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REVIEW ARTICLE

Association of Nrf2 with airway pathogenesis: lessons learned from genetic mouse models

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Abstract Nrf2 is a key transcription factor for antioxidant response element (ARE)-bearing genes involved in diverse host defense functions including redox balance, cell cycle, immunity, mitochondrial biogenesis, energy metabolism, and carcinogenesis. Nrf2 in the airways is particularly essential as the respiratory system continuously interfaces with environmental stress. Since Nrf2 was determined to be a susceptibility gene for a model of acute lung injury, its protective capacity in the airways has been demonstrated in experimental models of human disorders using *Nrf2* mutant mice which were susceptible to supplemental respiratory therapy (e.g., hyperoxia, mechanical ventilation), cigarette smoke, allergens, virus, environmental pollutants, and fibrotic agents compared to wild-type littermates. Recent studies also determined that Nrf2 is indispensable in developmental lung injury. While association studies with genetic *NRF2* polymorphisms supported a protective role for murine Nrf2 in oxidative airway diseases, somatic *NRF2* mutations enhanced NRF2–ARE responses, and were favorable for lung carcinogenesis and chemoresistance. Bioinformatic tools have elucidated direct Nrf2 targets as well as Nrf2-interacting networks. Moreover, potent Nrf2–ARE agonists protected oxidant-induced lung phenotypes in model systems, suggesting a therapeutic or preventive intervention. Further investigations on Nrf2 should yield greater understanding of its contribution to normal and pathophysiological function in the airways.

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Introduction

Nuclear factor, erythroid-derived 2, like 2 (Nfe2l2), or NF-E2-related factor 2 (Nrf2) is an indispensable transcriptional regulator of cytoprotective genes that acts through ARE (or electrophile response element, EpRE) binding (Chan et al. 1996; Itoh et al. 1995). Intersection between discovery of Nrf2 and increasing evidence for a role of oxidative stress in the pathogenesis of many critical diseases has highlighted research in this field during the last two decades. Nrf2 homeostasis is regulated by a cytoplasmic inhibitor Kelch-like ECH-associated protein 1 (KEAP1) in response to housekeeping proteolytic demands or against exogenous stimuli by oxidants, xenobiotics, carcinogens, antioxidants, and chemopreventive agents (Taguchi et al. 2011; Wakabayashi et al. 2003). Nrf2 functions have been characterized by well-established in vivo models with germ-line or conditionally *Nrf2*-deficient mice (Cho 2013), and many have been confirmed in clinical investigations. Nrf2 contributes not only to the maintenance of cellular redox balance but also to regulation of cell cycle and death, immunity, metabolism, selective protein degradation, development, and carcinogenesis.

Nrf2 is ubiquitous but known to be relatively more abundant in thyroid, immune cells, airways, uterus, and heart (http://biogps.org/#goto=genereport&id=4780). As the interface with the external environment, airways are routinely exposed to inhaled oxidants and environmental stresses and thus their redox balance needs to be tightly controlled. The airway epithelial lining fluids and tissues are rich in cellular and extracellular antioxidants (e.g.,

enzymes, thiols). Indication of oxidant stress including excess reactive oxygen species (ROS) or glutathione (especially reduced GSH) depletion has been detected in various acute and chronic respiratory diseases such as idiopathic pulmonary fibrosis (IPF), asthma, emphysema and chronic obstructive pulmonary disease (COPD), cystic fibrosis, bronchopulmonary dysplasia (BPD), acute lung injury (ALI), and lung malignancies (Fridovich 1998; Halliwell et al. 1992; Saugstad 2003). Supplemental antioxidant therapies with N-acetyl-L-cysteine (NAC) or vitamins C and E, or recombinant antioxidant enzymes such as superoxide dismutase (SOD) and catalase have been applied clinically and have demonstrated mild and inconsistent efficacy (Domej et al. 2014; Poggi and Dani 2014; Taniguchi and Kondoh 2013). Naturally occurring antioxidants that activate Nrf2–ARE pathways have been widely tested in recent in vivo and in vitro studies (Domej et al. 2014; Kumar et al. 2014), and some successful therapies have been shown to be effective in clinical settings, supporting the potential for therapeutic intervention (Egner et al. 2014; Fahey et al. 2012; Houghton et al. 2013). Genetic or somatic *NRF2* mutations and their significant association with human airway disorders, ALI, COPD, asthma, BPD, and lung cancer, have been extensively described in recent reviews (Cho et al. 2015b).

In this review, we describe current knowledge of the role for airway Nrf2 in disease pathogenesis. We address the functional roles of Nrf2 focusing on evidence from model systems (Table 1) using genetic or conditional *Nrf2*-deficient mice generated in three different genetic backgrounds [ICR, BALB/cJ, C57BL/6J or B6; (Chan et al. 1996; Itoh et al. 1997; Martin et al. 1998; Reddy et al. 2011)]. In addition to the polymorphisms and haplotypes determined in murine *Nrf2* (Fig. 1) and the genetic and somatic *NRF2* mutations relevant to human airway disorders (Table 2), we also review the molecular aspects of Nrf2 pathways revealed by global transcriptomic analyses and bioinformatic studies. Phytochemical or synthetic antioxidants which have been investigated in pulmonary disorders (Table 3) are yielding translational 'bench-to-bedside' Nrf2 research.

Role of Nrf2 in murine models

Acute lung injury (ALI)

Clinical ALI

ALI or the more severe form, acute respiratory distress syndrome (ARDS), is the major acute airway disease in adults, affecting up to 150,000 patients annually with reported mortality rates of 35–65 % in the USA alone (Goss et al. 2003; Ware and Matthay 2000). ALI is caused by direct pulmonary injury (e.g., pneumonia, trauma) or by secondary insults originated from various clinical conditions (e.g., sepsis, pancreatitis). It is defined by pulmonary edema, alveolar inflammation, and respiratory failure leading to severe systemic hypoxemia in seriously ill patients (Matthay et al. 2012). Many clinical trials have assessed pharmacologic interventions, innovative strategies for positive-pressure ventilation, and other supportive approaches to ALI and ARDS treatment (Matthay et al. 2012), and more recent advances in supportive care demonstrate therapeutic potential of mesenchymal stem cell incorporation in patients as well as in experimental ALI [Review (Walter et al. 2014)]. Oxygen (O_2) is one of the most commonly used supplemental therapeutic agents in various hypoxic clinical situations including respiratory failure. However, the margin of safety between effective and potentially toxic doses of O_2 is relatively narrow, and prolonged administration of high concentrations O_2 (hyperoxia) often paradoxically causes pulmonary toxicity or exacerbates pre-existing disorders.

Hyperoxia-induced lung injury

In laboratory rodents, inhalation of hyperoxia (>85 % oxygen) causes pulmonary injuries which resemble ALI phenotypes. This model thus has been used widely to determine the molecular mechanisms of ALI and oxidant-induced lung injury. Candidate gene approaches in animal models have determined that classical or direct antioxidant enzymes including SODs and heme oxygenase-1 (HO-1) protect lungs against hyperoxia-induced lung injury (Asikainen et al. 2002; Danel et al. 1998; Folz et al. 1999; Ho et al. 1998; Otterbein et al. 1999; Taylor et al. 1998; Tian et al. 1998). To further investigate the genetic basis of ALI pathogenesis, we conducted an inbred mouse strain screen and genome-wide linkage analyses of hyperoxia-induced murine ALI phenotypes and identified *Nrf2* in a significant quantitative trait locus on chromosome 2 (Cho et al. 2015a, b; Hudak et al. 1993). This finding followed the discovery of Nrf2 as a master regulator for antioxidant responses (Itoh et al. 1997). Support for the functional relevance of Nrf2 in ALI was further demonstrated when $Nrf2^{-/-}$ mice were found to be significantly more susceptible to the development of ALI-like phenotypes including protein edema and inflammation than similarly treated wild-type, $Nrf2^{+/+}$ mice (ICR) after hyperoxia exposure (Cho et al. 2002a). Enhanced susceptibility to hyperoxia and deferred recovery from hyperoxic injury were significantly greater in mice conditionally deficient in airway epithelial *Nrf2* ($Nrf2^{\Delta cc}$, B6) compared to the wild-type littermates as indicated by a persistent lung inflammation

Table 1 Role of airway Nrf2 in model disorders determined using gene-deficient mice

Model disorder	Insult	Phenotypes	Mechanisms examined	Nrf2–ARE agonist effect	References†
ALI	BHT	↓Mortality ↓Lung inflammation	CAT1, SOD1, NQO1, GCLC	na	Chan and Kan (1999)
	Hyperoxia	↓Lung edema, inflammation, apoptosis ↑Resolving lung injury	·AOEs/redox regulators (NQO1, etc.) ·Extracellular matrix (COL1a1, etc.) ·Immunity (*MARCO, etc.) ·Tissue remodeling/coagulationproteins.transcription factors (*PPARγ, etc.) ·Transporters (SLC7a11, etc.) ·Kinases and phosphatases	↓Lung injury by oral CDDO-Im in *Nrf2*$^{+/+}$	Cho et al. (2002a, 2005†, 2010), Pendyala et al. (2011), Reddy et al. (2009a, b, 2011)
	Mechanical ventilation (MV)	↓Lung edema and vascular leakage, inflammation, redox balance, impaired gas exchange	·Tissue remodeling (*AREG, etc.) ·AOEs/redox regulators (MT2, etc.) ·Inflammation (IL-6, etc.) ·Transporters (SLC38a4, etc.) ·Transcription factors (ATF3, etc.) ·Kinases and phosphatases	↓Lung injury by NAC in*Nrf2*$^{+/+}$	Papaiahgari et al. (2007)†, Reiss et al. (2014)
	Sepsis (LPS, CLP)	↓Mortality ↓Lung inflammation, chemokines, GSH	·Cytokines and chemokines (CXCL9, IL-6, etc.) ·Adhesion molecules (TRGM1, etc.) ·Immunoglobulins and MHC ·AOEs/redox regulators (GPx2, GCLC, p47Phox, etc.) ·NF-κB and its signal transducers	↓Lung inflammation and cytokine expression by NAC in *Nrf2*$^{-/-}$ ↓Mortality by NAC in*Nrf2*$^{+/+}$ and *Nrf2*$^{-/-}$ ↓Mortality and lung chemokine expression and ↑Lung AOEs by CDDO-Imin *Nrf2*$^{+/+}$	Thimmulappa et al. (2006a)†, Kim et al. (2014), Thimmulappa et al. (2006b)
	Sepsis (*Staphylococcus aureus*)	↓Lung edema, inflammation ↑Mitochondrial copy number	·Mitochondria biogenesis and autophagy markers (LC3-II, p62/SQSTM1, NRF-1, PGC-1a, TRAM, Claudin4, Bnip3, PDCD2, citrate synthase) ·Cytokines (IL-1β, TNF, CCL2, IL-10, SOCS3) ·HO-1, SOD2	na	Athale et al. (2012), Chang et al. (2015)
	Carrageenan (carrageenin)	↓Lung inflammation	·COX -2, 15d-PGJ$_2$ ·PRDX1, HO-1	↓Lung inflammation by 15d-PGJ2 in *Nrf2*$^{+/+}$	Mochizuki et al. (2005)
	Traumatic brain injury	↓Lung permeability, wet/dry weight ratio, alveolar apoptosis	·TNF-α, IL-1β, IL- ·NQO1, GST-α1	na	Jin et al. (2009)
	Hyperoxia + *Pseudomonas aeruginosa*	↓Lung inflammation, edema, bacterial burden ↓Lethality	MARCO, MSR1, GCLC, IL-6, and IL-1 in alveolar macrophages	na	Reddy et al. (2009c)

