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The Antioxidant Response Element and Oxidative Stress Modifiers in Airway Diseases

Sekhar P. Reddy*
The Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences/Division of Physiology, Rm. E7547, 615 N. Wolfe Street, Baltimore, MD 21205, USA

Abstract

Redox balance is particularly important in the airways because they are the first points of contact with environmental pollutants such as ozone, particles, and cigarette smoke, as well as pathogens such as bacteria and viruses. However, an imbalance between toxicant-induced reactive oxygen (ROS) and nitrogen (RNS) species and the antioxidant defense system leads to oxidative stress, which has been implicated in the development and/or perpetuation of airway diseases, including malignancy. Various antioxidant enzymes and proteins are critical to maintaining the reducing environment of the cell and preventing the damage to various biomolecules that is elicited by ROS/RNS. Emerging evidence indicates that transcriptional activation of the antioxidant response element (ARE) plays a crucial role in modulating oxidative stress and providing cytoprotection against prooxidant stimuli. This review focuses on the regulation and functional roles of key effectors that bind to the ARE and differentially (up- or down-) regulate gene expression in lung tissue/cell types in response to respiratory toxicants. It also provides a perspective on whether boosting ARE-mediated gene expression with dietary plants and synthetic plant products will offer a better therapeutic strategy for mitigating oxidative stress and respiratory pathogenesis.

Keywords

Lung; inflammation; redox imbalance; antioxidant enzymes; Nrf2; AP1

INTRODUCTION

Oxidative Stress and Lung Pathogenesis

Under physiologic conditions, reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion, and hydroxyl radical are generated as byproducts of the metabolism of oxygen [1]. Reactive nitrogen species (RNS), which include nitrite, nitrate and peroxynitrite, are generated by the products of NO metabolism. Many physiological conditions, including wound repair, host defense, blood vessel relaxation, and neurotransmission, require ROS/RNS for cell signaling and various biological processes [2]. However, elevated levels of ROS/RNS can have deleterious effects on a wide range of biomolecules. For example, the generation of lipid peroxides and DNA or protein adducts leads to a dysfunctional cellular protective response [2].

Various environmental pollutants such as cigarette smoke, asbestos, and diesel exhaust particles (DEP) are prooxidant in nature and contain electrophiles, free radicals, and

*Address correspondence to this author at The Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences/Division of Physiology, Rm. E7547, 615 N. Wolfe Street, Baltimore, MD 21205, USA; Tel: 410-614-5442; Fax: 410-955-0299; sreddy@jhsph.edu.

carcinogens, including quinones and polycyclic aromatic hydrocarbons, that have been implicated in airway diseases [1,3,4]. Moreover, excessive generation of ROS/RNS can occur when inflammatory cells such as neutrophils, eosinophils and macrophages are recruited to the alveolar spaces, either in response to toxins or antigens or as consequence of infection. Experimental evidence from cell culture studies indicates that lung epithelial and endothelial cells also generate ROS/RNS following exposure to prooxidants. Various experimental models strongly support the hypothesis that oxidative stress generated by these agents and inflammatory cells can lead to the development of many acute and chronic airway diseases, including fibrosis, asthma, emphysema, acute respiratory distress syndrome (ARDS), and bronchial carcinogenesis [1,3–5]. Consistent with these results obtained from experimental models, diminished levels of lung GSH and alteration of other antioxidants have often been observed in specimens from individuals afflicted with such diseases [6,7], further highlighting the contention that redox imbalance produced by environmental pollutants may be an important factor in perpetuating or contributing to the onset of lung pathogenesis.

