The Role of Nrf2 in Cellular Innate Immune Response to Inflammatory Injury

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Nuclear factor erythroid derived 2-related factor-2 (Nrf2) is a master transcription regulator of antioxidant and cytoprotective proteins that mediate cellular defense against oxidative and inflammatory stresses. Disruption of cellular stress response by Nrf2 deficiency causes enhanced susceptibility to infection and related inflammatory diseases as a consequence of exacerbated immune-mediated hypersensitivity and autoimmunity. The cellular defense capacity potentiated by Nrf2 activation appears to balance the population of CD4+ and CD8+ of lymph node cells for proper innate immune responses. Nrf2 can negatively regulate the activation of pro-inflammatory signaling molecules such as p38 MAPK, NF-κB, and AP-1. Nrf2 subsequently functions to inhibit the production of pro-inflammatory mediators including cytokines, chemokines, cell adhesion molecules, matrix metalloproteinases, COX-2 and iNOS. Although not clearly elucidated, the antioxidative function of genes targeted by Nrf2 may cooperatively regulate the innate immune response and also repress the expression of pro-inflammatory mediators.

Key words: Nrf2, Innate immunity, Inflammation

INTRODUCTION

The inflammatory stress which is implicated in the vast variety of pathogenic conditions, such as sepsis, is balanced by an array of counter-regulatory molecules that attempt to restore immunological equilibrium (Cohen, 2002). Such counter-inflammatory response should occur timely and appropriately to resolve the inflammatory injury. The innate immune system is recognized as the critical first line of host defense for sensing and neutralizing pathogenic infection (Thimmulappa et al., 2006), and dysregulation of innate immune response can result in persistent tissue damage and may propagate further infection. Host factors that regulate innate immunity may hence influence inflammatory response and susceptibility to infection. Few host genetic factors that are vital for controlling inflammation are known, and various studies have identified genes encoding proteins, such as Toll-like receptors (TLRs), tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS)-binding protein, CD14, and bactericidal/permeability-increasing protein (Thimmulappa et al., 2006), responsible for appropriate innate immune response.

Reactive oxygen species (ROS) are important mediators of inflammation. Nuclear factor-erythroid 2 (NF-E2)-related factor 2 (Nrf2) that belongs to the CNC ("cap 'n' collar") subfamily containing the basic leucine zipper region plays a pivotal role in cellular redox balance and stress response (Kobayashi and Yamamoto, 2005). Nrf2 has a pleiotropic role in regulating the constitutive and inducible expression of a battery of antioxidant and other cytoprotective genes by binding to the cis-acting enhancer sequence referred to as antioxidant response element (ARE) or electrophile response element (EpRE) (Kensler et al., 2007). The Nrf2-responsive antioxidant enzymes play roles in cellular defense by enhancing the removal of cytotoxic electrophilic species or ROS (Lee and Johnson, 2004).

Besides its role in cellular protection against electrophilic and oxidative stresses, many recent studies have demonstrated that Nrf2 responds to inflammatory stimulations and rescues cells/tissues from inflammatory injuries. Because of its anti-inflammatory as well as antioxidant functions, Nrf2 is known as an important
therapeutic target for the treatment and prevention of inflammation-associated disorders (Kim et al., 2009). The importance of Nrf2 as a novel regulator of the innate immune response by protecting against oxidative/inflammatory stress has been recently demonstrated in several animal models. Thus, disruption of this redox-sensitive transcription factor dramatically increases the mortality of mice in response to experimentally-induced septic shock. LPS as well as TNF-α stimulus resulted in greater lung inflammation in Nrf2-deficient mice (Kolls, 2006; Thimmulappa et al., 2006). Likewise, intraperitoneal administration of LPS caused expression of proinflammatory cytokines (e.g., TNF-α and IL-6) and enzymes (COX-2 and iNOS) more in the retina and iridociliary body of Nrf2-deficient mice than in the wild-type animals (Nagai et al., 2009). More recently, Reddy and colleagues (2009) have reported impaired innate immunity against bacterial infection following hyperoxia exposure in Nrf2-deficient mice.