Table 1 continued

Model disorder	Insult	Phenotypes	Mechanisms examined	Nrf2–ARE agonist effect	References†
COPD/ emphysema	Cigarette smoke	↓Emphysema symptoms (increases in alveolar diameter, mean linear intercept, static lung compliance) ↓Airway hyperreactivity ↓Alveolar macrophages ↓Mucous cell metaplasia ↓Lung 8-oxo-dG and 8-isoprostane ↑α1-Anti-trypsin ↓Neutrophil elastase activity ↑Macrophage phagocytosis ↓Apoptosis signaling (caspase-3) ↓Steroid resistance	·AOEs/redox regulators (*GPx2, *Srx1,*GR, NQO1, GLCC, GST-A1, HO-1, PRDX, TXNRD1, PGDH, etc.) ·CD36 ·α1-Anti-trypsin ·Protein degradation (UBC, etc.) ·Transcription factors (MAF-F, etc.) ·Kinases and phosphatases (PTP1, etc.) ·Cell cycle (CCNA2, CDCA5, etc.)	↓Lung edema and inflammation by NAC or vitamin E in *Nrf2*$^{-/-}$ ↓Lung emphysema, apoptosis, and DNA oxidation, and right ventricle function by CDDO-Im in *Nrf2*$^{+/+}$	Rangasamy et al. (2004)†, Adenuga et al. (2010), Iizuka et al. (2005), Sussan et al. (2009), Ying et al. (2014)†, Gebel et al. (2010), Messier et al. (2013a, b)
	Elastase	↓Alveolar destruction (mean linear intercept) ↓Lung edema and inflammation ↑α1-Anti-trypsin	·*SLPI ·PRDX1, HO-1, NQO1, GST-Yc	na	Ishii et al. (2005)
	Cigarette smoke +influenza virus/cigarette smoke + poly(I:C)	↓Mortality and body weight loss ↓Lung edema and inflammation ↓Lung 8-oxo-dG ↓Mucous cell metaplasia and Muc5ac	·TNF, KC ·NF-κB	na	Harvey et al. (2011), Yageta et al. (2011)
	Cigarette smoke + *P. aeruginosa*	↓Alveolar macrophage phagocytosis and lung inflammation	·MARCO	↓Alveolar macrophage phagocytosis and ↑MARCO by sulforaphane in *Nrf2*$^{+/+}$	Harvey et al. (2011)
Allergic asthma	Ovalbumin	↓Lung eosinophilia ↓Airway hyperresponsiveness ↓Lung lipid and protein oxidation ↓Bronchial mucous cell metaplasia	·Th2 cytokines (IL-4, IL-13) ·AOEs ·NF-κB	↓Lung eosinophilia by NAC in *Nrf2*$^{-/-}$	Rangasamy et al. (2005), Sussan et al. (2015)
	DEP	↓Lung DNA adduct formation ↓Lung 8-oxo-dG ↓Airway hyperresponsiveness ↓Lung lymphocytes and eosinophils	·IL-12, IL-13 ·GCLC, GCLM, GSTs (A3, P2, M1), HO-1, SOD2	na	Aoki et al. (2001), Li et al. (2008)
	Ovalbumin + DEP	↓Exacerbation of lung inflammation ↓Airway hyperresponsiveness ↓Bronchial mucous cell metaplasia ↓GSH oxidation	·IL-5, TARC	na	Li et al. (2010)
	Ovalbumin + UFP	↓Exacerbation of allergic inflammation and constriction ↓IgE/G1	·IL-13, IL-12, IL-6 ·Dendritic cells	na	Li et al. (2013)

Table 1 continued

Model disorder	Insult	Phenotypes	Mechanisms examined	Nrf2–ARE agonist effect	References†
BPD	Hyperoxia (newborn)	↓Mortality ↓Arrest of saccular-to-alveolar transition (mean linear intercept, histopathological score) ↓Lung growth retardation ↓Lung inflammation, apoptosis, edema ↓DNA damage, protein carbonyl, malondialdehyde ↑Surfactant protein C	·Cell cycle and growth (EGR2, etc.) ·Redox homeostasis (AKR1B8, GPx2*, NQO1, AOX3, etc.) ·Transport (Slc7a11, etc.) ·Organ development and morphology(ANG3, etc.) ·Immune response (MARCO*, etc.) ·TGF-β, VEGF, ANGPT2, IL-6, p21	na	Cho et al. (2012)†, McGrath-Morrow et al. (2009, 2014)†
Lung fibrosis	Bleomycin	↓Mortality ↓Body weight loss ↓Lung edema, inflammation, proliferation ↓Lung fibrotic markers (collagen, tenascin-C, TGF-β, MMP) ↓Lung lipid peroxidation	·AOEs ·Chemokines (TNF, MIP-2) ·TGF-β·NF-κB ·Th1/Th2 (IFNγ, IL-4, IL-13)	na	Cho et al. (2004), Kikuchi et al. (2010), Walters et al. (2008)
	Radiation	↑Life span ↑Number of alveoli ↓PAI-1, FSP-1	na	na	Travis et al. (2011)
Viral diseases	RSV	↓Lung viral load and replication ↓Lung inflammation ↓Nasal airway injury ↓Airway epithelial mucus ↓Lung protein and lipid oxidation	·AOEs ·NF-κB, AP-1 ·Cytokines (IL-6, IL-18, IL-10, IL-13, IFNγ)	↓Lung inflammation and viral replication by sulforaphane in *Nrf2*$^{+/+}$	Cho et al. (2009)
	Haemophilus influenzae	↓Lung B cell aggregate nodules ↓Peribronchovascular inflammation ↓Serum antibodies against viral membrane protein ↓Bone marrow P6-specific B cells	IL-6, IL-4, IL-17, TNF	na	Lugade et al. (2011)
Environmental toxicants	Ozone	↓Lung inflammation, edema ↓Airway mucous cell metaplasia, mucus (Muc5ac) hypersecretion ↓Lung protein and lipid oxidation ↑GSH	GPX2, HO-1, NQO1	na	Cho et al. (2013)
	Arsenic (As III)	↓Lung inflammatory cells ↓Lung chemokine expression ↓Lung histopathological change ↓Lung 8-oxo-dG	·NQO1, GCLC ·NF-κB	↓Lung inflammation, NF-kB, IL-13, and DNA oxidation by sulforaphane and tanshinone I in *Nrf2*$^{+/+}$	Tao et al. (2013), Zheng et al. (2012)
	TiO_2 nanoparticle	↓Lung injury and inflammation ↓Lung 3-NT and 4-HNE	·HO-1 ·TNF, IFNγ, TGF-β	na	

Table 1 continued

Model disorder	Insult	Phenotypes	Mechanisms examined	Nrf2–ARE agonist effect	References†
Pulmonary hypertension	Hypoxia	↓Right ventricular hypertrophy		↓Right ventricular hypertrophy and improved pulmonary vasculature by oltipraz in *Nrf2*$^{+/+}$	Eba et al. (2013)
Lung tumorigenesis	Benzo(a)pyrene	↓Lung DNA mutation frequency	GST-A, GST-P	na	Aoki et al. (2007)
	Lewis lung carcinomas (3LL)	↓Metastatic nodules in host lung ↓Lung weight gain ↓Incidence of tumor ↓Tumor cell proliferation ↑Tumor cell apoptosis ↓Lung 8-OHdG	·ROS in lung MDSC fraction ·CD4^{-}Cd8^{+} cells	na	Satoh et al. (2010)
	Urethane	↓Pre-neoplastic body weight loss ↓Pre-neoplastic lung cell necrosis, apoptosis, inflammation ↓Lung precancerous nodules ↑Lung tumor frequency, number, incidence ↑Tumor mucus ↑Tumor cell proliferation ↑Tumor engraft and growth in nude mice	·*Kras* mutation ·Kras signaling pathway ·Cell-to-cell signaling, connective tissue development/function, glutathione metabolism and oxidative stress, immune/inflammatory responses (e.g., MELA, GAS6, ITGA6, PTGIS, MMP2, CD34, ARG2, ATRNL1) at 12 weeks ·Lung development, cell growth and proliferation (e.g., SOX9, ID2, MYC, CCND1) at 16 weeks ·Drug metabolism, cell cycle and death and organismal survival, and tumor morphology (e.g., CKS1B, CCND1, ATRNL1, DTX4, GSTM1, AKR1B8, ANGPTL2, FGA) at 22 weeks	na	Satoh et al. (2013)†, Bauer et al. (2011)†

Nrf2$^{+/+}$ wild-type mice, *Nrf2*$^{-/-}$ *Nrf2* gene knockout mice, *Nrf2*$^{\Delta CC}$ *Nrf2* conditional knockout mice, *na* not available, *ALI* acute lung injury, *ANG* angiogenin, *ANGPTL2* angiopoietin-like 2, *AOE* antioxidant enzyme, *AOX3* aldehyde dehydrogenase 3, *ARE* antioxidant response element, *Arg2* arginase type II, *ATF* activating transcription factor, *ATRNL1* attractin like 1, *Bnip3* BCL2/adenovirus E1B 19 kDa protein-interacting protein 3, *BPD* bronchopulmonary dysplasia, *CCL* chemokine (C–C motif) ligand, *CCNA2* cyclin A2, *CDCA5* cell division cycle associated 5, *CDDO-Im* 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole, *CKS1B* CDC28 protein kinase 1b, *CLP* cecal ligation and puncture, *Col1a1* collagen, *type 1* alpha 1, *COPD* chronic obstructive pulmonary disease, *COX-2* cyclooxygenase 2, *CXCL* chemokine (C–X–C motif) ligand, *DEP* diesel exhaust particle, *DTX4* deltex 4 homolog, *Egr2* early growth response, *FGA* fibrinogen alpha chain, *FSP* fibroblast-specific protein, *GCLC* glutamate-cystein lygase, catalytic subunit, *GCLM* glutamate-cystein lygase, modifier subunit, *Gas6* growth arrest specific 6, *GPx2* glutathione peroxidase 2, *GST* glutathione-S-transferase, *HO-1* heme oxygenase-1, *ID2* inhibitor of DNA, *IFNγ* interferon gamma, *IL* interleukin, *IRF-3* interferon regulatory factor 3, *ITG* integrin, binding 2, *LPS* lipopolysaccharide, *MARCO* macrophage receptor with collagenous structure, *MDSC* myeloid-derived suppressor cells, *Mela* melanoma antigen, *MHC* major histocompatibility complex, *MMP2* matrix metalloproteinase 2, *MSR1* macrophage scavenger receptor, *MT* metallothionein, *NAC N*-acetyl-L-cysteine, *NQO1* NAD(P)H:oxidoreductase 1, *NRF-1* nuclear respiratory factor 1, *PAI* plasminogen activator inhibitor, *PDCD2* programmed cell death protein 2, *PGC-1a* peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *PGDH* 15-hydroxyproslandin dehydrogenase, *PM* particulate matter, *PRDX1* peroxiredoxin -1, *PTGIS* prostacyclin (prostaglandin I2) synthase, *PTP1* protein-tyrosine-phosphatase, *ROS* reactive oxygen species, *RSV* respiratory syncytial virus, *SLC* solute carrier family, *SLPI* secretory leukocyte peptide inhibitor, *SOD* superoxide dismutase, *SOCS3* suppressor of cytokine signaling 3, *SOX9* sex determining region Y-box 9, *SQSTM1* sequestosome-1, *SSLPI* secretory leukoprotease inhibitor, *TFAM* mitochondrial transcription factor-A TGF, transforming growth factor, *TRAM* thyroid hormone receptor activator molecule, *UBC* ubiquitin C, *UFP* ultrafine particle, *VEGF* vascular endothelial growth factor, *3-NT* 3-nitrotyrosine, *4-HNE* 4-hydroxynonenal, *15d-PGJ*$_2$ 15-deoxy-delta(12,14)-prostaglandin J$_2$

* Genes that were analyzed for their functional ARE(s)

