Nrf2 and the Nrf2-Interacting Network in Respiratory Inflammation and Diseases

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Abstract Atmospheric pollutants and cigarette smoke influence the human respiratory system and induce airway inflammation, injury, and pathogenesis. Activation of the NF-E2-related factor 2 (Nrf2) transcription factor and downstream antioxidant response element (ARE)-mediated transcriptions play a central role in protecting respiratory cells against reactive oxidative species (ROS) that are induced by airway toxins and inflammation. Recent studies have revealed that Nrf2 can also target and activate many genes involved in developmental programs such as cell proliferation, cell differentiation, cell death, and metabolism. Nrf2 is closely regulated by the interaction with kelch-like ECH-associated protein 1 (Keap1), while also directly interacts with a number of other proteins, including inflammatory factors, transcription factors, autophagy mediators, kinases, epigenetic modifiers, etc. It is believed that the multiple target genes and the complicated interacting network of Nrf2 account for the roles of Nrf2 in physiologies and pathogeneses. This chapter summarizes the molecular functions and protein interactions of Nrf2, as well as the roles of Nrf2 and the Nrf2-interacting network in respiratory inflammation and diseases, including acute lung injury (ALI), asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis (PF), cystic fibrosis (CF), viral/bacterial infections, and lung cancers. Therapeutic applications that target Nrf2 and its interacting proteins in respiratory diseases are also reviewed.

1 Introduction

Environmental toxins generated by modern industry significantly influence human health. The respiratory system is most susceptible to pollutants as it is directly exposed to atmospheric toxins such as airborne chemicals, O_3, particulate matter (PM), and cigarette smoke (CS). Respiratory diseases and relevant secondary symptoms, including ALI, asthma, COPD, bacterial/viral infections, lung fibrosis, and lung cancers, are among the most common causes of severe illness and death worldwide [1].

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Most of the airway pathogeneses are related to xenobiotic and oxidative stresses occurring in different types of respiratory cells. These stresses can be triggered directly by the inhaled toxins (xenobiotics) or by excess reactive oxygen species (ROS) that are induced by many of these toxins. Intracellular accumulation of ROS may damage macromolecules including DNA, proteins, and lipids, thereby inducing cell defects or death. Imbalanced redox homeostasis and cell damage lead to inflammatory responses, which further enhance oxidative stress. Cellular factors that can respond to and reduce oxidative/xenobiotic stresses are essential protective machinery against airway pathogenesis.

NF-E2-related factor 2 (Nrf2), a cap 'n' collar (CNC)-bZIP family transcription factor, is a central regulator that protects cells against ROS and xenobiotics [2, 3]. Activated by oxidative and xenobiotic stimuli, Nrf2 binds to antioxidant response elements (AREs) and activates a battery of antioxidant and detoxifying genes [4]. Nrf2 also activates many developmental genes, allowing Nrf2 to regulate programs that are related with cell proliferation, cell differentiation, cell death, and metabolism [4]. In addition, Nrf2 interacts with many other proteins and pathways, providing a complicated regulatory network for redox homeostasis and other cellular programs. Nrf2 is identified as the key player and important drug target for a number of human diseases including respiratory diseases [5]. Fully understanding the molecular partners and downstream functions of Nrf2 will significantly aid in the development of Nrf2-targeting therapies. This chapter first reviews the molecular mechanisms of Nrf2, focusing on the multiple interacting partners of Nrf2. Next, the molecular functions and therapeutic applications of Nrf2 and the Nrf2-interacting network in respiratory inflammation and diseases are summarized.

2 Molecular Mechanisms of the Nrf2 Network

2.1 Keap1-Nrf2 Pathway

The key regulator of Nrf2 activity in response to oxidative and xenobiotic stimuli is kelch-like ECH-associated protein 1 (Keap1). As an adaptor of the Cul3-based E3 ligase, Keap1 confines Nrf2 to the cytoplasm through mediating Nrf2 ubiquitination and degradation [6]. The Keap1–Nrf2 interaction is mediated by the Kelch repeats of the Keap1 dimer and the N-terminal DLG and ETGE motifs of Nrf2. Cysteine residues (e.g., C151, C273, C288) on Keap1 can be modified by a broad range of oxidative and xenobiotic compounds [7, 8]. According to the established model, cysteine modifications alter the conformation of the Keap1 dimer and interfere with the Keap1–Nrf2 interaction, thereby blocking the proteasomal degradation of Nrf2. The stabilized Nrf2 proteins accumulate in the nucleus and activate transcriptions [9, 10]. After induction, Keap1 can enter the nucleus and shuttle Nrf2 back to the cytosol [11]. The genetic interaction of Keap1 and Nrf2 has been verified in vivo. *Keap1$^{-/-}$* mice die before weaning due to a severe dysfunction of keratinocytes, while co-knockout of Nrf2 significantly rescues the viability of *Keap1$^{-/-}$* mice [12].

2.2   Nrf2-Downstream Genes and Functions

2.2.1   Antioxidant and Detoxifying Genes

Microarray analyses based on Nrf2 activators and *Nrf2* knockout mice have identified many antioxidant and detoxifying genes that are controlled by Nrf2-ARE [4, 13–15]. Most of these genes code for enzymes that catalyze the metabolism or removal of ROS and other toxins, including: (1) phase I antioxidant enzymes such as superoxide dismutases (SODs), glutathione peroxidase (GPx), and glutathione reductase (GR); (2) phase II detoxifying enzymes such as glutathione-S-transferase (GST), NADP(H):quinone oxidoreductase-1 (NQO1), glutamate-cysteine ligases (GCLM/GCLC); (3) phase III xenobiotic transporters such as multidrug resistance protein 1 (MRP1); and (4) other stress response proteins such as heme oxygenase-1 (HO-1). Protective roles of some of these enzymes against respiratory inflammation and damages have been demonstrated by several studies. For example, the lungs of *Sod2* mutant mice have enhanced sensitivity to hyperoxia [16], while overexpression of *Sod2* in the mouse airway reduces the hyperoxia-induced lung inflammation and injury [17]. Activation of the Nrf2-ARE antioxidant pathway is believed to be an efficient therapeutic strategy for redox-related lung diseases.

