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Review

Role of Nrf2 in the pathogenesis of respiratory diseases

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ABSTRACT

Nuclear factor erythroid 2-related factor (Nrf)2 is a transcription factor that integrates cellular stress signals by directing various transcriptional programs. As an evolutionarily conserved intracellular defense mechanism, Nrf2 and its endogenous inhibitor Kelch-like ECH-associated protein (Keap)1 inhibit oxidative stress in the lung, which is the internal organ that is continuously exposed to the environment. Oxidative stress is implicated in the pathogenesis of various lung diseases including asthma, acute lung injury, chronic obstructive pulmonary disease (COPD), and interstitial lung disease (ILD). Thus, Nrf2 is considered as a potential therapeutic target in lung diseases owing to its antioxidant effect. Nrf2 also plays a complex role in lung cancer, acting as a tumor suppressor and promoter; recent studies have revealed the tumor-promoting effects of Nrf2 in tumors that have undergone malignant transformation. Lung cancer-associated mutations in Keap1 disrupt Keap1–Nrf2 complex formation, resulting in the ubiquitination and degradation of Keap1, and the constitutive activation of Nrf2. In lung cancer cells, persistently high nuclear Nrf2 levels induce the expression of genes that contribute to metabolic reprogramming, and stimulate cell proliferation. In this review, we outlined the major functions of Nrf2, and discussed its importance in pulmonary diseases such as asthma, acute respiratory distress syndrome, and lung cancer. Elucidating the mechanisms through which Nrf2 modulates the initiation and progression of pulmonary diseases can lead to the development of therapeutics specifically targeting this pathway.

Abbreviations: AOX1, aldehyde oxidase 1; ARDS, acute respiratory distress syndrome; ARE, antioxidant response element; CDDO-Im, 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole; COPD, chronic obstructive pulmonary disease; GCL, glutamate-cysteine ligase; *GPx*, glutathione peroxidase; GSH, glutathione; *GST*, glutathione-S-transferase; *HO-1*, heme oxygenase-1; ILD, interstitial lung disease; Keap1, Kelch-like ECH-associated protein 1; LPS, lipopolysaccharide; MCC, mucociliary clearance; Nrf2, nuclear factor erythroid 2-related factor 2; *NQO1*, NAD(P)H quinine dehydrogenase1; ROS, reactive oxygen species; *SOD1*, superoxide dismutase 1; TXN, thioredoxin.

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Contents

1. Introduction

Nuclear factor erythroid 2-related factor (Nrf)2 is a transcription factor that regulates the expression of genes involved in protection against oxidative damage [1,2]. Kelch-like ECH-associated protein (Keap)1 inhibits the activity of Nrf2 by preventing its binding to the antioxidant response element (ARE) [3]. Single-particle electron microscopy analysis has revealed that a Keap1 homodimer binds one molecule of Nrf2 [4] through two binding sites—the DLG and ETGE motifs—within the Neh2 domain of Nrf2 [5]. Thus, under normal conditions, Nrf2 is continuously degraded in a Keap1-dependent manner through the proteasome pathway [6]. However, in the presence of reactive oxygen species (ROS) or electrophiles, Nrf2 is stabilized due to the disruption of Keap1-mediated repression, and accumulates in the nucleus, where it activates target genes related to cytoprotection by binding to AREs or electrophile-responsive elements as an Nrf2/small Maf heterodimer [2,7] (Fig. 1). Nrf2 regulates the glutathione (GSH)- and thioredoxin (TXN)-dependent antioxidant systems by targeting the catalytic and modulatory subunits of the GSH-synthesizing enzyme glutamate-cysteine ligase (GCLC and GCLM) [8], and regulating the expression of TXN1-associated factors such as thioredoxin reductase 1 [9]. Given the role of oxidative stress in the pathogenesis of asthma, acute lung injury, chronic obstructive pulmonary disease (COPD), and interstitial lung disease (ILD), Nrf2 has attracted attention as a potential therapeutic target in the treatment of lung diseases.