† References including microarray data

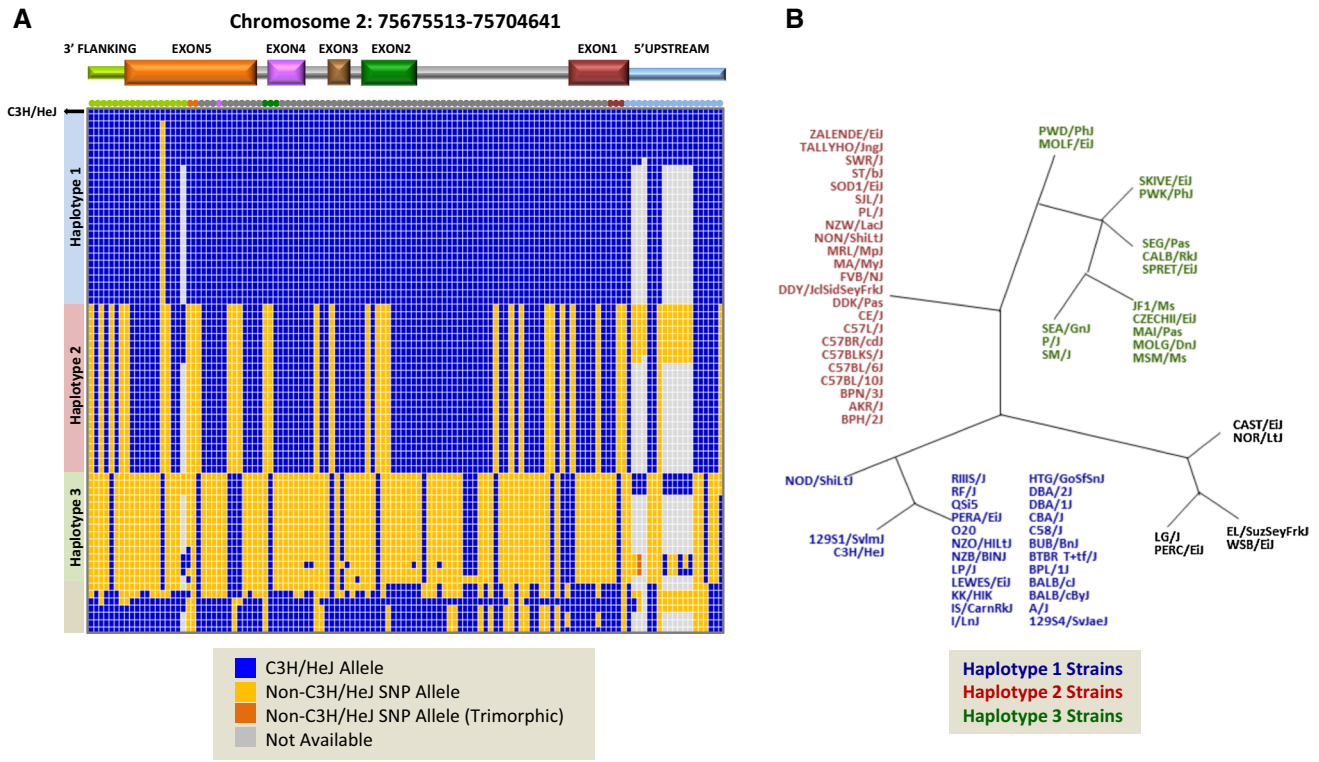


Fig. 1 Inbred mouse strains classified by *Nrf2* genetic variation. **a** An illustration of three major haplotypes of murine *Nrf2* (75.7–75.6 Mb) on chromosome 2 determined by hierarchical clustering of 173 single nucleotide polymorphism (SNP) from 72 inbred strains of mice (Cho et al. 2015a). *Each row of the matrix* represents a single inbred strain of mouse. The *blue* and *orange filled boxes* represent the panel of SNPs (*columns*) in *Nrf2*. *Blue* indicates the reference (C3H/HeJ) allele and *yellow* indicates the alternate allele. *Orange* indicates the third allele in the trimorphic SNP sites. *Colors of the circles* on top of the matrix indicate the SNP location in the structural regions (exon, intron, or flanking). **b** A phylogenetic tree for inbred strains of mice constructed from the *Nrf2* haplotype analysis

and retarded epithelium regeneration (Reddy et al. 2009a, 2011). Oral administration of a triterpenoid Nrf2–ARE inducer, 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole (CDDO-Im), in parallel with hyperoxia exposure, significantly increased ARE-responsive genes and mitigated lung edema, inflammation, and apoptosis in $Nrf2^{+/+}$ mice (ICR), but not in $Nrf2^{-/-}$ mice (Reddy et al. 2009b). However, pre-treatment of CDDO-Im did not protect $Nrf2^{+/+}$ mice from subsequent hyperoxia toxicity (Reddy et al. 2009b). Hyperoxia-exposed $Nrf2^{-/-}$ mice were also found to be highly vulnerable to bacterial infection, with severe alveolar macrophage accumulation and 50 % lethality 5 days after *Pseudomonas aeruginosa* (*P. aeruginosa*) challenge compared to $Nrf2^{+/+}$ mice (ICR) which survived hyperoxia and subsequent infection (Reddy et al. 2009c). The authors suggested that macrophage scavenger receptors including MSR1 and macrophage receptor with collagenous receptor (MARCO), glutamate-cystein lygase, catalytic subunit (GCLC), and interleukins 1 and 6 (IL-1, IL-6) contribute to the role of alveolar macrophages in alleviation of bacterial infection under oxidative stress.

Mechanical ventilation (MV)-induced lung injury

MV, in combination with hyperoxia, is an indispensable therapy for critically ill patients with ALI/ARDS. Conventional MV with high tidal volumes and low positive end expiratory pressure causes excessive alveolar distention, resulting in biotrauma characterized by lung edema and inflammation in patients (Ware et al. 2006). In response to experimentally applied MV, greater levels of lung permeability, inflammation, and disturbed redox balance and impaired gas exchange were found in $Nrf2^{-/-}$ mice compared to $Nrf2^{+/+}$ mice (ICR or B6) (Papaiahgari et al. 2007; Reiss et al. 2014). Supplementation of $Nrf2^{-/-}$ mice (ICR) with NAC, a precursor for glutathione biosynthesis, significantly attenuated ventilator-induced lung injury, indicating a role for Nrf2-mediated thiol homeostasis in this model (Papaiahgari et al. 2007).

Sepsis-induced ALI

Sepsis is characterized by dysregulation of inflammation that occurs primarily after systemic bacterial infection. Mortality due to sepsis is 50 % or greater in patients with

Table 2 Genetic and somatic *NRF2* mutations associated with disease risk

Domain	Variant description	Chromosome position	CDS mutation	Functional consequence	Associated disorders	References
Promoter	Genetic rs6706649 C>T	g.177265343	c.−767G>A	Upstream-212 variant	Asthma	Shaheen et al. (2010)
	Genetic rs6721961 T>G	g.177265309	c.−733A>C	Upstream-178 variant	Acute Lung Injury Annual FEV_1 decline Asthma BPD	Marzec et al. (2007), O'Mahony et al. (2012), Masuko et al. (2011a, b), Ungvari et al. (2012), Sampath et al. (2015)
Intron	Genetic rs2364723 G>C	g.177261818	c.45 + 2714 C>G	Intron 1 variant	FEV_1 decline in lung cancer	Sasaki et al. (2013b), Siedlinski et al. (2009)
	Genetic rs2364722 A>G	g.177260059	c.45 + 4473T>C	Intron 1 variant	Annual FEV_1 decline	Masuko et al. (2011a, b)
	Genetic rs1806649 C>T	g.177253424	c.45 + 11108G>A	Intron 1 variant	COPD COPD/asthma (PM 10) Annual FEV_1 decline	Figarska et al. (2014), Canova et al. (2012), Masuko et al. (2011a, b)
	Genetic rs1962142 A>G	g.177248756	c.46−14485T>C	Intron 1 variant	Annual FEV_1 decline	Masuko et al. (2011a, b)
	Genetic rs6726395 A>G	g.177238501	c.46−4230T>C	Intron 1 variant	FEV_1 decline in lung cancer Annual FEV_1 decline	Sasaki et al. (2013b), Siedlinski et al. 2009), Masuko et al. (2011a, b)
	Genetic rs2001350 C>T	g.177235697	c.46-1426G>A	Intron 1 variant	Annual FEV_1 decline	Masuko et al. (2011a, b)
Coding-DLG motif	Somatic COSM132852 COSM132989	–	c.72G>C c.72G>T	p.Trp24Cys	NSCLC	Ojesina et al. (2014), Shibata et al. (2011), Shibata et al. (2008)
	Somatic COSM132986	–	c.76C>G	p.Gln26Glu	NSCLC	Shibata et al. (2008, 2011)
	Somatic	–	c.80G>A	p.Asp27Gly	NSCLC	Hu et al. (2012)
	Somatic	–	c.83C>T	p.Ile28Thr	NSCLC	Shibata et al. (2008)
	Somatic COSM124726	–	c.85G>C	p.Ile29His	NSCLC	Hu et al. (2012), Kim et al. (2010), Sasaki et al. (2013a)
	Somatic COSM132854	–	c.88C>T	p.Leu30Phe	NSCLC	Shibata et al. (2008, 2011)
	Somatic COSM132956	–	c.92G>C	p.Gly31Ala	NSCLC	Hu et al. (2012), Kim et al. (2010), Sasaki et al. (2013a), Shibata et al. (2008, 2011) Tan et al. (2014)
	Somatic COSM132847	–	c.100C>G	p.Arg34Gly	NSCLC	Eichenmuller et al. (2014), Sasaki et al. (2013a)
	Somatic COSM132849	–	c.101G>A	p.Arg34Gln	NCSCL	Hu et al. (2012), Kim et al. (2010), Ojesina et al. (2014), Shibata et al. (2008)
	Somatic COSM132848	–	c.101G>C	p.Arg34Pro	NSCLC	Eichenmuller et al. (2014), Ojesina et al. (2014)
Coding-ETGE motif	Somatic COSM132859	–	c.230A>T	p.Asp77Val	NSCLC	Hu et al. (2012), Shibata et al. (2008, 2011)
	Somatic COSM132958	–	c.230A>C	p.Asp77Ala	NSCLC	Kim et al. (2010)
	Somatic COSM132987	–	c.232G>A	p.Glu78Lys	NSCLC	Shibata et al. (2008, 2011)
	Somatic COSM132851	–	c.235G>A	p.Glu79Lys	NSCLC	Kim et al. (2010), Sasaki et al. (2013a), Shibata et al. (2008, 2011)

Table 2 continued

Domain	Variant description	Chromosome position	CDS mutation	Functional consequence	Associated disorders	References
	Somatic COSM120958	–	c.235G>C	p.Glu79Gln	NSCLC	Hu et al. (2012), Kim et al. (2010), Shibata et al. (2008, 2011)
	Somatic COSM3961573	–	c.238A>G	p.Thr80Ala	NSCLC	Eichenmuller et al. (2014)
	Somatic COSM132964 COSM132861	–	c.239C>A c.239C>G	p.Thr80Lys	NSCLC	Kim et al. (2010), Shibata et al. (2008, 2011)
	Somatic COSM717624	–	c.241G>A	p.Gly81Ser	NSCLC	Sasaki et al. (2013a), Tanase et al. (2014)
	Somatic COSM132957	–	c.242G>A	p.Gly81Asp	NSCLC	Hu et al. (2012), Kim et al. (2010), Shibata et al. (2011)
	Somatic COSM131265	–	c.244G>C	p.Gly81Gln	NSCLC	Hu et al. (2012), Kim et al. (2010)
	Somatic COSM132853	–	c.245A>G	p.Gly81Gly	NSCLC	Shibata et al. (2008) Ooi et al. (2013)
	Somatic	–	c.247T>C	p.Phe83Leu	NSCLC	Hu et al. (2012)

Chromosomal location corresponds to GRCh38 annotation

More thorough information on *NRF2* somatic mutations is available at http://cancer.sanger.ac.uk/cosmic/search?q=nfe2l2

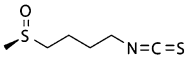
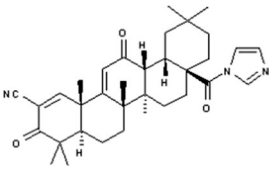
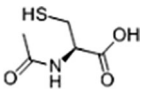
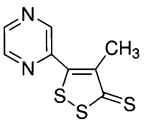
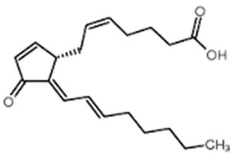
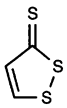
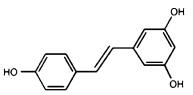
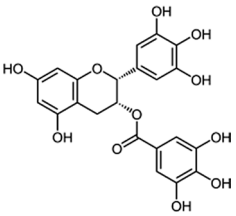
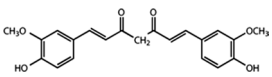
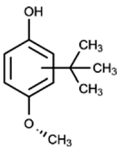
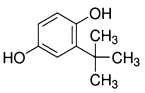
BPD bronchopulmonary dysplasia, *COPD* chronic obstructive pulmonary disease, FEV_1 forced expiratory volume in 1s, *NSCLC* non-small cell lung cancer, *COSMIC* catalogue of somatic mutations in cancer ID

the more severe syndrome, septic shock, and about 40 % of critically ill sepsis patients develop ALI (Angus et al. 2001; Hotchkiss and Karl 2003). Oxidative stress has a key role in the pathogenesis of sepsis, and host factors regulating innate immunity such as toll like receptors (TLRs), TNF-α, lipopolysaccharide (LPS)-binding protein, CD14, or bactericidal/permeability-increasing protein have been examined (Cohen 2002; Powers et al. 2006). In experimental sepsis as an indirect ALI model, systemic LPS and cecal ligation and puncture (CLP)-induced septic shock significantly augmented lung inflammation and innate immunity gene induction and nuclear factor kappa B (NF-κB) activation in $Nrf2^{-/-}$ mice compared to wild types (ICR) (Kim et al. 2014; Thimmulappa et al. 2006a, b). NAC treatment significantly suppressed lethality and increased lung GSH production and antioxidant enzyme expression during sepsis in $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice (Thimmulappa et al. 2006a). Pre-treatment with CDDO-Im also protected $Nrf2^{+/+}$ mice (ICR) from lethality caused by sepsis, but not $Nrf2^{-/-}$ mice, and enhanced lung ARE-responsive genes and decreased inflammatory chemokines may underlie this effect (Thimmulappa et al. 2006b). During acute pneumonia development by *Staphylococcus aureus (S. aureus)*-induced peritonitis, mitophagy (the elimination of damaged mitochondria by selective autophagy) was found to occur in an Nrf2-dependent manner (Chang et al. 2015). The authors demonstrated marked accumulation of autophagosome protein (p62) and diminished macrophage autophagy protein (LC3-II) in type 2 pneumocytes and alveolar macrophages in $Nrf2^{-/-}$ mice (B6), which may have caused decreased autophagic turnover of mitochondria and enhanced lung inflammation against oxidative stress in $Nrf2^{-/-}$ mice relative to $Nrf2^{+/+}$ mice (Chang et al. 2015). The authors also demonstrated that the absence of *Nrf2* suppressed mitochondria copy number increase and transcriptional networks of mitochondrial biogenesis represented by nuclear respiratory factor 1, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a), mitochondrial transcription factor-A, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) in type 2 pneumocytes after intranasal *S. aureus* infection, which may worsen lung edema and inflammation (Athale et al. 2012).

