The Relationship Between Oxidative Stress Responses and Lung Inflammation with Cigarette Smoking

Changcheng Song and Steven G. Kelsen

Introduction

Cigarette smoking represents a major world health hazard. In fact, chronic cigarette smoking is the leading risk factor for the development of chronic obstructive pulmonary disease (COPD), the world's third leading cause of death and accounts for 90 % of lung cancers [1, 2]. Cigarette smoking produces adverse respiratory effects by exposing the airways and lung parenchyma to a variety of reactive oxygen species (ROS) and other toxic compounds. Although the molecular mechanisms underlying lung and airway damage in response to cigarette smoke remain incompletely understood, ROS are believed to produce tissue injury by affecting the function and gene expression profiles of lung structural cells and inflammatory cells. In fact, cigarette smoke exposure alters the expression of >600 genes in human monocytes [3]. Specifically, ROS exert direct deleterious effects on cell structure and function by damaging protein, lipid and DNA macromolecules which impair cellular function, induce apoptosis, and stimulate dysfunctional matrix remodeling in the lung and in the respiratory tract. Furthermore, cigarette smoke-induced cell damage causes the release of

C. Song, Ph.D.
Division of Pulmonary and Critical Care Medicine and Center for Inflammation and Translational Lung Research (CILR), Departments of Medicine, Temple University School of Medicine, 3500 North Broad Street, Philadelphia, PA 19140, USA

S.G. Kelsen, M.D. (✉)
Division of Pulmonary and Critical Care Medicine and Center for Inflammation and Translational Lung Research (CILR), Departments of Medicine, Temple University School of Medicine, 3500 North Broad Street, Philadelphia, PA 19140, USA

Temple University Hospital, Room 761 Parkinson Pavilion, 3401 North Broad Street, Philadelphia, PA 19140, USA
e-mail: kelsen@temple.edu; Steven.Kelsen@tuhs.temple.edu

T.J. Rogers et al. (eds.), *Smoking and Lung Inflammation: Basic, Pre-Clinical and Clinical Research Advances*, DOI 10.1007/978-1-4614-7351-0_5,

alarmins, cytokines, chemokines and up-regulation of adhesion molecules by epithelial cells in the airway and lung which collectively serve to attract an inflammatory cell infiltrate. In addition, bacterial constituents present in cigarette smoke further shape the intensity and inflammatory response by activating PAMPs [pathogen associated molecular patterns] expressed by lung cells which, in turn, interact with the cell's oxidant defense mechanisms [4, 5]. Moreover, the inflammatory process generated by the innate immune system, in turn, increases oxidant stress in the lung through the production of the superoxide ion by infiltrating neutrophils. Accordingly, the inflammatory reaction in the lung induced by oxidants in cigarette smoke has the potential to act as a positive feedback loop or self-amplifying process which exacerbates both conditions.

This chapter will describe the composition of cigarette smoke and the mechanism by which its major constituents induce oxidant stress imposed in the respiratory tract. Furthermore, we will discuss the molecular mechanisms in the lung which deal with oxidant stress. Finally, we will discuss the manner in which antioxidant defense mechanisms in the respiratory tract interact with inflammatory signaling pathways to shape the intensity and nature of both responses to cigarette smoke.

Cigarette Smoke Composition

Cigarette smoke contains more than 4,700 separate compounds many of which are highly toxic and xenobiotic materials [6–9]. In particular, cigarette smoke contains a variety of aromatic and non-aromatic hydrocarbons (dioxin, benzopyrene); alpha and beta aldehydes (acrolein); heavy metals (cadmium, zinc, iron); toxic gases (nitrogen dioxide, nitric oxide, carbon monoxide); and bacterial-derived substances (lipopolysaccharides [LPS]) which induce important biological effects on the innate and adaptive immune systems.

The effects of many of these compounds on oxidant defenses and the immune system have been well characterized (see below). Of considerable importance, the effects of individual compounds of smoke produce effects which in some cases are opposite in sign to those produced by cigarette smoke per se suggesting that the effects of cigarette smoke cannot be predicted from the study of its individual components but are best assessed using cigarette smoke itself.

Reactive Oxygen Species (ROS)

Smoke from a burning cigarette contains approximately 10^{15} ROS per puff [6]. It has been estimated that the concentration of total reactive oxygen species contained in the average cigarette ranges from 16 to 55 nmol H_2O_2/L [10]. Chemical studies of cigarette smoke performed in the 1980s and 1990s have characterized the nature of the ROS generated by both the particulate phase which is retained on a filter (termed cigarette "tar") and the gas or vapor phase which passes through the filter. In fact,

the differing materials in these two phases of cigarette smoke exert different biological effects. The majority of the toxic chemicals generated in the particulate phase are semiquinones, phenol, catechol and nicotine [11]. The tar phase also retains several heavy metal ions including cadmium, nickel, and zinc. The major chemicals in gaseous phase are carbon monoxide, nitrogen oxides, ammonia, formaldehyde, acrolein (aldehyde), toluene, benzene, hydrogen cyanide, and the short-chained amides, acrylamide, acetamide [11, 12].

Cigarette smokers deposit up to 20 mg of tar in their lungs per cigarette smoked and the levels of ROS in cigarette smoke, which correlate closely with the level of tar phase materials [10]. Semiquinones cause sustained production of superoxide ($.O_2-$), hydroxyl radical (OH.), singlet oxygen ($1O_2$) and hydrogen peroxide (H_2O_2) [9]. In the aqueous phase of the surface lining fluid of the respiratory tract, superoxide radicals react quickly to oxidize proteins, lipids or nucleic acid macromolecules. Superoxide also can be enzymatically dismuted to form the more stable oxidant, hydrogen peroxide, by superoxide dismutase. Hydrogen peroxide can also form the very highly reactive hydroxy radical via the Fenton reaction via the iron present in cellular fluids and cigarette tar.

Although free radicals in the gas phase are short-lived and affect primarily the upper respiratory tract, radical concentrations are maintained at high levels in gas phase cigarette smoke for more than 10 min and seem to increase in concentration as smoke ages. In fact, radicals are continuously formed and destroyed. Based on nitrogen oxide chemistry, nitric oxide is slowly oxidized to form the more reactive nitrogen dioxide which reacts with unsaturated compounds such as isoprene to form carbon radicals which then rapidly react with oxygen to form peroxyl radicals. These in turn react with nitric oxide to produce more nitrogen dioxide. In general, the gas phase is believed to be less harmful to the lung than the tar phase [13].

CS also promotes the generation of ROS and reactive nitrogen species (RNS) in resident lung structural and inflammatory cells by activation of endogenous NADPH oxidase (NOX). NOX isoforms transport electrons from cytoplasmic high energy electron donor NADPH to generate O_2^- and hydrogen peroxide (H_2O_2) [14]. Of the family of NADPH oxidase (NOX) isoforms, NOX1, 2, 4, 5 and Duox 1 and 2 are expressed in lung epithelial and other cell types [14–19]. Moreover, NADPH oxidase (NOX) isoforms are activated by cigarette smoke in a variety of cell types including alveolar macrophages, airway smooth muscle and pulmonary artery endothelial cells and are believed to contribute importantly to oxidant stress in the lung [14, 20, 21]. LPS in cigarette smoke acting on TLR4 [21] and cytokines secreted from airway and alveolar epithelial cells and inflammatory cells in the lung such as TNF–α and IL-1β, activate NADPH oxidase. Of considerable importance, NADPH oxidase-derived ROS can induce mitochondrial ROS production indicating the possibility of a positive feedback loop [22, 23]. Of interest, tar- and nicotine-free cigarette smoke is capable of activating NADPH oxidase in PKC-dependent fashion indicating that gaseous phase ROS in addition to cigarette tar may contribute to NADPH-induced oxidant stress in some cell types [24]. In fact, acrolein, a gas phase constituent of cigarette smoke (see below), activates NADPH oxidase and superoxide production in human pulmonary endothelial cells [25].

An additional mechanism can contribute to endogenous ROS production in the lung. Nitric oxide (NO) in cigarette smoke can be transformed to NO by nitric oxide synthase which is expressed by a variety of respiratory cell types. In turn, ·NO can be oxidized to the more potent peroxynitrite (ONOO-) by superoxide anion. NO synthase expression can be up-regulated by TNF–α and IL-1β.

Acrolein

Acrolein, a highly water-soluble gas which is highly irritating to eyes, nose and respiratory passages, is deposited mainly in the aqueous lining fluid of the lower respiratory tract when cigarette smoke is inhaled. Acrolein contains a highly reactive alpha carbon which forms carbonylated macromolecules and depletes reduced glutathione and antioxidants in the respiratory tract [26–29]. As such acrolein and other α, β unsaturated aldehydes are major contributors to the oxidative damage induced by gas phase cigarette smoke [30, 31].

Of interest, the concentration of acrolein in sidestream smoke is actually 17-fold greater than in mainstream smoke due to altered combustion chemistry and lower temperatures [32, 33]. Acrolein is also formed endogenously in the lung during inflammation via three separate pathways: (1) by myeloperoxidase oxidation of the amino acid, threonine; by the oxidation of membrane fatty acids; and by thiamine oxidase mediated catabolism of the polyamines, spermine and spermidine. In fact, acrolein concentrations in expired breath condensate and in induced sputum are higher in smokers and ex-smoking subjects with COPD than in healthy nonsmokers [34, 35].

Acrolein induces a variety of deleterious effects on the respiratory tract which mimic the effects of chronic cigarette smoke exposure. On balance, acrolein has a proinflammatory effect but its effects on individual components of the inflammatory response are complex. In rodents, chronic exposure to acrolein increases infiltration of macrophages, neutrophils, and CD8+ T cell into the lungs [36]. Moreover, acrolein exposure increases IFN-γ, IP-10 (CXCL10), IL-12, MCP-1 (CCL2), RANTES (CCL5) and metalloproteinase (MMP)-12 in BAL [36]. Of interest, macrophage accumulation, production of these cytokines and increased MMP12 in response to acrolein does not occur in CD8 deficient mice suggesting that the pro-inflammatory effect of acrolein is initiated by effects on CD8 cells. Acrolein inhibits neutrophil apoptosis [36] but augments alveolar macrophage apoptosis [37]. Acrolein also has novel pro-inflammatory effects by generating chemoattractant peptides from breakdown of lung connective tissue. For example, acrolein acetylates the proline-glycine-protein (PGP) tri-peptide degradation product of collagen breakdown rendering it resistant to breakdown by the aminopeptidase activity of LTA4 hydroxylase [38]. PGP is chemoattractive for neutrophils by acting as a ligand for CXCL1 and CXCL2 [38]. Acrolein does not affect the hydrolyase activity of LTA4 hydroxylase which converts LTA4 to the powerful neutrophil chemoattractant, LTB4 [38].

Acrolein also has direct effects on the master inflammatory gene transcription factor, NF-κB which appear to be anti-inflammatory [39]. For example, acrolein induces alkalization of the p50 subunit of NF-κB which inhibits its

binding to NF-κB consensus DNA sequences. This effect appears to account for acrolein-mediated inhibition of cytokine gene expression induced by endotoxin and TNF-α treatment [40].

Acrolein also has complex effects on lung oxidant defense. It irreversibly inhibits important antioxidant proteins including thioredoxin reductase, thioredoxin 1 and thioredoxin 2 but increases heme oxygenase-1 (HO-1) expression in human pneumocytes [41, 42].

Cadmium

Cadmium (Cd^{2+}) is abundant in tobacco and, depending on tobacco growing conditions, may be present in μg amounts in each cigarette [43, 44]. In general, the higher binding affinity of heavy metal ions leads to replacement of physiological divalent ions like Zn^{2+} in native proteins thereby altering their structure and function [45, 46]. Cd^{2+} is highly toxic to the lung when inhaled as a vapor or fume or directly instilled [47–50]. In human subjects [51–53] and animal models [54–56], acute exposure induces lung inflammation; chronic exposure induces centrilobular emphysema [47, 48, 57] and pulmonary fibrosis [58]. Cd^{2+} is retained in the body for long periods with a half-life of >10 years [59]. Cd^{2+} binding to metallothioneins in lung cells mitigates its toxicity [60, 61].