As oxidative stress caused by overproduction and/or inefficient elimination of ROS is implicated in diverse human diseases associated with infection and inflammation, understanding the mechanism by which the Nrf2 protects against oxidative damage can provide the therapeutic and preventive strategies. This review mainly focuses on the role of Nrf2-modulated enzymes in innate immunity and inflammation.

OXIDATIVE STRESS-INDUCED ACTIVATION OF NRF2

In the resting state, the nuclear level of Nrf2 is low as it is sequestered and also degraded in the cytoplasm by the cytoskeleton-associated protein, Kelch-like ECH-associated protein 1 (Keap1) (Kensler et al., 2007; Kobayashi and Yamamoto, 2005). Keap1 functions as a negative regulator of Nrf2 by modulating ubiquitination and proteasomal degradation of Nrf2.

Fig. 1. Nrf2-Keap1 signaling. The cytoplasmic repressor Keap1, bound to actin filaments, acts as a sensor for oxidative and electrophilic stresses through cystein residues. In the absence of stimuli, Keap1 sequesters the transcription factor Nrf2 and acts as an adaptor for Nrf2 ubiquitination and proteasomal degradation. Once electrophiles, ROS or ARE inducers interact with sulfhydryl groups of cysteins in Keap1, ubiquitination switches from Nrf2 to Keap1, leading to the degradation of Keap1 and stabilization and concurrent activation of Nrf2 protein. The phosphorylation of Nrf2 at serine and threonine residues by kinases such as MAPKs, PI3K and PKC is speculated to facilitate the dissociation of Nrf2 from Keap1 and subsequent translocation to the nucleus. After translocation to nucleus, Nrf2 transactivates the expression of genes, which contain AREs in their promoter region. The major function of gene products induced by Nrf2 is cellular detoxification, protection against oxidant damage and regulation of GSH biosynthesis and utilization.
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(Kobayashi et al., 2004). Oxidative stress facilitates the dissociation of Nrf2 from Keap1 and subsequent translocation of Nrf2 into the nucleus where it forms a heterodimer with a small Maf (sMaf) protein (Kensler et al., 2007). The Nrf2-sMaf dimer then binds to ARE/EpRE, a cis-acting DNA regulatory element with a core nucleotide sequence of 5'-GTGACNNNGCN-3', resulting in enhanced transcriptional activation of target genes, which in turn confers adaptive survival response to ongoing or subsequent stresses (Fig. 1).

A widely accepted model for nuclear accumulation and activation of Nrf2 upon oxidative stress involves alteration of the Keap1 structure by oxidation of cysteines (Cys) contained in Keap1 (Na and Surh, 2006). In this context, reactive sulfhydryl groups present in some critical Cys residues of Keap1 function as sensors for ROS (Dinkova-Kostova et al., 2002). Alternatively, ROS can activate Nrf2 directly by phosphorylating the specific serine and threonine residues of this transcription factor. The following session deals with Nrf2-induced transcriptional activation of some representative genes whose protein products are involved in innate immunity and protection against inflammatory damage.

NRF2-MEDIATED INDUCTION OF ANTIOXIDANT ENZYMES AND THEIR ROLES IN INNATE IMMUNITY AND ANTI-INFLAMMATION

Heme oxygenase (HO-1). HO-1 has both antioxidant and anti-inflammatory properties. HO-1 promoter contains ARE, and the activation of Nrf2 enhances HO-1 expression in several cell types (Kim et al., 2009). It was reported that regulation of the HO-1 gene expression by Nrf2 is based on the availability of free-heme (Srisook et al., 2005). Under normal cellular conditions, a mammalian transcriptional repressor, Bach1 heteromerizes with sMaf, binds to ARE, and subsequently interferes with Nrf2-binding to ARE. However, under conditions producing oxidative/nitrosative stresses, cellular hemeprotein releases heme, which preferentially binds to Bach1 at its heme-binding motif and thus hampers Bach1 binding to ARE (Srisook et al., 2005). Free heme-derived de-repression of Bach1 was suggested to facilitate Nrf2 to activate the transcription of HO-1 gene (Fig. 2A) (Sun et al., 2002; Srisook et al., 2005).