Contrastingly, it is becoming increasingly clear that Nrf2 has more than a cytoprotective function; it also regulates genes associated with lipid, amino acid, carbohydrate, and nucleotide metabolism [10,11]. A recent study also highlighted the oncogenic role of Nrf2; persistently high nuclear Nrf2 levels were shown to enhance the expression of genes in anabolic pathways, which promotes cancer cell proliferation [12]. In this review, we outlined the principal functions of Nrf2, and discussed its importance in pulmonary diseases such as asthma, acute respiratory distress syndrome, and lung cancer.

2. Role of Nrf2 in asthma

Asthma is an inflammatory airway disease that results in airflow limitation, hyper-reactivity, and remodeling. Nrf2 in macrophages and epithelial cells protects against the proinflammatory and oxidizing effects of diesel exhaust chemicals [13], and thus has a protective function in asthma. Indeed, disrupting *Nrf2* enhanced the susceptibility of mice to severe airway inflammation and asthma [14]. Nrf2 was also found to regulate the antioxidant response and proliferation in airway smooth muscle cells, which exhibit aberrant function in asthma [15].

Although asthma is primarily an inflammatory airway disease associated with type 2 helper T cells, epithelial dysfunction is also relevant to its pathogenesis [16]. Specifically, impaired barrier function caused by disruption of epithelial tight junctions, allows inhaled substances to pass more easily into the airway wall and interact with immune and inflammatory cells, thereby increasing susceptibility to air pollution and respiratory virus infection [16]. We previously reported that steroids can potentiate airway epithelial barrier integrity through mechanisms that have not yet been elucidated [17]. Through global gene expression profiling, we determined that Nrf2-mediated oxidative stress response is critical for maintaining epithelial barrier integrity upon steroid treatment [18] (Fig. 2). We further demonstrated that aldehyde oxidase (AOX)1 functions downstream of Nrf2 in the formation of the airway epithelial barrier [18]. These data suggest that therapeutics targeting the Nrf2/AOX1 pathway can alleviate asthma by enhancing airway epithelial barrier integrity.

3. Role of Nrf2 in acute lung injury

Acute respiratory distress syndrome (ARDS) is a life-threatening syndrome characterized by a rapid-onset bilateral pulmonary infiltration and hypoxemia. ROS play an important role in the pathogenesis of ARDS associated with sepsis, hyperoxia, trauma, pharmaceutical or xenobiotic exposure, and mechanical ventilation [19]. Nrf2-deficient mice were more susceptible to ARDS than their wild-type counterparts [20], and a non-lethal dose of lipopolysaccharide (LPS) induced lung inflammation in Nrf2-deficient lungs, suggesting that Nrf2 protects against sepsis-induced acute lung injury [21]. NADPH oxidase-dependent ROS, proinflammatory cytokine (tumor necrosis factor-α and interleukin-6), and chemokine (macrophage inflammatory protein 2 and magnesium-dependent phosphatase 1) levels