Other ALI models

In the butylated hyroxytoluene-induced ALI model, $Nrf2^{-/-}$ mice (B6) were significantly more susceptible to mortality and lung inflammation compared with $Nrf2^{+/+}$ mice (Chan and Kan 1999). Expression of pulmonary antioxidants including catalase, SOD1, NAD(P)H:oxidoreductase 1 (NQO1), and GCLC was suppressed in $Nrf2^{-/-}$ mice at baseline and after treatment relative to similarly treated wild types (Chan and Kan 1999).

Table 3 Nrf2 agonists for potential airway therapeutic intervention

Classification	Compound	Original source	Structure	Suggested therapeutic indication from *Nrf2*-deficient mouse model
Isothiocyanates	R-sulforaphane	Crucifers		Nrf2-dependent bacterial infection following cigarette smoke-induced COPD, RSV disease, and As toxicity
Triterpenoid	CDDO-Im (1[2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole)	Synthetic		Nrf2-dependent hyperoxia- and sepsis-induced induced ALI and cigarette smoke-induced COPD
Thiol precursor	NAC (*N*-acetyl-L-cysteine)	Endogenous		Nrf2-dependent MV-induced ALI. Nrf2-independent sepsis-induced ALI, cigarette smoke-induced COPD, and ovalbumin-induced allergic asthma
Organosulfur	Oltipraz	Synthetic		Nrf2-dependent hypoxia-induced pulmonary hypertension
Cyclopentenone prostaglandin	15d-PGJ_2 (15-deoxy-delta12,14-prostaglandin J_2)	Endogenous		Nrf2-dependent carrageenan-induced ALI
Dithiolethiones	D3T (3H-1,2,-dithiole-3-thione)	Crucifers		Not examined
Polyphenol	Resveratrol	Grapes, mulberries, blueberries, soybeans, peanuts		Not examined
	EGCG (epigallocatechin-3-gallate)	Tea		Not examined
Diarylheptanoid	Curcumin	Turmeric		Not examined
Phenol	BHA (butylated hydroxyanisole)	Synthetic		Not examined
	tBHQ (2-tert-butylhydroquinone)	Synthetic		Not examined

Carrageenan-induced acute lung inflammation was also exacerbated in *Nrf2*$^{-/-}$ mice compared with *Nrf2*$^{+/+}$ mice (ICR) (Mochizuki et al. 2005). A selective cyclooxygenase (COX)-2 inhibitor (NS-398) suppressed anti-inflammatory 15-deoxy-delta(12,14)-prostaglandin J_2 (15d-PGJ_2) accumulation and ARE response or ARE-mediated transcription (HO-1, PRDX1), and increased lung injury in *Nrf2*$^{-/-}$ mice more than in *Nrf2*$^{+/+}$ mice; 15d-PGJ_2 treatment attenuated carrageenan toxicity coincident with antioxidant production in *Nrf2*$^{+/+}$ mice, but not in *Nrf2*$^{-/-}$ mice (Mochizuki et al. 2005). The results indicated that 15d-PGJ_2 and Nrf2 signaling pathways interact to protect against acute pulmonary injury. Investigators have also identified a role of murine Nrf2 in the regulation of experimental traumatic brain injury-induced ALI. After moderately severe weight-drop impact head injury, pulmonary capillary permeability, wet/dry weight ratio, and alveolar cell apoptosis were greater in *Nrf2*$^{-/-}$ mice than in *Nrf2*$^{+/+}$ mice (ICR) (Jin et al. 2009). Exacerbated lung injury in *Nrf2*$^{-/-}$ mice was associated with increased pulmonary expression of inflammatory cytokines including tumor necrosis factor (TNF)-α, IL-1β, and IL-6 and with decreased pulmonary antioxidant and detoxifying enzymes including NQO1 and glutathione-S-transferase (GST)-α1 relative to those in *Nrf2*$^{+/+}$ mice.

Downstream effector mechanisms of model ALI

Global transcriptome analyses were conducted in *Nrf2*$^{-/-}$ and *Nrf2*$^{+/+}$ mice (ICR) to study underlying mechanisms of Nrf2-mediated protection in model ALI (Cho et al. 2005; Papaiahgari et al. 2007). These studies showed that pulmonary thiol homeostasis machinery, including well-known phase 2 detoxification enzymes and classical antioxidants, is under the control of Nrf2 basally and during development of ALI. Although GSH was known to protect the lung from oxidative injury, the role for thiol-related phase 2 antioxidant enzymes in oxidative lung injury was not well understood until Nrf2 was identified. A protective role for the phase 2 antioxidant enzyme in the lung was demonstrated first in a study with mice that lack gamma glutamyltranspeptidase (*Ggt*) and had more diffuse lung injury and lower survival rate after hyperoxia exposure compared with wild-type mice (Barrios et al. 2001; Jean et al. 2002). Thioredoxin peroxidase (TDX or PRDX) which catalyzes the reduction of a broad spectrum of peroxides using thiols as reductants was abundantly expressed in the lung (Kim et al. 2003), and more severe lung edema, epithelial cell injury, and ROS production were found in *Prdx6*-deficient mice after hyperoxia (Wang et al. 2004). Importantly, various genes in other pathways such as transporters, growth and tissue remodeling factors, and inflammatory mediators were also found to be modulated in an Nrf2-dependent manner during development of ALI-like phenotypes. A computational analysis based on SNPs in ARE transcription factor binding sites using a position weight matrix (PWM) approach determined novel effector genes differentially regulated by Nrf2 (Wang et al. 2007). For example, lung peroxisome proliferator-activated receptor gamma (*Pparg* or *Nr1c3*) was suppressed in *Nrf2*$^{-/-}$ mice relative to *Nrf2*$^{+/+}$ mice basally and after hyperoxia, and found to possess a functional ARE (−784_−764), and further analysis indicated that activated PPARγ (through Nrf2 binding) contributes to the Nrf2-mediated protection in hyperoxic lung injury (Cho et al. 2010). In addition, hyperoxia-inducible NADPH oxidase 4 (*Nox4*) contained functional AREs (−438_−458, −619_−636) and was also regulated by Nrf2 in mouse lungs for endothelial cell function and signaling (Pendyala et al. 2011). Moreover, MV caused Nrf2-dependent induction of amphiregulin (*Areg*), a ligand for epidermal growth factor (EGF) receptor (Papaiahgari et al. 2007), and it had a functional ARE (-760) for Nrf2 biding (Reiss et al. 2014). Interestingly, *Pparg* or *Areg* are not only Nrf2 downstream targets but their protein products also mutually activate *Nrf2* (Cho et al. 2010; Reiss et al. 2014).

Nrf2 variation and ALI susceptibility

From murine strain screening studies for hyperoxia-induced ALI susceptibility (Cho et al. 2015a; Hudak et al. 1993) and the profile of genetic variations in *Nrf2* (Cho 2013), a significant correlation of *Nrf2* single nucleotide polymorphisms (SNP) haplotypes with hyperoxia susceptibility was found. Three major *Nrf2* haplotypes were classified by hierarchical clustering as shown in Fig. 1 (Cho et al. 2015a). Compared to haplotype 1 strains (e.g., C3H/HeJ), haplotype 2 strains (e.g., B6) bearing a promoter SNP −103T/C, and haplotype 3 strains (e.g., P/J) containing nonsynonymous coding SNPs (1862A/T, H543Q and 1417T/C, T395I) were more susceptible to oxidative lung injury. The −103 T/C SNP causes addition of an Sp1 binding site to decrease Nrf2 promoter activation and ARE response, and Nrf2 proteins with the nonsynonymous coding SNPs in and near the DNA-binding Neh1 domain have reduced transactivation and ARE response (Cho et al. 2015a). These findings further supported Nrf2 as a genetic determinant for model ALI and provided additional insights into the function and genetic regulation of Nrf2. It also provides a useful tool for investigators who use mouse strains classified by *Nrf2* haplotypes to elucidate the role for Nrf2 in oxidative stress models.

Bench-to-bedside

A role for Nrf2 in clinical ALI was first determined by Marzec et al. (Marzec et al. 2007) in a population of

Europeans and African-Americans. A significant increase in the risk of ALI following major trauma (OR 6.44; 95 % CI 1.34, 30.8; $p = 0.021$) was found in patients bearing a promoter SNP at −178 (formerly −617, rs6721961 G/T) relative to the patients with wild type. This SNP has been further characterized as an at-risk allele in another ALI population (O'Mahony et al. 2012) as well as in many other diseases (Cho et al. 2015b). The translational investigations not only supported *NRF2* as a signature transcription factor associated with ALI risk but also may help to identify patients who are predisposed to develop ALI under at-risk conditions, such as trauma and sepsis.

Chronic obstructive pulmonary disease (COPD)/ emphysema

Clinical relevance

Emphysema gradually damages the alveoli, leading to loss of pulmonary elasticity and progressively more shortness of breath. COPD includes emphysema, chronic bronchitis, or both, and affects millions of people worldwide and is associated with a high incidence of morbidity and mortality. Cigarette smoke is the major contributor to COPD pathogenesis and also has been the dominant risk factor for lung cancer. In multiple human studies, pulmonary NRF2-KEAP1-BACH1 equilibrium was found to be lowered in lung tissues and in alveolar macrophages of aged smokers and COPD patients (Malhotra et al. 2008; Singh et al. 2009; Suzuki et al. 2008). In association studies from multiple Japanese and European cohorts, interactions between *NRF2* SNPs or SNP haplotypes (e.g., rs6726395, rs1806649) and smoking-related lung function reduction or COPD survival rate have been found (Canova et al. 2012; Figarska et al. 2014; Masuko et al. 2011a, b; Siedlinski et al. 2009). In another investigation of COPD and NRF2, Wang et al. (Wang et al. 2010) examined bronchial airway cells from smokers with or without lung cancer, and found significantly lowered ARE response genes associated with MAFG expression in smokers with lung cancer. Importantly, ARE SNPs in MAFG-binding antioxidant genes including aldo–keto reductase family 1, member C1 (*AKR1C1*), epoxide hydrolase 1 (*EPHX1*), *GCLC*, and *DUSP1* were significantly associated with their expression and lung cancer status, suggesting their roles in lung cancer risk among the smokers.

