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Nrf2 protects against airway disorders

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ABSTRACT

Nuclear factor-erythroid 2 related factor 2 (Nrf2) is a ubiquitous master transcription factor that regulates antioxidant response elements (AREs)-mediated expression of antioxidant enzyme and cytoprotective proteins. In the unstressed condition, Kelch-like ECH-associated protein 1 (Keap1) suppresses cellular Nrf2 in cytoplasm and drives its proteasomal degradation. Nrf2 can be activated by diverse stimuli including oxidants, pro-oxidants, antioxidants, and chemopreventive agents. Nrf2 induces cellular rescue pathways against oxidative injury, abnormal inflammatory and immune responses, apoptosis, and carcinogenesis. Application of *Nrf2* germ-line mutant mice has identified an extensive range of protective roles for Nrf2 in experimental models of human disorders in the liver, gastrointestinal tract, airway, kidney, brain, circulation, and immune or nerve system. In the lung, lack of *Nrf2* exacerbated toxicity caused by multiple oxidative insults including supplemental respiratory therapy (e.g., hyperoxia, mechanical ventilation), cigarette smoke, allergen, virus, bacterial endotoxin and other inflammatory agents (e.g., carrageenin), environmental pollution (e.g., particles), and a fibrotic agent bleomycin. Microarray analyses and bioinformatic studies elucidated functional AREs and Nrf2-directed genes that are critical components of signaling mechanisms in pulmonary protection by Nrf2. Association of loss of function with promoter polymorphisms in *NRF2* or somatic and epigenetic mutations in *KEAP1* and *NRF2* has been found in cohorts of patients with acute lung injury/acute respiratory distress syndrome or lung cancer, which further supports the role for NRF2 in these lung diseases. In the current review, we address the role of Nrf2 in airways based on emerging evidence from experimental oxidative disease models and human studies.

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Abbreviations: ALI, acute lung injury; ARACNE, Algorithm for the Reconstruction of Accurate Cellular Networks; ARDS, acute respiratory distress syndrome; ARE, antioxidant response element; ATF, activating transcription factor; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; BPD, bronchopulmonary dysplasia; CDDO, 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole; CNC-bZIP, Cap'n'collar-basic region leucine zipper; COPD, chronic obstructive pulmonary disease; CBP, CREB binding protein; CLR, context likelihood of relatedness; Cul3, culin-3; DC, dendritic cell; DEP, diesel exhaust particle; 15d-PGJ_2, 15-deoxy-delta (12,14)-prostaglandin J2; D3T, 1,2-dithiole-3-thiones; EFL, epithelial lining fluid; ERK, extracellular signal-regulated kinase; FTH, ferritin heavy chain; FTL, ferritin light chain; GCLc, gamma glutamyl cysteine ligase, catalytic subunit; GCLm, gamma glutamyl cysteine ligase, modifier subunit; GGT, gamma glutamyltranspeptidase; G6PD, glutathione-6-phosphate dehydrogenase; GPx, glutathione peroxidase; GS, glutathione synthetase; GSH, glutathione; GST, glutathione-S-transferase; HO-1, heme oxygenase-1; H_2O_2, hydrogen peroxide; *Hsl1*, hyperoxia susceptibility locus 1; HVt, high tidal volume; IPF, idiopathic pulmonary fibrosis; Keap1, kelch-like ECH-associated protein 1; LPS, lipopolysaccharide; LVt, low tidal volume; MMP, matrix metalloproteinase; MV, mechanical ventilation; NAC, N-acetylcysteine; NF-E2, nuclear factor-erythroid 2; NQO1, NADP(H):quinone oxidoreductase 1; Nrf2, nuclear factor-erythroid 2 related factor 2; $O_2^{\bullet-}$, superoxide anion; $OH^{\bullet}$, hydroxyl radical; PK-C, protein kinase-C; PM, particulate matter; Prx, thioredoxin peroxidase; PWM, position weight matrix; Rbx1, ring box 1; ROS, reactive oxygen species; RSV, respiratory syncytial virus; RXR, retinoic acid X receptor; SNP, single nucleotide polymorphism; SOD, superoxide dismutase; TFBS, traditional transcription factor binding sites; TGF-β, transforming growth factor-beta; Trx, thioredoxin; TXNRD, thioredoxin reductase; UGT, UDP-glucuronyl transferase; VILI, ventilator-induced lung injury; XRE, xenobiotic response element.

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Introduction

Oxidative stress and antioxidant defense

Molecular oxygen (O_2) and its homeostasis are essential for the survival of all aerobic organisms. Under normal physiological conditions, partially reduced O_2 metabolites including hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{-\bullet}$), and hydroxyl radical ($OH^{\bullet}$) are generated as metabolic by-products. These reactive oxygen species (ROS) have the potential to damage cellular DNA, lipids, or proteins, to generate inorganic or organic peroxidation products. ROS are also known to activate signal transduction pathways through receptor protein tyrosine kinase and protein tyrosine phosphatase (Denu and Tanner, 1998; Uings and Farrow, 2000). Endogenous antioxidant systems cope with the oxidative burden and limit potential toxicity of ROS. Excess ROS, however, overwhelm antioxidant capacity to perturb the balance in this reduction-oxidation (redox) equilibrium, and eventually lead to oxidative stress of cells and tissues. Phagocytic cells (e.g., neutrophils, macrophages) and a variety of non-phagocytic cells can produce ROS via NADPH oxidase, a membrane-bound enzyme complex, or other oxidases including cytochrome P450 and lipoxygenase (Terui et al., 2000; Thannickal and Fanburg, 2000). Over the last two decades, investigations have associated increased oxidative stress with pathogenesis of various human diseases including cancer, atherosclerosis, ischemia–reperfusion injury, neurodegenerative disorders, and the aging process.