Protein-bound heme catalyzes electron transfer reactions in cells and is integral to aerobic life. On the other hand, the free heme released as a consequence of degradation of heme proteins by oxidative stress is toxic, because it can catalyze the Fenton reaction and cause oxidative damage to cellular macromolecules. In this respect, HO-1 plays an important role in removal of the cytotoxic pro-oxidant free heme accumulated in cells. HO-1 catalyzes the oxidation of free heme and generates equimolar amounts of ferrous iron (Fe^{2+}), carbon monoxide (CO), and biliverdin (Wagener et al., 2003). Biliverdin is then reduced in bilirubin, a lipid-soluble antioxidant, by biliverdin reductase, in mammals (Fig. 2B) (Wagener et al., 2003). Pro-oxidant Fe^{2+} produced by HO-1 is quickly eliminated by iron storage protein ferritin, which is induced by Nrf2 as well (Wagener et al., 2003; Chen and Kunsch, 2004). The heme-derived metabolites, including CO, biliverdin and bilirubin generated by HO-1, have been shown to scavenge ROS such as lipid peroxyl radicals (LOO*/LOOH),...
superoxide anion \( \left( \text{O}_2^- \right) \), hydrogen peroxide \( \left( \text{H}_2\text{O}_2 \right) \), and peroxynitrite \( \left( \text{ONOO}^- \right) \) (Wagener et al., 2003).

Recent research has revealed that HO-1 is a critical modulator of the innate immunity and inflammation. For instance, HO-1 levels are frequently elevated in acute inflammatory disorders, and it was suggested that monocyte HO-1 production confers potent anti-inflammatory effects against excessive cell or tissue injury caused by oxidative stress and cytokinemia (Yachie et al., 2003). Up-regulation of HO-1 ameliorated the inflammatory damages in an endotoxic shock model (Tamion et al., 2007), airway mucus (Almolki et al., 2008), vascular endothelium (Lin et al., 2008), colon (Horvath et al., 2008), and brain (Cuadrado and Rojo, 2008; Syapin, 2008). Moreover, HO-1 attenuated skin inflammation and contact hypersensitivity, and also accelerated wound healing after epithelial injury in mice (Pae et al., 2008; Patil et al., 2008).

The antioxidative properties of heme-derived metabolites CO, biliverdin, and bilirubin were suggested to account for potent anti-inflammatory effects of HO-1. CO was reported to inhibit LPS-induced activation of endothelial cells (Sun et al., 2008a). CO attenuated the inflammatory insult in the liver of thermally injured mice (Sun et al., 2008b). Treatment with a CO-releasing molecule ameliorated joint inflammation and erosion in collagen-induced murine arthritis (Ferrandiz et al., 2008) and enhanced the host defense response to microbial sepsis in mice (Chung et al., 2008). The HO-1/CO-biliverdin pathway was found to down-regulate neutrophil rolling, adhesion and migration in acute inflammation (Freitas et al., 2006). Biliverdin was shown to protect against polymicrobial sepsis (Overhaus et al., 2006), endotoxin-induced acute lung injury (Sarady Andrews et al., 2005) and colitis (Berberat et al., 2005). Bilirubin, another metabolite derived by HO-1, also exerts an important role in innate immunity and inflammatory response. Bilirubin suppressed experimental autoimmune encephalomyelitis and autoimmune hepatitis (Sawada et al., 1997; Liu et al., 2003). It also inhibited iNOS expression and NO production and protected against endotoxic shock in mice and rats (Wang et al., 2004; Lanone et al., 2005; Kadl et al., 2007). Bilirubin attenuated vascular endothelial activation and dysfunction and inhibited VCAM-1-mediated transendothelial leukocyte migration (Kawamura et al., 2005; Keshavan et al., 2005).