In vivo models

Functions of the Nrf2 pathway in models of COPD and emphysema have been studied extensively. A microarray analysis using rats with acute (~hour) and sub-chronic (~week) exposure to mainstream cigarette smoke indicated that Nrf2 and several detoxifying enzyme genes encoding HO-1, NQO1 and GCLC were found to be involved in nasal and pulmonary pathogenesis (Gebel et al. 2004). Chronic exposure (1.5–6 months) to cigarette smoke caused more severe emphysema symptoms, greater degree of oxidative DNA adduct formation, neutrophil elastase activation, and apoptosis as well as suppressed α1-antitrypsin (A1AT) and antioxidant enzymes in lungs of $Nrf2^{-/-}$ mice compared to those in $Nrf2^{+/+}$ controls (ICR) (Iizuka et al. 2005; Rangasamy et al. 2004). Marked dose-dependent alveolar destruction was also demonstrated in $Nrf2^{-/-}$ mice (ICR) after chronic cigarette smoke exposure (Gebel et al. 2010). Short-term exposure to cigarette smoke (up to 14 days) induced more severe lung inflammation, airway hyperreactivity, mucous cell metaplasia, and a mucin gene *Muc5ac* overexpression in $Nrf2^{-/-}$ mice than in $Nrf2^{+/+}$ mice (ICR) (Ying et al. 2014). Another model of COPD uses elastase to induce lung inflammation and subsequently results in alveolar destruction in mice similar to the pathogenesis of emphysema. The severity of elastase-induced emphysema assessed by alveolar destruction and lung edema and inflammation was evident in $Nrf2^{-/-}$ mice relative to $Nrf2^{+/+}$ mice (BALB/cJ) (Ishii et al. 2005). Wild-type bone marrow transplantation rescued the $Nrf2^{-/-}$ mice from initial lung inflammation and subsequent emphysema, and the improvement was attributed to suppressed levels of anti-protease, secretory leukoprotease inhibitor (SLPI), and antioxidant enzyme (PRDX1, HO-1, NQO1, GST-Yc) in alveolar macrophages from $Nrf2^{-/-}$ mice (Ishii et al. 2005). Supporting this notion, functional AREs in murine GPx2 (Singh et al. 2006b), GR (Harvey et al. 2009), and sulfiredoxin-1 (Srx1) (Singh et al. 2009) were discovered after acute or sub-chronic exposure to cigarette smoke. $Nrf2^{-/-}$ mice (B6) did not respond to the inhibiting effect of budesonide on inflammation caused by short-term cigarette smoke exposure or LPS (Adenuga et al. 2010), and authors speculated that heightened oxidant status in these mice may decrease lung histone deacetylase 2 activity to cause steroid resistance. Inflammation and edema caused by short-term cigarette smoke exposure (3–4 days) were significantly reduced by NAC or water-soluble vitamin C analogue in $Nrf2^{-/-}$ mice (B6), indicating non-specific protection in more severely injured lung (Messier et al. 2013b). However, CDDO-Im significantly reduced lung emphysema phenotypes (e.g., mean linear length of alveoli), DNA oxidation, and apoptosis as well as right ventricular dysfunction in $Nrf2^{+/+}$ mice (B6) exposed to chronic cigarette smoke (Sussan et al. 2009). NRF2 as a potential therapeutic target for COPD has been well described in recent reviews (Biswal et al. 2012; Domej et al. 2014). Overall, NRF2-mediated activation of thiol metabolism,

antioxidant and detoxification enzymes, proteasome system, and scavenger receptors have been suggested as key underlying mechanisms.

Concomitant infection

When mice were infected with influenza virus following short-term cigarette smoke exposure (4 days), mortality and body weight loss, lung edema and inflammation as well as bronchial mucous cell metaplasia were exacerbated relative to mice exposed to only cigarette smoke (Yageta et al. 2011). In addition, lung oxidation products and chemokine induction (TNF and KC) accompanying NF-κB increase by cigarette smoke were significantly enhanced by the post-viral infection. Compared to wild-type mice (B6), *Nrf2*$^{-/-}$ mice developed significantly more severe lung injury phenotypes after the co-exposure (Yageta et al. 2011). Macrophages from *Nrf2*$^{-/-}$ mice were also significantly more susceptible to poly(I:C), a synthetic analogue of viral dsRNA, than those from *Nrf2*$^{+/+}$ mice after cigarette smoke stimulation (Yageta et al. 2011). Harvey et al. (Harvey et al. 2011) demonstrated that *P. aeruginosa* infection exacerbated cigarette smoke-induced lung inflammation and altered macrophage phagocytosis in *Nrf2*$^{-/-}$ mice than in wild types (B6). Sulforaphane treatment prevented the cigarette smoke-induced decrease in alveolar macrophage phagocytosis in *Nrf2*$^{+/+}$ mice, but not in *Nrf2*$^{-/-}$ mice, and enhanced MARCO expression on *Nrf2*$^{+/+}$ alveolar macrophages suggested a role for the scavenger receptor in this model (Harvey et al. 2011). Data therefore indicated Nrf2 as a potential target for the prevention of exacerbation of inflammatory lung diseases such as COPD by microbial infection.

Downstream mechanisms in models

A microarray analysis of human airway epithelium obtained by bronchoscopy demonstrated a significant upregulation of 16 out of 44 antioxidant-related genes tested in smokers compared to nonsmokers (Hackett et al. 2003), indicating association of redox signaling in the pathogenesis. In mice, Ragasamy et al. (Rangasamy et al. 2004) identified pulmonary genes changed by acute cigarette smoke (5 h) in a Nrf2-dependent manner and determined potential AREs in their promoter. They included well-known Nrf2 effectors as well as novel genes encoding PRDX1, TXNRD1, 15-prostaglandin dehydrogenase (PGDH), A1AT, and ubiquitin C (UBC). Gebel et al. (Gebel et al. 2010) investigated lung transcriptome changed by three different chronic doses of cigarette smoke in *Nrf2*$^{+/+}$ and *Nrf2*$^{-/-}$ mice (ICR). They determined differentially suppressed ARE-bearing genes including cyclin A2 (*Ccna2*) and cell division cycle associated 5 (*Cdca5*) in *Nrf2*$^{-/-}$ mice relative to *Nrf2*$^{+/+}$ mice, which suggested a key role for cell loss–regeneration imbalance during the development of emphysema.

Allergic airway diseases

In vivo ovalbumin model

Asthma is a complex genetic and environmental disorder characterized by chronic airway inflammation and bronchoconstriction. Despite decades of extended research efforts, the disease pathogenesis is not fully understood. Growing evidence indicates that oxidative stress is involved, and highly oxidized thiols and suppressed Nrf2–ARE responses have been found in airway lavage cells, peripheral blood mononuclear cells, or airway smooth muscle cells from severe asthmatics (Fitzpatrick et al. 2011; Michaeloudes et al. 2011). A role for Nrf2 in a murine model of allergic asthma was described by Rangasamy et al. (Rangasamy et al. 2005). *Nrf2* deficiency (ICR) caused augmented ovalbumin-driven airway allergic responses including airway hyperresponsiveness, lung mucus cell hyperplasia, eosinophilic infiltration, and release of Th2 cytokines IL-4 and IL-13 concurrently with suppressed multiple antioxidants (Rangasamy et al. 2005). Supporting these results, the authors found in the same model significant reduction of all allergic asthma phenotypes and oxidative stress in mice with overactivated pulmonary Nrf2 due to epithelial deletion of *Keap1* (CC10-*Keap1*$^{-/-}$) compared to wild-type mice (Sussan et al. 2015).

In vivo environmental allergen models

Environmental oxidants including particulate matter (PM) such as diesel exhaust particles (DEP) may cause asthma symptoms and exacerbate pre-existing airway diseases including asthma and COPD. DEP contains various pro-oxidants and carcinogens (e.g., quinones, polycyclic aromatic hydrocarbons) and have been implicated in the pathogenesis of asthma (McClellan 1987). Aoki et al. (2001) reported a significant role for Nrf2–ARE pathway in DEP-induced pulmonary oxidative injury. They found accelerated DNA adduct formation and DNA oxidation in *Nrf2*$^{-/-}$ mice relative to *Nrf2*$^{+/+}$ mice (ICR) after high-dose DEP exposure (3 mg/m^3, 4 weeks). Low-dose DEP (100 μg/m^3) significantly enhanced airway hyperresponsiveness and lymphocytic and eosinophilic inflammation in *Nrf2*$^{-/-}$ mice compared to *Nrf2*$^{+/+}$ mice (B6) (Li et al. 2008). Low-dose DEP exposure also exacerbated ovalbumin-induced airway inflammation and hyperreactivity significantly more in *Nrf2*$^{-/-}$ mice than in *Nrf2*$^{+/+}$ mice (Li et al. 2010). Similarly, ambient Los Angeles ultrafine particles augmented ovalbumin-caused allergic airway inflammation as indicated by enhanced eosinophils,

lymphocytes, immunoglobulins E and G1, and IL-13, and the exacerbation was significantly greater in *Nrf2*$^{-/-}$ mice than in *Nrf2*$^{+/+}$ mice (Li et al. 2013). Transfer of bone marrow-derived *Nrf2*$^{-/-}$ dendritic cells (DCs) treated with ovalbumin/ultrafine particles developed significantly stronger allergic inflammation in normal mice, compared to DCs from similarly treated *Nrf2*$^{+/+}$ (Li et al. 2013). In primary cell culture models, heightened inflammatory cytokine production was found in lung DCs from *Nrf2*$^{-/-}$ mice constitutively and after ambient Baltimore PM stimulation, relative to lung DCs from *Nrf2*$^{+/+}$ mice (ICR) (Williams et al. 2008). Highly Th2-skewed immune response (IL-13, IL5), enhanced oxidant production, and suppressed antioxidant induction were also found in DCs from *Nrf2*$^{-/-}$ mice compared to DCs from *Nrf2*$^{+/+}$ mice, and NAC treatment attenuated PM-induced markers for DC activation (CD80 and CD86) in *Nrf2*$^{-/-}$ DCs (Williams et al. 2008). Similarly, allergen ragweed extract triggered cell maturation, major histocompatibility complex (MHC) II expression, ROS production, and inflammatory cytokine secretion in DCs (bone marrow-derived or lung) from *Nrf2*$^{-/-}$ mice compared to those from *Nrf2*$^{+/+}$ mice (ICR), and NAC inhibited ragweed extract effects on the *Nrf2*$^{+/+}$ DCs (Rangasamy et al. 2010). Overall, these investigations suggest that Nrf2 has important roles in pro-allergic, Th2-mediated immunity for airway protection against environmental allergens, and DCs may play a key role in orchestrating the effects.

Clinical associations

Many studies have shown that ROS influences asthma susceptibility or allergic airway responses through association with functional SNPs in ARE-responsive antioxidant enzymes GST (e.g., *GSTM1, GSTP1*), catalases (*CAT1*), and SODs (e.g., *SOD2*) (Mak et al. 2006; Mapp et al. 2002). More recent studies indicate the effect of NRF2 SNPs with asthma, and the promoter *NRF2* SNP rs6721961 G as well as an intronic SNP (rs1806649 C) have been associated with infection-induced asthma in Hungarian children (OR 0.290; $p = 0.015$) (Ungvari et al. 2012) and increased hospital admission during high-level PM_{10} exposure (OR 1.35; CI 1.04–1.76) (Canova et al. 2012). Evidence has also indicated an effect of pre-natal stimuli on post-natal asthmatic symptoms in *NRF2* variants in the Avalon Longitudinal Study (UK). That is, early gestational acetaminophen exposure significantly enhanced the risk of asthma (OR 1.73; CI 1.22–2.45) and wheezing (OR 1.53; CI 1.06–2.20) at age 7 in more than 5000 children when maternal copies of the -212 *NRF2* SNP (rs6706649 T, formerly -651 or -684) was present (Shaheen et al. 2010).

Bronchopulmonary dysplasia (BPD)

Neonate mouse model

BPD is a chronic lung disease of infancy, developing in about 20 % of very low-birth-weight (<1 kg) premature infants. Lung injury in BPD is thought to result from early developmental arrest probably associated with prenatal exposure and/or genetic factors that interrupt alveolar growth in extreme prematurity (new BPD) or from structural damage of relatively more developed lungs characterized by surfactant deficiency (Baraldi and Filippone 2007). Major pathological features of BPD are alveolarization failure, inflammation, and respiratory distress (Baraldi and Filippone 2007; Jobe and Bancalari 2001). BPD survivors often suffer from lifelong consequences of respiratory symptoms. Relevance of Nrf2 with lung maturation and injury in underdeveloped lung has been recently investigated using new born mice. Lungs of full-term rodents are in the saccular stage of lung development and thus are similar to premature human lungs. *Nrf2* deficiency (ICR) did not affect saccular-to-alveolar transition of normal lung development, but augmented pulmonary injury and arrest of alveolarization when the lung was exposed to hyperoxia during the saccular phase (Cho et al. 2012; McGrath-Morrow et al. 2009). That is, hyperoxia-induced BPD-like phenotypes including mortality, arrest of saccular-to-alveolar transition (determined by mean linear intercept and histopathological score), lung cell apoptosis, lung edema and inflammation, and protein and lipid oxidation were significantly more severe in *Nrf2*$^{-/-}$ neonates than in *Nrf2*$^{+/+}$ neonates. Hyperoxia-induced impairment of lung growth and decreased surfactant-producing type 2 cell numbers were also found in 2-week-old *Nrf2*$^{-/-}$ pups, but not in *Nrf2*$^{+/+}$ pups (McGrath-Morrow et al. 2009). These findings suggest a therapeutic potential for Nrf2 inducers in prevention of BPD in preterm infants and management of lifelong consequences of BPD.

