; TRX2, thioredoxin 2; TTC, tricarboxylate carrier; TXNRD2, thioredoxin reductase 2; UCP3, uncoupling protein 3; xCT, cystine/glutamate transporter.

Table 2.

Potential antioxidant response elements (AREs) in mouse lung genes involved in mitochondrial function and energy metabolism.

Gene Symbol	Gene Title	ARESequence/Orientation*	Distance to TSS§	PWM†	MS‡	Total AREs¶	FI∞
Acaa2	acetyl-Coenzyme A acyltransferase 2	gggtcTGCtatGTCACctggc/R	−4463	10.8	0.89	6 (5)	1.48
Acadl[1]	acyl-Coenzyme A dehydrogenase, long-chain	gtggcTGCtgtGTCACaagtg/R	−3512	11.6	0.90	15 (7)	1.75
Acadm	acyl-Coenzyme A dehydrogenase, medium chain	gcaagATGACtctGACaacat/F	−4871	8.1	0.765	9 (7)	1.66
Acads	acyl-Coenzyme A dehydrogenase, short-chain	ccaggATGACtgaGCAcccgg/F	−4060	14.6	0.936	8 (3)	1.41
Acadvl	acyl-Coenzyme A dehydrogenase, very long chain	tagtaCTGACtgaGGAgttgg/F	−2518	8.8	0.796	10 (4)	1.62
Acat1	acetyl-Coenzyme A acetyltransferase 1	attttGGCtaaTTCAGctgga/R	−2339	8.8	0.816	6 (2)	1.35
Aco2	aconitase 2, mitochondrial	gagggATGAGgaaGCGattac/F	−3839	6.9	0.784	9 (1)	1.74
Acsf2	acyl-CoA synthetase family member 2	tttctCTGACtcaGAAatgga/F	−1557	8.7	0.815	9 (5)	1.30
Acsl1	acyl-CoA synthetase long-chain family member 1	ttcatTGCtgtGTCATaacta/R	−2001	14.5	0.916	12 (8)	1.66
Adhfe1	alcohol dehydrogenase, iron containing, 1	aaataATTACtttGCAttcta/F	−3812	8.3	0.783	4 (3)	1.34
Adipoq[1]	adiponectin, C1Q and collagen domain containing	agagaGTGAT acaGCTttgag/F	−1461	8.7	0.817	10 (6)	8.73
Adlh1l2	aldehyde dehydrogenase 1 family, member L2	acaccATGACcaaGGCagctc/F	−4967	8.1	0.764	12 (2)	1.66
Alox15	arachidonate 15-lipoxygenase	tcactCTGACaatGCTcatgt/F	−4763	10.7	0.838	9 (5)	2.33
Atp2a2[1]	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	ttgcaTTGAAtttGCAaatat/F	−1200	6.8	0.796	11 (1)	2.07
Atp5c1[1]	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	cagaccTGACgctGCAgttta/F	−2442	10.1	0.855	9 (6)	1.44
Atp5g1[1]	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c1	tatgtTGCtatGTAACcccgg/R	−3067	11.4	0.807	9 (3)	1.83
Atp5k[1]	ATP synthase, H+ transporting, mitochondrial F1F0 complex, subunit	aaaacCTGACaaaGCAtacat/F	−2805	12.3	0.895	16 (9)	1.49
Bckdha	branched chain ketoacid dehydrogenase E1, alpha polypeptide	tagatAGCatgGTCAGggcca/R	−3157	7.8	0.81	6 (3)	1.26
Bckshb	branched chain ketoacid dehydrogenase E1, beta polypeptide	gacaaATAACtcaGCAgggat/F	−118	8.7	0.825	9 (5)	1.38
Bdh1	3-hydroxybutyrate dehydrogenase, type 1	tgtgaAGCtgtGTCACcaagg/R	−4175	9.6	0.877	14 (6)	1.95
Car5b	carbonic anhydrase 5b, mitochondrial	taagaATGAGcctGCAattga/F	−3847	10.8	0.808	6 (3)	1.46
Casq2	calsequestrin 2	aggacTGCttaGTCACacaca/R	−3816	13.5	0.923	8 (5)	5.03