It has been suggested that Cd^{2+} present in cigarettes contributes to the development of lung dysfunction and COPD in chronic smokers [62, 63]. In fact, Cd^{2+} concentrations are greater in the emphysematous lung (fourfold) compared to smokers without emphysema and never smokers [64] and in alveolar macrophages from smokers compared to non-smokers [65]. Epidemiological studies indicate that urinary Cd^{2+} levels are greater in smokers and ex-smokers than in never smokers [63, 66]. Moreover, FEV1 and FVC are inversely related urinary Cd^{2+} levels and correlate better with Cd^{2+} concentrations than pack years of smoking [63]. Studies of the effects of Cd^{2+} in cultured pneumocytes, airway epithelial cells and lung fibroblasts indicate that Cd^{2+} causes protein misfolding and may induce an unfolded protein response (UPR) response [see below] [67]. Specifically, Cd^{2+} induces heat shock 70 chaperone expression in rat pneumocytes and human airway epithelial cells [68, 69]. Moreover, Cd^{2+} dose-dependently decreases total protein synthesis in rat type II pneumocytes and a human pneumocyte cell line and procollagen and proteoglycan mRNA and protein expression in human lung fibroblasts [69].

Polycyclic Aromatic Hydrocarbons

Cigarette smoke contains a variety of biologically active, poly-aromatic hydrocarbons which act as ligands for an endogenous receptor, the aryl hydrocarbon receptor (AhR). The AhR is a member of the basic helix-loop-helix family of transcription factors which mediates the biologic and toxic effects of its xenobiotic ligands [70].

The AhR induces expression of a variety of genes including phase I and II enzymes which detoxify toxins contained in cigarette smoke, cytochrome P450 and other monooxygenase activities [71]. The AhR also regulates cell apoptosis and transition through the cell cycle [71].

When bound to its polycyclic aromatic hydrocarbon ligands such as dioxin (tetrachloro-dibenzo-dioxin [TCDD]) or benzopyrene, the AhR translocates from the cytoplasm to the nucleus, heterodimerizes with the AhR nuclear translocator (ARNT) and activates transcription through the xenobiotic response element (XRE). The XRE consists of a canonic motif of 5′-TNGCGTG-3′. After nuclear export, the AhR is degraded via the proteasome. Of interest, the lung contains the highest concentration of AhRs of any other organ in the body [72–74]. In fact, secretory proteins such as surfactant protein A (SP-A) and clara cell secretory protein (CC10) are highly regulated by the AhR [75].

TCDD the best characterized AHR ligand induces a variety of effects in cultured respiratory cells and the lungs of rodents. For example, TCDD induces expression of MUC5AC, COX-2, IL-1β and MCP-1 mRNA in cultured clara cells [75]. When injected intraperitoneally, TCDD produced similar effects in the whole lung lysate of mice along with increases in TNF-α and reductions in SP-A mRNA. Of interest, the results obtained using AhR knock-out mice exposed to cigarette smoke suggest that the AhR exerts an anti-inflammatory effect. For example, $AhR^{-/-}$ mice demonstrate increased numbers of total cells, neutrophils and lymphocytes in BAL in response to acute cigarette smoke inhalation [76]. Similar results were obtained with LPS treatment, which does not contain AhR ligands. Moreover, $AhR^{-/-}$ fibroblasts demonstrate heightened COX-2 expression and prostaglandin production in response to cigarette smoke which could be rescued by transient expression of AhR [77]. These data suggest that AhR deficient animals are inflammation prone and that the inflammatory responses to TCDD may be a manifest of non-AhR mediated effects.

Nicotine

Nicotine is present in milligram amounts in cigarette tar and, like acetylcholine, is a cholinergic agonist that binds to and activates nicotinic acetylcholine receptors [78]. In cell types which express the α7 nicotinic acetylcholine receptor, nicotine exerts diverse, cell type specific effects on immune cells [79].

In general, nicotine induces anti-inflammatory effects in vivo [79–82]. For example, nicotine inhibits LPS-induced elevation of serum TNF-α in mice, an effect which is eliminated in α7 knockout mice [79]. Similar effects were produced in alveolar macrophages in vitro [79]. Nicotine treatment also reduced LPS-induced leukocyte infiltration and myeloperoxidase activity in the mouse lungs, and reduced lung MIP-1a, MIP-2, eotaxin, IL-1, IL-6, and TNF-α [81]. In contrast, nicotine augments dendritic cell capacity to stimulate T-cell proliferation and release TH1 cytokines like interleukin-12 by increasing expression of the co-stimulatory molecules, CD86, CD40, MHC class II receptor, and the adhesion molecules, LFA-1 and

CD54 [83]. These latter results suggest that in contrast to its effects on innate immune responses activated by toll-like receptor stimulation, nicotine may augment adaptive immune responses. The mechanisms by which nicotine exerts these cell type specific effects and the signaling processes involved are unknown.

Antioxidant Defense Systems Involved in the Response to Cigarette Smoke

The respiratory system contains a variety of non-enzymatic and enzymatic antioxidant systems which protect against the injurious effects of oxidants. These systems function to affect electron transfer, enzymatically degrade the chemical compound, as well as scavenge and sequester transition metal ions. The non-enzymatic system scavenges free radicals via electron transfer to electrophilic thiol or carbon groups. This system is comprised of low molecular weight molecules and proteins including glutathione, thioredoxin, peroxiredoxin, α-tocopherol (vitamin E), uric acid, and vitamin C in the extracellular compartment of the lung. Cigarette smoke alters the concentrations of anti-oxidants in lung and other tissues [6, 30, 84 and reviewed by 85]. For example, smokers have lower concentrations of vitamin C in their blood plasma and vitamin E in lung lavage than nonsmokers [86–88].

The enzymatic system comprises the superoxide dismutase (SOD) family, catalase, glutathione peroxidase and heme oxygenase-1 and is also altered by chronic cigarette smoke exposure. SOD which transforms superoxide into hydrogen peroxide includes copper/zinc SOD (Cu^{2+}/Zn^{2+})-SOD in the cytoplasm, manganese (Mn^{2+})-SOD in mitochondria, and extracellular SOD in the interstitial space of the lung [89, 90]. Catalase and glutathione peroxidase catalyze hydrogen peroxide to oxygen and water. Catalase is primarily located in peroxisomes, and glutathione peroxidase is distributed in cytoplasm and extra-cellularly [91]. Heme oxygenase-1 (HO-1) inactivates redox generating heme groups by converting heme to biliverdin-IXα, carbon monoxide (CO) and iron in the presence of O_2 and an electron donor, NADPH/cytochrome p450 reductase [92]. Heme cleavage by HO-1 prevents hydroxyl radical formation through iron. In fact, the importance of the enzymatic systems involved in anti-oxidant stress in the prevention of cigarette smoke induced lung inflammation and injury has been demonstrated repeatedly. The expression of SOD, catalase, glutathione peroxidase, and HO-1 is inducible by chronic oxidative stress including cigarette smoke exposure [93–95] and proinflammatory cytokines [96, 97]. Finally, the phase I enzymes which decarbonylate endogenous proteins and detoxify reactive aldehydes such as acetaldehyde and acrolein and other xenobiotics, such as aldehyde dehydrogenase, aldo-keto reductase, and NQO1 reductase, are also up-regulated in response to chronic cigarette smoke exposure [98]. Up-regulation of these enzymes in response to cigarette smoke-induced oxidant stress is largely accomplished via the transcriptional activity of the master antioxidant transcription factor, Nrf2 (nuclear factor-erythroid 2-related factor-2) [99].

The importance of the enzymatic anti-oxidant enzymes in protecting the respiratory system from cigarette smoke-induced injury has been demonstrated repeatedly. For example, exogenous expression of Cu^{2+}-Zn^{2+} – SOD protects CS, elatase and ceramide-induced emphysema in mice [100, 101]. Conditional knockout of extracellular SOD results in the elevation of lung superoxide levels, infiltration of inflammatory cells, and histological changes similar to those observed in adult respiratory distress syndrome [102]. In contrast, increased expression of extracellular SOD attenuates CS-mediated lung inflammation and emphysema in mice [103]. Administration of the SOD mimetic (i.e., MnTBAP) and intranasal administration of SOD-containing microparticles which act to increase lung superoxide levels, reduces mortality and prevents histological alterations [102].

The relevance of antioxidant enzymes in the prevention of cigarette smoke-induced chronic obstructive pulmonary disease in man is strongly suggested by epidemiologic data as well as observations that levels of antioxidant enzymes such as HO-1 are reduced in COPD [104]. Moreover, COPD patients demonstrate a high frequency of mutation of several genes of antioxidant enzymes such as extracellular SOD [105, 106], glutathione S-transferase M1 (GSTM1), GSTT1, GSTP1 and glutamate cysteine ligase (GCL) [107–112].

Nrf2/Keap

Nrf2, a transcription factor that mediates a broad-based set of adaptive responses to intrinsic and extrinsic cellular stresses, regulates expression of enzymes that inactivate oxidants; increase NADPH synthesis; and enhance toxin degradation and export [113, 114]. Nrf2 also enhances the recognition, repair and removal of damaged proteins; augments nucleotide repair; regulates expression of other transcription factors, growth factors, receptors and molecular chaperones; and inhibits cytokine–mediated inflammation [113, 114]. Of particular interest in the setting of cigarette smoke exposure, Nrf2 binds to anti-oxidant response elements in the promoter region of a variety of genes coding for important anti-oxidant enzymes (e.g., heme oxygenase-1 [HO-1], glutathione-S-transferase [GST], glutathione peroxidase [GP], superoxide dismutase [SOD], etc.) [99, 115–117]. In fact, Nrf2 regulates two major redox systems, the glutathione and thioredoxin systems, by promoting expression of enzymes involved in glutathione synthesis, transfer and reduction and thiodoxin synthesis and reduction [99, 115–117]. In addition, Nrf2 regulates several glutathione-dependent (e.g., UDP-glucuronosyl transferase) and glutathione independent enzymes (e.g., NAD(P)H:quinone oxidoreductase1[NQO1]), which are important in the detoxification of tobacco smoke products [116, 117].

Nrf2 and its actin-tethered redox-sensitive inhibitor, Keap1 (Kelch like-ECH-associated protein 1), are widely expressed in bodily tissues [118]. When present in the cytoplasm attached Keap1, Nrf2 has a short half-life as a result of its susceptibility to ubiquitination and proteasomal degradation [118]. Oxidation of Keap1 allows

Nrf2 to dissociate and migrate to the nucleus where it binds to a specific DNA consensus sequence found in the antioxidant response element [5′- NTGAG/CNNNGC-3′] [119]. Nrf2 activity is also regulated by the cytosolic protein, DJ-1, and the nuclear protein, Bach1 [120]. DJ-1 enhances Nrf2 expression by preventing its degradation by the proteasome thereby acting to stabilize Nrf2 in the cytoplasm [121]. The transcriptional inhibitor, Bach-1, on the other hand, inhibits Nrf2 transcriptional activity [122] by competing with Nrf2 for available transcriptional co-factors in the nucleus such as Maf K [119, 120, 123]

Of importance, post-translational modifications of Nrf2 (i.e., phosphorylation and acetylation) affect its functional activity in terms of binding to its inhibitors, its nuclear import and export, and its DNA binding affinity and transcriptional activity [124–127]. For example, phosphorylation of Nrf2 facilitates its dissociation from KEAP1 and its translocation to the nucleus [124–126]. Kinases which phosphorylate Nrf2 include PKC, PI3K and PERK [PKR-like ER resident kinase] [124–127]. At present, however, the Nrf2 phosphorylation sites targeted by kinases which may be activated by cigarette smoke e.g., PERK, PKC, PI3K, etc. and their functional consequences are completely unstudied.

Nrf2 is also acetylated by histone acetyltransferase (HAT) and deacetylated by histone deacetylase (HDAC) 2 [128]. Acetylation of Nrf2 diminishes its transcriptional activity and enhances its export from the nucleus [128]. Accordingly, increases in the level of acetylated Nrf2 are associated with decreases in Nrf2 activity. Specifically, reductions in HDAC2 expression or activity which occur in the setting of cigarette smoke exposure, reduce Nrf2-regulated HO-1 expression and increase sensitivity to oxidative stress in BEAS2B cells and mice [128]. Moreover, HDAC2 knock-down by RNA interference reduces H_2O_2-induced Nrf2 protein stability and activity in cells.