2.2.2   Other Nrf2-Target Genes and Functions

Recent studies in different model systems have revealed that Nrf2 can target genes independent of oxidative and xenobiotic responses, demonstrating that Nrf2 can act as more than just a detoxifying factor. For examples, a ChIP-seq study combining microarray assays using mouse embryonic fibroblasts with either enhanced or reduced levels of Nrf2 identified around 1000 Nrf2-target genes, more than half of which were involved in cell proliferation [4]. Nrf2 can control adipogenesis through binding to and activating *peroxisome proliferator-activated receptor γ* (*PPARγ*), *retinoid X receptor α* (*RXRα*), and *small heterodimer partner* (*SHP*) nuclear receptor genes as well as some lipid metabolism genes [18–21]. The Nrf2-activated *RXRα* can also control the differentiation of acute myeloid leukemia cells [22]. In addition, Nrf2 can regulate neuronal stem cell fate through activating genes that inhibit self-renewal or promote differentiation [23]. Another essential Nrf2-target gene is *p62*, which mediates the role of Nrf2 in the regulation of autophagy [24]. In a human lung cancer cell line, NRF2 activates transcripts of anti-apoptotic factor Bcl-2 and glucose metabolic enzymes [25, 26], indicating the promotive role of Nrf2 in cancer cell proliferation. It is notable that Nrf2 can target and inhibit some proinflammatory cytokine genes such as *IL6* through an ARE-independent manner, revealing a novel mechanism that mediates the anti-inflammation function of Nrf2 [27].

Structural and functional conservation of the Keap1-Nrf2 signaling has been revealed in mammals, zebrafish, and *Drosophila* [8, 28]. Studies using non-mamalian model organisms provide additional insights into the biological functions of Nrf2. In *Drosophila*, CncC and dKeap1 (homologs of Nrf2 and Keap1, respectively) regulate metamorphosis through targeting and activating ecdysteroid biosynthetic genes

and response genes in a tissue-specific manner [29], indicating a potential role of
Nrf2 family proteins in the regulation of steroid hormones. CncC and dKeap1 also
regulate intestinal stem cell proliferation in *Drosophila* [30]. It is believed that mul-
tiple developmental functions of Nrf2 and Keap1 in specific tissues contribute to
their complicated roles in diseases [31, 32]. How Nrf2 selectively targets and acti-
vates developmental genes remains to be elucidated, but it is likely that it acts
through mechanisms that differ from the classic ARE-dependent antioxidant path-
way. Notably, recent studies showed that dKeap1 can directly bind to chromatin and
function as a transcription coactivator with CncC through interaction with CncC at
specific genomic loci [29, 33].

2.3   Other Nrf2-Interacting Proteins

Besides Keap1, a number of proteins that interact with Nrf2 have been identified.
These protein partners can regulate the activity, subcellular localization, degrada-
tion, and chromatin-binding specificity of Nrf2. This places Nrf2 in the center of a
multi-layer regulatory network for redox homeostasis and other cellular programs
(Fig. 1). These interactions are believed to mediate the complicated roles of Nrf2 in
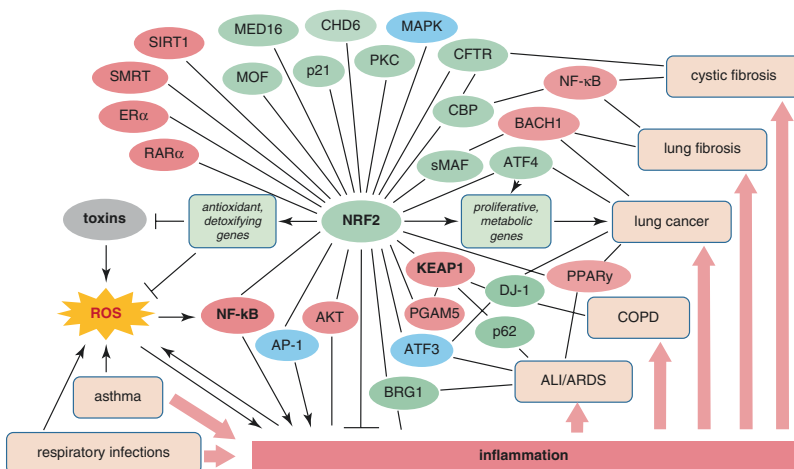pathogeneses, including respiratory diseases.