Gene Symbol	Gene Title	ARESequence/Orientation*	Distance to TSS§	PWM†	MS‡	Total AREs¶	FI∞
ChkbCpt1b	carnitine palmitoyltransferase 1b, muscle	caaatCTCtgtGTCAGgagta/R	−2798	8.9	0.767	5 (4)	3.98
Cidea[1]	cell death-inducing DNA fragmentation factor, alpha subunitlike effector A	atgacTTCtgaGTCAAggggc/R	−1960	11	0.832	7 (2)	6.88
Clybl[1]	citrate lyase beta like	cccCTTGATtctGCAatcca/F	−3233	9.7	0.831	8 (5)	1.76
Coq3[1]	coenzyme Q3 homolog, metliyltransferase	tgacaGTGAT agaGCAgctgg/F	−1014	10.6	0.835	7 (5)	1.37
Coq7	demethyl-Q 7	caactTGCtgtGTCATccagg/R	−1516	16.1	0.927	17 (9)	1.32
Coq9[1]	coenzyme Q9 homolog	ctaccCTGACtcaGCAaaaag/F	−4217	17.4	0.96	10 (8)	1.62
Cox4i1[1]	cytochrome c oxidase subunit IV isol'onn 1	aggccTGCgtaGTCAGcttca/R	−276	8.8	0.87	5 (3)	1.30
Cox5a[1]	cytochrome c oxidase, subunit Va	gagagATGACttaGCTgctct F	−2459	9.4	0.878	11 (4)	1.49
Cox5b	cytochrome c oxidase, subunit Vb	tgctcCTGACtgaGCTcatga F	−2340	9.7	0.875	11 (7)	1.52
Cox6b1	cytochrome c oxidase, subunit VIb polypeptide 1	aaaccCTGACtaagCTGggtg/F	−4713	12.3	0.899	11 (8)	1.34
Cox7a1	cytochrome c oxidase, subunit Vila 1	agatcCTGACttgGCCctgag/F	−304	9.1	0.845	11 (7)	4.06
Cox7c	cytochrome c oxidase, subunit VIIc	tcatgGTGACtctgTCCttta/F	−3785	10.7	0.781	5 (4)	1.30
Cox8b	cytochrome c oxidase, subunit VIIIb	gttagTGCtgtGTTAT cctgc/R	−463	13.2	0.84	5 (3)	3.70
Cpt2[1]	carnitine palmitoyl transferase 2	caaaaGTGACtgaGCCacaaa/F	−2058	11.3	0.90	9 (3)	1.52
Crat	carnitine acetyltransferase	ccaatTGCagaCTCAGgctga/R	−3648	14.6	0.86	5 (3)	1.29
Cs[1]	citrate synthase	gagagATGACtcaGCAgttaa/F	2727	16.2	0.947	11(0)	1.43
Cycl	cytochrome c-1	actccAGCtgtGTCAAgttga/R	−4786	12	0.892	8 (3)	1.68
Cycs[1]	cytochrome c, somatic	acccaGGCttgGTCAAggctl/R	−421	6.7	0.84	8 (3)	1.70
Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1	taagaTTGACtcaGCCtgagc/F	−2573	12.3	0.911	6 (5)	3.52
Decrl[1]	2,4-dienoyl CoA reductase 1, mitochondrial	ggagcTGCtgaATCAGgtcct/R	−4367	9.5	0.854	8 (5)	1.58
Dgat2	diacylglycerol O-acyltransferase 2	gcagcATCAGtcaGCAtgtct/F	−1343	9.8	0.788	10 (5)	2.47
Dhrs7c	dihydrolipoamide S-acetyltransferase	gactcATAACtcaGCAgttcc/F	−2314	10.8	0.838	10 (4)	3.51
Dlat[1]	dihydrolipoamide S-acetyltransferase	ctagtGTCACtgaGCAgcatc/F	−1643	7.9	0.819	7 (1)	1.49
Dut	deoxyuridine triphosphatase, mitochondrial	aataaTGCcctGTCATgtgta/R	−3266	10.8	0.85	8 (4)	1.30
Echl	enoyl coenzyme A hydratase 1, peroxisomal	tcttgTGCtgcCTCATggagg/R	−4260	11.2	0.847	8 (6)	1.56
Echsl	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	caaccCTGACacaGCAggaca/F	−852	15.2	0.925	9 (7)	1.43
Eci1	dodecenoyl-Coenzyme A delta isomerase	agaagCTGACtctGCCtgaga/F	−643	9.2	0.859	7 (4)	1.34