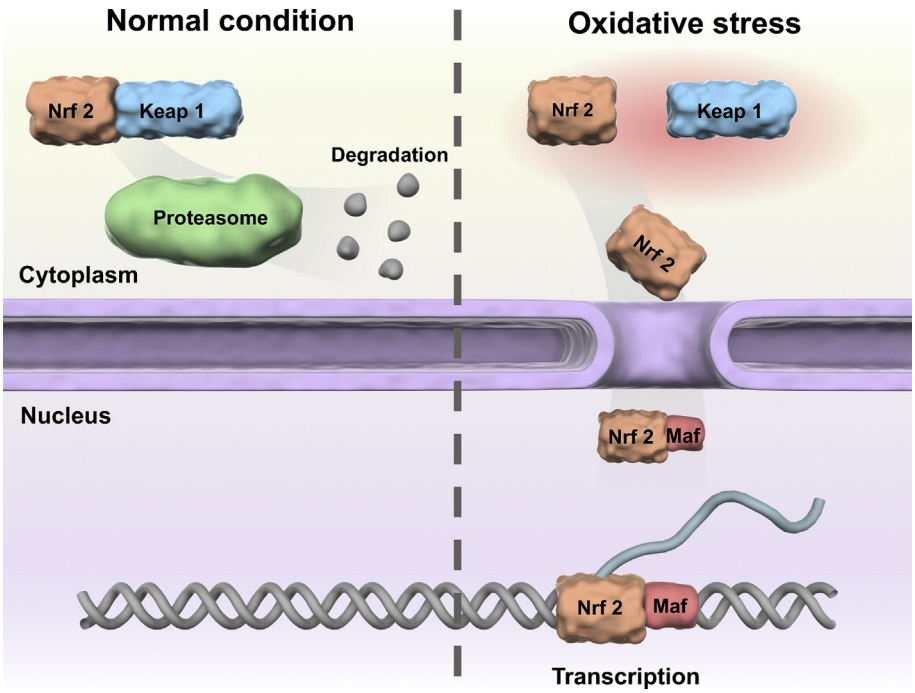

Fig. 1 – Activation of Nrf2. Nrf2 is continuously degraded through the Keap1-dependent proteasome pathway under normal conditions. However, in the presence of ROS, Nrf2 is stabilized by the alleviation of Keap1 repression and accumulates in the nucleus, leading to activation of target genes related to cytoprotection through the binding of the Nrf2/small Maf heterodimer to ARE.

were increased upon LPS treatment in Nrf2-deficient peritoneal neutrophils, but not in wild type cells [22]. In humans, *NRF2* gene polymorphisms increase the risk of acute lung injury [23]; however, there is no direct evidence indicating that increasing Nrf2 activity leads to clinical improvement of acute lung injury. Thus, although Nrf2 is a promising candidate for therapeutic interventions in ARDS, further investigation is required to translate the experimental findings to a clinical setting.

4. Role of Nrf2 in lung cancer

Nrf2 plays a critical role in cancer pathophysiology by acting both as a tumor suppressor and an oncogene. Sulforaphane is a phytochemical present in broccoli sprouts that was shown to activate the Nrf2-dependent antioxidant pathway [24], and suppress carcinogenesis in multiple organs including the lungs [25]. Sulforaphane–Nrf2-mediated induction of phase 2 antioxidant enzymes is thought to promote cellular defense against oxidative damage and carcinogen removal. However, as sulforaphane also induces other cellular responses including apoptosis and cell cycle arrest [26,27], the mechanism underlying these regulated steps remains to be elucidated.

Lung cancer-associated mutations in *KEAP1* have been identified that result in constitutive activation of Nrf2 [28–30] through disruption of Keap1–Nrf2 complex formation, which stabilizes Nrf2. Cancer cells take advantage of the overexpression of Nrf2 target genes by proliferating and resisting cellular defense mechanisms; for instance, cancer cell lines expressing lower levels of KEAP1 and mutant KEAP1 showed greater resistance to cisplatin than those with normal KEAP1 levels, suggesting that resistance to chemotherapeutic agents is enhanced by constitutive Nrf2 activation [30]. Conversely, silencing *NRF2* in non-small lung cancer cells reduced colony formation on soft agar relative to control cells [31], and *NRF2* depletion in non-small lung cancer cells completely suppressed tumor formation in athymic mice [31]. Activation of Nrf2 through the binding of cyclin-dependent kinase 20 to Keap1 also increased the resistance of lung cancer cells to radio-/chemotherapy [32], while small molecule inhibitors of *NRF2* exhibited anti-tumor activity in *KEAP1*-deficient non-small lung cancer [33].

The dual roles of Nrf2 in cancer may be attributed to its target genes; Nrf2 activators protect normal cells from carcinogens, whereas its inhibitors suppress the proliferation of cancer cells that exhibit aberrant Nrf2 activation (Fig. 3). In normal cells, low levels of Nrf2 are sufficient for maintaining cellular homeostasis; Nrf2 blocks tumor initiation and cancer metastasis by removing carcinogens, ROS, and other DNA-damaging agents. However, in cancer cells, accumulation of DNA damage can lead to mutations in *KEAP*, and consequently, the constitutive activation of Nrf2, which then activates metabolic genes that stimulate cell proliferation.