The Antioxidant Defense System

The endogenous cellular defense system consists of a number of antioxidant enzymes and proteins that maintain the cellular redox status that is critical for various biological processes. Many of these classical antioxidant enzymes, such as superoxide dismutases (SODs), catalase, glutathione peroxidases (Gpxs) and peroxiredoxins, directly inactivate ROS/RNS. Other antioxidant enzymes, such as glutathione reductase (Gsr), NAD(P)H:quinone oxidoreductase 1(Nqo1), UDP-glucuronyl transferase (Ugt), and thioredoxin (Txn) reductase (Txrnd), sulfiredoxin (Srx) and glutathione-S-transferases (Gsts), recycle thiols or facilitate the excretion of oxidized and reactive secondary metabolites (e.g., quinones, epoxides, aldehydes, and peroxides) through reduction/conjugation reactions. γ-Glutamyl cysteine synthase (γGCS), which is composed of glutamate cysteine ligase (Gcl) catalytic (Gclc) and modifier (Gclm) subunits, is essential for the biosynthesis of glutathione (GSH), a major intracellular antioxidant. In addition to GSH, several small thiol-containing compounds, such as thioredoxins, glutaredoxins, and periredoxins, serve as substrates for antioxidant enzymes in redox cycles and undergo a rapid oxidization and regeneration to maintain the intracellular redox status. Other molecules such as vitamin C (L- ascorbate) and vitamin E quench ROS levels and thereby play key roles in modulating oxidative stress. In addition to the antioxidant enzymes/proteins described above, stress response proteins such as heme oxygenase -1 (hmox1) and -2 (hmox2), [8], metallothioneins [9], and heat shock proteins [10] provide cellular protection against various oxidant or pro-oxidant insults. Experimental evidence from genetic mouse models strongly supports a protective role for several of these proteins in preventing oxidative lung damage and the subsequent development of lung disease [11,12]. Thus, it is likely that a dysregulation of the expression of the cellular antioxidant defense system by respiratory toxicants could potentially enhance the susceptibility of the lungs to oxidative stress, thereby contributing to the development of airway diseases.

Transcriptional Activation of the Antioxidant Defense System

The AP-1 dimeric transcription factor binding site, TPA response element (TRE, TGAC/GTCA), was originally thought to play a key role in the regulation of antioxidant gene expression [13]. However, evidence obtained in recent years has suggested that the transcriptional induction of several antioxidant genes requires the presence of a functional antioxidant response element (ARE) with a core sequence 5′-TGAG/CnnnGC -3′ (see Fig. 1), also referred to as the electrophile response element (EpRE) [14,15]. The ARE, with an embedded AP-1 or AP-1-like binding site, is commonly found in the regulatory region of genes encoding various antioxidant and cytoprotective detoxifying enzymes and proteins. Experimental evidence suggests that the flanking GC nucleotides in the ARE are essential for

antioxidant gene expression, but the AP-1 does not require the flanking GC residues to bind to the DNA.

Recent studies have unequivocally demonstrated a crucial role for NF-E2 related factor 2 (Nrf2), a cap'n'collar basic leucine zipper (CNC-bzip) transcription factor, in the induction of the ARE-mediated transcriptional response [15]. Nrf2 binds to the ARE and up-regulates the expression of several antioxidant genes in response to a variety of stimuli [15]. Some of the well-known targets of Nrf2 include Gpx2, Nqo1, Trxdn, Gclc, and Gclm [15]. Disruption of this gene greatly diminishes the expression levels of several antioxidant genes both *in vitro* and *in vivo* [16]. Nrf2 alone can not bind to the DNA, but after selective heterodimerization with the MAF, activation transcription factor (ATF), and/or AP-1 family of leucine zipper proteins, it occupies the ARE and drives transcription [13]. Thus, the MAF, ATF and AP-1 protein families fundamentally contribute to Nrf2-regulated gene expression [17].

Transcriptional Repression of the Antioxidant Defense System

The cell contains several proteins that act as "repressors" to dampen unwarranted levels of Nrf2-dependent ARE-driven transcriptional responses induced by toxic and oxidant stimuli, thereby reducing the antioxidant gene expression to physiological levels. These "repressors" modulate Nrf2 activity at multiples levels. One of the major repressors of the ARE-mediated transcription is a cytoplasmic actin-bound protein known as Kelch-like ECH-associated protein 1 (Keap1) or inhibitor of Nrf2 (iNrf2). In unstressed cells, Keap1 sequesters Nrf2 in the cytoplasm by binding to the N-terminal Neh2 domain and facilitating the degradation of Nrf2 via the ubiquitin-dependent proteosomal degradation pathway [18]. Disruption of the Keap1 gene enhances the stability and nuclear accumulation of Nrf2 [17] as well as the overproduction of Nrf2 target genes [19].