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Endog	endonuclease G	tggctGTGATcctGCGcttgt F	−2877	7.4	0.768	8 (2)	1.23
Etfa	electron transferring llavoprotein, alpha polypeptide	actctTGCtgaGTCATctggt R	−2545	16.4	0.951	10 (6)	1.52
Etfb	electron transferring flavoprotein, beta polypeptide	atatcTGCtgcCTCACagaga/R	−3260	11.3	0.849	7 (3)	1.48
Fabp3[1]	fatly acid binding protein 3, muscle and heart	tgagtGTGACcatGCCctgaa/F	−1115	8.1	0.82	13 (6)	3.10
Fh1	fumarate hydratase 1	gcagtCTGACacaGTAaacat F	−4197	10.5	0.808	7 (4)	1.35
Gatd3a	Glutamine amidotransferase-like class 1 domain-containing protein 3A, mitochondrial	tcagcATGAGttgGCAcctgt/F	−1503	12.5	0.833	10 (5)	1.48
Gpd1	glycerol-3-phosphate dehydrogenase 1	taaccTGCtgtCTCAGtgtcc R	−4600	12	0.851	7 (5)	2.19
Gpx2[1]	glutathione peroxidase 2	ccgggATGACttaGCAaaaaa F	59	16.6	0.937	13 (11)	1.78
Gstm4[1]	glutathione S-transferase, mu 4	tacctGTGACtcaGCAtcttc F	−2627	19	0.992	1 1 (3)	1.30
Gsto1[1]	glutathione S-transferase omega 1	tggatAGCtgaGTCACtgccc/R	−2726	13.1	0.916	9 (6)	2.22
Hadhb	hydroxyacyl-Coenzyme A dehydrogenase	taaagGTGAAttaGCAcccag/F	−1398	10.3	0.853	10 (6)	1.50
Hibadh	3-hydroxyisobutyrate dehydrogenase	caaagGGCtaaG'l CATagttt R	−2325	14.7	0.914	6 (4)	1.27
Hibch	3-hydroxyisobutyryl-Coenzyme A hydrolase	ccacaATGACacaGTTcctct F	−2170	9.4	0.787	9 (4)	1.45
Hk2	hexokinase 2	gccacCGCcgcGTCAGgctca R	−265	9.3	0.836	7 (4)	1.38
Hrc	histidine rich calcium binding protein	ctcagGGCtctGTCACtgata R	−4140	10.1	0.862	11 (7)	3.75
Idh3a[1]	isocitrate dehydrogenase 3 (NAD +) alpha	tttctCTGACtcaGCActttg/F	−2163	14.9	0.942	12 (6)	1.56
Ifi27l2a	interferon, alpha-inducible protein 27 like 2A	tatttTTCtgtGTCATccata/R	−3889	11.4	0.803	7 (5)	5.07
Ldhb[1]	lactate dehydrogenase B	gccacTGCaaaGTCAGcaggc/R	65	10.1	0.869	13 (8)	1.43
Macrodl[1]	MACRO domain containing 1	ttaacTGCtgaGTCATctctc/R	−1327	16.2	0.947	8 (5)	1.58
Mdh2	malate dehydrogenase 2, NAD (mitochondrial)	ttgctTTCtctGTCACtgtcc/R	−1518	7.6	0.782	13 (3)	1.37
Me3	malic enzyme 3, NADP(+)-dependent, mitochondrial	actatTGCaaaGTCAActagg/R	−4844	10.4	0.873	7 (2)	1.92
Mfn2	mitofusin 2	ctctgTGCtgaTTCAGgtcca R	1472	11.2	0.861	4 (2)	1.42
Mpcl[1]	mitochondrial pyruvate carrier 1	tgtagTGCtgaTTAATgatta R	−3305	11.1	0.788	11 (7)	1.41
Mrpl12	mitochondrial ribosomal protein L12	gattcTGCagaCTCATctggt R	−559	10.9	0.833	4 (3)	1.4
Mybpc3	myosin binding protein C, cardiac	tgcacATGACttaGGAgcagg/F	−899	9.3	0.801	10 (3)	4.90
Myh7	myosin, heavy polypeptide 7, cardiac muscle, beta	tcaagCTGACttaGACaattc/F	−4150	9.4	0.789	12 (4)	3.63
Myl3	myosin, light polypeptide 3	gagtcTGCtgtGTCAAggggt/R	−2096	14.1	0.92	10 (1)	5.90