Nrf2 also interacts with the NF-κB family of transcription factors which regulate the innate and adaptive inflammatory response and cell apoptosis (see below). For example, Nrf2 knockout mice demonstrate increased NF-κB activity after treatment with TNF-α, LPS and respiratory syncytial virus [129, 130]. Nrf2 attenuates IκB phosphorylation and increases IKK activity in response to TNF-α or LPS [130]. It is not clear if greater expression of NF-κB and its targets in Nrf2 deficient animals is a result of diminished ability to scavange ROS or to a direct interaction between the two transcription factors.

Direct evidence of the importance of Nrf2 in the pathogenesis of cigarette smoke-induced lung inflammation and emphysema has been provided in animal models and in cultured lung cells and human subjects [99, 131]. For example, Nrf2 knockout mice are more susceptible to CS-induced emphysema and inflammation while transcriptional induction of Nrf2 by CDDO (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oicacid) reduces oxidative stress and alveolar destruction in wild-type mouse but not in Nrf2 knockout mice [15, 99, 132, 133]. Mice deficient in Nrf2 demonstrate increased numbers of macrophages in BAL and lung tissue following cigarette smoke exposure [119]. Nrf2 knockout mice demonstrate increased lung infiltration with macrophages, lymphocytes, eosinophils, and neutrophils after

ovalbumin inhalation [134]. Moreover, type II pneumocytes from Nrf2 knockout mice demonstrate impaired growth and increased sensitivity to oxidant-induced cell death [99, 131]. In addition, deletion of KEAP1 in Clara cells in the airways of mice attenuates CS-induced inflammation and oxidative stress [135]. On the other hand, knockdown of DJ-1 in mouse lungs, mouse embryonic fibroblasts and human airway epithelial cells (BEAS2B) impairs antioxidant induction in response to CS [133]. Of considerable interest, expression of Nrf2 and several Nrf2-regulated antioxidant enzymes e.g. NQO1, HO-1 and glutamate cysteine ligase modifier subunit, is reduced in subjects with advanced COPD [133]. The potential importance of Nrf2 in cigarette smoke-induced lung inflammation and tissue injury have recently prompted trials of substances which increase anti-oxidant gene expression in respiratory cells in subjects with COPD. For example, sulforaphane, a derivative of broccoli sprouts, and resveratrol, a polyphemolic phytoalexin in grapes, regulate Nrf2 expression [136, 137].

Heat Shock Proteins (HSP)

The HSP family of proteins (e.g., HSP27, 60, 70, 90 and 100) participate in protein homeostasis in the cytoplasm and mitochondria and interact closely the ER chaperones in protein folding and transport [138]. For example, HSP90 interacts with and stabilizes IRE1 and PERK kinases [139].

Hsps (e.g., Hsp27, 60, 70, 90 and 100) are induced in response to cigarette smoke and are highly expressed in the lungs of chronic smokers and subjects with COPD [140]. In addition, serum levels of Hsp27 [141], Hsp70 and Hsp90 are elevated in COPD [142]. In vitro, ROS induce HSP expression in lung structural cells. For example, H_2O_2 increases the levels of Hsp60 in bronchial epithelial cells through a pathway involving NF-κB-p65 [143]. Of interest, however, in animal models, the effects of cigarette smoke exposure on the expression of Hsps are complex and appear to be related to the duration of CS exposure. For example, 1- month exposure of rats to CS increases expression of Hsp70 in airway smooth muscle while a 3-month exposure dramatically reduced it [144]. Hsp expression is also controlled by corticosteroids. For example, dexamethasone increases Hsp72 mRNA and protein expression in the presence of cigarette smoke extract resulting in increased survival of alveolar epithelial cells [145].

Release of HSPs into the extracellular milieu may promote inflammation. For example, HSP60 released by epithelial cells in the setting of oxidative stress stimulates neutrophil activity in COPD patients [143]. Hsp60 also is a key target of T cell responses in chronic inflammation and induces expression of TNF-α and Th1-promoting cytokines, IL-12 and IL-15 in macrophages [146]. In addition, oxidative stress inducers such as CS induce the secretion of Hsp70 from lung structural cells and promote IL-8 release [147, 148], probably through acting as ligand for TLR4 [149].

Molecular Mechanisms of Oxidant Stress Induced Inflammation

Mitogen Activated Protein Kinases (MAPK)

The major redox sensitive signaling system presented in the lung is the MAPK system. MAPKs affect molecular targets which ultimately alter gene transcription in response to environmental stress. MAPK kinases include extracellular signal-regulated kinases (ERK), c-Jun-terminal kinases (JNKs), and p38 kinases. These kinases target a variety of immune effector molecules. For example, MAPK signaling pathways affect T-cell activation and differentiation [150, 151]. MAPK signaling regulates the influx of inflammatory cells into the respiratory tract. Specifically, p38 activation enhances lung inflammation by increasing the expression of inter-cellular adhesion molecule-1, tumor necrosis factor (TNF)-α, and MIP-2. P38 MAPK inhibitors decrease the expression of these pro-inflammatory cytokines and inhibit neutrophil influx in animal models of COPD [152].

Reactive oxygen species in cigarette smoke such as superoxide, hydrogen peroxide and peroxynitrite induce phosphorylation and activation of ERK [153], P38 [154], and JNK [155]. In part, MAPK activation is mediated by activation of the epidermal growth factor receptor and its tyrosine kinase activity [156]. Oxidants can also enhance MAPK signaling by inactivating tyrosine protein tyrosine phosphatase is such as PP2a which inactivate MAPK such as JNK and p38 [157]. The importance of ROS-induced activation of the protein tyrosine phosphatases (PTPS) has been demonstrated recently in studies in which PP2a knockdown increases the intensity of cigarette smoke-induced inflammation in the lungs of mice. PTPS may be an activated as a result of oxidation of cysteine residues within their catalytic domains. In addition MAPK phosphatases (MKPs) which inactivate MAPK are also inactivated by ROS [158].

Nuclear Factor Kappa B (NF-κB)

A redox sensitive transcriptional factor NF-κB, is an important regulator of the inflammatory and cell stress responses [159, 160]. Specifically, NF-κB regulates expression of a variety of cytokines, chemokines, immunoreceptors, cell-adhesion molecules, stress response genes, regulators of apoptosis, growth factors, and transcription factors. NF-κB is a family of homo- or heterodimers which contain a conserved Rel homology domain responsible for dimerization and binding to the consensus sequence [5′-GGGRNNYYCC-3′] [159, 160]. The NF-κB family of proteins can be divided into two distinct families based on the presence of a transactivation domain. RelA (p65), RelB and c-Rel all contain transactivation domains while p50 and p52 do not and require heterodimerization with the Rel proteins for

this function. In the absence of stimulation, NF-κB is inhibited in the cytosol by association with IκB [161–163]. In response to appropriate stimuli, IκB is phosphorylated by IκB kinases (IKKs) at two separate serine residues which leads its ubiquitination and subsequent proteasomal degradation. Release of NF-κB from IκB allows its translocation to the nucleus and subsequent binding to the promoter region of over 100 target genes [164]. In particular, NF-κB regulates the expression of over 30 cytokines and chemokines, immune recognition receptors and cell adhesion molecules required for neutrophil migration including TNF-α, inducible NOS (iNOS), interleukin-1 (IL-1), intra-cellular adhesion molecule-1 (ICAM-1), and cyclooxygenase (COX-2) [165]. A wide range of agents involved in oxidant stress, immune system activation and bacterial infection stimulate IKK to activate NF-κB including H_2O_2, TNF-α, IL-1, phorbol esters, microbial infection or PAMPs [165].

The importance of NF-κB in the inflammatory response of the lung is demonstrated by the fact that NF-κB knockout mice manifest less lung inflammation and cytokine levels in the BAL compared to wild-type animals in response to inhaled toxic substances. For example, NF-κB knockout mice demonstrate less neutrophil infiltration and cytokine expression in the lung in response to LPS [166]. Moreover, ROS in cigarette smoke such as hydrogen peroxide activate NF-κB in several cell lines in vitro [167–169]. In fact, H_2O_2 treatment leads to phosphorylation and activation of IKK. Oxidants may also also directly phosphorylate the p65 subunit of NF-κB.

Of interest, NF-κB is also regulated by Nrf2. For example, NF-κB activity is increased in Nrf2 knockout mice after treatment with TNF-α, LPS and respiratory syncytial virus [129, 130]. In fact, Nrf2 attenuates IκB phosphorylation and increases IKK activity in response to TNF-α or LPS [130]. In addition, Nrf2 appears to regulate expression of at least subsets of the NF-κB family directly. For example, p50 and p65 are reduced in $Nrf2^{-/-}$ fibroblasts while c-Rel is increased in $Nrf2^{-/-}$ fibroblasts [170]. Greater expression of NF-κB and its targets in Nrf2 deficient animals may be a result of diminished ability to scavenge ROS and, hence, to greater oxidant stress or to more direct interactions between the two transcription factors. Of note, since a variety of stimuli such as ROS and LPS induce both Nrf2 and NF-κB activity, an entirely antagonistic relationship between the two transcription factors under all circumstances is unlikely [171–174].

Of note, NF-κB appears to be negatively regulated by the AhR. For example, $AhR^{-/-}$ mice demonstrate increased NF-κB DNA binding activity in whole lung lysates [76]. Morever, heightened prostaglandin responses to cigarette smoke in $AhR^{-/-}$ fibroblasts appear to be explained in part by loss of RelB protein. These data suggest that the AhR represses the NF-κB complex by interacting with RelB.

AP-1

AP-1, another redox sensitive transcription factor, exerts a pro-inflammatory effect by inducing the expression of a variety of chemokines, in particular, C-X-C chemokines [175] in alveolar macrophages [176] and lung epithelial cells [177, 178].

AP-1 is a heterodimer composed of Fos, Jun and activating transcription factor (ATF) subunits interacted with c-Jun, the most potent activator of the group. Fos stabilizes Jun thereby enhancing its binding to promoter region in target genes.

MAPK signaling is an important pathway for AP-1 activation by phosphorylation of Fos, JUN, or ATF subunits [179, 180]. PERK (protein kinase R-like ER resident kinase) activation also induces Fos expression [181]. ROS can activate AP-1. For example, hydrogen peroxide induces phosphorylation of FOS and JUN and increases the expression of Fos, an effect which is attenuated by the use of ERK or JNK inhibitors indicating the importance of the MAPK signaling pathway [182]. Cigarette smoke induces phosphorylation of c-Jun which in turn promotes the expression of CXCL8.

Histone Deacetylases (HDAC)

The HDAC enzymes deacetylate lysine groups on histones thereby interfering with the binding of transcriptional activators. As such, the HDAC family of enzymes generally inhibits immune responses in the lung [183]. In fact, corticosteroids act by recruiting HDACs to transcriptional co-activators such as p65-CBP thereby inhibiting their activity by inducing deacetylation of the histone complex. HDACs also attenuate inflammation by deacetylating and, hence, inactivating the RelA subunit of NF-κB [184]. Acetylation of RelA inhibits IκB−α binding [184] and augments binding to IKKα causing export of the NF-κB complex from the nucleus [184]. The effect of HDAC activity, therefore, is to attenuate NF-κB transcriptional activity.

Of considerable interest, HDAC activity is affected by the redox state of the cell and is inhibited under conditions of oxidative stress. For example, cigarette smoke and H2O2 augment histone acetylation by decreasing the expression and activity of HDAC in human bronchial epithelial cells [185–187]. In the rodent model, cigarette smoke exposure increases histone acetylation, decreases HDAC activity and enhances NF-κB mediated signaling [188, 189]. In contrast, cigarette smoke increases HAT activity contributing to increased acetylation of histone proteins [186]. Of interest, reduced HDAC2 activity in COPD may contribute to increases in Nrf2 acetylation, reduced Nrf2 stability and impaired anti-oxidant defense [128].

The Unfolded Protein Response (UPR)

The UPR alters the activity of signaling pathways which control protein synthesis, transport and degradation [125, 126, 190]. Moreover, the UPR up-regulates expression of a wide array of genes vital for cell survival including genes which promote oxidant defense (e.g., Nrf2, ATF4, HO-1). The UPR is activated in response to ROS and protects against oxidant-induced cell injury and death while defective function of UPR activity impairs the response to oxidant stress, increases ROS burden and diminishes cell survival [125, 126, 190–195]. Of considerable importance, signaling

pathways activated by the UPR also enhances the activity of pro-inflammatory pathways which regulate the immune response and thus have the potential to augment the innate inflammatory response to cigarette smoke [196].