In contrast to Keap1, the Bach family of CNC-b-Zip transcription factors represses Nrf2 function in the nucleus. Like Nrf2, Bach proteins such as Bach1 are themselves unable to bind to the DNA; instead, they require heterodimerization with other proteins such as small Mafs in order to bind to the ARE. In recent years, experimental evidence has indicated that Bach1 and Nrf2 are mainly localized to the cytoplasm in unstressed cells [20,21]. However, prooxidant exposure promotes nuclear accumulation of both proteins. Because the nuclear accumulation of Bach1 is significantly delayed when compared to that of Nrf2, it has been proposed that heterodimerization of Bach1 with Maf proteins turns off or dampens the transcription activation from the ARE, mainly by limiting the accessibility of Nrf2 to the ARE. Consistent with this notion, Bach1 overexpression has been shown to negatively regulate Nrf2-ARE-mediated Nqo1 and hmox-1 gene expression in response to antioxidants [21]. Thus, it appears that interplay between Nrf2 and Maf proteins and Bach and Maf proteins dictates the outcome (up- or down-regulation) of ARE-mediated gene expression. The status of these combinatorial interactions in various airway diseases and whether respiratory toxicants modulate such interactions, and thereby diminish the lung antioxidant levels, warrant further study.

AP-1 family members have been shown to regulate various biological processes, including cellular defense in response to oxidant and toxic stimuli [22–24]. A cooperative or antagonistic role for AP-1 proteins in the modulation of Nrf2-ARE-mediated gene transcription has been documented: The Jun family of proteins, c-Jun and Jun-D, heterodimerize with Nrf2 and positively regulate the induction of ARE-mediated gene expression [25,26]. For example, Jun-D, acting *via* the ARE element, regulates H_2O_2 and t-BHQ-induced ferritin gene expression [27] and protects cells from oxidative stress-induced cell death [25,28]. In contrast, members of the FOS family (c-Fos and Fra-1) suppress ARE-mediated gene expression [29]. For example, overexpression of Fos and Fra-1, but not c-Jun, suppresses the induction of the Nrf2 target genes Nqo1 [30] and Gclc [31] by t-BHQ. Under basal conditions, the expression levels of c-Fos and Fra-1 are low, but these proteins are highly inducible by respiratory pathogens

such as DEP [32], asbestos, and cigarette smoke [33,34] and by inflammatory cytokines such as TNFα [35] in lung epithelial cells. Thus, it is likely that FOS family of proteins, acting in either a Bach-independent or -dependent manner, dampens the ARE-mediated transcriptional response provoked by environmental toxicants. The use of lung-specific genetic models will be necessary to define the specific roles of these proteins in mouse models of various human airway diseases.

It is clear from the studies described above that a wide variety of combinatorial interactions may interfere with, or enhance, the binding of positive and negative regulators of ARE-mediated gene expression [13,36]. Characterizing these combinatorial interactions involving the ARE in a variety of lung cell types, both in culture in response to toxicants and *in vivo* in disease models, is a challenging and daunting task. However, such studies are necessary if we are to obtain additional mechanistic insight into the development and perpetuation of respiratory pathogenesis.

Role of Nrf2 in Respiratory Pathogenesis

In 1999, Chan and coworkers [37] reported for the first time that Nrf2 confers pulmonary protection in a mouse of model of acute lung injury (ALI). When treated with the oxidant butylated hydroxytoluene (BHT), Nrf2-deficient (*Nrf2*$^{-/-}$) mice displayed severe lung injury and inflammation, accompanied by diminished levels of antioxidant enzyme expression, when compared to wildtype *(Nrf2*$^{+/+}$*)* mice. Moreover, the disruption of Nrf2 enhanced the susceptibility of the mice to BHT-induced toxicity at low doses.

Using an inbred strain of mice and genome-wide linkage analyses, we have identified Nrf2 as a candidate hyperoxia susceptibility gene that modulates ALI *in vivo* [38]. Oxygen supplementation (hyperoxia) is used therapeutically to treat critically ill patients with airway diseases; however, exposure to hyperoxia causes lung injury and airway inflammation. In Nrf2-deficient mice, alveolar permeability and injury associated with elevated levels of macrophage infiltration were increased in response to hyperoxic insult [38]. As anticipated, these mice displayed significantly lower levels of expression of several antioxidant enzymes, including Nqo1, GST-Ya, and hmox-1, in response to hyperoxia than did wildtype mice [38,39]. Other studies have shown that Nrf2 deficiency enhances cell death, while its overexpression confers protection against hyperoxia [40] and other pro-apoptotic stimuli [18,41].