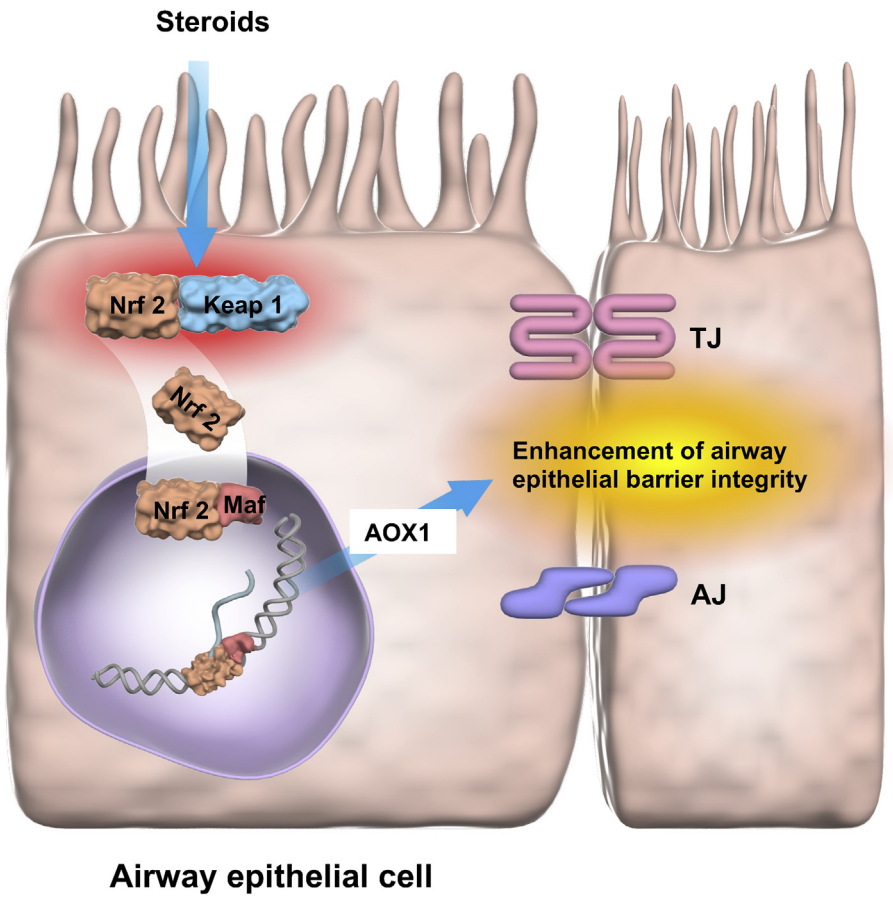

Fig. 2 – Nrf2 pathway and airway epithelial barrier integrity. Steroids induce AOX1 expression through the Nrf2 pathway, resulting in the formation of the airway epithelial barrier and enhancement of its integrity.

5. Role of Nrf2 in chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by persistent respiratory symptoms and airflow limitation, caused by a mixture of small airway disease and pulmonary emphysema. Oxidative stress plays a crucial role in the pathogenesis of COPD through the activation of the proinflammatory transcription, impairment of antiprotease defenses, cellular senescence, DNA damage, autoantibody generation, and corticosteroid resistance via inactivation of histone deacetylase 2 [34]. Therefore, the Nrf2-antioxidant response is considered to be a promising candidate for the antioxidant therapy of COPD. Aged smokers and patients with COPD exhibit a reduction in the expression of Nrf2 in their pulmonary macrophages [35]. Genetic disruption of Nrf2 in mice caused early onset and severe emphysema [36]. Furthermore, in mice fed a diet containing the potent Nrf2 activator 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole (CDDO-Im) reduced lung oxidative stress, alveolar cell apoptosis, and alveolar destruction caused by chronic exposure to cigarette smoke (CS) [36]. These results suggest that Nrf2 contributes to a pathogenic process leading to pulmonary emphysema induced by CS exposure, and this process can be reversed by a chemical activator of Nrf2.