Fig. 1 Nrf2-interacting network and its correlation with respiratory inflammation and diseases.
Nrf2 plays a protective role against respiratory inflammation and respiratory diseases through
mediating the transcriptional responses to oxidative and xenobiotic stresses. Nrf2 can also promote
lung oncogenesis through activating genes that facilitate cell proliferation. In addition, Nrf2 inter-
acts with many proteins and pathways, placing Nrf2 in the center of a network that controls redox
homeostasis, inflammation, and pathogenesis. The ovals represent proteins that can interact with
Nrf2. The positive and negative regulators of Nrf2 activity are colored in green and red, respec-
tively. Proteins in blue ovals can either activate or inhibit Nrf2 activity. The identified roles of Nrf2
and some of the Nrf2-interacting proteins in respiratory inflammation and diseases are represented
with line connections

2.3.1 Inflammatory Factors

Nuclear factor kappa light chain enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1) are classic transcription factors that induce inflammation in response to oxidative stress through the activation of genes encoding proinflammatory cytokines and chemokines. The essential roles of NF-κB and AP-1 proteins in inflammation and chronic lung diseases (e.g., asthma, IPF, and COPD) have been identified by many studies [34–36]. It is noted that oxidative stress can co-activate NF-κB and Nrf2 pathways. For example, PM exposures co-induce NF-κB and Nrf2-HO-1 in both mouse lungs and human bronchial epithelial cells [37, 38]. NF-κB can inactivate Nrf2 by competing with the CBP–Nrf2 interaction or by recruiting the transcription repressor HDAC3 to ARE [39]. The AP-1 subunit c-Jun can dimerize with Nrf2 at AREs and co-activate Nrf2-induced transcription, while another AP-1 subunit c-Fos can suppress Nrf2-induced transcription [40, 41]. Crosstalk between Nrf2 and these inflammatory factors establishes a mechanism whereby Nrf2 regulates inflammation.

2.3.2 Small Maf Transcription Factors

Nrf2 can form heterodimers with small Maf (sMaf) transcription factors, including MafF, MafG, and MafK [42]. These interactions are mediated by their leucine zipper domains. It has been well established that the Nrf2-sMaf complexes recognize ARE and the sMaf proteins can support Nrf2-mediated transcriptional activation [43]. Combinatory knockouts of MafF and MafG can rescue the lethality of *Keap1* null mice, suggesting the cooperative roles of sMaf proteins with Nrf2 in vivo [44]. ChIP-Seq analyses of Nrf2-MafG-binding sites indicate that Nrf2-sMaf heterodimers globally regulate antioxidant and metabolic genes [45].

2.3.3 ATF/CREB Transcription Factors and Cofactors

A number of transcription factors, coactivators, and mediators can form complexes with Nrf2 at AREs and control Nrf2-mediated transcription. The mediator MED16 directly interacts with Nrf2 and mediates 75% of Nrf2-dependent transcription [46]. CREB-binding protein (CBP) and its cofactor p300 can bind the Neh4/5 domains of Nrf2. It was observed that p300 and Nrf2 can be co-recruited to ARE in response to oxidative stress [47, 48]. The Nrf2–CBP interaction can be regulated by a $Cl^-/HCO3^-$ channel protein cystic fibrosis transmembrane conductance regulator (CFTR), which also interacts with Nrf2 and promotes Nrf2-dependent transcription in human bronchial epithelial cells [49]. Activating transcription factor 4 (ATF4) can dimerize with Nrf2 at ARE and co-activate *HO-1* [50]. Formation of the ATF3-Nrf2 dimer can compete with the Nrf2–CBP interaction at ARE and suppress Nrf2-induced transcription [51]. On the other hand, ATF3 depletion in human bronchoalveolar epithelial cells enhances NRF2 degradation through KEAP1 and DJ-1 pathways [52]. Therefore, ATF3 can either positively or negatively control Nrf2 activity.

2.3.4   Nuclear Receptors

Several nuclear receptors can enhance or inhibit Nrf2 activity through interactions with Nrf2. PPARγ can directly bind to Nrf2 [53], while Nrf2 and PPARγ also regulate the transcription of each other in the lungs of hyperoxia-induced mice [54, 55]. Estrogen receptor α (ERα) can form a complex with Nrf2 and suppress the Nrf2 pathway in a ligand-dependent manner [56]. Retinoic acid receptor α (RARα) can inhibit Nrf2 binding to ARE [57]. Direct interactions between DNA-binding domains of RARα and the Neh7 domain of NRF2 have been reported recently in acute promyelocytic leukemia, in which RARα fuses with promyelocytic leukemia protein (PML) [58].

2.3.5   p62 and Autophagy

Nrf2 can directly activate gene expression of p62, a selective adaptor that targets ubiquitinated cargos to the autophagosome during autophagy [24, 59]. In addition, phosphorylated p62 can interact with the Kelch repeats domain of Keap1 and compete with the Keap1–Nrf2 interaction, resulting in stabilization of Nrf2 and activation of antioxidant genes [24, 60, 61]. Inhibition of autophagy by arsenic can induce accumulation of autophagosomes and p62, resulting in prolonged activation of Nrf2 [62]. On the other hand, since p62 is an autophagy target, induction of autophagy can enhance the degradation of p62-Keap1 complexes, thereby activating the Nrf2 pathway [63].

2.3.6   Kinases and Nrf2 Phosphorylation

In response to specific compounds, Nrf2-ARE activity can be controlled by protein kinases such as PKC, Akt, ERK, and p38 MAPK in specific cell types, including pulmonary epithelial cells [64]. Nrf2 can be directly phosphorylated by PKC and MAP kinases [65, 66]. How this phosphorylation controls Nrf2 function remains to be elucidated. ERK/MAPK is required for the oxidative-induced NRF2 nuclear localization in HepG2 cells [67]. However, another study indicates that phosphorylation of Nrf2 by MAPK at multiple sites only moderately enhances Nrf2 nuclear accumulation [66]. The p38 MAPK inhibits Nrf2-mediated *HO-1* expression in HepG2 and MEF cells [68, 69]. In *C. elegans*, phosphorylation of the Nrf2 homolog SKN-1 by p38 MAPK or by Akt have opposite effect on SKN-1 nuclear accumulation and transcription activity [70, 71]. In *Drosophila*, CncC functions downstream of the Ras/ERK pathway to activate ecdysone-synthetic genes in the prothoracic gland [29, 72]. In addition, the constitutively activated Ras^{V12} modulates CncC binding at specific chromatic loci, indicating that MAPK may directly control the chromatin binding specificity of Nrf2 [29].