An important cross-talk between HO-1 and the TLR system was reported (Shen et al., 2005; Tsuchihashi et al., 2007). Over-expression of HO-1 down-regulated the activation of STAT-1 via the type-1 interferon (IFN) pathway, downstream of TLR-4, indicating that HO-1 exerts adaptive, cytoprotective and anti-inflammatory functions in the context of innate TLR-4 activation (Tsuchihashi et al., 2007). In studying the mechanism of innate immunity by which HO-1 attenuated the pro-inflammatory responses that are triggered via TLR4 signaling, it was found that TLR4 also negatively modulated HO-1 expression (Shen et al., 2005). In many cases, compounds which enhance the expression of HO-1 also inhibit the pro-inflammatory activation of NF-κB (Chaea et al., 2007; Pang et al., 2008). In fact, HO-1 over-expression, HO-1 induction, or treatment with heme metabolites reversed interleukin (IL)-18-mediated activation of p38 mitogen-activated protein kinase (MAPK) and NF-κB in endothelial cells (Zabalgoitia et al., 2008). HO-1 inhibited the expression of adhesion molecules associated with endothelial cell activation by removing iron and ROS and blocking NF-κB RelA phosphorylation at serine 276 (Seldon et al., 2007). HO-1 activity also influences regulatory T cells in the lungs and prevents smoke-induced B-cell infiltrates (Brandsma et al., 2008). HO-1 augmented production of IL-10 and transforming growth factor (TGF)-β and foxp3+CD4+CD25+ Treg cell function, thereby leading to attenuation of airway inflammation (Xia et al., 2007).

**NAD(P)H:quinone oxidoreductase (NQO1) and NRH:quinone oxidoreductase (NQO2).** Nir2 is a major transcription factor regulating the gene expression of NQO1 and NQO2 (Venugopal and Jaiswal, 1996; Wang and Jaiswal, 2006). NQO1 and NQO2 are cytosolic flavoproteins that oxidize NAD(P)H and NRH, respectively, and catalyze two-electron reduction of quinones to less reactive forms of these molecules (hydroquinones) (Fig. 3) (Ross, 2004; Halliwell and Guttridge, 2007). The obligatory two-electron reduction of quinones catalyzed by NQO1 competes with the one-electron reduction catalyzed by cytochrome P450 reductases and other flavoprotein enzymes, which generate unstable semiquinones (Jaiswal, 2000). NQO1 also keeps coenzyme Q (ubiquinone) in a reduced antioxidant state (ubiquinol) to retain membrane-stabilizing activity (Ross et al., 2000). Ubiquinols can readily inactivate the oxygen free radicals and thus, prevent the oxygen free radical-derived damage to biomolecules and also prevent the initiation of lipid peroxidation (Niki, 1997). The NQO1 and NQO2 proteins hence detoxify endogenous and exogenous quinones and their derivatives, thereby preventing oxidative stress that may arise as a result of their redox cycling (Ross, 2004).

Both NQO1-null and NQO2-null mice showed enhanced susceptibility to autoimmune diseases and predisposition to collagen-induced arthritis (Iskander et al.,
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Electron Metabolism of Menadione by One-Electron Reductive Processes Leading to Production of Unstable Semiquinone Radical

**Fig. 3.** Detoxification of quinone by NQO1. Metabolism of menadione (vitamin K3) by an one-electron (1e/W) reducing enzyme generates an unstable semiquinone radical, with further one-electron (1e/W) reduction to the stable hydroquinone. Back oxidation generates ROS (O2) when oxygen is present. NQO1 metabolizes menadione by one-step, two-electron (2e/2W) reduction directly to the hydroquinone, with no ROS production. Inhibition of NQO1 may cause preferential metabolism of menadione by one-electron reductive processes leading to production of unstable semiquinone radical which generates ROS (Cridle et al., 2007).