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Mylk3	myosin light chain kinase 3	acacaGTAACccaGCAcatta/F	−2004	12.5	0.834	9 (8)	4.25
Mylk4	myosin light chain kinase family, member 4	tcttgGGCagtGTCACcatag/R	−1053	9.7	0.843	10 (7)	2.49
Myom2	myomesin 2	cagatTGCtatGTCACactga/R	−3988	16.3	0.924	17 (10)	4.29
Ndrg4[1]	N-myc downstream regulated gene 4	cggcaATGAGtgtGCAgaaag/F	−2645	8.7	0.805	17 (11)	5.77
Ndufa1[1]	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1	gtgagATGACtcaGCGggtaa/F	−2810	11.1	0.901	11 (6)	1.31
Ndufa10[1]	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	cacaaATGAAtcaGCAcaaat/F	−2392	12.1	0.867	7 (3)	1.47
Ndufa4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4	aagctGTGACaaaGTAtcata/F	−4369	7.9	0.778	4 (3)	1.35
Ndufa5[1]	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5	gcttcTGCttaGTAAT cgtgt/R	−3593	14.8	0.855	6 (4)	1.59
Ndufa8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8	tcaggCTGATcttGCAaataa/F	−1464	8	0.781	7 (2)	1.34
Ndufa9[1]	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9	agtttGGCggaGTCATtctca/R	−2217	12.5	0.879	10 (6)	1.48
Ndufab1[1]	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1	cattcTGCcttGTCATggtca/R	−3775	15.3	0.88	8 (4)	1.61
Ndufb5[1]	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5	gcgacTGCaagGTCACgctct/R	−531	10.4	0.852	11 (7)	1.37
Ndufb7[1]	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7	aggagGGCtcaGTCATtactg/R	−776	9.9	0.881	11 (4)	1.43
Ndufb8	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8	gagttGCCttgGTCATggtgt/R	−1792	9	0.768	9 (5)	1.50
Ndufb9[1]	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9	ccacaATAACtcaGCActccg/F	−3274	13.7	0.866	13 (5)	1.46
Ndufs1[1]	NADH dehydrogenase (ubiquinone) Fe-S protein 1	ctggcCGCtaaGTCAAtatgg/R	171	9.7	0.889	11 (6)	1.63
Ndufs2[1]	NADH dehydrogenase (ubiquinone) Fe-S protein 2	ttcacTGCttaGTCACactct/R	−1449	15.3	0.937	14 (5)	1.44
Ndufs3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	gctttTGCcgaGTCACtaagg/R	−2898	14.2	0.907	7 (4)	1.44
Ndufs6[1]	NADH dehydrogenase (ubiquinone) Fe-S protein 6	tctgaTGCcaaGTAACcttga/R	−2794	9.5	0.794	7 (4)	1.43
Ndufv1[1]	NADH dehydrogenase (ubiquinone) flavoprotein 1	ccttcTGCtttGTCAGacaga/R	−846	12.7	0.894	11 (5)	1.41
Ndufv2	NADH dehydrogenase (ubiquinone) flavoprotein 2	gcttcGGCtgtGTAAGagtca/R	−4523	10	0.793	6 (3)	1.44
Ndufv3	NADH dehydrogenase (ubiquinone) flavoprotein 3	gcatgGTGAGactGCCttaaa/F	−3718	8.5	0.786	3 (2)	1.50