Clinical BPD

Clinical trials for antioxidant therapies in management of respiratory disease in preterm newborns have largely been unsuccessful although recombinant human SOD administration has been applied with marginal efficacy as summarized previously (Poggi and Dani 2014). Genetic influence of antioxidant enzymes on the outcome of prematurity has not been well investigated except in a small BPD population ($n = 35$) in which mutant *GSTP1* (encoding the lower activity enzyme than wild-type *GSTP1*) was positively associated with the BPD risk (Manar et al. 2004). Sampath

et al. (Sampath et al. 2015) recently demonstrated that the homozygote *NRF2* rs6721961 C/C genotype was significantly ($p < 0.01$) associated with decreased severe BPD in very low-birth-weight infants ($n = 659$). This study also indicated association of an *NQO1* SNP with increased BPD, suggesting that genetic variants in Nrf2–ARE axis may contribute to the variance in liability to BPD in prematurity.

Underlying mechanisms

A transcriptome analysis found that hyperoxia exposure of the saccular phase lungs caused Nrf2-dependent suppression of genes for gene expression machinery and cell cycle/growth (DNA replication/repair, apoptosis, transcription, translation, cell cycle) and redox homeostasis including multiple ARE-responsive antioxidant/defense genes (Cho et al. 2012). The Nrf2-dependent genes may have functions in acute phase organ injury, cell growth and proliferation, vasculature development, immune response, transforming growth factor (TGF)-β signaling, and hematological system development and function (Cho et al. 2012). Consistent with the suppressed transcriptome for DNA replication and cell growth machinery in $Nrf2^{-/-}$ neonates, genomic and mitochondrial DNA base lesions induced by hyperoxia were evident in these mice (Cho et al. 2012). Putative AREs or ARE-like motifs in the potential Nrf2 target genes were analyzed using the PWM statistical model (Wang et al. 2007). Novel genes such as solute carrier family 7 (anionic amino acid transporter light chain, xc-system), member 11 (*Slc7a11*) encoding cysteine/glutamate exchange transporter (xCT), aldehyde dehydrogenase 3 (*Aox3*), angiogenin, ribonuclease A family, member 2 (*Ang2*), and integrin alpha 4 (*Itga4*) were found to be direct Nrf2 effectors that may modulate aberrant alveolarization in preterm lung. *Gpx2* and *Marco* were also differentially expressed in $Nrf2^{-/-}$ and $Nrf2^{+/+}$ neonates, and targeted deletion of each of these genes enhanced hyperoxia-induced lung injury and therefore suggested protective roles in the underdeveloped lung (Cho et al. 2012).

Pulmonary fibrosis

Clinical relevance

Tissue fibrosis characterized by uncontrolled deposition of extracellular matrix molecules (e.g., collagen and elastin) with persistence of myofibroblast proliferation, is an irreversible end-state process manifested in many chronic diseases of kidney, liver, and lung. Idiopathic pulmonary fibrosis (IPF) in humans is the most frequent idiopathic interstitial pneumonia with a prevalence ranging from 5 to 15 per 100,000 persons, and above 175 per 100,000 in older populations. The manifestation of IPF symptoms progressively deteriorates respiratory function and ultimately leads to death. The etiology of IPF remains unknown, but oxidative stress is known to play a role in alveolar epithelial cell injury and fibrogenesis. Interestingly, heightened NRF2 expression and oxidant markers were found in a population of IPF patients (Markart et al. 2009).

In vivo models

Bleomycin has been used as a combined therapy for carcinomas and lymphomas, but it causes IPF-like lung fibrosis in susceptible patients. In laboratory rodents, bleomycin-induced lung fibrosis has been widely used to study the mechanisms of IPF pathogenesis. Bleomycin triggers production of ROS and DNA damage in the lung (Ishida and Takahashi 1975), and NAC administration significantly attenuated bleomycin-induced fibrosis in rodents (Mata et al. 2003). The role of oxidative stress in pulmonary fibrogenesis was further supported by studies that demonstrated protective effects of exogenously administered antioxidant enzymes (e.g., SOD3, catalase, peroxiredoxin) or transgenes against lung fibrogenesis (e.g.(Fattman et al. 2003; Hoshino et al. 2003; Kikuchi et al. 2011; Machtay et al. 2006; Van Rheen et al. 2011)). In another study, significantly higher lethality and suppressed body weight gain were found in $Nrf2^{-/-}$ mice (ICR or B6) and demonstrated their enhanced susceptibility to bleomycin (Cho et al. 2004; Kikuchi et al. 2010). Compared to $Nrf2^{+/+}$, $Nrf2^{-/-}$ mice also developed more severe lung inflammation and widespread fibrosis by bleomycin (Cho et al. 2004; Kikuchi et al. 2010; Walters et al. 2008). Attenuated induction of ARE-responsive genes and augmented lipid peroxide, chemokines/cytokines, and fibrotic markers including TGF-β and tenascin-C in $Nrf2^{-/-}$ mice suggested that the Nrf2-mediated antioxidant pathway is essential in limiting bleomycin-induced inflammation and fibrosis (Cho et al. 2004). Pulmonary injury and fibrosis are also secondary to radiation therapy and can cause significant morbidity and mortality among cancer survivors. Thoracic irradiation significantly reduced life span of $Nrf2^{-/-}$ mice (176 vs 212 days), compared to similarly treated wild types (Travis et al. 2011). Although fibrosis score did not differ between the two genotypes, decreased numbers of alveoli and enhanced pulmonary localization of plasminogen activator inhibitor (PAI)-1, a TGF-β target, and fibroblast-specific protein (FSP)-1 indicated augmented fibrogenesis in $Nrf2^{-/-}$ mice compared to $Nrf2^{+/+}$ mice (Travis et al. 2011). Activation of Nrf2–ARE responses by a polyphenol epigallocatechin-3-gallate treatment also reduced bleomycin-induced lung injury and inflammation in rats (Sriram et al. 2008). Overall, these studies contribute to our understanding of potential actions of Nrf2–ARE pathway on anti-fibrogenesis mechanisms.

Downstream mechanisms

Because TGF-β is known to engage in ROS generation and suppress transcription of genes encoding antioxidants (e.g., GST, SOD) through interaction of Smad3-ATF3 with Nrf2 (Bakin et al. 2005; Jardine et al. 2002; Liu et al. 2004a), the lack of *Nrf2* and concomitant overproduction of TGF-β may synergistically accelerate pathogenesis as seen in $Nrf2^{-/-}$ mice (Cho et al. 2004). The higher levels of Th2 cytokines were also evident in the lungs of $Nrf2^{-/-}$ mice relative to $Nrf2^{+/+}$ mice during bleomycin-induced fibrogenesis (Kikuchi et al. 2010). Interestingly, GGT, an Nrf2 target and a key enzyme in GSH synthesis, seems to promote fibrosis in the bleomycin model (Pardo et al. 2003). This deviates from what would be predicted given the evidence that GSH is protective; however, the attenuating effect of *Ggt* deletion appears to be related to the immunomodulating effects of GGT, particularly a lack of neutrophilic inflammation soon after bleomycin exposure (Pardo et al. 2003). A recent study indicated that impaired capacity of pulmonary fibrosis in aged mice was associated with reduced Nrf2 and enhanced NADPH oxidase 4, which drove myofibroblast proliferation, and NOX4-NRF2 imbalance in lung tissues from IPF patients provided evidence consistent with this observation (Hecker et al. 2014).

Viral airway diseases

Respiratory syncytial virus (RSV) in clinic

RSV is a global seasonal pathogen. Most (>95 %) children are known to be infected by the virus by age 2 (Foy et al. 1973). While most infected individuals experience mild upper airway symptoms, high risk groups including infants, young children, and immune compromised adults and the elderly may develop severe lower respiratory illness characterized by bronchiolitis and respiratory failure. RSV infection is the leading cause of infant hospitalization (Lozano et al. 2012). Severe RSV disease is associated with increased virus titers in the lungs leading to epithelial damage and sloughing, mucus production, and augmented inflammation linked to decreased Th1 and increased Th2 cytokine production (Becker 2006; Moore and Peebles 2006). Extensive research on host immune responses to RSV has been conducted in humans and in laboratory animals, and roles for innate immune receptors including TLR4, chemokines such as CX3CL1, Th1 (interferon gamma or IFNγ) and Th2 (IL-4) cytokines, and intracellular adhesion molecule-1 have been suggested in RSV pathogenesis (Behera et al. 2001; Boelen et al. 2002; Caballero et al. 2015; Kurt-Jones et al. 2000; Tripp et al. 2001; van Schaik et al. 2000). ROS production and lipid peroxidation have also been implicated in RSV toxicity to lung cells and tissues (Casola et al. 2001; Liu et al. 2004b). Moreover, antioxidant treatment has been suggested to provide some protection against the RSV disease (Castro et al. 2006). Because airway epithelial cells are not only the major source of antioxidant enzymes/defense proteins but also the primary targets for RSV, it is important to determine the role of cellular antioxidant mechanisms in RSV pathogenesis. Nasopharyngeal secretion samples from RSV-infected children and infants with bronchiolitis were examined (Hosakote et al. 2011), and more clinically advanced cases (hypoxic bronchiolitis or under ventilator support) were associated with diminished antioxidant enzymes (SODs, catalase, GST-M) and enhanced lung lipid peroxidation compared to relatively mild cases (upper respiratory tract infection alone or non-hypoxic bronchiolitis). These observations support a role for oxidative stress in RSV pathogenesis and suggest Nrf2–ARE may protect against RSV disease.

In vivo RSV disease model

A role for Nrf2 and ARE-driven downstream mechanisms in airway RSV pathogenesis was tested in mice. RSV disease phenotypes were found to be significantly enhanced in $Nrf2^{-/-}$ mice (ICR) compared to $Nrf2^{+/+}$ mice 1–7 days after a single dose of RSV. Nrf2 deficiency not only heightened viral load, but also augmented lung injury and inflammation, and bronchial mucous cell metaplasia and mucus hypersecretion (Cho et al. 2009). Suppressed ARE-responsive antioxidant enzyme induction and increased pulmonary oxidative stress markers in $Nrf2^{-/-}$ mice may contribute to the underlying mechanisms of augmentation. A role for Nrf2 in nasal airway was also examined, and $Nrf2^{-/-}$ mice had more severe protein exudates, inflammatory infiltration and epithelial damage and metaplastic changes, and increased mucous production and secretion (Cho et al. 2009). Importantly, pretreatment with sulforaphane significantly attenuated RSV-induced acute lung inflammation and pulmonary viral expression in $Nrf2^{+/+}$ mice (Cho et al. 2009). Results from the study confirmed an association of oxidant stress in RSV pathogenesis and provide compelling evidence for an important regulatory role of Nrf2–ARE as a host defense mechanism against RSV disease.

Influenza virus infection

Haemophilus influenza (*H. influenza*) infection is a major cause of acute sino-pulmonary infection and can exacerbate existing pulmonary disorders such as of COPD. Nrf2 interrupted influenza virus entry and replication as determined in cultured human nasal epithelial cells, and influenza virus titer and expression was significantly increased with *Nrf2* knockdown by short hairpin RNA treatment (Kesic et al.

2011). Antiviral activity of murine Nrf2 was further provided by Lugade et al. (Lugade et al. 2011) in an *H. influenza*-induced chronic lung bronchitis model (4–16 weeks). Compared to wild-type mice (B6), more severe lung inflammation and sustained increase in serum antibodies for the viral membrane protein (P6) and bone marrow P6-specific B cells were found in *Nrf2*$^{-/-}$ mice. BAL pro-inflammatory cytokines (IL-6, IL-4, IL-17, TNF) were also significantly greater in *Nrf2*$^{-/-}$ mice than in *Nrf2*$^{+/+}$ mice (Lugade et al. 2011). These studies demonstrated that Nrf2 could potentially modulate immune responses to *H. influenzae* infection.