2.3.7   Acetylation of Nrf2

Nrf2 can be acetylated by CBP/p300 [48]. Upon oxidative stimuli, acetylation of Nrf2 at Lys-588/591 by CBP/p300 enhances the binding of Nrf2 to ARE promoters, supporting CBP/p300 as a coactivator of Nrf2 in oxidant responses [48]. Alternative reading frame (ARF), a tumor suppressor that triggers apoptosis, directly interacts with Neh1/3 domains of NRF2 in a human non-small cell lung carcinoma cell line [73]. It was proposed that the ARF–Nrf2 interaction prevents acetylation of NRF2 by CBP and suppresses Nrf2-mediated transcription [73, 74]. Males absent on the first (MOF), a histone acetyltransferase, can acetylate Nrf2 at Lys-588 and is required for Nrf2-induced transcription [75]. Nrf2 can be de-acetylated by sirtuin 1 (SIRT1) at Lys-588/591, which suppresses Nrf2-dependent transcription [76]. These studies indicate acetylation as a positive regulatory mechanism for Nrf2 activity. Alternatively, since both CBP/p300 and MOF are histone acetyltransferases [75, 77], these proteins may cooperate with Nrf2 indirectly through the epigenetic regulation of chromatin structure.

2.3.8   Epigenetic Machinery

Increasing lines of evidences reveal the interactions of Nrf2 with epigenetic modifiers. Brahma-related gene 1 (Brg1), the ATPase of SWI/SNF complex, forms a complex with Nrf2 selectively at the *HO-1* promoter and activates *HO-1* transcription through Brg1-mediated Z-DNA formation [78, 79]. Chromodomain helicase DNA-binding protein-6 (CHD6) interacts with the Neh3 domain of Nrf2 and facilitates Nrf2-induced *NQO1* expression [80]. Silencing mediator for retinoid and thyroid hormone receptor (SMRT), a transcriptional repressor that mediates histone deacetylation, can interact with Nrf2 Neh4/5 domains and inhibit Nrf2-induced *GSTA2* expression [81]. Notably, a recent study in *Drosophila* showed that depletion of CncC or dKeap1 suppresses pericentric silencing and decreases the level of heterochromatin marker H3K9me2, providing direct evidence in support of the epigenetic role of Keap1/Nrf2 family proteins in chromatin remodeling [82].

2.3.9   Other Nrf2-Interacting Proteins

BTB domain and CNC homolog 1 (Bach1) is a CNC family transcription factor that is essential in controlling heme level through the inhibition of *HO-1* gene. Bach1 can dimerize with sMaf and compete with the Nrf2–sMaf interaction at ARE sites and inhibit the activation of *HO-1* [83]. NRF2-induced *HO-1* expression requires inactivation of BACH1 [84]. BACH1 can promote invasion and metastasis by activating metastatic genes [85, 86]. Recent studies showed that Nrf2 activation can inhibit the F-box Protein 22 (Fbxo22)-induced Bach1 degradation, revealing a novel mechanism whereby the Nrf2-Bach1 pathway promotes carcinogenesis [87, 88].

The p21 tumor suppressor can interact with the DLG and ETGE motifs of Nrf2, compete with the Keap1–Nrf2 interaction and stabilize Nrf2 [89]. This discovery adds one more mechanism to the multiple functions of the p53-p21 pathway in cytoprotection and cell survival, indicating a potential role of the Nrf2-p21 network in oncogenesis [90].

DJ-1/PARK7 is a protein that plays multiple antioxidant and cytoprotective functions and is associated with cancer and neurodegeneration. DJ-1 can stabilize Nrf2 by inhibiting the Keap1–Nrf2 interaction and proteasomal degradation of Nrf2 [91]. High levels of both DJ-1 and NRF2 were seen in a large set of lung carcinomas [92].

Phosphoglycerate mutase 5 (PGAM5) is a mitochondrion-binding protein whose ESGE motif can interact with the Kelch domain of Keap1 [93]. It was proposed that a Keap1 dimer can simultaneously interact with one Nrf2 and one PGAM5 molecule. This PGAM5-Keap1-Nrf2 complex can be anchored to mitochondrial surface and likely serve as an antioxidant mechanism specifically targeting ROS leaked from mitochondria [94].

3   The Roles of Nrf2 and Nrf2-Interacting Proteins in Respiratory Diseases

3.1   ALI/ARDS

Acute lung injury (ALI) is a syndrome manifesting as lung edema, inflammation, and alveolar hemorrhaging. If not treated, ALI can progress into its severe form, acute respiratory distress syndrome (ARDS). ALI can be induced by aspiratory injuries caused by various stimuli. For example, mechanical ventilation (MV) along with hyperoxia is used as a therapy for patients with respiratory dysfunction, but can induce lung inflammation and ventilator-induced lung injury (VILI), which directly causes or exacerbates ALI. ROS play a central role in the pathogenesis of lung injury and ALI/ARDS [95]. Protective roles of Nrf2-ARE-activated genes against respiratory injury have been revealed in mouse models with induced lung injury. For examples, overexpression of SOD2 in airways of mice can reduce the hyperoxia-induced lung injury and inflammation [17, 96]. In support of a protective role of Nrf2 in the lungs, enhanced lung injuries and inflammatory responses to MV were found in *Nrf2*⁻/⁻ mice compared with wild-type mice, and these responses can be restored by supplementing *Nrf2*⁻/⁻ mice with antioxidant N-acetylcysteine (NAC) [97]. Sulforaphane (SFN), an activator of Nrf2, can reduce pulmonary injury in wild-type mice but not in *Nrf2*⁻/⁻ mice [98]. CDDO-imidazolide (CDDO-Im), another Nrf2 activator, protects the lungs of mice against CS-induced oxidative stress and alveolar damage through the Nrf2 pathway [99]. These studies support the use of Nrf2-activating antioxidant therapeutics as a way to attenuate pulmonary inflammation and damage in ALI/ARDS patients.