Oxidant stress in general and cigarette smoke exposure, in particular, cause protein oxidation and misfolding in the lungs [197, 198] and cultured respiratory cells [197, 199]. The effects of cigarette smoke on protein oxidation appear to be largely due to the action of acrolein, superoxide and H_2O_2. In addition, nicotine induces a UPR response in several cell types presumably by increasing cytosolic calcium [200]. Protein misfolding induced by cigarette smoke can be attenuated by ROS scavengers and by pre-treatment with an anti-oxidant, the glutathione precursor, n-acetyl cysteine [190, 194, 201–204].

Misfolded proteins are non-functional and potentially cytotoxic when present in sufficient amount. Accordingly, cells have evolved mechanisms to refold misfolded proteins using a variety of chaperones, protein disulfide isomerases and oxidoreductases to isomerize, oxidize and reduce thiol groups on target proteins [196]. The processes involved in protein refolding are energy dependent and require oxidation of thiol groups and the formation of intramolecular and intermolecular disulfide bonds [205]. Electron transport during disulfide bond formation involves two ER-resident enzymes: protein disulfide isomerase [PDI] and ER oxidoreductase 1 [ERO1] [206]. PDI accepts electrons resulting in cysteine oxidation and disulfide bond formation. Electrons are then transferred by ERO1 to reduce molecular oxygen (O_2) and form H_2O_2, thereby increasing the oxidant burden of the cell.

The presence of misfolded proteins is sensed by a triad of ER resident proteins [i.e., PERK (protein kinase R like-ER resident kinase); ATF6 (activating transcription factor 6); and IRE1 (inositol requiring enzyme-1)] [191, 192, 207–209]. Although the precise mechanism by which an increase in the load of misfolded proteins is sensed is uncertain, dissociation of an inhibitor protein, the chaperone, GRP78, from the luminal surface of the sensors increases their activity and triggers a UPR. IRE1α and IREβ (which is present in the lung and gut only), are transmembrane kinases with RNase activity, which splice XBP1 mRNA into a transcription factor (sXBP1) which also up-regulates the above ER resident chaperones, as well as genes involved in protein ubiquitination and degradation, lipid biosynthesis and expansion of ER mass. Activation of IRE1 induces a conformational change which leads to its formation of a complex with the adapter protein, TRAF2 [TNF-α receptor associated factor-2] which recruits IKK leading to the phosphorylation and degradation of IkB [210, 211]. The IRE1-TRAF2 complex can also recruit JNK which phosphorylates and activates AP-1 [212].

PERK is a transmembrane kinase which phosphorylates and thereby inhibits eIF2α, the eukaryotic translation initiation factor-2α. Phosphorylation of eIF2α is a crucial feature of the UPR since it inhibits protein translation globally, but facilitates translation of selected mRNAs containing appropriate open reading frames. In fact, inhibition of eIF2α up-regulates translation of Nrf2 and ATF4, a basic zipper transcription factor which enhances ER chaperone expression, and which up-regulates expression of HO-1 and NQO1metabolizing enzymes [116, 117]. Of interest, phosphorylation of eIF2α and attenuated translation increases expression of NF-κB [213]. Since IκB, which has a much shorter half-life than NF-κB, attenuating expression of

IkB increases the ratio of NF-κB to IkB therby freeing NF-κB to translocate to the nucleus. PERK also directly phosphorylates Nrf2, which facilitates its dissociation from the cytoplasmic inhibitor, KEAP1, and its translocation to the nucleus [124–126]. ATF4 also induces expression of the pro-apoptotic transcription factor, CHOP (CCAAT/enhancer protein-homologous protein) which contributes to a UPR driven apoptosis pathway of cell death in lung structural and inflammatory cells [214, 215].

ATF6 is a proto-transcription factor, which upon proteolytic cleavage of its N-terminal transcriptionally active form in the Golgi apparatus, traffics to the nucleus where in conjunction with sXBP1, it activates genes encoding GRP78, calreticulin, calnexin, and PDI (protein disulfide isomerase).

Cigarette smoke induces a UPR response in the lungs of chronic cigarette smokers and in cultured human airway epithelial cells as reflected by up-regulation of expression of the hallmark UPR effector proteins, GRP78, calreticulin, calnexin and PDI [197]. Of interest, the UPR response to cigarette smoke appears to be partially reversible with smoking cessation since expression of these proteins is significantly less in ex-smokers than in active smokers. Cigarette smoke exposure also increases the expression of genes involved in protein folding and the ubiquitin-proteosome pathway in human monocytes suggesting impaired protein folding in this cell type [3]. Moreover, in vitro studies in human airway epithelial cells [202, 203, 215, 216] indicate that the cigarette smoke induced UPR is rapid in onset (within hours) and dose-dependent [197, 202, 203, 215, 216]. Furthermore, PERK activity is increased since phospho-eIF2α, ATF4 and Nrf2 are up-regulated [197, 203]. In contrast, the IRE1 signaling pathway is not activated by cigarette smoke since XBP1 mRNA splicing is unchanged [202, 203, 215]. Activation of the PERK pathway without increase in activity of the IRE1 pathway in the setting of cigarette smoke exposure appears to be explained by active suppression of XBP1 splicing by cigarette smoke [203].

Of considerable interest, cross-talk between components of the UPR and components of both the MAPK pathway (i.e., JNK) and Toll-like receptor pathways (i.e., TLR4) affect the intensity of the inflammatory response and inflammatory cell survival [4, 5, 217]. These interactions may augment the intensity of the inflammatory process in the lung. For example, activation of the kinase activity of the IRE-1 arm of the UPR by misfolded proteins activates both JNK and NF-κB and increases IL-8 mRNA and protein in human alveolar pneumocytes and airway epithelial cells [218–220]. Moreover, the IRE1 arm of the UPR can be activated by PAMPs to amplify the intensity of the innate immune response to pathogens. For example, LPS-induced activation of TLR 2 or 4 acting through the TRAF6 adapter protein activates the endonuclease activity of IRE1 with resultant increases in sXBP1 [5]. In turn, sXBP1 augmented production of IL-6 thereby magnifying the innate immune response to microbial infection in human monocytes [221]. Prior activation of the UPR by inducing protein misfolding using the canonical stimulus, thapsigargin, potentiated IRE activation of potentiated the IL-6 response to LPS. Of interest, the PERK and ATF6 arms of the UPR were inhibited. These data suggest that the UPR may potentiate the innate inflammatory response to PAMPs. Specifically, the combination of cigarette smoke exposure and TLR activation may act cooperatively to increase lung inflammation. In fact, mice treated with a combination of cigarette smoke and the viral PAMP and TLR3 ligand, poly (I:C),

demonstrate synergistic augmentation of lung inflammation and emphysema compared to either treatment alone [222].

Of interest, TLR4 activation by LPS also inhibits translation of ATF4 through a TRIF-dependent pathway in mouse monocytes [4, 217]. Inhibition of ATF4 expression diminishes expression of its downstream target, the pro-apoptosis transcription factor, CHOP, thereby promoting cell survival. Of interest, CHOP also appears to induce IL-8 expression at the transcriptional level by binding to the IL-8 promoter in human airway epithelial cells [218]. Inhibition of CHOP expression by LPS may therefore reduce IL-8 expression. Nonetheless, enhanced survival of TLR4-activated monocytes in the lung is likely to augment the inflammatory response to LPS present in cigarette smoke. Moreover, the combination of cigarette smoke exposure and activation of a TLR may act cooperatively to increase lung inflammation. In fact, mice treated with a combination of cigarette and the TLR3 ligand, poly (I:C), demonstrated synergistic augmentation of lung inflammation and emphysema compared to either treatment alone [222].

Oxidant stress is heightened in subjects with COPD and persists for prolonged periods even after subjects have stopped smoking [104, 133, 223]. In part, oxidant stress is heightened because Nrf2 expression is reduced in subjects with COPD [15, 122, 133, 223]. Reductions in Nrf2 in lung tissue and in alveolar macrophages appears to explain reductions in both glutathione dependent and glutathione-independent anti-oxidant defense, in particular, HO-1, which is transcriptionally regulated by Nrf2 and ATF4 [92, 224]. Nrf2 up-regulates the expression of the components of the 26 S proteosome [223]. Accordingly, decreased Nrf2 expression decreases proteasomal activity, impairs protein degradation and leads to accumulation of misfolded proteins in the lung of subjects with COPD [223]. Accumulation of misfolded proteins in the lungs of subjects with COPD may be expected to enhance UPR activity and contribute to the NF-κB-induced inflammatory process. However, UPR activity in the lungs of subjects with COPD is unstudied.

Variations in Antioxidant Gene Expression and Susceptibility to Lung Inflammation

Of considerable importance, the propensity to develop lung disease varies widely across cigarette smokers and correlates only weakly with the smoking history as reflected in the number of cigarette pack years [225, 226]. In fact, it is estimated that only a minority (i.e., 15–35 %) of chronic, continuous cigarette smokers develop COPD [226, 227]. That the majority of long-term smokers do not develop lung damage or COPD suggests that compensatory mechanisms protect the lung from RONS or xenobiotic materials. In this regard, the magnitude of up-regulation of mRNA for several anti-oxidant genes e.g., glutathione peroxidase, glutathione synthase, HO-1, etc., varies considerably across individual cigarette smokers [228, 229]. The mechanism(s) underlying this inter-individual variability in important anti-oxidant gene expression is unknown.

In this regard, the expression of UPR related genes in response to pharmacological stimuli like thapsigargin or tunicamycin also varies widely in healthy human subjects but is concordant in monozygotic twins [230]. Moreover, polymorphisms in the PERK promoter affect PERK function and expression [231]. These findings suggest that UPR responses are genetically determined and that inter-individual differences in UPR function may affect the response to cigarette smoke and the development of lung inflammation in chronic smokers. This issue is unstudied, however.

Conclusion

The complex mix of compounds present in cigarette smoke exposes the respiratory tract to oxidant stress. Many of these compounds induce an inflammatory response by activating redox sensitive, pro-inflammatory pathways including NF-κB, AP-1 and MAPKs and by inhibiting redox sensitive anti-inflammatory pathways such as HDACs. Conversely, an elaborate network of protein and small molecule anti-oxidants exist to scavenge ROS in the respiratory tract and maintain redox balance in the cell. The regulation of anti-oxidant defense is largely under the control of the redox sensitive transcription factor, Nrf2. Moreover, the UPR which is activated when proteins are oxidized and misfolded in the ER, regulates anti-oxidant defense per se by both Nrf2 dependent and Nrf2-indpendent mechanisms. Of considerable importance, the NF-κB mediated pro-inflammatory and Nrf2 mediated anti-oxidant pathways interact to shape the intensity of the inflammatory response to cigarette smoke. Moreover, the UPR acting through its IRE1 arm appears to paradoxically have a pro-inflammatory aspect as well by affecting cytokine expression directly and indirectly via the NF-κB and AP-1 signaling pathways. Of considerable importance, genetically determined inter-individual responses to oxidant stress and UPR activation vary considerably and are likely to contribute to differences in susceptibility to cigarette smoke-induced lung inflammation and lung damage. Further studies will be required to characterize the effects of cigarette smoke on the Nrf2 and UPR systems in the lung and their role in the development of cigarette smoke-induced lung inflammation and tissue damage.