The endogenous cellular antioxidant-defense system consists of a number of proteins or peptides (e.g., enzymes) and small molecules (e.g., vitamins C and E) that maintain the reducing environment of the body. Among these, classical antioxidant enzymes including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) directly inactivate ROS and prevent ROS-initiated reactions. In addition, two biologically important small thiol-containing compounds, glutathione (GSH) and thioredoxin (Trx) participate in antioxidant defense by serving as substrates for antioxidant enzymes such as GPx and Trx peroxidase in redox cycles. The ratio of GSH to GSH disulfide (2GSH:GSSG) has served as a parameter of cellular redox status. Trx is located in the inner mitochondrial membrane where it scavenges ROS, and is also known to activate mitochondrial antioxidants such as SOD2 (Hirota et al., 2002). Overexpression of Trx or exogenous administration of Trx increased protection against oxidative stress and inflammation (Yamamoto et al., 2003; Liu et al., 2004c). Phase 2 detoxifying enzymes contribute to biosynthesis/recycling of thiols or facilitate excretion of oxidized, reactive secondary metabolites (e.g., quinones, epoxides, aldehydes, peroxides) through reduction/conjugation reactions during xenobiotic detoxification. They are represented by glutathione-S-transferase (GST) isozymes and NADP(H):quinone oxidoreductase (NQO1). In addition, stress response proteins such as heme oxygenase (HO)-1 and heavy (FTH) and light (FTL) chains of ferritin are cytoprotective against various oxidant or pro-oxidant insults (Otterbein et al., 1999; Thompson et al., 2003). HO-1 contributes to degradation of the pro-oxidant heme molecule, which also generates the antioxidative products, carbon monoxide and bilirubin, with release of iron (II). Ferritin is inducible by iron (II) and exerts its antioxidant function by sequestering iron from participation in free radical formation.

Keap1 and Nrf2

Nrf2 belongs to the cap'n'collar (CNC)-basic region/leucine zipper (bZIP) transcription factor family. Nrf2 expression is relatively abundant in tissues such as the intestine, lung, and kidney where detoxification reactions occur routinely (Itoh et al., 1997). Nrf2 is critical in cytoprotection by induction of antioxidant and detoxifying enzymes and proteins via its binding to the *cis*-acting antioxidant response element (ARE). Keap1 is a cytoplasmic, cysteine-rich, actin-bound protein that represses Nrf2 in many species including mouse, human, and zebrafish (Itoh et al., 1999; Kobayashi et al., 2002); the rat homologue is termed INrf2 (Dhakshinamoorthy and Jaiswal, 2001). Nrf2 is known to be activated by phosphorylational modification via several protein kinase pathways leading to Keap1·Nrf2 dissociation, nuclear Nrf2 translocation, and ARE responsiveness. Recent investigation has focused on the regulatory mechanisms of Keap1-dependent turnover and translocation of Nrf2. Keap1 sequesters Nrf2 in the cytoplasm by binding to the N-terminal Neh2 domain, and thus prevents nuclear accumulation under normal cellular conditions. This role of Keap1 has been ascertained by investigations of *Keap1*$^{-/-}$ mice in which constitutive transactivation of Nrf2 and overproduction of its target genes lead to esophagus and forestomach hyperkeratosis (Wakabayashi et al., 2003). *KEAP1* silencing in non-transformed human keratinocytes showed similar pre-adaptation responses by marked antioxidant inductions without redox stimuli (Devling et al., 2005). *Nrf2–Keap1* double mutant mice reverse the phenotypes and rescue the *Keap1*$^{-/-}$ mice from lethality, which further confirms negative regulation of Nrf2 directly by Keap1 (Wakabayashi et al., 2003). The Kelch repeat domain (Kelch1–Kelch6) of Keap1 binds directly to Nrf2 and actin filaments for cytoplasmic sequestration of Nrf2 (Itoh et al., 2004; Kang et al., 2004). The N-terminal BTB/POZ domain of Keap1 may mediate dimerization of Keap1 (Zipper and Mulcahy, 2002). In addition, cysteine residues of Keap1 (e.g., C259, C273, C288, C297) in the intervening region between BTB and Kelch repeat domains are known to have a role in Keap1·Nrf2 complex formation through the Neh2 domain (ETGE and DLG motifs) of Nrf2 (Dinkova-Kostova et al., 2002; Kobayashi et al., 2002; Zhang and Hannink, 2003; Katoh et al., 2005; McMahon et al., 2006). Investigators have also proposed Nrf2 stabilization by Keap1; Keap1 may bind to culin-3 (Cul3)-ring box 1 (Rbx1) to form core E3 ubiquitin ligase complex, which renders Nrf2 ubiquitination for targeting of proteasomal degradation (McMahon et al., 2003; Furukawa and Xiong, 2005). Keap1 also contributes to cytoplasmic-nuclear shuttling of Nrf2 for repression after ARE transactivation (Itoh et al., 2003). Moreover, it is suggested that disruption of actin cytoskeleton is required to dysregulate Keap1 for nuclear accumulation of Nrf2 (Kang et al.,