Cooperative roles of Nrf2 with several interacting partners, including ATF3, PPARγ, Brg1, p62, and Akt, in lung inflammation and injury have been revealed. ATF3-deficient mice have enhanced susceptibility to ALI and VILI, which is associated with the recruitment of inflammatory cells and loss of junctions among resident cells [52, 100]. It was proposed that ATF3 can reduce lung injury through inhibiting Nrf2 degradation via Keap1 and DJ-1 pathways [52]. The protective role of Nrf2 in hypoxia-induced ALI can also be mediated by PPARγ, which can be transcriptionally induced by Nrf2 and activate several anti-inflammatory and antioxidant genes [55]. In a hepatic ischemia/reperfusion (HIR)-induced ALI mouse model, overexpression of Brg1 increases Nrf2 activity and reduces ROS and inflammatory factors in lung tissues [101]. Autophagy plays both a positive and negative role in ALI, and this dual role is suggested to be mediated by the Keap1-Nrf2-p62 pathway [102]. The protective role of the PI3K/Akt-dependent activation of the Nrf2-HO-1 pathway against lipopolysaccharide (LPS)-induced lung inflammation and injury was revealed in mice treated with desoxyrhapontigenin [103].

3.2   Asthma

Asthma is a lung disorder that is characterized by the swelling of airways, which causes breathing difficulty, coughing, wheezing, and chest tightness. The swelling of the airways can be initiated or exacerbated by environmental allergens (pollen, mold, etc.) and irritants (smoke, dust, gas, etc.). In a mouse model, challenging by the allergen ovalbumin increases inflammation and asthma symptoms by inducing the expression of proinflammatory factors and increasing mucus secretion and airway hyperresponsiveness. In these allergen-challenged mice, Nrf2 knockout resulted in a worsening of asthma symptoms, represented by a higher number of inflammatory cells in bronchoalveolar lavage (BAL) fluid and an increase in mucus-producing cells in the proximal airways [104]. When being exposed to acetylcholine, a molecule involved in asthma pathogenesis, the lungs of $Nrf2^{-/-}$ mice are more prone to asthmatic symptoms than the wild-type mice, represented by the less stretch and the greater resistance of air passage through the airways [104]. In humans, a recent study on childhood bronchial asthma in Egyptian children found that children with lower levels of NRF2 in the serum had more severe asthma [105].

Given the role of Nrf2 in protection against asthma, a number of drugs targeting Nrf2 have been tested in rats and mice and been found to reduce asthma symptoms. For example, polydatin treatment of asthmatic mice showed upregulated Nrf2 signaling and reduced oxidative stress in the lungs as well as attenuation of asthma symptoms [106]. Treatment of asthmatic rats with methanolic extract from Artemisia pallens increased Nrf2 levels in the lungs and reduced oxidative stress and inflammation [107]. Taken together, these studies suggest that Nrf2 plays an important role in protecting the lungs against asthma and can be a target for asthma treatment.

3.3 COPD

Chronic obstructive pulmonary disease (COPD) is usually caused by exposure to cigarette smoke (CS) or occupational exposure to various chemicals and smoke, and characterized by airway inflammation and emphysema. Emphysema is mainly caused by damage to alveolar walls and enlargement of air sacs, making it difficult to exhale and inhale. Several studies in mice and humans have indicated that Nrf2 appears to play a protective role against emphysema, and the loss of Nrf2 activity correlates with the progression of COPD. Emphysema can be induced in mice by elastase, which is an enzyme that breaks down elastin, a protein that is vital to the elasticity of the lungs. Although both wild-type and $Nrf2^{-/-}$ mice have alveolar wall damage and enlarged airspaces upon elastase treatment, these symptoms are exacerbated in the $Nrf2^{-/-}$ mice [108]. Elastase-treated mice had a significant increase in expression of Nrf2-regulated antioxidant genes in the alveolar macrophages. Transplantation of wild-type bone marrow to $Nrf2^{-/-}$ mice significantly reduced emphysema symptoms, indicating that the Nrf2-induced antioxidant pathway in alveolar macrophages plays a protective role against lung inflammation and emphysema [108]. In *Drosophila,* drug-induced increase in Nrf2/CncC activity partially rescued the flies that had been chronically exposed to CS [109]. Fisetin, a plant flavonoid compound, has been suggested as a potential therapeutic to COPD since it can activate the Nrf2 pathway and reduced the oxidative stress, inflammation, and lung damage caused by CS exposure to rats [110]. In human studies, it has been found that initial exposure to CS results in activation of NRF2 in alveolar macrophages. However, in older smokers with COPD, NRF2 expression was reduced in alveolar macrophages [111]. Genetic polymorphisms in NRF2-regulatory genes or NRF2-target genes have also been found to contribute to COPD [112]. These studies demonstrate the importance of Nrf2 in protecting the lungs against oxidative stress and damage from CS while suggesting Nrf2 as a potential therapeutic target in COPD treatment.

The role of Nrf2 dysfunction in COPD is likely mediated by the loss of DJ-1. In support of this model, DJ-1 overexpression activates Nrf2 and inhibits apoptosis of alveolar type II cells that are treated with CS extract, suggesting the protective role of the DJ-1-Nrf2 pathway against the CS-induced oxidative stress and inflammatory response [113]. Crosstalk of Nrf2 and NF-κB in inflammation and COPD have been revealed by many studies [35]. In particular, a number of nature products or pharmacological compounds that can simultaneously activate the Nrf2 pathway and inhibit the NF-κB pathway have shown therapeutic potency against respiratory inflammation and COPD [114–119].