Several recent studies have revealed that Nrf2 deficiency is associated with greater susceptibility to various experimental models of other human pulmonary diseases, such as emphysema, sepsis, asthma, and fibrosis. For example, chronic exposure to cigarette smoke causes more severe emphysema symptoms, such as alveolar air space enlargement, in the lungs of *Nrf2*$^{-/-}$ mice than in those of *Nrf2*$^{+/+}$ mice [42]. These symptoms are accompanied by greater levels of inflammation, oxidative stress, and endothelial and epithelial cell apoptosis in the deficient mice [42]. In a different study using the elastase-induced mouse model of emphysema, Nrf2 deficiency was found to lead to an imbalance between proteases and anti-proteinases in the lung [43]. Similarly, exposure of *Nrf2*$^{-/-}$ mice to DEP enhances oxidative DNA adduct formation in the lung [44].

We have recently shown that disruption of Nrf2 dramatically increases the susceptibility of mice to lipo-polysaccharide (LPS)-induced septic shock and results in earlier death when compared to wildtype mice [45]. Non-lethal exposure to LPS results in greater lung inflammation and injury in *Nrf2*$^{-/-}$ mice, in part because of the LPS-induced increases in NF-κB activation and Myd88-independent signaling. Nrf2-regulated cellular GSH and antioxidant levels are indispensable in bringing about optimal NF-κB activation in response to LPS and TNFα [45]. In a mouse model of asthma, disruption of Nrf2 resulted in severe airway inflammation and airway hyper-responsiveness, with elevated levels of T helper type 2

cytokines in bronchoalveolar lavage fluid and in splenocytes after allergen challenge [46]. Intervention with N-acetylcysteine, a precursor of GSH, decreased the emphysematic and asthmatic symptoms in *Nrf2*$^{-/-}$ mice, suggesting that redox status modulates the severity of airway diseases. We have shown that in response to bleomycin, *Nrf2*$^{-/-}$ mice display greater levels of lung inflammation, injury, edema, and extracellular matrix deposition than do wildtype mice [47]. These fibrogenic effects are correlated with diminished levels of antioxidant gene expression and with enhanced levels of ROS and DNA damage. Consistent with this result, the pulmonary fibrogenic effects of bleomycin are antagonized by antioxidant enzymes (e.g., SODs) in rodents [48,49]. We found elevated levels of TGF-β, a fibrogenic factor that induces fibrosis, in the lungs of *Nrf2*$^{-/-}$ mice treated with bleomycin. This factor α has been to shown to enhance ROS generation by suppressing antioxidant gene expression [50–52], in part through the modulation of Smad3, ATF3, and Nrf2 interactions [53]. Collectively, these data suggest that Nrf2 confers protection against oxidant-induced fibrosis, at least in part through its modulation of TGF-β-mediated effects.

The results of the studies described above highlight the importance of the Nrf2-driven transcriptional response in pulmonary protection against prooxidants. Microarray analyses have revealed that Nrf2 deficiency results in low levels of basal and inducible expression of several antioxidant genes in response to various oxidant and toxic stimuli, leading to a redox imbalance in the lungs [54]. Intervention with antioxidants such as N-acetyl L-cysteine (NAC) markedly attenuates pro-oxidant-induced lung injury and inflammatory responses in *Nrf2*$^{-/-}$ mice, supporting the hypothesis that a lowered antioxidant status enhances the severity of lung diseases. These results, obtained from various experimental models of human airway diseases, are consistent with the enhanced levels of alveolar oxidative stress and decreased levels of antioxidants that are found in patients with various clinical syndromes, including interstitial pulmonary fibrosis [55,56], allergic asthma [57,58], COPD [59,60], and ARDS/sepsis [61, 62]. Thus, further defining the mechanism(s) by which Nrf2 confers protection against lung diseases is likely to contribute to the development of novel therapeutic strategies and improved outcomes.