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Nnmt	nicotinamide N-methyltransferase	catttTGCtgaATGAT catgc/R	−2776	10.8	0.79	7 (4)	1.74
Nppa	natriuretic peptide type A	atattTGCagtGTGACtcgta/R	−2299	9.5	0.772	14 (6)	31.7
Nudt8	nudix (nucleoside diphosphate linked moiety X)-type motif 8	tcacaGTCACacaGCAaagta/F	−878	14.4	0.846	7 (1)	2.12
Oxct1	3-oxoacid CoA transferase 1	tgaggGTCAGtcaGCAcgctg/F	−279	9.5	0.78	6 (2)	1.31
Pdha1	pyruvate dehydrogenase E1 alpha 1	gaagaGTGACtgaGGAagact/F	−1276	10	0.814	6 (4)	1.34
Pdhb[1]	pyruvate dehydrogenase beta	ctgatTGCtgaGTCATctaag/R	−1822	13.3	0.934	9 (5)	1.44
Pfkfb1	6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 1	tcttgTGCtgtGTAAGgaggg/R	−4051	11.2	0.815	10 (5)	1.55
Phgdh	3-phosphoglycerate dehydrogenase	ctccgGTGACtggGCAgggtt/F	59	9.8	0.871	11 (3)	1.98
Phyh	phytanoyl-CoA hydroxylase	catttTGCtgtCTCACaggtg/R	−2798	12.9	0.853	8 (5)	1.79
Pkm[1]	pyruvate kinase, muscle	tctgcTGCtgaGTCATtactg/R	−2757	15.1	0.939	10 (4)	1.24
Pla2g16	phospholipase A2, group XVI	gggagGTGACtgaGCAgagcc/ F	−4218	10.9	0.909	8 (5)	1.61
Plin4	perilipin 4	tctggGTGACacaGCAgctcg/F	94	14.9	0.924	5 (3)	2.74
Plin5	perilipin 5	taatcTGCtgtTTAAT cctga/R	−1 119	12.1	0.773	12 (9)	2.80
Pln	phospholamban	taaagTGCtgaATCATaatgc R	−4526	14.8	0.895	9 (4)	10.1
Pnpla3	patatin-like phospholipase domain containing 3	gtccaGGCtgaGTCACtgaag R	−813	10.3	0.901	4 (3)	3.03
Ppal	pyrophosphatase (inorganic) 1	aaaccATAACcaaGCActaga/F	−2110	10.3	0.804	9 (4)	1.85
Ppargcla	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	actagATAACtctGCAttatt/F	−2195	9.5	0.802	8 (6)	1.76
Ppargcab	peroxisome proliferative activated receptor, gamma, coactivator 1 beta	gctgcTGCtgaATCAActtgg/R	−2602	11	0.874	10 (6)	1.50
Prdx3[1]	peroxiredoxin 3	ccaacATGAAgcaGCAtatga/F	−4476	10.9	0.851	11 (8)	1.34
Pxmp2[1]	peroxisomal membrane protein 2	acaaaCGCttaGTCAGcgcgg R	−3633	14.7	0.909	7 (5)	1.50
Qsox1[1]	quiescin Q6 sufhydryl oxidase 1	atagtTGCttgGTCAGgtgcc R	−2189	9.9	0.872	10 (1)	1.56
Sdha	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	tatcgGTGACttaGAGataaa F	−1070	9.4	0.785	10 (3)	1.31
Sdhb[1]	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	gaaagCTGACgcaGCCcagtg/F	−3662	9.1	0.868	12 (6)	1.58
Sdhd	succinate dehydrogenase complex, subunit D, integral membrane protein	aaaaaGTAACagaGCAaatgt/F	−1373	8.8	0.797	7 (5)	1.34
Slc16a1[1]	solute carrier family 16 (monocarboxylic acid transporters), member 1	cgcggCGCcggGTCACgtggc/ R	−212	6.5	0.825	10 (6)	1.33
Slc25a18	solute carrier family 16 (monocarboxylic acid transporters), member	aaataCGCtgtGTCACtttat/R	−1773	12.5	0.894	15 (6)	1.44