3.4 Lung Fibrosis/IPF

Lung fibrosis is generally caused by the proliferation of fibroblasts from profibroblasts and the subsequent accumulation of extracellular matrix proteins. Idiopathic pulmonary fibrosis (IPF) is a disease that is characterized by progressive scarring

of the lung tissue around the alveoli. This results in thickening and stiffness of the alveolar walls and makes it difficult for oxygen to penetrate into the bloodstream, leading to symptoms that include dry coughing, breathing difficulty, and finger clubbing. Although the causes of IPF remain to be fully understood, oxidative stresses that are induced by stimuli such as CS are thought to be a contributing factor [120]. IPF patients have higher than normal levels of ROS in the BAL fluid and lower than normal levels of antioxidant enzymes (e.g., SOD) and antioxidants in the lungs. Bleomycin-induced pulmonary injury, inflammation, and fibrosis in mice has been used as a model to study lung fibrosis. $Nrf2^{-/-}$ mice have significantly higher sensitivity to the bleomycin-induced fibrogenesis than wild-type mice [121], suggesting that Nrf2 plays a protective role against pulmonary fibrosis.

It is well-known that the herbicide paraquat (PQ) can cause pulmonary fibrosis in animals and human [124]. Rapamycin is known to protect against PQ-induced lung injury and has been suggested as a potential treatment for pulmonary fibrosis [122]. Treatment of rats or mice with rapamycin led to an increase in Nrf2 levels and a decrease of PQ-induced ROS and fibrosis-related factors, and the effect of rapamycin can be reversed by an Nrf2 knockdown [123, 124]. In several in vitro assays, the PQ-induced fibroblast-to-myofibroblast transition (FMT) or epithelial-mesenchymal transition (EMT), both important cellular processes in fibrosis, were inhibited by treatment with rapamycin or Nrf2 activator [123, 124]. Rosavin, emodin, and pirfenidone are other drugs that have recently been proposed as treatments for pulmonary fibrosis [125–127]. Treatment of mice or rats with these compounds increases the expression and/or activity of Nrf2 and reduces the bleomycin-induced lung fibrosis. It is also noticed that these drugs can simultaneously inhibit NF-κB or Bach1, suggesting that the unbalanced Nrf2/NF-κB or Nrf2/Bach1 equilibrium may contribute to the development of IPF.

3.5   Cystic Fibrosis

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-dependent and ATP-gated chloride channel that regulates epithelial surface fluid secretion in respiratory and gastrointestinal tracts. In the lung of a CF patient, the mucus blocks the airways and traps bacteria, leading to infections, inflammation, and respiratory dysfunctions. CF is commonly associated with the F508del mutation of CFTR. It was found that CFTR interacts with Nrf2 in human bronchial epithelial (hBE) cells, and that CFTR–NRF2 interaction decreases in CF hBE cells [49]. VX809 and VX661, the approved correctors for CFTR-F508del, restores Nrf2 phosphorylation and its interaction with CBP [49]. Excessive inflammation triggered by the activation of NF-κB by oxidative stress is highly related with the morbidity and mortality of CF [128]. Roles of CBP-Nrf2-NF-κB network in CF have been revealed in airway epithelial cells both in vitro and in vivo. Dramatic decrease

of Nrf2 activity is detected in CF cell lines as well as lungs and excised nasal epithelia of CF mice [129]. The defective Nrf2 in CF cells is associated with cAMP signaling, and inhibition of cAMP signaling can interfere in CBP interactions with Nrf2 and NF-κB, resulting in enhanced Nrf2 activity and reduced NF-κB activity in CF cells [130]. Combined, these studies implicate that reducing inflammation in CF by strengthening CBP–Nrf2 or weakening CBP–NF-κB interactions might be potential therapies for CF.

3.6   Respiratory Infections

There are many different viral and bacterial infections that can occur in airways. The most common pathogens are viruses, including respiratory syncytial virus (RSV), influenza A virus (IAV), adenovirus, coronavirus, etc. The less common but very present bacterial pathogens include *Streptococcus pneumoniae, Haemophilus influenzae,* and *Mycobacterium tuberculosis* (TB). Both viral and bacterial infections occur in the upper and lower respiratory tract as well as the esophagus. Infection-induced symptoms such as coughing and mucus build up can cause damage to the epithelial tissue and create ROS.

RSV targets the epithelial airway tissues and has been found to activate deacetylation and proteasomal degradation of Nrf2 [131]. *Nrf2$^{-/-}$* mice that are infected with RSV show more severe lung inflammation and injury as well as attenuated viral clearance than wild-type mice [132]. IAV mainly targets the epithelial tissue and specifically alveolar type I and II epithelial cells. An in vitro study shows that infection of these cells by IAV increases NRF2/HO-1 level. NRF2 knockdown causes these cells to be more sensitive to IAV-induced injury, while overexpression of NRF2 shows a protective effect against IAV [133]. It was found that a NRF2 knockdown is correlated with an increase in viral entry and replication while SFN-induced activation of NRF2 in human nasal epithelial cells significantly decreases IAV entry and replication, directly supporting a protective role of Nrf2 against IAV infection [134]. The effectiveness of entry and replication of a virus can be altered with the cleavage of viral HA surface proteins, which allows the virus to enter the targeted host cell. It has been shown that an increase in NRF2 expression correlates with a lowered expression of the viral HA gene [134]. Several Nrf2 activators have shown protective effect against RSV and IAV. For example, SFN or emodin treatment significantly reduces virus-induced lung inflammation and viral replication [132, 135].