Cellular Signaling that Controls the Activation of the NRF2-ARE Transcriptional Response

In unstressed cells, Nrf2 has been clearly shown to be predominantly localized to the cytoplasm, where it associates with Keap1, an actin-binding cytoskeletal protein [63]. Oxidant and toxic insults disrupt the sequestration of Nrf2 by Keap1, thereby triggering the translocation of Nrf2 from the cytoplasm to the nucleus [63], where it binds to the ARE and regulates transcription (see Fig. 1). Accumulating evidence obtained using pharmacologic and genetic inhibitors has pointed to the involvement of various protein kinase pathways in regulating Nrf2 phosphorylation and nuclear accumulation as well as ARE-mediated transcriptional responses [64]. However, the upstream signals that control the translocation of Nrf2 from the cytoplasm to the nucleus appear to vary according to the inducer and/or cell type [63]. Only a very limited number of studies have analyzed in detail the mechanisms by which respiratory toxins transduce signals that affect the Nrf2 nuclear accumulation in non-malignant lung epithelial cells. For example, acrolein [65], the aldehyde component of cigarette smoke, and DEP [66, 67] have been shown to enhance Nrf2 DNA binding activity and ARE-mediated gene transcription in airway epithelial cells and macrophages, but the precise upstream signaling mechanisms by which these toxicants regulate Nrf2 activity remain unclear.

However, the important contribution of PI3K and ERK signaling to controlling the proxidant hyperoxia-induced Nrf2-ARE transcriptional response in pulmonary epithelial cells has been well established [40]. Hyperoxia activates PI3K and ERK signaling in NADPH oxidase-dependent, EGFR-induced signaling. A well-established role for Akt and ERK1/2 kinases in conferring protection against oxidative stress has been demonstrated in lung epithelial cells.

Given that an Nrf2 deficiency enhances cell death, and its overexpression confers cellular protection against pro-apoptotic stimuli [18,41] that include hyperoxia [40], it is likely that the PI3K and ERK pathways modulate cell death pathways *via* Nrf2-ARE signaling. Dysfunctional EGFR signaling has been linked to the abnormal airway remodeling that is frequently seen in airway diseases such as asthma and COPD [68,69]. In addition to these known effector kinases, other signal transduction pathways can regulate nucleocytoplasmic shuttling of Nrf2, thereby affecting ARE-mediated transcription. The question of whether chronic or elevated levels of oxidative stress dysregulate signaling pathways, and thereby limit Nrf2-activated functions and the subsequent development of airway disease, warrants thorough investigation.

Direct modulation of the redox status of the cysteine residues of Keap1 by oxidative and electrophilic stress has been implicated in the dissociation of Nrf2 from Keap1 [41,70,71]. Some of the regulatory mechanisms that regulate the turnover of Nrf2 by Keap1, such as ubiquitin- and proteasome-dependent pathways, may be of clinical significance. Such mechanisms govern the activity of various transcription factors that regulate oxidative stress. For example, the PI3K-Akt pathway regulates proteasomal degradation of the FoxO1 transcriptional regulator [72]. In contrast, the PI3K-Akt pathway stabilizes the HIF1α transcription factor *via* the induction of heat shock proteins [73]. Although DEP chemicals induce the accumulation of Nrf2 by inhibiting its proteasomal degradation in macrophages [67], the precise mechanism(s) by which various respiratory toxicants modulate the upstream signals that affect Nrf2 expression and activity are still unclear and require further study.

Therapeutic Use of Antioxidants or Boosters of Nrf2 Activity in Airway Diseases: Which has Promise?

As we have already mentioned, antioxidant intervention using NAC has been tested extensively in various experimental models of airway diseases in which oxidant stress plays an important pathogenic role. Several of these studies have demonstrated reduced oxidant injury and improved organ and system function following antioxidant intervention [59]. Because of the problems associated with the intracellular bioavailability of exogenous GSH, various forms of GSH or its precursor NAC, procysteine, have been tested as potential therapeutic agents in various clinical trials [62,74–76]. The clinical trials of these compounds and other antioxidants such as SOD mimetics, and porphyrins, as well as vitamins C and E, have yielded both promising and discouraging results with regard to the treatment of airway diseases. These studies are not discussed here but reviewed extensively elsewhere [6,77–81].