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Slc27a1[1]	solute carrier family 27 (fatty acid transporter), member 1	gagcaCTGACtgtGCTagctt/F	−1175	7	0.831	5 (4)	1.57
Slc27a2	solute carrier family 27 (fatty acid transporter), member 2	ttcctGTTACtcaGCAggctt/F	−4469	11.3	0.844	14 (8)	3.50
Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4	gagaaTGCctaTTCATcctcc/R	−2266	9.5	0.817	4 (3)	2.18
Slc47a1	solute carrier family 47, member 1	ccctgATGACtaaGCAttcca/F	−1687	13.3	0.925	9 (4)	4.02
Sucla2	succinate-Coenzyme A ligase, ADP-forming, beta subunit	gaagtAGCagcGTCACagttc/R	−220	8.7	0.841	7 (2)	1.35
Tecrl	trans-2,3-enoyl-CoA reductase-like	gaatcATAACgctGCAaaata/F	−213	7.5	0.768	5 (1)	2.28
Thrsp	thyroid hormone responsive SPOT14	atcagGGCtaaGTCAGactca/R	−3275	12.4	0.896	8 (5)	4.11
Tnnc1	troponin C, cardiac slow skeletal	gcccaGTGAGgcaGCAccagt F	−3469	10.4	0.843	9 (6)	4.85
Tnni3	troponin I, cardiac 3	accttTACtgaGTCATctctc R	−3438	10.8	0.823	9 (5)	6.94
Tomm5[1]	translocase of outer mitochondrial membrane 5	ccaggGTGACacaGAAaaatc F	−906	14.3	0.83	5 (2)	1.36
Tmp1	tropomyosin 1, alpha	tggggGTGACtggGCAtcctc/F	−2579	10.4	0.872	13 (8)	1.69
Trf	transferrin	ccaatTGCccaATCACcccgc/R	−71	9.9	0.816	9 (1)	1.29
Uox[1]	urate oxidase	taacgGGCtttGTCATccctt/R	−4600	11.2	0.863	14 (7)	1.64
Uqcr10	ubiquinol-cytochrome c reductase, complex III subunit X	ttaatTACttaGTCAAtttaa/R	−2159	7.9	0.804	3 (2)	1.44
Uqcr11	ubiquinol-cytochrome c reductase, complex III subunit XI	ttttcTGCttgGTCACtgggg/R	−3126	13.7	0.901	10 (4)	1.50
Uqcrc1	ubiquinol-cytochrome c reductase core protein 1	tttttTGCttaGTTATtttat/R	−593	9.1	0.822	4 (3)	1.55
Uqcrfs1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	tctacTGCagaGTGATgcttc/R	−2866	11.6	0.814	6 (2)	1.58
Uqcrh	ubiquinol-cytochrome c reductase hinge protein	gtgtgTGCtgaCTCACacctg/R	−3276	11.9	0.869	7 (4)	1.38
Uqcrq	ubiquinol-cytochrome c reductase, complex III subunit VII	tctccTGCtcaGTCACtctag/R	−3503	12.9	0.925	11 (4)	1.50
Usp13	ubiquitin specific peptidase 13	cccgcCTGACgctGCAgctgg/F	−106	9.9	0.857	9 (3)	1.36

Selected putative AREs in the upstream of each gene are shown. Full list of potential AREs and details are in the supplemental Table 3 of previous publication (Cho et al. 2019). Bioinformatic analysis (Wang et al. 2007) done for Nrf2-dependent, sulforaphane-induced lung genes in ICR mice (Cho et al. 2019). Mouse genome build mm9 used.

*ARE core-like sequence 5'-RTKAYnnnGCR-3' (R=A or G, K=G or T, Y=C or T, n=A, C, G, or T), forward (F) or reverse complementary (R) orientation.

§transcription start site.

†position weight matrix score. The minimal PWM of functional ARE=6.4 and the maximal PWM of functional ARE=21.8 (the median PWM of all known functional AREs= 15) in the current bioinformatic model (Wang et al. 2007).

‡matrix similarity score.

[¶] Total number of ARE-like sequences determined in the gene loci (including up to 5 kb of upstream and downstream sequences). Number of the potential AREs in the upstream region including 5'-UTR is in the parenthesis.

[∞] Fold increase over PBS-pretreatment control in *Nrf2* wild-type (ICR) mouse lung at day 9 after sulforaphane treatment (oral, 9 μmol) on days 1, 3, and 5 (Cho et al. 2019).

[1] Genes with Nrf2/sMaf-bound functional AREs based on mouse ENCODE ChIP-seq data (Mouse et al. 2012; Yue et al. 2014).