Lung tumorigenesis

Lung carcinomas are the leading cause of cancer-related deaths worldwide. Increased risk of lung cancer is associated with environmental factors including cigarette smoking, exposure to polycyclic aromatic hydrocarbons, asbestos, ionizing radiation, and COPD with airflow obstruction (Tan and Spivack 2009). Overexpression of phase 2 antioxidant enzymes (e.g., GSTs, NQO1, UDP-glucuronosyltransferase or UGT, HO-1) has been reported in a variety of cancers (Siegel and Ross 2000). In addition, a large body of evidence indicates that polymorphisms in these antioxidant genes are associated with risk of cancers in the lung, bladder, gastrointestinal tract, and prostate (Lin et al. 2003; Matthias et al. 1998; Ockenga et al. 2003).

Role in pre-carcinogenic stage

While a protective effect of Nrf2 has been found in many pulmonary diseases as described above, the role of Nrf2 in cancer pathogenesis is less clear. Experimental tumorigenesis models of non-pulmonary tissues such as liver, stomach, colon, skin, prostate, bladder, and mammary have indicated that tumor incidence and/or size was increased in *Nrf2*$^{-/-}$ mice relative to *Nrf2*$^{+/+}$ mice (Slocum and Kensler 2011). These studies support the hypothesis that Nrf2-mediated induction of phase 2 detoxification enzymes is pivotal in opposing mutagenesis and carcinogenesis. *Nrf2* deficiency (B6) also significantly increased DNA mutation frequency spontaneously and in response to intratracheal benzo(a)pyrene in the transgenic guanine phosphoribosyltransferase delta mouse system, indicating a protective role of Nrf2 against a genotoxic carcinogen (Aoki et al. 2007). Experimentally implanted highly metastatic lung cancer cells (Lewis lung carcinomas, 3LL) produced significantly more pulmonary metastatic nodules in *Nrf2*$^{-/-}$ mice than in *Nrf2*$^{+/+}$ mice (Satoh et al. 2010). Consistent with this finding, suppressed metastatic nodule formation was found in *Keap1*-knockdown mice compared to *Keap1* normal mice (Satoh et al. 2010). After cancer cell implantation, recipient *Nrf2*$^{+/+}$ mice transplanted with *Nrf2*$^{-/-}$ bone marrow, which bears more ROS-myeloid-derived suppressor cells than *Nrf2*$^{+/+}$ bone marrow, had increased metastatic lung nodules and suppressed splenic CD8^{+} cells than the recipients received *Nrf2*$^{+/+}$ bone marrow (Satoh et al. 2010). The authors suggested that Nrf2 in host lung tissues prevents metastasis through the stabilization of redox balance in the hematopoietic and immune cells by increasing immunosuppressive cells which can cause a decrease in CD8^{+} T cell immunity.

Oncogenic activity in lung cancer

The potential detrimental effects of Nrf2 in health and disease were first observed in *Keap1*-deficient mice which die by 3 weeks of age (Wakabayashi et al. 2003). Yamamoto and colleagues found that the premature death was due to malnutrition resulting from hyperkeratosis in esophagus and fore-stomach which, in turn, was caused by nuclear accumulation and transactivation of Nrf2 leading to aberrant expression of squamous cell genes as well as cytoprotective genes (Wakabayashi et al. 2003). They hypothesized that an abnormality in the NRF2-KEAP1 system may also facilitate the growth of cancer cells. Importantly, their laboratory and others found a high incidence/frequent occurrence of loss of KEAP1 function in patients with lung cancer, and identified nonsynonymous somatic mutations in *KEAP1* associated with reduced KEAP1 levels in lung cancer tissues and in lung cancer cells (Ohta et al. 2008; Padmanabhan et al. 2006; Singh et al. 2006a). Somatic or missense mutations occur in the nuclear DNA of dividing cells under normal and neoplastic conditions, likely due to mis-incorporation during DNA replication or exposure to mutagens. 'Driver' mutations in the cancer genome (as shown in *KEAP1* above) confer growth advantage in the cell in which they occur, are causally implicated in cancer development, and thus have been positively selected. Lung cancer cells with low KEAP1 activity have enhanced transactivation of Nrf2 and expression of cytoprotective genes and drug efflux pumps. This cascade of events conferred resistance to a chemopreventive agent, cisplatin, as determined by siRNA silencing of *NRF2* (Ohta et al. 2008; Singh et al. 2006a). Following this observation, many clinical investigations of lung cancer patients found that aberrant activation of NRF2 by somatic mutations in *NRF2* as well as in *KEAP1* was associated with increased risk of non-small cell lung carcinoma (NSCLC) cases including squamous cell lung carcinoma, large cell carcinoma, and adenocarcinoma [Review: (Cho et al. 2015b)]. Those studies demonstrated that multiple somatic mutations in cancer cells are clustered in N-terminal DLG and ETGE motifs of *NRF2* (Table 2) which are critical in Keap1-Cul3 E3 ligase-mediated ubiquitination and rapid turnover of NRF2

(Shibata et al. 2008). The mutations caused aberrant cellular accumulation of NRF2 due to impaired NRF2-KEAP1 binding for NRF2 suppression and KEAP1-mediated NRF2 degradation, and persistent, aberrant transactivation of NRF2. Consequently, uncontrolled overexpression of ARE-responsive genes involved in antioxidation and detoxification, pentose phosphate pathway, nucleotide synthesis, and drug efflux may give selective growth advantage and chemoresistance of the metastatic cells. Importantly, smoking history was correlated with mutation occurrence in all the cases. Investigators suggest that in chemotherapy-resistant cancers, *KEAP1* may be a potential 'tumor suppressor' gene while *NRF2* may conversely be 'oncogenic' by its excess 'gain of function'.

Aberrant DNA methylation is another important mechanism for gene silencing. For example, Arisawa et al. (Arisawa et al. 2008) demonstrated a relationship between *NRF2* promoter polymorphisms (−214, formerly −686/−684) and the CpG island methylation of cell cycle genes in humans with or without malignancies, suggesting that the promoter polymorphisms of *NRF2* may affect the methylation status of tumor-related genes. *KEAP1* suppression by epigenetic mutations in the *KEAP1-NRF2* system is also associated with lung tumor and cancer cells, and results in uncontrolled NRF2 activation, which provides growth advantage and chemoresistance of neoplastic cells [Review: (Hayes and McMahon 2009)]. These findings justify further epigenetic investigations on *NRF2* and *KEAP1* in lung cancer to provide new insights into pulmonary carcinogenesis and chemoprevention.

In vivo chemical carcinogenesis

Consistent with clinical observations, a murine lung carcinogenesis study with chronic urethane treatment demonstrated a detrimental function of Nrf2. Compared to wild-type animals, targeted deletion of *Nrf2* (BALB/cJ) enhanced body weight loss, lung cell necrosis and apoptosis, and lung inflammation and injury during the early, preneoplastic stage (8 or 11 weeks), and led to significantly reduced lung tumor development concurrent with lowered cell proliferation at 12 or 22 weeks (Bauer et al. 2011). Similar findings were reported by Satoh et al. (Satoh et al. 2013). Following urethane administration, the numbers of lung nodules were more abundant in $Nrf2^{-/-}$ mice (ICR) during the precancerous stage while lung surface tumor size and number as well as mucus production in the tumors were significantly lower in $Nrf2^{-/-}$ mice than in $Nrf2^{+/+}$ mice. Tumors from $Nrf2^{-/-}$ mice failed to engraft and grow in immunodeficient nude mice while tumors from $Nrf2^{+/+}$ mice increased 50-fold in volume in nude mice, indicating $Nrf2^{-/-}$ tumors are deficient in autonomous growth (Satoh et al. 2013). Interestingly, they also found an activating somatic mutation at codon 61 (c.182A>G, Q61R) of *Kras* in urethane-induced $Nrf2^{+/+}$ tumors (15/15) which was scarce in $Nrf2^{-/-}$ tumors (1/13), and the finding was consistent with induced expression of Kras downstream transducers in $Nrf2^{+/+}$ mice (Satoh et al. 2013). This supports the role for Kras signaling in the activation of Nrf2–ARE in tumorigenesis (DeNicola et al. 2011) and suggests a beneficial role for mutation of *Kras* and downstream activation including Nrf2 in lung tumor formation. Together, murine studies suggest that, contrary to the initiation stage when Nrf2-mediated defense processes are essential for tissue protection, enhanced Nrf2–ARE activity in association with the Kras signaling pathway in the advanced stages of carcinogenesis may create a favorable environment to provide resistance to chemotherapy and promote malignancy. These results suggest a new paradigm for cancer treatment in which NRF2 inhibition could enhance chemotherapeutic sensitivity.

Downstream mechanisms of chemical carcinogenesis

Transcriptome analyses for tumor tissues and uninvolved control tissues from $Nrf2^{+/+}$ or $Nrf2^{-/-}$ mice were performed in both urethane studies to characterize Nrf2-dependent lung tumor transcriptomics (Bauer et al. 2011; Satoh et al. 2013). In the early stage (12 weeks), tumor genes in pathways including cell-to-cell signaling and interaction, glutathione metabolism and oxidative stress, and immune/inflammatory responses were changed Nrf2-dependently, and genes including melanoma antigen (*Mela*), growth arrest specific 6 (*Gas6*), integrin alpha 6 (*Itga6*), prostacyclin synthase (*Ptgis*), matrix metalloproteinase 2 (*Mmp2*), CD34 antigen (*Cd34*), and TGF-β induced (*Tgfbi*) were relatively highly induced in $Nrf2^{-/-}$ mice compared to $Nrf2^{+/+}$ mice (Bauer et al. 2011). In contrast, ARE-responsive antioxidants, solute carrier family, chemokines, arginase type II (*Arg2*), and attractin like 1 (*Atrnl1*) were suppressed in these mice (Bauer et al. 2011). Heightened *Ptgis* expression in the early stage tumors of $Nrf2^{-/-}$ mice suggested that the tumor microenvironment suppresses the normal inflammatory response in these mice. Importantly, a 92 % reduction in tumor multiplicity was found in *Ptgis*-overexpressing mice (Keith et al. 2010) and prostacyclin analogs are currently in use for clinical trials in human NSCLC by the National Cancer Institute Lung Cancer Biomarker and Chemoprevention Consortium. At 16 weeks, expression of genes involved in lung development and cell growth and proliferation such as sex determining region Y-box 9 (*Sox9*), inhibitor of DNA binding 2 (*Id2*), *Myc,* and *Ccnd1* were higher in $Nrf2^{+/+}$ mice than in $Nrf2^{-/-}$ mice, and they were related to cancer progression pathways including Wnt/β-catenin and notch signaling (Satoh et al. 2013). Nrf2-dependent gene expression

in the late stages of tumorigenesis (22 weeks) was associated with cell cycle regulation (e.g., CDC28 protein kinase 1b or *Cks1b*, *Ccnd1*), organismal injury/survival (e.g., attractin like 1 or *Atrnl1*, deltex 4 homolog or *Dtx4*), xenobiotic/thiol metabolism (e.g., *Gstm1*, *Akr1b8*), and cell-to-cell signaling (e.g., angiopoietin-like 2 or *Angptl2*, fibrinogen alpha chain or *Fga*) (Bauer et al. 2011). The largest family of genes encoding thiol metabolism enzymes, solute carrier family members, and transmembrane proteins were included, and they were closely associated with drug metabolism, cell cycle and death and organismal survival, and tumor morphology. Although major functional categories of Nrf2-dependent transcripts were similar in early neoplastic lungs and in tumors, only 21 individual genes (e.g., *Cd34*, *Txnrd1*, phosphogluconate dehydrogenase or *Pgd, Ugt1*a1, G protein-coupled receptor 137B or *Gpr137b*, dynein light chain Tctex-type 1B or *Dynlt1*) connected in a molecular network of cancer-cell cycle-cell death were found to be common at these two stages.

Other airway disorders

Chronic hypoxia in high altitude causes pulmonary hypertension characterized by right ventricular hypertrophy and vascular remodeling. Pulmonary hypertension exacerbates preexisting pulmonary disease such as COPD and IPF (Minai et al. 2010). *Nrf2*$^{-/-}$ mice developed more severe right ventricular hypertrophy than *Nrf2*$^{+/+}$ mice (B6) in experimental hypoxia, and *Keap1* knockdown mice (Taguchi et al. 2010) were significantly protected against hypoxic insult (Eba et al. 2013). In addition, the Nrf2–ARE agonist oltipraz significantly diminished right ventricular hypertrophy and improved regeneration of vessels in *Nrf2*$^{+/+}$ mice (Eba et al. 2013), indicating the beneficial role for Nrf2 in pulmonary hypertension development.