Since the benefits of antioxidant therapies have been uncertain at best, the current focus of research has shifted to dietary plant products that appear to have beneficial effects in experimental models of various diseases. Although a wide variety of plant products have been used in tissue culture and animal models by various investigators (see recent reviews [82, 83]), this review will focus on three compounds: i) curcumin, ii) the broccoli extract sulforaphane, and iii) triterpenoids. These compounds appear to be most effective in activating Nrf2-ARE-driven antioxidant gene expression in lung tissue/cell types. The activity of these compounds appears to be mediated by the Nrf2 regulatory protein, Keap1, which limits ARE-mediated gene transcription by promoting Nrf2 degradation in the cytosol (see Fig. 1).

i) Curcumin—It is a yellow/gold-colored spice commonly used in curries, has been shown to have beneficial effects against a wide variety of diseases, including pulmonary disorders [84]. For example, curcumin supplementation markedly attenuates lung eosinophil accumulation and inflammation in a murine model of latex allergy [85] and cecal-ligation induced tissue injury and mortality in experimental models of sepsis [86]. Oral administration of curcumin (20 mg/kg body weight/daily) prior to ovalbumin challenge is effective in attenuating airway hyperresposiveness in the ovalbumin-sensitized guinea pigs [87]. Evidence

indicates that curcumin exerts its antioxidant function in part by activating Nrf2 activity, thereby enhancing ARE-mediated gene expression [88,89].

In contrast to its activation of Nrf2 and induction of antioxidant activity, curcumin can also exhibit anti-inflammatory effects: It suppresses NF-*k*B activity, which is required for toxin- and oxidant-induced pro-inflammatory cytokine gene transcription in lung epithelial cells [90]. NF-kB activation by curcumin appears to be regulated at the level of IκBα, a key cytosolic inhibitor of NF-kB. In addition to its effects on Nrf2 and NF-kB, curcumin modulates the activity of other key transcription factors such as AP-1, STATs, and β-catenin [91]. However, despite curcumin's clear antioxidant and anti-inflammatory activity, no experimental evidence has thus far been published to indicate that these compounds or metabolites are actually present in the lung after oral administration. Moreover, the requirement for high doses of this compound, which have proapoptotic effects [92,93], to induce antioxidant gene expression in the lung tissue may pose difficulties in using this compound therapeutically in airway disease.

ii) Sulforaphane—Several isothiocyanates isolated from the broccoli extracts, including sulforaphane and indoles, have been shown to quench free radicals and reduce the risk of occurrence of various malignancies that originate in prostate, colon, or breast tissue (see recent reviews [94,95]). Various studies have shown that sulforaphane has a protective effect against the damage caused by free radicals induced by pro-oxidants [94]. Exposure of cells to sulforaphane disrupts the Keap1:Nrf2 interactions, resulting in Nrf2 nuclear accumulation and subsequent induction of ARE-mediated cytoprotective phase 2 detoxification enzymes, which mitigate the effects of oxidative stress and damage to biomolecules. Sulforaphane attenuates DEP-induced airway inflammation and cytokine production in respiratory epithelial cells, which is associated with elevated levels of antioxidant gene expression [96]. Sulforaphane is a more potent activator of Nrf2 than is curcumin [97]; however, the use of sulforaphane in experimental models of lung diseases has been limited. Furthermore, several concerns may limit the broad use of this isothiocynate in clinics, including 1) its limited bioavailability and the requirement for large daily consumption of broccoli to achieve optimal levels of sulforaphane in lung tissues/cells, 2) its potential non-specific effects on other cysteine-rich proteins, and 3) its ability to induce apoptosis and cell cycle arrest [95].