References

1. Shapiro SD, Ingenito EP (2005) The pathogenesis of chronic obstructive pulmonary disease: advances in the past 100 years. Am J Respir Cell Mol Biol 32(5):367–372
2. Centers for Disease Control and Prevention (CDC) (2006) Tobacco use among adults-united states, 2005. MMWR Morb Mortal Wkly Rep 55:1145–1148
3. Wright WR, Parzych K, Crawford D, Mein C, Mitchell JA, Paul-Clark MJ (2012) Inflammatory transcriptome profiling of human monocytes exposed acutely to cigarette smoke. PLoS One 7(2):e30120

4. Woo CW, Cui D, Arellano J, Dorweiler B, Harding H, Fitzgerald KA, Ron D, Tabas I (2009) Adaptive suppression of the ATF4-CHOP branch of the unfolded protein response by toll-like receptor signalling. Nat Cell Biol 11(12):1473–1480
5. Martinon F, Chen X, Lee AH, Glimcher LH (2010) TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. Nat Immunol 11(5):411–418
6. Church DF, Pryor WA (1985) Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 64:111–126
7. MacNee W (2005) Pathogenesis of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2(4):258–266, discussion 290–251
8. Pryor WA, Stone K (1993) Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann N Y Acad Sci 686:12–27, discussion 27–18
9. Pryor WA, Stone K, Zang LY, Bermudez E (1998) Fractionation of aqueous cigarette tar extracts: fractions that contain the tar radical cause DNA damage. Chem Res Toxicol 11(5):441–448
10. Huang MF, Lin WL, Ma YC (2005) A study of reactive oxygen species in mainstream of cigarette. Indoor Air 15(2):135–140
11. Committee on Passive Smoking, National Research Council (1986) Environmental tobacco smoke: measuring exposures and assessing health effects. National Academy Press, Washington, DC
12. Diekmann J, Wittig A, Stabbert R (2008) Gas chromatographic-mass spectrometric analysis of acrylamide and acetamide in cigarette mainstream smoke after on-column injection. J Chromatogr Sci 46(7):659–663
13. Seagrave J (2000) Oxidative mechanisms in tobacco smoke-induced emphysema. J Toxicol Environ Health A 61(1):69–78
14. Lambeth JD (2004) Nox enzymes and the biology of reactive oxygen. Nat Rev Immunol 4(3):181–189
15. Suzuki M, Betsuyaku T, Ito Y, Nagai K, Nasuhara Y, Kaga K, Kondo S, Nishimura M (2008) Down-regulated NF-E2-related factor 2 in pulmonary macrophages of aged smokers and patients with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 39(6): 673–682
16. Ranjan P, Anathy V, Burch PM, Weirather K, Lambeth JD, Heintz NH (2006) Redox-dependent expression of cyclin D1 and cell proliferation by Nox1 in mouse lung epithelial cells. Antioxid Redox Signal 8(9–10):1447–1459
17. Fink K, Duval A, Martel A, Soucy-Faulkner A, Grandvaux N (2008) Dual role of NOX2 in respiratory syncytial virus- and sendai virus-induced activation of NF-kappaB in airway epithelial cells. J Immunol 180(10):6911–6922
18. Zhang WJ, Wei H, Tien YT, Frei B (2011) Genetic ablation of phagocytic NADPH oxidase in mice limits TNFalpha-induced inflammation in the lungs but not other tissues. Free Radic Biol Med 50(11):1517–1525
19. Cheng G, Cao Z, Xu X, van Meir EG, Lambeth JD (2001) Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. Gene 269(1–2):131–140
20. Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87(1):245–313
21. Lin CC, Lee IT, Yang YL, Lee CW, Kou YR, Yang CM (2010) Induction of COX-2/PGE(2)/IL-6 is crucial for cigarette smoke extract-induced airway inflammation: role of TLR4-dependent NADPH oxidase activation. Free Radic Biol Med 48(2):240–254
22. Hawkins BJ, Madesh M, Kirkpatrick CJ, Fisher AB (2007) Superoxide flux in endothelial cells via the chloride channel-3 mediates intracellular signaling. Mol Biol Cell 18(6):2002–2012
23. Zinkevich NS, Gutterman DD (2011) ROS-induced ROS release in vascular biology: redox-redox signaling. Am J Physiol Heart Circ Physiol 301(3):H647–H653
24. Asano H, Horinouchi T, Mai Y, Sawada O, Fujii S, Nishiya T, Minami M, Katayama T, Iwanaga T, Terada K et al (2012) Nicotine- and tar-free cigarette smoke induces cell damage through reactive oxygen species newly generated by PKC-dependent activation of NADPH oxidase. J Pharmacol Sci 118(2):275–287

25. Jaimes EA, DeMaster EG, Tian RX, Raij L (2004) Stable compounds of cigarette smoke induce endothelial superoxide anion production via NADPH oxidase activation. Arterioscler Thromb Vasc Biol 24(6):1031–1036
26. Reddy S, Finkelstein EI, Wong PS, Phung A, Cross CE, van der Vliet A (2002) Identification of glutathione modifications by cigarette smoke. Free Radic Biol Med 33(11):1490–1498
27. Grafstrom RC, Dypbukt JM, Willey JC, Sundqvist K, Edman C, Atzori L, Harris CC (1988) Pathobiological effects of acrolein in cultured human bronchial epithelial cells. Cancer Res 48(7):1717–1721
28. Nardini M, Finkelstein EI, Reddy S, Valacchi G, Traber M, Cross CE, van der Vliet A (2002) Acrolein-induced cytotoxicity in cultured human bronchial epithelial cells. Modulation by alpha-tocopherol and ascorbic acid. Toxicology 170(3):173–185
29. Jia L, Zhang Z, Zhai L, Bai Y (2009) Protective effect of lipoic acid against acrolein-induced cytotoxicity in imr-90 human fibroblasts. J Nutr Sci Vitaminol (Tokyo) 55(2):126–130
30. Reznick AZ, Cross CE, Hu ML, Suzuki YJ, Khwaja S, Safadi A, Motchnik PA, Packer L, Halliwell B (1992) Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. Biochem J 286(Pt 2):607–611
31. O'Neill CA, Halliwell B, van der Vliet A, Davis PA, Packer L, Tritschler H, Strohman WJ, Rieland T, Cross CE, Reznick AZ (1994) Aldehyde-induced protein modifications in human plasma: protection by glutathione and dihydrolipoic acid. J Lab Clin Med 124(3):359–370
32. Faroon O, Roney N, Taylor J, Ashizawa A, Lumpkin MH, Plewak DJ (2008) Acrolein environmental levels and potential for human exposure. Toxicol Ind Health 24(8):543–564
33. Leikauf GD (2002) Hazardous air pollutants and asthma. Environ Health Perspect 110 (Suppl 4):505–526
34. Corradi M, Pignatti P, Manini P, Andreoli R, Goldoni M, Poppa M, Moscato G, Balbi B, Mutti A (2004) Comparison between exhaled and sputum oxidative stress biomarkers in chronic airway inflammation. Eur Respir J 24(6):1011–1017
35. Deshmukh HS, Shaver C, Case LM, Dietsch M, Wesselkamper SC, Hardie WD, Korfhagen TR, Corradi M, Nadel JA, Borchers MT et al (2008) Acrolein-activated matrix metalloproteinase 9 contributes to persistent mucin production. Am J Respir Cell Mol Biol 38(4):446–454
36. Borchers MT, Wesselkamper SC, Harris NL, Deshmukh H, Beckman E, Vitucci M, Tichelaar JW, Leikauf GD (2007) CD8+ T cells contribute to macrophage accumulation and airspace enlargement following repeated irritant exposure. Exp Mol Pathol 83(3):301–310
37. Li L, Hamilton RF Jr, Taylor DE, Holian A (1997) Acrolein-induced cell death in human alveolar macrophages. Toxicol Appl Pharmacol 145(2):331–339
38. Snelgrove RJ, Jackson PL, Hardison MT, Noerager BD, Kinloch A, Gaggar A, Shastry S, Rowe SM, Shim YM, Hussell T et al (2010) A critical role for LTA4H in limiting chronic pulmonary neutrophilic inflammation. Science 330(6000):90–94
39. Bein K, Leikauf GD (2011) Acrolein – a pulmonary hazard. Mol Nutr Food Res 55(9): 1342–1360
40. Lambert C, Li J, Jonscher K, Yang TC, Reigan P, Quintana M, Harvey J, Freed BM (2007) Acrolein inhibits cytokine gene expression by alkylating cysteine and arginine residues in the NF-kappaB1 DNA binding domain. J Biol Chem 282(27):19666–19675
41. Myers CR, Myers JM (2009) The effects of acrolein on peroxiredoxins, thioredoxins, and thioredoxin reductase in human bronchial epithelial cells. Toxicology 257(1–2):95–104
42. Zhang H, Forman HJ (2008) Acrolein induces heme oxygenase-1 through PKC-delta and PI3K in human bronchial epithelial cells. Am J Respir Cell Mol Biol 38(4):483–490
43. Elinder CG, Kjellstrom T, Lind B, Linnman L, Piscator M, Sundstedt K (1983) Cadmium exposure from smoking cigarettes: variations with time and country where purchased. Environ Res 32(1):220–227
44. Scherer G, Barkemeyer H (1983) Cadmium concentrations in tobacco and tobacco smoke. Ecotoxicol Environ Saf 7(1):71–78
45. Huang YH, Shih CM, Huang CJ, Lin CM, Chou CM, Tsai ML, Liu TP, Chiu JF, Chen CT (2006) Effects of cadmium on structure and enzymatic activity of Cu, Zn-SOD and oxidative status in neural cells. J Cell Biochem 98(3):577–589

46. Ahmed MJ, Chowdhury MT (2004) A simple spectrophotometric method for the determination of cadmium in industrial, environmental, biological and soil samples using 5,7-dibromo-8-hydroxyquinoline. Anal Sci 20(6):987–990
47. Hayes JA, Snider GL, Korthy AL (1973) Lesions resembling centrilobular emphysema induced in rats by exposure to aerosolized solutions of cadmium chloride. Chest 63(Suppl): 68S–69S
48. Snider GL, Hayes JA, Korthy AL, Lewis GP (1973) Centrilobular emphysema experimentally induced by cadmium chloride aerosol. Am Rev Respir Dis 108(1):40–48
49. Thurlbeck WM, Foley FD (1963) Experimental pulmonary emphysema: the effect of intratracheal injection of cadmium chloride solution in the guinea pig. Am J Pathol 42(4): 431–441
50. Asvadi S, Hayes JA (1978) Acute lung injury induced by cadmium aerosol. II. Free airway cell response during injury and repair. Am J Pathol 90(1):89–98
51. Nemery B (1990) Metal toxicity and the respiratory tract. Eur Respir J 3(2):202–219
52. Patwardhan JR, Finckh ES (1976) Fatal cadmium-fume pneumonitis. Med J Aust 1(25): 962–966
53. Barnhart S, Rosenstock L (1984) Cadmium chemical pneumonitis. Chest 86(5):789–791
54. Zhang W, Fievez L, Cheu E, Bureau F, Rong W, Zhang F, Zhang Y, Advenier C, Gustin P (2010) Anti-inflammatory effects of formoterol and ipratropium bromide against acute cadmium-induced pulmonary inflammation in rats. Eur J Pharmacol 628(1–3):171–178
55. McKenna IM, Waalkes MP, Chen LC, Gordon T (1997) Comparison of inflammatory lung responses in Wistar rats and C57 and DBA mice following acute exposure to cadmium oxide fumes. Toxicol Appl Pharmacol 146(2):196–206
56. Yamano T, DeCicco LA, Rikans LE (2000) Attenuation of cadmium-induced liver injury in senescent male fischer 344 rats: role of Kupffer cells and inflammatory cytokines. Toxicol Appl Pharmacol 162(1):68–75
57. Davison AG, Fayers PM, Taylor AJ, Venables KM, Darbyshire J, Pickering CA, Chettle DR, Franklin D, Guthrie CJ, Scott MC et al (1988) Cadmium fume inhalation and emphysema. Lancet 1(8587):663–667
58. Snider GL, Lucey EC, Faris B, Jung-Legg Y, Stone PJ, Franzblau C (1988) Cadmium-chloride-induced air-space enlargement with interstitial pulmonary fibrosis is not associated with destruction of lung elastin. Implications for the pathogenesis of human emphysema. Am Rev Respir Dis 137(4):918–923
59. Ellis KJ, Vartsky D, Zanzi I, Cohn SH, Yasumura S (1979) Cadmium: in vivo measurement in smokers and nonsmokers. Science 205(4403):323–325
60. Webb M, Verschoyle RD (1976) An investigation of the role of metallothioneins in protection against the acute toxicity of the cadmium ion. Biochem Pharmacol 25(6):673–679
61. Kayaalti Z, Aliyev V, Soylemezoglu T (2011) The potential effect of metallothionein 2A–5A/G single nucleotide polymorphism on blood cadmium, lead, zinc and copper levels. Toxicol Appl Pharmacol 256(1):1–7
62. Hendrick DJ (2004) Smoking, cadmium, and emphysema. Thorax 59(3):184–185
63. Mannino DM, Holguin F, Greves HM, Savage-Brown A, Stock AL, Jones RL (2004) Urinary cadmium levels predict lower lung function in current and former smokers: data from the third national health and nutrition examination survey. Thorax 59(3):194–198
64. Hirst RN Jr, Perry HM Jr, Cruz MG, Pierce JA (1973) Elevated cadmium concentration in emphysematous lungs. Am Rev Respir Dis 108(1):30–39
65. Grasseschi RM, Ramaswamy RB, Levine DJ, Klaassen CD, Wesselius LJ (2003) Cadmium accumulation and detoxification by alveolar macrophages of cigarette smokers. Chest 124(5):1924–1928
66. Hotz P, Buchet JP, Bernard A, Lison D, Lauwerys R (1999) Renal effects of low-level environmental cadmium exposure: 5-year follow-up of a subcohort from the Cadmibel study. Lancet 354(9189):1508–1513
67. Kitamura M, Hiramatsu N (2010) The oxidative stress: endoplasmic reticulum stress axis in cadmium toxicity. Biometals 23(5):941–950