Streptococcus pneumoniae is the most common cause of pneumonia and sepsis. Nrf2 knockout has an increase of proinflammatory mediators and neutrophils present in lung digests from mice treated with *Streptococcus* [136]. Activation of NRF2 by resveratrol significantly reduced oxidative stress in human lung epithelial cells infected by *Streptococcus* [137]. Another common bacterium that infects the lungs is *Tuberculosis* (TB). The Nrf2 activator NAC has been shown

to decrease the bacteria counts and lung injury in guinea pig model infected by TB, suggesting that NAC could be used as an optional aid along with treatment [138].

3.7   Lung Cancers

The double-sided sword roles of Nrf2 in carcinogenesis has been revealed in many studies and discussed in several reviews [10, 139]. On the one hand, Nrf2-deficient mice treated with different carcinogens show increased severities of inflammation-associated carcinogenesis such as the AOM/DSS-induced colonic tumor [140]. On the other hand, enhanced levels of NRF2 or mutations that are predicted to activate NRF2 are found in many human cancers especially lung cancers [10, 141–144]. In support of the promotive role of Nrf2 in lung cancer, targeted deletion of Nrf2 reduces urethane-induced lung tumorigenesis in mice [145]. NRF2 depletion increases chemosensitivity of human lung cancer A549 cells both in vitro and in vivo [146]. Lung tumorigenesis induced by constitutively active K-Ras^{G12D} is suppressed in Nrf2-deficient mice [147]. It is proposed that Nrf2 facilitates cancer cell resistance to chemotherapeutic drugs and radiation through the activation of the antioxidant pathway. Enhanced NRF2-HO-1 pathway is increasingly seen in lung adenocarcinoma [148]. In addition, Nrf2 can promote cancer cell proliferation through activating proliferating and metabolic genes [139]. In human lung cancer cell lines, NRF2 activates transcriptions of anti-apoptotic factor Bcl-2 and glucose metabolic enzymes [25, 26], which to some extent accounts for the role of NRF2 in promoting lung cancers.

Recent studies have revealed that the Nrf2-interacting networks also contribute to the oncogenic role of Nrf2 in lung cancers. For example, single nucleotide polymorphisms (SNPs) in MAF-G have been found to be associated with lung cancers or airway transcription response to CS [149]. Enhanced levels of both MOF and NRF2-target transcriptions were revealed in human non-small cell lung cancer [75]. Co-upregulation of both DJ-1 and NRF2 were observed in a large set of lung carcinomas [92]. Depletion of KEAP1 in human non-small cell lung carcinomas cell lines increases their sensitivity to chemotherapeutic agents, which is likely mediated by enhanced PPARγ levels and its activation of differentiation genes [150]. Novel mechanisms of the Nrf2-Bach1 pathway in lung cancer metastasis has been recently identified [87, 88]. In normal cells, free heme inhibit Bach1 activity through Fbxo22-mediated ubiquitination. In cells with enhanced Nrf2, activation of HO-1 decreases the cellular level of heme thus stabilizing Bach1. Enhanced Bach1 promotes lung cancer metastasis through the activation of metastatic genes and glycolysis genes. Another protein that cooperates with Nrf2 in the promotion of lung cancer is ATF4. Nrf2-ATF4 dimer can directly target and activate *HO-1* gene [50]. Cooperative activations of *HO-1* by Nrf2 and ATF4 promotes fibrosarcoma lung colonization [151]. Nrf2 and ATF4 can also co-activate biosynthetic enzyme genes involved in serine/glycine metabolism, which plays a promotive role in non-small cell lung cancer [152].

4 Lung Disease Therapies Targeting Nrf2-Interacting Network

Given the protective role of Nrf2 in airway inflammation, injury and pathogenesis, designing drugs that target Nrf2 and its essential regulator Keap1 is valuable for lung disease therapies. Many nature products and pharmaceutical compounds have been identified as activators or inhibitors of Nrf2 and Keap1 [153]. Protective roles of these drugs against lung inflammation, injuries, and diseases have been verified in mouse models, and some drugs are in clinical trials. For example, SFN (an isothiocyanate isolated from cruciferous plants) treatment enhances bacteria removal and phagocytosis in the alveolar macrophages from both mice and COPD patients, supporting the therapeutic effect of SFN in treating COPD [154, 155]. A drug screen in human lung fibroblasts identified a group of synthetic small molecules (HPP-4382) that induced Nrf2-dependent HO-1 expression [156]. Among these compounds, HPP971 has completed phase 1 studies and shown potential therapeutic benefits in diseases, including respiratory injuries (vTv Therapeutics).

The roles of Nrf2 in respiratory inflammation and diseases can be mediated by many other proteins that interact with Nrf2 (Fig. 1). Therefore, based on knowledge of the molecular functions of the Nrf2 network in pathogeneses, drugs targeting the Nrf2-interacting networks can increase the efficiency and selectivity of therapies. Scientists are screening for compounds that directly target Nrf2-associated protein complexes especially the Keap1-Nrf2 complex. In addition, compounds that simultaneously target Nrf2 and its interacting partners have shown therapeutic potency for lung diseases.

4.1 Compounds Targeting Nrf2–Keap1 Interaction

Several groups have successfully identified compounds that directly interfere with the Nrf2–Keap1 interaction. These include ML334 by Hu and colleagues and monoacidic compounds by GlaxoSmithKline Pharmaceuticals [157, 158]. These compounds have been shown to induce the expression of NRF2-regulated genes in bronchial epithelial cells derived from COPD patients and reverse ozone-induced lung inflammation in rats [158].