iii) Triterpenoids—Recent studies have indicated that triterpenoid compounds can potently activate Nrf2-dependent ARE-mediated gene expression. Triterpenoids are synthetic derivatives of sqaulene, an aliphatic hydrocarbon and a precursor of steroids. These compunds are found in shark liver oil and in botanical species, including amaranth seed, rice bran, wheat germ, and olives. Triterpenoids have been shown to have beneficial effects, at least in experimental animal models, in inflammation and carcinogenesis (see review [98]). The synthetic derivatives of squalene are effective at low doses, and they are roughly 10 to 100 times more potent than sulforaphane and curcumin in inducing Nrf2-ARE-mediated transcriptional responses. Among the various synthetically derived triterpenoid compounds, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) has recently been shown to potently activate antioxidant gene expression at nanomolar concentrations in various tissues, including the lungs [45,97,99]. The induction of antioxidants appears to be Nrf2-dependent, since CDDO-Im failed to induce the expression of the Nrf2 target genes Nqo1 and Gclc in Nrf2-deficient, but not in Nrf2-sufficient, cells and tissues [45,100]; these data suggest that CDDO-Im activates ARE-mediated gene transcription *via* Nrf2. The activation of Nrf2 by triterpenoids such as CDDO-Im appears to be regulated at the level of the Keap1:Nrf2 interaction (see Fig. 1). Specific activation of Nrf2-dependent gene transcription by CDDO-Im in the lung has shown promising results in an experimental model of sepsis [45]. Also, use of CDDO-Im to endogenous Nrf2 activity has been shown to dampen LPS-induced airway inflammation [45].

Studies using chemical activators of Nrf2 are not straightforward, because these compounds also activate other transcription factors that regulate airway inflammation. For example, triterpernoids suppresses both constitutive and IL6-induced Stat 3/5 phosphorylation and upregulate the expression of suppressor of cytokine signaling-1 [101]. It is unclear whether cross-talk exists between the Nrf2-dependent antioxidant transcriptional response and pro-inflammatory cytokine gene transcription. Nevertheless, these compounds are the most potent activators of Nrf2 that have been studied thus far, and they exert both pro-antioxidant and anti-inflammatory effects at nanomolar concentrations. However, their clinical applicability remains an open question.

SUMMARY AND FUTURE PERSPECTIVES

The data presented here collectively suggest that maintenance of a proper redox balance, as regulated by the ARE-mediated transcriptional response, is critical to the integrity and function of many lung cellular components of the lung and to the outcome of a variety of airway diseases. Increased susceptibility to respiratory pathogenesis resulting from a lack of proper redox balance in the lungs may be caused by dysregulation or variation in the signaling pathway(s) that converge at the ARE and are regulated by an Nrf2-dependent transcriptional program. In order to further define the pathologic mechanisms underlying airway disease development, future studies are needed to decipher the cellular signals that control the activation of Nrf2-ARE-mediated gene transcription by pro-oxidants and to determine whether these pathways are dysfunctional in lung tissues and cell types following chronic or high level exposure of respiratory toxins. Although compounds that potently boost Nrf2 activity have shown promising results in experimental models, their effective doses, potential risks and benefits, and clinical relevance all remain unclear at this stage and require further study before these molecules can be considered promising preventive and therapeutic agents for modulating airway disease. In addition, the question of whether targeting transcription factor(s), using emerging tools such as RNAi or pharmocologic inhibitors, that dampen ARE-mediated transcriptional activation, is an attractive strategy in the treatment of airway disease remains to be explored.

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ABBREVIATIONS

ALI	acute lung injury
ARE	antioxidant response element
CDDO-Im	1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole
COPD	chronic obstructive pulmonary disease
DEP	diesel exhaust particles
ERK	extracellular signal-regulated kinase
MAP kinase	mitogen-activated protein (MAP) kinase
Nrf2	nuclear factor erythroid 2- like 2
PI3K	Phosphoinositide-3-kinase
ROS	reactive oxygen species
RNS	reactive nitrogen species