68. Somji S, Todd JH, Sens MA, Garrett SH, Sens DA (1999) Expression of the constitutive and inducible forms of heat shock protein 70 in human proximal tubule cells exposed to heat, sodium arsenite, and CdCl(2). Environ Health Perspect 107(11):887–893
69. Tatrai E, Kovacikova Z, Hudak A, Adamis Z, Ungvary G (2001) Comparative in vitro toxicity of cadmium and lead on redox cycling in type II pneumocytes. J Appl Toxicol 21(6): 479–483
70. Gu YZ, Hogenesch JB, Bradfield CA (2000) The PAS superfamily: sensors of environmental and developmental signals. Annu Rev Pharmacol Toxicol 40:519–561
71. Stockinger B, Hirota K, Duarte J, Veldhoen M (2011) External influences on the immune system via activation of the aryl hydrocarbon receptor. Semin Immunol 23(2):99–105
72. Dolwick KM, Schmidt JV, Carver LA, Swanson HI, Bradfield CA (1993) Cloning and expression of a human Ah receptor cDNA. Mol Pharmacol 44(5):911–917
73. Li W, Donat S, Dohr O, Unfried K, Abel J (1994) Ah receptor in different tissues of C57BL/6J and DBA/2J mice: use of competitive polymerase chain reaction to measure Ah-receptor mRNA expression. Arch Biochem Biophys 315(2):279–284
74. Carver LA, Hogenesch JB, Bradfield CA (1994) Tissue specific expression of the rat Ah-receptor and ARNT mRNAs. Nucleic Acids Res 22(15):3038–3044
75. Wong PS, Vogel CF, Kokosinski K, Matsumura F (2010) Arylhydrocarbon receptor activation in NCI-H441 cells and C57BL/6 mice: possible mechanisms for lung dysfunction. Am J Respir Cell Mol Biol 42(2):210–217
76. Thatcher TH, Maggirwar SB, Baglole CJ, Lakatos HF, Gasiewicz TA, Phipps RP, Sime PJ (2007) Aryl hydrocarbon receptor-deficient mice develop heightened inflammatory responses to cigarette smoke and endotoxin associated with rapid loss of the nuclear factor-kappaB component RelB. Am J Pathol 170(3):855–864
77. Baglole CJ, Maggirwar SB, Gasiewicz TA, Thatcher TH, Phipps RP, Sime PJ (2008) The aryl hydrocarbon receptor attenuates tobacco smoke-induced cyclooxygenase-2 and prostaglandin production in lung fibroblasts through regulation of the NF-kappaB family member RelB. J Biol Chem 283(43):28944–28957
78. Brioni JD, Decker MW, Sullivan JP, Arneric SP (1997) The pharmacology of (-)-nicotine and novel cholinergic channel modulators. Adv Pharmacol 37:153–214
79. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L et al (2003) Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421(6921):384–388
80. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405(6785):458–462
81. Mabley J, Gordon S, Pacher P (2011) Nicotine exerts an anti-inflammatory effect in a murine model of acute lung injury. Inflammation 34(4):231–237
82. Zhou Y, Zuo X, Li Y, Wang Y, Zhao H, Xiao X (2012) Nicotine inhibits tumor necrosis factor-alpha induced IL-6 and IL-8 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Rheumatol Int 32(1):97–104
83. Aicher A, Heeschen C, Mohaupt M, Cooke JP, Zeiher AM, Dimmeler S (2003) Nicotine strongly activates dendritic cell-mediated adaptive immunity: potential role for progression of atherosclerotic lesions. Circulation 107(4):604–611
84. Penn A, Snyder CA (1993) Inhalation of sidestream cigarette smoke accelerates development of arteriosclerotic plaques. Circulation 88(4 Pt 1):1820–1825
85. Pryor WA (1997) Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ Health Perspect 105(Suppl 4):875–882
86. Pacht ER, Kaseki H, Mohammed JR, Cornwell DG, Davis WB (1986) Deficiency of vitamin E in the alveolar fluid of cigarette smokers. Influence on alveolar macrophage cytotoxicity. J Clin Invest 77(3):789–796
87. Wurzel H, Yeh CC, Gairola C, Chow CK (1995) Oxidative damage and antioxidant status in the lungs and bronchoalveolar lavage fluid of rats exposed chronically to cigarette smoke. J Biochem Toxicol 10(1):11–17

88. Thome GR, Spanevello RM, Mazzanti A, Fiorenza AM, Duarte MM, da Luz SC, Pereira ME, Morsch VM, Schetinger MR, Mazzanti CM (2011) Vitamin e decreased the activity of acetylcholinesterase and level of lipid peroxidation in brain of rats exposed to aged and diluted sidestream smoke. Nicotine Tob Res 13(12):1210–1219
89. Zelko IN, Mariani TJ, Folz RJ (2002) Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 33(3):337–349
90. Bowler RP, Nicks M, Warnick K, Crapo JD (2002) Role of extracellular superoxide dismutase in bleomycin-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 282(4):L719–L726
91. Weydert CJ, Cullen JJ (2010) Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 5(1):51–66
92. Ryter SW, Alam J, Choi AM (2006) Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev 86(2):583–650
93. Knorr-Wittmann C, Hengstermann A, Gebel S, Alam J, Muller T (2005) Characterization of Nrf2 activation and heme oxygenase-1 expression in NIH3T3 cells exposed to aqueous extracts of cigarette smoke. Free Radic Biol Med 39(11):1438–1448
94. Li N, Venkatesan MI, Miguel A, Kaplan R, Gujuluva C, Alam J, Nel A (2000) Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. J Immunol 165(6):3393–3401
95. Slebos DJ, Ryter SW, van der Toorn M, Liu F, Guo F, Baty CJ, Karlsson JM, Watkins SC, Kim HP, Wang X et al (2007) Mitochondrial localization and function of heme oxygenase-1 in cigarette smoke-induced cell death. Am J Respir Cell Mol Biol 36(4):409–417
96. Camhi SL, Alam J, Wiegand GW, Chin BY, Choi AM (1998) Transcriptional activation of the HO-1 gene by lipopolysaccharide is mediated by 5′ distal enhancers: role of reactive oxygen intermediates and AP-1. Am J Respir Cell Mol Biol 18(2):226–234
97. Yee EL, Pitt BR, Billiar TR, Kim YM (1996) Effect of nitric oxide on heme metabolism in pulmonary artery endothelial cells. Am J Physiol 271(4 Pt 1):L512–L518
98. Rangasamy T, Misra V, Zhen L, Tankersley CG, Tuder RM, Biswal S (2009) Cigarette smoke-induced emphysema in A/J mice is associated with pulmonary oxidative stress, apoptosis of lung cells, and global alterations in gene expression. Am J Physiol Lung Cell Mol Physiol 296(6):L888–L900
99. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tuder RM, Biswal S (2004) Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. J Clin Invest 114(9):1248–1259
100. Foronjy RF, Mirochnitchenko O, Propokenko O, Lemaitre V, Jia Y, Inouye M, Okada Y, D'Armiento JM (2006) Superoxide dismutase expression attenuates cigarette smoke- or elastase-generated emphysema in mice. Am J Respir Crit Care Med 173(6):623–631
101. Petrache I, Medler TR, Richter AT, Kamocki K, Chukwueke U, Zhen L, Gu Y, Adamowicz J, Schweitzer KS, Hubbard WC et al (2008) Superoxide dismutase protects against apoptosis and alveolar enlargement induced by ceramide. Am J Physiol Lung Cell Mol Physiol 295(1):L44–L53
102. Gongora MC, Lob HE, Landmesser U, Guzik TJ, Martin WD, Ozumi K, Wall SM, Wilson DS, Murthy N, Gravanis M et al (2008) Loss of extracellular superoxide dismutase leads to acute lung damage in the presence of ambient air: a potential mechanism underlying adult respiratory distress syndrome. Am J Pathol 173(4):915–926
103. Yao H, Arunachalam G, Hwang JW, Chung S, Sundar IK, Kinnula VL, Crapo JD, Rahman I (2010) Extracellular superoxide dismutase protects against pulmonary emphysema by attenuating oxidative fragmentation of ECM. Proc Natl Acad Sci USA 107(35):15571–15576
104. Maestrelli P, Paska C, Saetta M, Turato G, Nowicki Y, Monti S, Formichi B, Miniati M, Fabbri LM (2003) Decreased haem oxygenase-1 and increased inducible nitric oxide synthase in the lung of severe COPD patients. Eur Respir J 21(6):971–976
105. Young RP, Hopkins R, Black PN, Eddy C, Wu L, Gamble GD, Mills GD, Garrett JE, Eaton TE, Rees MI (2006) Functional variants of antioxidant genes in smokers with COPD and in those with normal lung function. Thorax 61(5):394–399

106. Dahl M, Bowler RP, Juul K, Crapo JD, Levy S, Nordestgaard BG (2008) Superoxide dismutase 3 polymorphism associated with reduced lung function in two large populations. Am J Respir Crit Care Med 178(9):906–912
107. Harju T, Kaarteenaho-Wiik R, Soini Y, Sormunen R, Kinnula VL (2002) Diminished immunoreactivity of gamma-glutamylcysteine synthetase in the airways of smokers' lung. Am J Respir Crit Care Med 166(5):754–759
108. He JQ, Ruan J, Connett JE, Anthonisen NR, Pare PD, Sandford AJ (2002) Antioxidant gene polymorphisms and susceptibility to a rapid decline in lung function in smokers. Am J Respir Crit Care Med 166(3):323–328
109. Kinnula VL (2005) Focus on antioxidant enzymes and antioxidant strategies in smoking related airway diseases. Thorax 60(8):693–700
110. Juul K, Tybjaerg-Hansen A, Marklund S, Lange P, Nordestgaard BG (2006) Genetically increased antioxidative protection and decreased chronic obstructive pulmonary disease. Am J Respir Crit Care Med 173(8):858–864
111. Mak JC, Ho SP, Yu WC, Choo KL, Chu CM, Yew WW, Lam WK, Chan-Yeung M (2007) Polymorphisms and functional activity in superoxide dismutase and catalase genes in smokers with COPD. Eur Respir J 30(4):684–690
112. Siedlinski M, Postma DS, van Diemen CC, Blokstra A, Smit HA, Boezen HM (2008) Lung function loss, smoking, vitamin C intake, and polymorphisms of the glutamate-cysteine ligase genes. Am J Respir Crit Care Med 178(1):13–19
113. Hayes JD, McMahon M (2009) NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. Trends Biochem Sci 34(4):176–188
114. Kensler TW, Wakabayashi N, Biswal S (2007) Cell survival responses to environmental stresses via the Keap1-Nrf2-are pathway. Annu Rev Pharmacol Toxicol 47:89–116
115. He CH, Gong P, Hu B, Stewart D, Choi ME, Choi AM, Alam J (2001) Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. J Biol Chem 276(24):20858–20865
116. Cho HY, Reddy SP, Kleeberger SR (2006) Nrf2 defends the lung from oxidative stress. Antioxid Redox Signal 8(1–2):76–87
117. Cho HY, Reddy SP, Debiase A, Yamamoto M, Kleeberger SR (2005) Gene expression profiling of NRF2-mediated protection against oxidative injury. Free Radic Biol Med 38(3):325–343
118. Wakabayashi N, Slocum SL, Skoko JJ, Shin S, Kensler TW (2010) When NRF2 talks, who's listening? Antioxid Redox Signal 13(11):1649–1663
119. Nguyen T, Huang HC, Pickett CB (2000) Transcriptional regulation of the antioxidant response element. Activation by Nrf2 and repression by MafK. J Biol Chem 275(20):15466–15473
120. Venugopal R, Jaiswal AK (1996) Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H: quinone oxidoreductase1 gene. Proc Natl Acad Sci USA 93(25):14960–14965
121. Clements CM, McNally RS, Conti BJ, Mak TW, Ting JP (2006) DJ-1, a cancer- and parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. Proc Natl Acad Sci USA 103(41):15091–15096
122. Goven D, Boutten A, Lecon-Malas V, Marchal-Somme J, Amara N, Crestani B, Fournier M, Leseche G, Soler P, Boczkowski J et al (2008) Altered Nrf2/Keap1-Bach1 equilibrium in pulmonary emphysema. Thorax 63(10):916–924
123. He X, Chen MG, Lin GX, Ma Q (2006) Arsenic induces NAD(P)H-quinone oxidoreductase i by disrupting the Nrf2 × Keap1 × Cul3 complex and recruiting Nrf2 × Maf to the antioxidant response element enhancer. J Biol Chem 281(33):23620–23631
124. Apopa PL, He X, Ma Q (2008) Phosphorylation of nrf2 in the transcription activation domain by casein kinase 2 (CK2) is critical for the nuclear translocation and transcription activation function of Nrf2 in IMR-32 neuroblastoma cells. J Biochem Mol Toxicol 22(1):63–76
125. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA (2003) Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol 23(20):7198–7209
126. Cullinan SB, Diehl JA (2004) PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. J Biol Chem 279(19):20108–20117