Exacerbation of COPD is an important event in the natural history of the disease. Respiratory infection is one of the most common causes of exacerbation of COPD [37]. The mucociliary escalator contributes to a primary innate defense mechanism in the lungs, in which motile ciliated epithelial cells eliminate particles and pathogens trapped in the mucus from the airways. CS exposure disrupts airway epithelial cell function and shortens airway cilia, resulting in impaired mucociliary clearance (MCC) [38]. In patients with COPD, impaired airway clearance may promote susceptibility to respiratory infections [37]. We previously reported that *Nrf2*$^{-/-}$ mice had considerably impaired basal MCC compared with WT mice exposed to room air [39]. *Nrf2*$^{-/-}$ mice exposed to CS had no MCC after 3 weeks of exposure [39]. It has also been reported that the activation of Nrf2 by the phytochemical sulforaphane, restored bacterial recognition and phagocytosis in alveolar macrophages from patients with COPD [40]. Furthermore, sulforaphane treatment enhanced pulmonary bacterial clearance by alveolar macrophages, and reduced inflammation in wild type mice, but not in Nrf2-deficient mice exposed to CS for 6 months [40]. These findings demonstrate the importance of Nrf2 in improving antibacterial defenses, and provide a rationale for targeting this pathway to prevent the exacerbation of COPD.

Contrary to these experimental findings, daily oral administration of sulforaphane to patients with COPD did not