TRE	TPA response element

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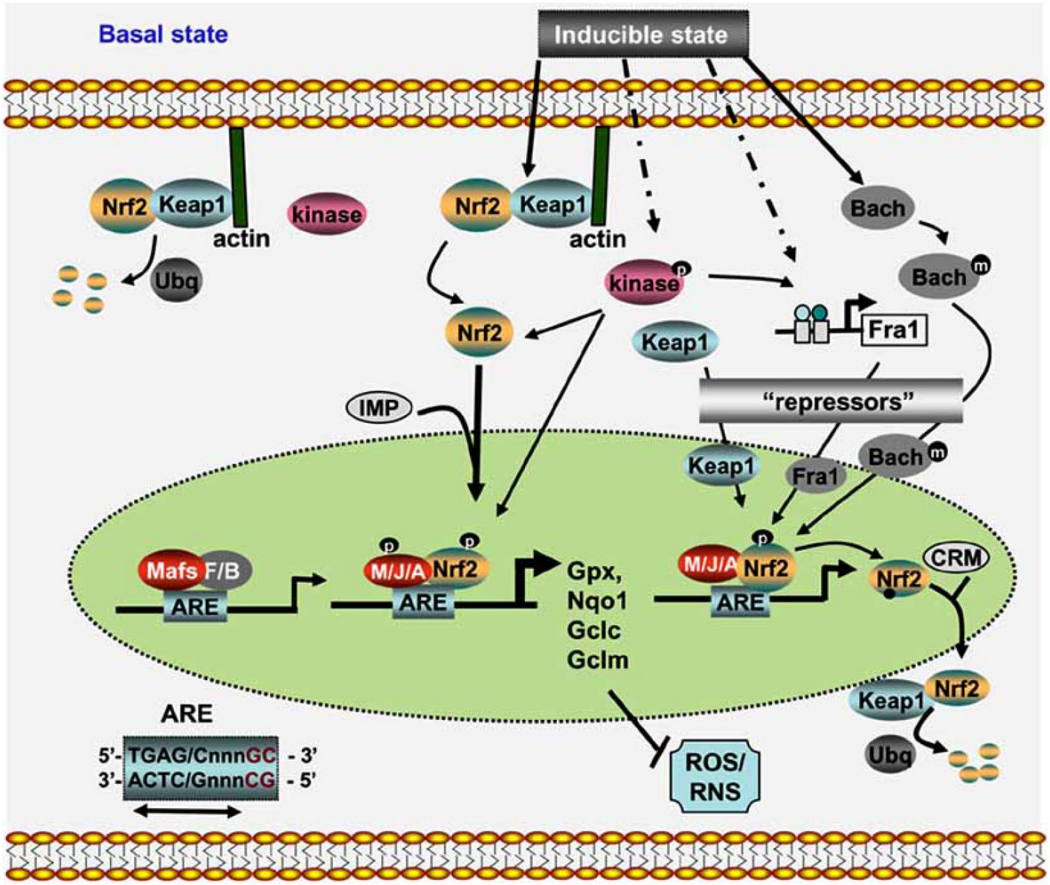

Fig. 1. Mechanism of the Nrf2-ARE-mediated transcriptional response

This scheme is based upon data from the studies summarized in this article, which provide a mechanistic framework for pathways that regulate intracellular Nrf2-ARE responses. Under basal conditions, Nrf2 is mainly localized to the cytosol and undergoes Keap1-mediated, ubiquitin (Ubq)-dependent proteosomal degradation. In the inducible state, toxicants or proxidants disrupt the interaction between Nrf2 and Keap1, either directly or indirectly through the generation of elevated levels of ROS by NADPH oxidase or mitochondria, thereby facilitating nuclear accumulation of Nrf2. Upon dimerization with the MAF, JUN, or ATF family of proteins, Nrf2 binds to the ARE sequence. This binding promotes or favors the displacement of negative factors, such as the Bach and Fos proteins, that bind to the ARE and the subsequent recruitment of transcriptional co-activators such as CBP/p300. The activation of kinases (PKC and MAP kinases) by toxicants affects both the nuclear accumulation and recruitment of Nrf2 and other transcription factors that bind at the ARE. Late induction of Fos proteins, such as Fra1, or modification of Bach proteins by oxidative stress and their subsequent nuclear accumulation dampens the ARE-mediated transcriptional activation either by competition with Nrf2 or displacement of Nrf2 from the promoter. Exportin Crm1 (CRM) and Keap1 facilitate the export of Nrf2 from the nucleus to the cytoplasm, where the latter undergoes Ubq-mediated degradation. M/J/A represents the MAF, JUN, or ATF protein. The double arrow indicates the position of the TRE-like AP-1 binding sequence. p, phosphorylation; M, modification such as oxidation.