Inorganic arsenic (As III) is an environmental toxicant emitted into air and then deposited into water and soil during industrial operations such as ore mining and smelting, and during volcanic eruptions and forest fires. As III affects multiple organs (Jiang et al. 2009) and its lung carcinogenic activity has been reported (Putila and Guo 2011). Sub-chronic inhalation (14 days) of As particles caused more severe inflammation and DNA oxidation in *Nrf2*$^{-/-}$ mice than in *Nrf2*$^{+/+}$ mice (Zheng et al. 2012). Nrf2 activation by concurrent, systemic (*i.p.*) treatment of sulforaphane prevented the lung histopathological changes and inflammatory cell infiltration in *Nrf2*$^{+/+}$ mice but not in *Nrf2*$^{-/-}$ mice (Zheng et al. 2012). Similarly, the authors also demonstrated that an herbal extract tanshinone I was protective against As-induced lung inflammation and injury only in *Nrf2*$^{+/+}$ mice (Tao et al. 2013).

Titanium dioxide (TiO_2) nanoparticles have been used widely in industries, but are also known to have oxidative properties and may be carcinogenic. Biweekly treatment of TiO_2 nanoparticles caused lung injury and lipid oxidation after 28 days in mice, and compared to *Nrf2*$^{+/+}$ mice (B6), lung inflammation and injury as well as TNF, IFNγ, and TGF-β were markedly greater in *Nrf2*$^{-/-}$ mice (Delgado-Buenrostro et al. 2014).

Sub-acute (0.3 ppm for 3 days) and acute (2 ppm for 3 h) exposures to ozone cause lung inflammation and mucous cell metaplasia in rodents. Lung inflammation and edema, epithelial proliferation in terminal bronchioles, and mucous cell metaplasia accompanying mucus hypersecretion were significantly greater in *Nrf2*$^{-/-}$ mice compared to wild types (ICR) (Cho et al. 2013). Augmented protein and lipid oxidation and retarded GSH pool explained were consistent with severe oxidative stress in *Nrf2*$^{-/-}$ mice (Cho et al. 2013).

ARE and Nrf2 network analysis tools

Investigators have explored novel genes that have little known antioxidant function but are differentially expressed in tissues or cells from *Nrf2*$^{+/+}$ and *Nrf2*$^{-/-}$ mice. The consensus sequence for Nrf2 binding promoter AREs has been considered as 5′-RGTGACnnnGC-3′ (where n = A, C, G, or T, R = A or G) following mutagenesis studies of the rat *Gsta2* and *Nqo1* gene enhancers (Rushmore et al. 1991). However, subsequent mutagenesis studies identified deviations from the consensus ARE (Erickson et al. 2002; Wasserman and Fahl 1997), and identification of a 'true' ARE sequence has been elusive. A computational method based on bioinformatics approaches in mammals was developed by Wang et al. (Wang et al. 2007) to identify potential polymorphic AREs in the human genome with improved predictive specificity, relative to traditional time-consuming and low-throughput experimental methods for exploring DNA–protein interaction. In this system, they constructed the PWM statistical model, based on a set of functional ARE sequences curated from published experimental studies. Using this PWM model and other computational tools, they predicted fidelity of AREs sequences and mapped ARE SNPs to the upstream regions of genes, and examined the evolutionary conservation of putative AREs by phylogenetic footprinting. Then they prioritized candidate SNPs based on microarray expression profiles from tissues in which the transcription factor of interest is either deleted or over-expressed and analyzed association of SNP genotypes with gene expression phenotypes. Using this novel apparatus, a set of polymorphic AREs with functional evidence was identified (Wang et al. 2007). This tool has been applied to the Nrf2-dependent genes elucidated from the microarray analysis of the hyperoxia model, and identified multiple potential AREs in 5 or 10 Kb upstream region of

genes such as *Pparg*, *Marco*, activating transcription factor 4 gene (*Atf4*), *Slc7a11,* and *Prdx1* in models ALI, BPD, and COPD (Cho et al. 2005, 2012; Wang et al. 2010).

Tayor et al. (2008) constructed independent information-theoretic algorithms, which included two network inference algorithms: the Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNE), and Context Likelihood of Relatedness (CLR). To find direct regulatory targets of Nrf2, these two computational approaches were applied to the analysis of mouse lung gene expression datasets to identify key regulatory relationships among the proteins that respond to oxidative stress. They also used a support vector machine classification algorithm to characterize promoter sequences of Nrf2 regulatory targets to corroborate ARACNE and CLR predictions. Inferred networks were analyzed, compared, and integrated to identify a number of previously known and novel targets of Nrf2 transcriptional activation. They determined genes predicted as novel Nrf2 targets (e.g., *Srx1*), and confirmed their predictions by various molecular methods in $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mouse lungs exposed to cigarette smoke (Singh et al. 2009; Taylor et al. 2008). The results of this approach demonstrate the promise of network inference algorithms in identifying transcriptional regulatory and other signaling relationships implicated in human disease.

Korcsmáros and colleagues introduced 'NRF2-ome (http://nrf2.elte.hu/)', an integrated online resource and discovery tool for protein interaction and regulatory networks of NRF2 (Turei et al. 2013). The authors extended the previously published NRF2 interactome and regulome (Papp et al. 2012), which led to a total of 7777 manually curated, integrated, and predicted interaction data for NRF2 along with its first neighbor interactors, target genes, NRF2 regulating transcription factors, and microRNAs as well as NRF2 signaling pathways. NRF2-ome allows the users not only to search data from multiple sources in a single resource, but also to analyze protein interaction and regulatory/signaling data simultaneously. The NRf2-related regulatory loops and pathway connections listed in NRF2-ome could also be a great resource for network pharmacology to investigate therapeutic intervention for oxidant-associated disorders.

Therapeutic potential of Nrf2–ARE agonists

The precursor of GSH biosynthesis, NAC, has been most widely used in cellular and animal models as well as clinically to investigate cellular antioxidant capacity in various disease cases. Exogenous administration of NAC significantly rescued lung phenotypes in murine models of ALI, COPD, and allergic asthma, but its action was in general Nrf2-independent, as $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice or only $Nrf2^{-/-}$ mice responded to the NAC treatment (See Table 1). Initial insight into chemical inducers for phase 2 detoxifying enzymes was obtained from studies with butylated hydroxyanisole (BHA), a chemoprotective antioxidant used as a food additive, and its metabolite tert-butylhydroquinone (tBHQ). Subsequently, diverse antioxidant inducers have been characterized, including various electrophiles, pro-oxidants/oxidants, phytochemical antioxidants, or chemoprotectors, and they have been investigated as direct (specific) or indirect (non-specific) Nrf2 agonists in model systems or in clinical settings (Domej et al. 2014; Feitelson et al. 2015; Kumar et al. 2014; Kwak and Kensler 2010). Nrf2 agonists highlighted in current respiratory studies are listed in Table 3.

Epidemiological studies have reported that consumption of cruciferous vegetables (*Cruciferase brassica*) including broccoli and cauliflower decreases cancer risk in lung, prostate, bladder, or breast (London et al. 2000; Talalay and Fahey 2001; Tan and Spivack 2009). Of the powerful phytochemical Nrf2 agonists, isothiocyanates and its special form sulforaphane have received the most attention. Fahey and Talalay (Talalay et al. 1995) initially recognized their role in induction of ARE-bearing phase 2 enzymes when the identity of ARE-binding transcription factors was unclear. Sulforaphane and a number of other isothiocyanates were thought to be an essential strategy for anti-carcinogenesis and anti-mutagenesis (Fahey et al. 1997), and they indeed effectively blocked chemical carcinogenesis in rodents (Gerhauser et al. 1997; Tan and Spivack 2009; Zhang et al. 1994). Metabolism of sulforaphane requires ARE-responsive proteins, including GSTs for conjugation and GSH S-conjugate transporting ATPase (MRP-1) for excretion of the conjugates (Zhang and Callaway 2002). Given as either pure sulforaphane or standardized broccoli sprout extracts (as the precursor glucosinolates), the metabolites including conjugates with cysteinyl-glycine, cysteine, GSH, and NAC, were detected in plasma and all the tissues showing particularly higher accumulation in lung, small intestine, prostate, and kidney, suggesting systemic beneficial effects (Bricker et al. 2014; Clarke et al. 2011). A commercially available composition of five phytochemical NRF2 activators including sulforaphane caused marked and significant induction of disease-related, ARE-bearing genes (e.g., *SLC7a11*, *HO1*) in human cells (Hybertson et al. 2011).

As addressed above, sulforaphane attenuated lung inflammation and increased phagocytosis in Nrf2-dependent manner against bacterial infection following emphysema, RSV infection, and inhaled As (See Table 1). Nrf2 targets such as MARCO are proposed to be associated with the protective activity of sulforaphane. When alveolar macrophages from bronchoscopy of COPD patients were cultured with bacteria (*H. influenza*, *P. aeruginosa*),

sulforaphane treatment decreased plaque forming units (Harvey et al. 2011). Moreover, inhibition of MARCO decreased sulforaphane effects on phagocytosis (Harvey et al. 2011). In a controlled human exposure study ($n = 29$, a pre–post experimental study design), consumption of sulforaphane-containing juice suppressed the number of nasal lavage cells after challenge with DEP (Heber et al. 2014). Kensler and colleagues (Kensler et al. 2013) are conducting a series of clinical intervention trials with broccoli sprouts in a rural China region, where exposure to food- and airborne carcinogens has been considerable. They determined that participants receiving sulforaphane-containing broccoli sprout beverage had rapid and sustained excretion of the toxic adducts with aflatoxin B1, benzene, or acrolein, compared to the placebo group (Egner et al. 2014; Kensler et al. 2013). Results indicate that preparations of broccoli sprouts may enhance detoxification of toxicants. In addition, benzene-derived mercapturic acid was higher in GST-T1-positive participants than participants with GST-T1 null genotype (Egner et al. 2014). Overall, human studies have suggested a therapeutic potential of sulforaphane in reducing adverse effects of the airway toxicants.

CDDO-Im is a synthetic Nrf2-specific agonist, and an Nrf2-dependent protective effect of CDDO-Im was demonstrated in murine models of ALI caused by hyperoxia or sepsis (See Table 1). CDDO-Im also alleviated lung emphysema phenotypes and associated cardiac functional changes caused by chronic cigarette smoke (See Table 1). Oral oltipraz, another Nrf2 agonist, diminished pulmonary hypertension in an Nrf2-dependent manner, while a vitamin E analogue had Nrf2-independent protection against cigarette smoke-induced lung injury (see Table 1). Curcumin, a polyphenol epigallocatechin gallate, resveratrol, and others have also been shown to have pulmonary protection effects experimentally and epidemiologically. Exogenously given prostaglandin 15d-PGJ_2 showed anti-inflammatory effect by exploiting the Nrf2–ARE response in an ALI model (Mochizuki et al. 2005). Furthermore, studies are ongoing to discover novel specific Nrf2 agonists. For example, a traditional herbal medicine from the fruit *Gleditsia sinensis* suppressed sepsis-induced lung inflammation and injury in $Nrf2^{+/+}$ mice but not $Nrf2^{-/-}$ mice (Kim et al. 2014), and similar Nrf2-dependent pulmonary protection was observed in As toxicity by another herbal medicine component, tanshinone I (Tao et al. 2013). Additional investigations on Nrf2 agonists should yield greater insight to alternative treatment strategies to protect against oxidative lung diseases.

Conclusion

Extensive research during the last decade or more has demonstrated that Nrf2 has essential roles in airways against various oxidative environmental toxicants and pollutants, medicinal agents, allergens, and pathogens. Further research on NRF2 in pulmonary biology may fall into several major scopes of application including lung carcinogenesis, energy metabolism, and intervention strategies for critical disorders. Accumulating evidence in lung cancer indicates that 'gain of function' *NRF2* mutations may be considered as predictive markers for poor responsiveness to chemotherapy and radiation therapy. The Nrf2–ARE pathway has also been linked to nutrient intake, energy metabolism and mitochondrial biogenesis, and redox balance (Itoh et al. 2015; Vomhof-Dekrey and Picklo 2012), and may have important implications for understanding lung responses to environmental stimuli. Additional discoveries of Nrf2-specific, potent agonists as preventive medicines, from phytochemicals or synthetics, may be especially beneficial for RSV disease, COPD, and asthma in which Nrf2 contributes to the pathogenesis but no efficient therapies are currently available. In association with the acquired knowledge and understanding of NRF2-mediated molecular and cellular events from in vitro, in vivo, and clinical studies, continued investigation of scenarios in which Nrf2 orchestrates host defense mechanisms will provide better insights into the intervention and prevention strategies against critical airway disorders.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

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