127. Bloom DA, Jaiswal AK (2003) Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from INrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H: quinone oxidoreductase-1 gene expression. J Biol Chem 278(45):44675–44682
128. Mercado N, Thimmulappa R, Thomas CM, Fenwick PS, Chana KK, Donnelly LE, Biswal S, Ito K, Barnes PJ (2011) Decreased histone deacetylase 2 impairs Nrf2 activation by oxidative stress. Biochem Biophys Res Commun 406(2):292–298
129. Cho HY, Imani F, Miller-DeGraff L, Walters D, Melendi GA, Yamamoto M, Polack FP, Kleeberger SR (2009) Antiviral activity of Nrf2 in a murine model of respiratory syncytial virus disease. Am J Respir Crit Care Med 179(2):138–150
130. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, Biswal S (2006) Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest 116(4):984–995
131. Iizuka T, Ishii Y, Itoh K, Kiwamoto T, Kimura T, Matsuno Y, Morishima Y, Hegab AE, Homma S, Nomura A et al (2005) Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. Genes Cells 10(12):1113–1125
132. Sussan TE, Rangasamy T, Blake DJ, Malhotra D, El-Haddad H, Bedja D, Yates MS, Kombairaju P, Yamamoto M, Liby KT et al (2009) Targeting Nrf2 with the triterpenoid CDDO-imidazolide attenuates cigarette smoke-induced emphysema and cardiac dysfunction in mice. Proc Natl Acad Sci USA 106(1):250–255
133. Malhotra D, Thimmulappa R, Navas-Acien A, Sandford A, Elliott M, Singh A, Chen L, Zhuang X, Hogg J, Pare P et al (2008) Decline in NRF2-regulated antioxidants in chronic obstructive pulmonary disease lungs due to loss of its positive regulator, DJ-1. Am J Respir Crit Care Med 178(6):592–604
134. Nioi P, Hayes JD (2004) Contribution of NAD(P)H: quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. Mutat Res 555(1–2):149–171
135. Blake DJ, Singh A, Kombairaju P, Malhotra D, Mariani TJ, Tuder RM, Gabrielson E, Biswal S (2010) Deletion of Keap1 in the lung attenuates acute cigarette smoke-induced oxidative stress and inflammation. Am J Respir Cell Mol Biol 42(5):524–536
136. Dinkova-Kostova AT, Kostov RV (2012) Glucosinolates and isothiocyanates in health and disease. Trends Mol Med 18(6):337–347
137. Speciale A, Chirafisi J, Saija A, Cimino F (2011) Nutritional antioxidants and adaptive cell responses: an update. Curr Mol Med 11(9):770–789
138. Buchberger A, Bukau B, Sommer T (2010) Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol Cell 40(2):238–252
139. Marcu MG, Doyle M, Bertolotti A, Ron D, Hendershot L, Neckers L (2002) Heat shock protein 90 modulates the unfolded protein response by stabilizing IRE1alpha. Mol Cell Biol 22(24):8506–8513
140. Hu R, Ouyang Q, Dai A, Tan S, Xiao Z, Tang C (2011) Heat shock protein 27 and cyclophilin a associate with the pathogenesis of COPD. Respirology 16(6):983–993
141. Ankersmit HJ, Nickl S, Hoeltl E, Toepker M, Lambers C, Mitterbauer A, Kortuem B, Zimmermann M, Moser B, Bekos C et al (2012) Increased serum levels of HSP27 as a marker for incipient chronic obstructive pulmonary disease in young smokers. Respiration 83(5): 391–399
142. Hacker S, Lambers C, Hoetzenecker K, Pollreisz A, Aigner C, Lichtenauer M, Mangold A, Niederpold T, Zimmermann M, Taghavi S et al (2009) Elevated HSP27, HSP70 and HSP90 alpha in chronic obstructive pulmonary disease: markers for immune activation and tissue destruction. Clin Lab 55(1–2):31–40
143. Cappello F, Caramori G, Campanella C, Vicari C, Gnemmi I, Zanini A, Spanevello A, Capelli A, Rocca GL, Anzalone R et al (2011) Convergent sets of data from in vivo and in vitro methods point to an active role of Hsp60 in chronic obstructive pulmonary disease pathogenesis. PLoS One 6(11):e28200

144. Xie J, Zhao J, Xiao C, Xu Y, Yang S, Ni W (2010) Reduced heat shock protein 70 in airway smooth muscle in patients with chronic obstructive pulmonary disease. Exp Lung Res 36(4):219–226
145. Gal K, Cseh A, Szalay B, Rusai K, Vannay A, Lukacsovits J, Heemann U, Szabo AJ, Losonczy G, Tamasi L et al (2011) Effect of cigarette smoke and dexamethasone on Hsp72 system of alveolar epithelial cells. Cell Stress Chaperones 16(4):369–378
146. Chen W, Syldath U, Bellmann K, Burkart V, Kolb H (1999) Human 60-kDa heat-shock protein: a danger signal to the innate immune system. J Immunol 162(6):3212–3219
147. Dick CA, Dennekamp M, Howarth S, Cherrie JW, Seaton A, Donaldson K, Stone V (2001) Stimulation of IL-8 release from epithelial cells by gas cooker PM(10): a pilot study. Occup Environ Med 58(3):208–210
148. Li CJ, Ning W, Matthay MA, Feghali-Bostwick CA, Choi AM (2007) MAPK pathway mediates EGR-1-HSP70-dependent cigarette smoke-induced chemokine production. Am J Physiol Lung Cell Mol Physiol 292(5):L1297–L1303
149. Doz E, Noulin N, Boichot E, Guenon I, Fick L, Le Bert M, Lagente V, Ryffel B, Schnyder B, Quesniaux VF et al (2008) Cigarette smoke-induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling dependent. J Immunol 180(2):1169–1178
150. Yamashita M, Kimura M, Kubo M, Shimizu C, Tada T, Perlmutter RM, Nakayama T (1999) T cell antigen receptor-mediated activation of the Ras/mitogen-activated protein kinase pathway controls interleukin 4 receptor function and type-2 helper t cell differentiation. Proc Natl Acad Sci USA 96(3):1024–1029
151. Dong C, Yang DD, Wysk M, Whitmarsh AJ, Davis RJ, Flavell RA (1998) Defective T cell differentiation in the absence of Jnk1. Science 282(5396):2092–2095
152. Underwood DC, Osborn RR, Bochnowicz S, Webb EF, Rieman DJ, Lee JC, Romanic AM, Adams JL, Hay DW, Griswold DE (2000) SB 239063, a p38 MAPK inhibitor, reduces neutrophilia, inflammatory cytokines, MMP-9, and fibrosis in lung. Am J Physiol Lung Cell Mol Physiol 279(5):L895–L902
153. Milligan SA, Owens MW, Grisham MB (1998) Differential regulation of extracellular signal-regulated kinase and nuclear factor-kappa B signal transduction pathways by hydrogen peroxide and tumor necrosis factor. Arch Biochem Biophys 352(2):255–262
154. Jope RS, Zhang L, Song L (2000) Peroxynitrite modulates the activation of p38 and extracellular regulated kinases in PC12 cells. Arch Biochem Biophys 376(2):365–370
155. Lee K, Esselman WJ (2002) Inhibition of PTPs by H(2)O(2) regulates the activation of distinct MAPK pathways. Free Radic Biol Med 33(8):1121–1132
156. Mendelsohn J, Baselga J (2000) The EGF receptor family as targets for cancer therapy. Oncogene 19(56):6550–6565
157. Walters MJ, Paul-Clark MJ, McMaster SK, Ito K, Adcock IM, Mitchell JA (2005) Cigarette smoke activates human monocytes by an oxidant-AP-1 signaling pathway: implications for steroid resistance. Mol Pharmacol 68(5):1343–1353
158. Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M (2005) Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 120(5):649–661
159. Rahman A, Fazal F (2011) Blocking NF-kappaB: an inflammatory issue. Proc Am Thorac Soc 8(6):497–503
160. Wullaert A, Bonnet MC, Pasparakis M (2011) NF-kappaB in the regulation of epithelial homeostasis and inflammation. Cell Res 21(1):146–158
161. Thompson JE, Phillips RJ, Erdjument-Bromage H, Tempst P, Ghosh S (1995) I kappa B-beta regulates the persistent response in a biphasic activation of NF-kappa B. Cell 80(4):573–582
162. Whiteside ST, Epinat JC, Rice NR, Israel A (1997) I kappa B epsilon, a novel member of the I kappa B family, controls RelA and cRel NF-kappa B activity. EMBO J 16(6):1413–1426
163. Zabel U, Baeuerle PA (1990) Purified human I kappa B can rapidly dissociate the complex of the NF-kappa B transcription factor with its cognate DNA. Cell 61(2):255–265
164. Pahl HL (1999) Activators and target genes of Rel/NF-kappab transcription factors. Oncogene 18(49):6853–6866