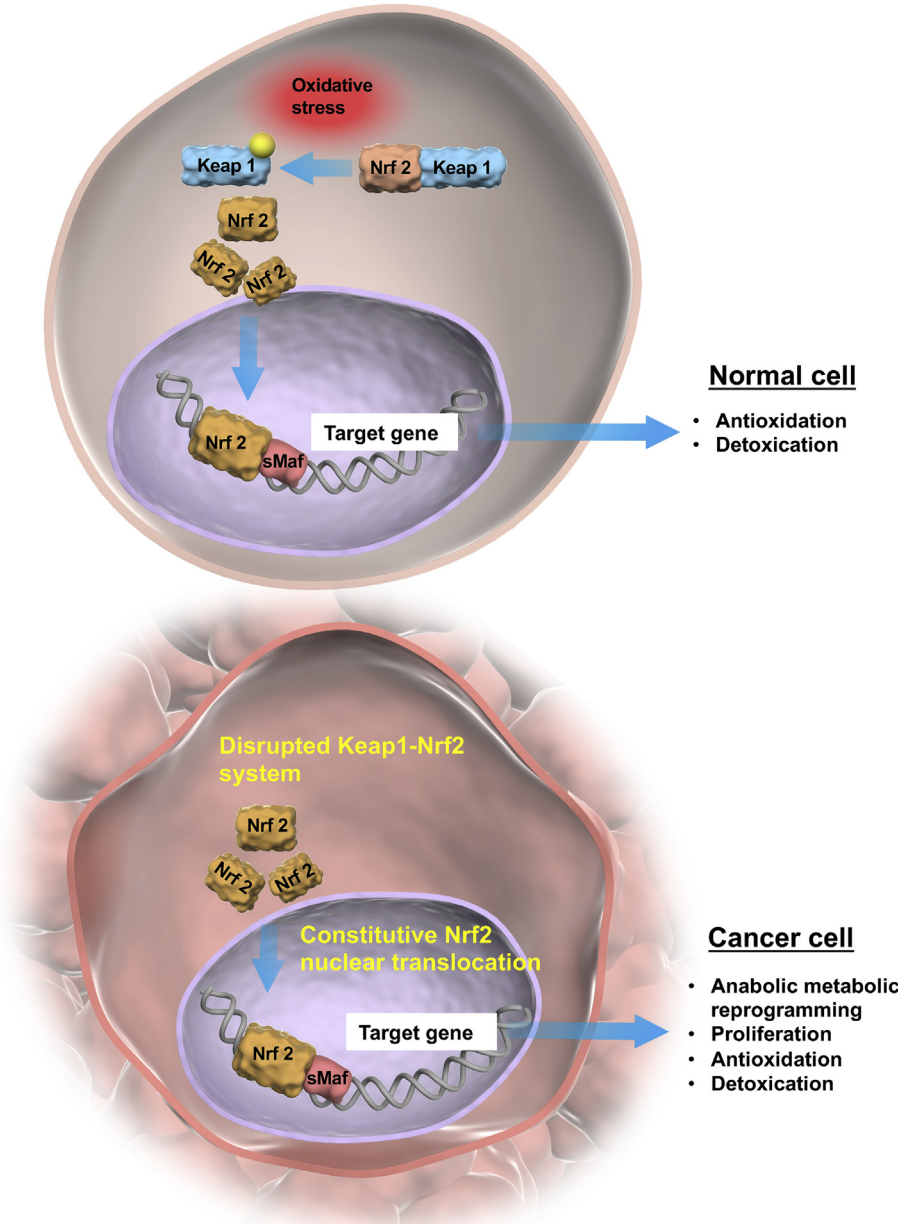

Fig. 3 – Nrf2 in cancer cells. In normal cells, NRF2 is activated by oxidative stress. It induces the expression of genes encoding antioxidant proteins that maintain cellular homeostasis and provide cellular protection. In cancer cells, NRF2 also activates genes associated with metabolism, which may promote cell proliferation.

result in consistent changes in Nrf2-dependent gene expression or markers of inflammation in alveolar macrophages and bronchial epithelial cells at the doses used [41]. Further investigation is required to translate the experimental findings to a clinical setting of COPD.

6. Role of Nrf2 in interstitial lung disease

Interstitial lung disease (ILD) is a group of lung diseases that cause fibrosis of the lungs. Although the molecular mechanisms of ILD remain poorly understood, ROS are thought to play an important role in the regulation of this disease [42]. It has been reported that indices of lung fibrosis are significantly greater in bleomycin-treated *Nrf2*$^{-/-}$ mice than in *Nrf2*$^{+/+}$ mice [43]. Bleomycin induced Nrf2 expression in mouse lungs, and upregulation of several NRF2-inducible antioxidant enzyme genes, including superoxide dismutase 1 (*SOD1*), glutathione-S-transferase (*GST*), glutathione peroxidase (*GPx*), NAD(P)H quinine dehydrogenase 1 (*NQO1*), and heme oxygenase-1 (*HO-1*) [43]. Bleomycin-induced expression of all these enzyme genes were significantly higher in *Nrf2*$^{+/+}$ mice than in *Nrf*$^{-/-}$ mice [43]. The activation of Nrf2-Keap1 signaling by epigallocatechin-3-gallate (EGCG) inhibited inflammation during bleomycin-induced experimental pulmonary fibrosis [44]. In the lung tissues obtained from human

subjects with idiopathic pulmonary fibrosis (IPF), while Nrf2 was expressed in alveolar epithelial cells, it was largely absent in fibroblasts within fibroblastic foci [45]. These results suggest that excessive oxidative stress has various deleterious effects that might contribute to the pathogenesis of ILD.

Pulmonary hypertension (PH) is a common complication of ILD, resulting in reduced exercise capacity and poor prognosis [46]. However, it is thought that PH-targeted therapy should not be used for most patients with PH-associated lung diseases including ILD and COPD as it may be harmful [47]. Therefore, it is critical to find new therapies for PH. In an experimental model, PH caused by chronic exposure to CS was reduced in mice fed a diet containing the potent Nrf2 activator, CDDO-Im [36]. For clinical application, a phase II study used bardoxolone methyl, an activator of the Nrf2 pathway, to treat patients with PH associated with ILD (LARIAT/NCT02036970) [48]. Further investigations are needed to evaluate therapies and strategies to improve outcomes in patients with ILD.

7. Conclusions

Nrf2 plays important roles in the pathogenesis of human lung diseases. Accumulating evidence indicates that Nrf2 activation can be a safe and effective strategy for the treatment of inflammatory lung diseases. The fact that Nrf2 suppresses tumor initiation and cancer metastasis in normal cells, but can increase proliferation when overexpressed in cancer cells suggests that its activities are protective in the early stages of tumorigenesis, but detrimental in the later stages. Although the detailed mechanisms through which Nrf2 exerts both protective and deleterious effects remain to be determined, therapeutics that target Nrf2 activators and inhibitors in different contexts are promising for the treatment of lung diseases.

Conflicts of Interest

The authors declare no conflicts of interest.

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