4.2 Compounds Co-Targeting Nrf2 and NF-κB

NF-κB is an essential factor and therapy target for airway inflammation and chronic lung diseases [159]. Natural products that have both antioxidant and anti-inflammatory effects were seen in recent studies as potential therapies for

inflammation-related lung injuries and diseases. For example, tyrosol (a natural phenolic antioxidant) treatment of LPS-induced ALI mice significantly inhibited NF-κB and AP-1 and reduced inflammatory cytokines, while at the same time activating Nrf2 and HO-1 [160]. Oridonin (isolated from *Rabdosia rubescens*) and bardoxolone (a synthetic triterpenoid based on oleanolic acid) inhibit the NF-κB pathway and activate the Nrf2 pathway in LPS-induced ALI mice [161, 162], supporting the antioxidant and anti-inflammatory effects of these compounds for ALI protection and therapy. Investigations of several compounds targeting the Nrf2-NF-κB network in both mouse and rat models have shown their potential therapeutic effects against CS-induced inflammation, emphysema, and COPD. These include natural products such as forsythiaside (a hydroxycinnamic acid from *Forsythia suspensa*) [114], eucalyptol (a monoterpenoid oil isolated from *Eucalyptus*) [115], platycodin D (a saponin from the root of *Platycodon grandiflorus*) [116], and isoliquiritigenin (a flavonoid from *Glycyrrhizae* species) [117], as well as pharmacological compounds such as Sul-121 [118] and 15d-PGJ2 [119]. All these drugs can reduce respiratory inflammation and CS-induced emphysema through both the activation of the Nrf2 pathway and the down-regulation of the NF-κB pathway. In addition, rosavin (found in *Rhodiola rosea*) and emodin (isolated from rhubarb *Rheum palmatum*), nature products that activate Nrf2 and inhibit NF-κB, have recently been proposed as treatments for pulmonary fibrosis [125, 126]. Dimethyl fumarate (DMF) was approved by the FDA for the treatment of multiple sclerosis. It was found that DMF can both activate Nrf2 and inhibit the NF-κB pathway and prevent the development of bleomycin-induced lung fibrosis in mice [163]. Notably, DMF can control Nrf2 activity in a dose-dependent manner. In several cancer cells, low concentrations of DMF activate Nrf2, while high concentrations of DMF reduce the nuclear Nrf2 level, likely through the down-regulation of the Nrf2 stabilizer DJ-1 [164].

4.3   Compounds Targeting Nrf2 Phosphorylation Pathways

Desoxyrhapontigenin (isolated from rhubarb plants), a compound that shows significant anti-inflammatory and antioxidant effects, can inhibit MAPK, Akt, and NF-κB and activate Nrf2 [103, 165]. It was found that desoxyrhapontigenin pretreatment reduces LPS-induced lung inflammation and injury in mice through the PI3K/Akt-dependent activation of the Nrf2-HO-1 pathway [103]. Metformin reduces the risk of lung cancer during anti-diabetic treatment [166]. A recent study showed that metformin suppresses proliferation and induces apoptosis of the human lung carcinoma A549 cells through the inhibition of the Akt and ERK1/2 signaling pathways and activation of Nrf2 [167]. These studies indicate the potency of Nrf2 phosphorylation pathways as therapeutic targets for lung diseases.

4.4   Other Compounds Targeting Nrf2 Network

Interacting with the Keap1-Nrf2 pathway, autophagy can play both positive and negative roles in chronic lung diseases, CS exposure, and ALI [102]. It was found that vitamin D can protect against PM-induced lung injury in mice through the degradation of p62 and Keap1 and the activation of the Nrf2 pathway [168]. Pirfenidone (PFD), an approved drug for IPF treatment, can suppress bleomycin-induced lung fibrosis through both the activation of the Nrf2 pathway and the inhibition of Bach1 [127, 169].

5   Conclusions

As a master antioxidant factor, the protective roles of Nrf2 against respiratory inflammation and pathogenesis, as well as the brilliant potency of Nrf2-targeting therapies for lung diseases, have been verified and supported by numerous studies. However, with the revealing of more and more Nrf2-interacting proteins/pathways and Nrf2-targeting genes/functions, the complexity of the Nrf2 network in physiology and pathology needs to be considered when designing therapies targeting Nrf2. On the bright side, selectively targeting Nrf2-related protein interactions or regulatory networks could increase the efficiency and specificity of therapies. On the negative side, off-target effects that are related to the multiple interacting partners and biological functions of Nrf2 need to be considered when modifying Nrf2 level or activity in different types of cells. Many recent studies have focused on the potential application of natural antioxidant-rich foods that have limited side effects. Although the protective effects of many of the natural products against lung inflammation and pathogenesis have been verified in animal models, their therapeutic efficiency for lung diseases as well as long-term biological effects in humans remain to be evaluated.

Given that activation of the Nrf2 pathway is a strategy for treating most of the respiratory diseases, we should be mindful of the tumorigenic risk of Nrf2 activators. Chemoprevention effects of some Nrf2 inducers, such as SFN and CDDO-Im, have been studied in both animal models and clinical trials [170, 171]. It is necessary to determine the safe thresholds of Nrf2 level in specific cells as well as the safe doses of Nrf2 activators that will not induce oncogenesis. It would also be useful to design therapies that differentially target the oncogenic role and protective role of Nrf2, which may be achieved by targeting specific Nrf2-related protein interactions in the future. Doubtlessly, revealing the complete range of molecular mechanisms and biological functions of the Nrf2 network will contribute to the development of Nrf2-targeting therapies.

Competing Interest Statement The authors declare that they have no competing interest.

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