165. Pan MH, Lin-Shiau SY, Lin JK (2000) Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of IkappaB kinase and NFkappaB activation in macrophages. Biochem Pharmacol 60(11):1665–1676
166. Poynter ME, Irvin CG, Janssen-Heininger YM (2003) A prominent role for airway epithelial NF-kappa B activation in lipopolysaccharide-induced airway inflammation. J Immunol 170(12):6257–6265
167. Schreck R, Rieber P, Baeuerle PA (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J 10(8):2247–2258
168. True AL, Rahman A, Malik AB (2000) Activation of NF-kappab induced by H(2)O(2) and TNF-alpha and its effects on ICAM-1 expression in endothelial cells. Am J Physiol Lung Cell Mol Physiol 279(2):L302–L311
169. Vollgraf U, Wegner M, Richter-Landsberg C (1999) Activation of AP-1 and nuclear factor-kappaB transcription factors is involved in hydrogen peroxide-induced apoptotic cell death of oligodendrocytes. J Neurochem 73(6):2501–2509
170. Yang H, Magilnick N, Lee C, Kalmaz D, Ou X, Chan JY, Lu SC (2005) Nrf1 and Nrf2 regulate rat glutamate-cysteine ligase catalytic subunit transcription indirectly via NF-kappaB and AP-1. Mol Cell Biol 25(14):5933–5946
171. Ahn KS, Aggarwal BB (2005) Transcription factor NF-kappaB: a sensor for smoke and stress signals. Ann N Y Acad Sci 1056:218–233
172. Anwar AA, Li FY, Leake DS, Ishii T, Mann GE, Siow RC (2005) Induction of heme oxygenase 1 by moderately oxidized low-density lipoproteins in human vascular smooth muscle cells: role of mitogen-activated protein kinases and Nrf2. Free Radic Biol Med 39(2): 227–236
173. Carayol N, Chen J, Yang F, Jin T, Jin L, States D, Wang CY (2006) A dominant function of IKK/NF-kappaB signaling in global lipopolysaccharide-induced gene expression. J Biol Chem 281(41):31142–31151
174. Rushworth SA, Chen XL, Mackman N, Ogborne RM, O'Connell MA (2005) Lipopolysaccharide-induced heme oxygenase-1 expression in human monocytic cells is mediated via Nrf2 and protein kinase C. J Immunol 175(7):4408–4415
175. Yasumoto K, Okamoto S, Mukaida N, Murakami S, Mai M, Matsushima K (1992) Tumor necrosis factor alpha and interferon gamma synergistically induce interleukin 8 production in a human gastric cancer cell line through acting concurrently on AP-1 and NF-kB-like binding sites of the interleukin 8 gene. J Biol Chem 267(31):22506–22511
176. Nicholson WJ, Slight J, Donaldson K (1996) Inhibition of the transcription factors NF-kappa B and AP-1 underlies loss of cytokine gene expression in rat alveolar macrophages treated with a diffusible product from the spores of aspergillus fumigatus. Am J Respir Cell Mol Biol 15(1):88–96
177. Zhou L, Tan A, Iasvovskaia S, Li J, Lin A, Hershenson MB (2003) Ras and mitogen-activated protein kinase kinase kinase-1 coregulate activator protein-1- and nuclear factor-kappaB-mediated gene expression in airway epithelial cells. Am J Respir Cell Mol Biol 28(6): 762–769
178. Romeo G, Ronchetto P, Luo Y, Barone V, Seri M, Ceccherini I, Pasini B, Bocciardi R, Lerone M, Kaariainen H et al (1994) Point mutations affecting the tyrosine kinase domain of the ret proto-oncogene in hirschsprung's disease. Nature 367(6461):377–378
179. Gruda MC, Kovary K, Metz R, Bravo R (1994) Regulation of Fra-1 and Fra-2 phosphorylation differs during the cell cycle of fibroblasts and phosphorylation in vitro by MAP kinase affects DNA binding activity. Oncogene 9(9):2537–2547
180. Cavigelli M, Dolfi F, Claret FX, Karin M (1995) Induction of c-fos expression through JNK-mediated TCF/Elk-1 phosphorylation. EMBO J 14(23):5957–5964
181. Hill CS, Wynne J, Treisman R (1994) Serum-regulated transcription by serum response factor (SRF): a novel role for the DNA binding domain. EMBO J 13(22):5421–5432
182. Iles KE, Dickinson DA, Watanabe N, Iwamoto T, Forman HJ (2002) AP-1 activation through endogenous H(2)O(2) generation by alveolar macrophages. Free Radic Biol Med 32(12): 1304–1313

183. Barnes PJ, Adcock IM, Ito K (2005) Histone acetylation and deacetylation: importance in inflammatory lung diseases. Eur Respir J 25(3):552–563
184. Chen L, Fischle W, Verdin E, Greene WC (2001) Duration of nuclear NF-kappaB action regulated by reversible acetylation. Science 293(5535):1653–1657
185. Tomita K, Barnes PJ, Adcock IM (2003) The effect of oxidative stress on histone acetylation and IL-8 release. Biochem Biophys Res Commun 301(2):572–577
186. Moodie FM, Marwick JA, Anderson CS, Szulakowski P, Biswas SK, Bauter MR, Kilty I, Rahman I (2004) Oxidative stress and cigarette smoke alter chromatin remodeling but differentially regulate NF-kappaB activation and proinflammatory cytokine release in alveolar epithelial cells. FASEB J 18(15):1897–1899
187. Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM (2004) Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. Biochem Biophys Res Commun 315(1):240–245
188. Rahman I, Marwick J, Kirkham P (2004) Redox modulation of chromatin remodeling: impact on histone acetylation and deacetylation, NF-kappaB and pro-inflammatory gene expression. Biochem Pharmacol 68(6):1255–1267
189. Marwick JA, Kirkham PA, Stevenson CS, Danahay H, Giddings J, Butler K, Donaldson K, Macnee W, Rahman I (2004) Cigarette smoke alters chromatin remodeling and induces proinflammatory genes in rat lungs. Am J Respir Cell Mol Biol 31(6):633–642
190. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R et al (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 11(3):619–633
191. Marciniak SJ, Ron D (2006) Endoplasmic reticulum stress signaling in disease. Physiol Rev 86(4):1133–1149
192. Schroder M, Kaufman RJ (2005) The mammalian unfolded protein response. Annu Rev Biochem 74:739–789
193. Gorlach A, Klappa P, Kietzmann T (2006) The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. Antioxid Redox Signal 8(9–10):1391–1418
194. Hung CC, Ichimura T, Stevens JL, Bonventre JV (2003) Protection of renal epithelial cells against oxidative injury by endoplasmic reticulum stress preconditioning is mediated by ERK1/2 activation. J Biol Chem 278(31):29317–29326
195. Liu H, Miller E, van de Water B, Stevens JL (1998) Endoplasmic reticulum stress proteins block oxidant-induced Ca2+ increases and cell death. J Biol Chem 273(21):12858–12862
196. Zhang K, Kaufman RJ (2008) From endoplasmic-reticulum stress to the inflammatory response. Nature 454(7203):455–462
197. Kelsen SG, Duan X, Ji R, Perez O, Liu C, Merali S (2008) Cigarette smoke induces an unfolded protein response in the human lung: a proteomic approach. Am J Respir Cell Mol Biol 38(5):541–550
198. Min T, Bodas M, Mazur S, Vij N (2011) Critical role of proteostasis-imbalance in pathogenesis of COPD and severe emphysema. J Mol Med (Berl) 89(6):577–593
199. Tagawa Y, Hiramatsu N, Kato H, Sakoh T, Nakajima S, Hayakawa K, Saito Y, Johno H, Takahashi S, Gu L et al (2011) Induction of CCAAT/enhancer-binding protein-homologous protein by cigarette smoke through the superoxide anion-triggered PERK-eIF2alpha pathway. Toxicology 287(1–3):105–112
200. Yang YM, Liu GT (2004) Damaging effect of cigarette smoke extract on primary cultured human umbilical vein endothelial cells and its mechanism. Biomed Environ Sci 17(2):121–134
201. Liu Q, Xu WG, Luo Y, Han FF, Yao XH, Yang TY, Zhang Y, Pi WF, Guo XJ (2011) Cigarette smoke-induced skeletal muscle atrophy is associated with up-regulation of USP-19 via p38 and ERK MAPKs. J Cell Biochem 112(9):2307–2316
202. Hengstermann A, Muller T (2008) Endoplasmic reticulum stress induced by aqueous extracts of cigarette smoke in 3T3 cells activates the unfolded-protein-response-dependent perk pathway of cell survival. Free Radic Biol Med 44(6):1097–1107
203. Jorgensen E, Stinson A, Shan L, Yang J, Gietl D, Albino AP (2008) Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. BMC Cancer 8:229

204. van der Vlies D, Pap EH, Post JA, Celis JE, Wirtz KW (2002) Endoplasmic reticulum resident proteins of normal human dermal fibroblasts are the major targets for oxidative stress induced by hydrogen peroxide. Biochem J 366(Pt 3):825–830
205. Tu BP, Weissman JS (2004) Oxidative protein folding in eukaryotes: mechanisms and consequences. J Cell Biol 164(3):341–346
206. Tu BP, Weissman JS (2002) The FAD- and O(2)-dependent reaction cycle of Ero1-mediated oxidative protein folding in the endoplasmic reticulum. Mol Cell 10(5):983–994
207. Ron D, Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 8(7):519–529
208. Szegezdi E, Logue SE, Gorman AM, Samali A (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 7(9):880–885
209. Malhotra JD, Kaufman RJ (2007) The endoplasmic reticulum and the unfolded protein response. Semin Cell Dev Biol 18(6):716–731
210. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 287(5453):664–666
211. Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH (2006) Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. Mol Cell Biol 26(8):3071–3084
212. Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. Cell 103(2): 239–252
213. Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N, Harding HP, Ron D (2004) Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. Mol Cell Biol 24(23):10161–10168
214. Tabas I (2011) Pulling down the plug on atherosclerosis: finding the culprit in your heart. Nat Med 17(7):791–793
215. Tagawa Y, Hiramatsu N, Kasai A, Hayakawa K, Okamura M, Yao J, Kitamura M (2008) Induction of apoptosis by cigarette smoke via ROS-dependent endoplasmic reticulum stress and CCAAT/enhancer-binding protein-homologous protein (CHOP). Free Radic Biol Med 45(1):50–59
216. Jung EJ, Avliyakulov NK, Boontheung P, Loo JA, Nel AE (2007) Pro-oxidative dep chemicals induce heat shock proteins and an unfolding protein response in a bronchial epithelial cell line as determined by dige analysis. Proteomics 7(21):3906–3918
217. Woo CW, Kutzler L, Kimball SR, Tabas I (2012) Toll-like receptor activation suppresses ER stress factor CHOP and translation inhibition through activation of eIF2B. Nat Cell Biol 14(2):192–200
218. Vij N, Amoako MO, Mazur S, Zeitlin PL (2008) CHOP transcription factor mediates IL-8 signaling in cystic fibrosis bronchial epithelial cells. Am J Respir Cell Mol Biol 38(2):176–184
219. Vij N, Fang S, Zeitlin PL (2006) Selective inhibition of endoplasmic reticulum-associated degradation rescues DeltaF508-cystic fibrosis transmembrane regulator and suppresses interleukin-8 levels: therapeutic implications. J Biol Chem 281(25):17369–17378
220. Woischnik M, Sparr C, Kern S, Thurm T, Hector A, Hartl D, Liebisch G, Mulugeta S, Beers MF, Schmitz G et al (2010) A non-brichos surfactant protein c mutation disrupts epithelial cell function and intercellular signaling. BMC Cell Biol 11:88
221. Neurath MF, Finotto S, Glimcher LH (2002) The role of Th1/Th2 polarization in mucosal immunity. Nat Med 8(6):567–573
222. Kang MJ, Lee CG, Lee JY, Dela Cruz CS, Chen ZJ, Enelow R, Elias JA (2008) Cigarette smoke selectively enhances viral PAMP- and virus-induced pulmonary innate immune and remodeling responses in mice. J Clin Invest 118(8):2771–2784
223. Malhotra D, Thimmulappa R, Vij N, Navas-Acien A, Sussan T, Merali S, Zhang L, Kelsen SG, Myers A, Wise R et al (2009) Heightened endoplasmic reticulum stress in the lungs of patients with chronic obstructive pulmonary disease: the role of Nrf2-regulated proteasomal activity. Am J Respir Crit Care Med 180(12):1196–1207

224. Morse D, Choi AM (2005) Heme oxygenase-1: from bench to bedside. Am J Respir Crit Care Med 172(6):660–670
225. Burrows B, Knudson RJ, Cline MG, Lebowitz MD (1977) Quantitative relationships between cigarette smoking and ventilatory function. Am Rev Respir Dis 115(2):195–205
226. Rennard SI, Vestbo J (2006) Copd: the dangerous underestimate of 15%. Lancet 367(9518): 1216–1219
227. Fletcher CM (1976) The natural history of chronic bronchitis and emphysema: an eight-year study of early chornic obstructive lung disease in working men in London. Oxiford University Press, New York
228. Hackett NR, Heguy A, Harvey BG, O'Connor TP, Luettich K, Flieder DB, Kaplan R, Crystal RG (2003) Variability of antioxidant-related gene expression in the airway epithelium of cigarette smokers. Am J Respir Cell Mol Biol 29(3 Pt 1):331–343
229. Spira A, Beane J, Shah V, Liu G, Schembri F, Yang X, Palma J, Brody JS (2004) Effects of cigarette smoke on the human airway epithelial cell transcriptome. Proc Natl Acad Sci USA 101(27):10143–10148
230. Dombroski BA, Nayak RR, Ewens KG, Ankener W, Cheung VG, Spielman RS (2010) Gene expression and genetic variation in response to endoplasmic reticulum stress in human cells. Am J Hum Genet 86(5):719–729
231. Delepine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C (2000) EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet 25(4):406–409