2006). The double knockout mice deficient in NQO1 and NQO2 showed the increased number and the size of bronchial-associated lymphoid tissue (BALT) with age and exacerbated infiltration of neutrophils and macrophages in BALT (Das et al., 2006). These mice also exhibited significantly increased levels of cytokines, such as TNF-α, IL-6, and IL-1β in the serum, and iNOS and nitric oxide (NO) in lung macrophages (Das et al., 2006). The disruption in intracellular redox balance resulting from the loss of NQO1 and NQO2 was suggested to cause alterations in the levels of cytokines and chemokines that control inflammation and homing of lymphocytes and neutrophils (Das et al., 2006). Transfecting the airway epithelial cells with NQO1 reduced diesel extract-induced chemokine IL-8 production (Ritz et al., 2007). A close correlation between NQO1 induction and suppression of pro-inflammatory iNOS was observed in murine hepatoma cells, indicating that these processes are functionally linked (Dinkova-Kostova et al., 2005b).

**Glutamate Cysteine Ligase (GCL).** Nrf2 plays a key role in modulating the intracellular reduced glutathione (GSH) level by regulating the expression of GCL in aerob...
Fig. 4. (A) Schematic diagram for GSH synthesis. GSH biosynthesis involves two enzymatic steps catalyzed by GCL and glutathione synthetase (Rahman, 2005). Among the cytosolic GCL proteins, 80% is inactive under physiological conditions due to binding to GSH. A decreased GSH level triggers less binding of GSH to GCL, makes GCL physiologically active, and hence enhances GSH synthesis. GSH synthetase rapidly converts GCL-ligated γ-glutamyl-cysteine to γ-glutamyl-cysteinyl-glycine, GSH. (B) Action of GSH and TRX-dependent enzymes regulated by Nrf2. GSH peroxidase metabolizes H$_2$O$_2$, generating H$_2$O and oxidized GSH (GSSG). GSH reductase regenerates GSH. Peroxiredoxin uses GSH and/or TRX as an electron donor for peroxidation of H$_2$O$_2$, resulting in generation of GSSG and/or oxidized TRX, respectively. Oxidized glutathione (GSSG) and/or oxidized TRX are converted to their reduced forms by GSH reductase and/or TRX reductase, respectively.

GSH depletion led to enhanced susceptibility to oxidative stress, increased inflammatory cytokine release and impaired T cell-responses (Hartl et al., 2005). Over-expression of GCL in rat hepatoma cells completely inhibited TNF-α-induced activation of NF-κB, AP-1 and JNK (Manna et al., 1999). GSH negatively regulates TNF-α-induced p38 MAPK activation and RANTES production in human bronchial epithelial cells (Hashimoto et al., 2001). Enhanced GSH synthesis inhibited the activation of NF-κB and the release of TNF-α in LPS-stimulated alveolar type II (AT-II) epithelial cells of rat lung (Zhang et al., 2008). GSH-mediated inhibition of p38 activation was also observed in LPS-stimulated rat peritoneal macrophages, together with lowered iNOS and COX-2 expression (Sun et al., 2006). It was reported that GSH-mediated redox-status determines Th1/Th2 balance in innate immune response (Peterson et al., 1998; Murata et al., 2002a, 2002b). In general, the Th1 pattern is characterized by IL-12 and interferon-γ (IFN-γ) production and the Th2 response pattern is characterized by IL-4, IL-10 and IL-13 produc-
tion (Romagnani, 1994). GSH depletion inhibited Th1-associated cytokine production and/or favored Th2-associated responses (Peterson et al., 1998). Increases in GSH attenuated Th2-responses including production of IL-4 (Jeannin et al., 1995) and IL-12 (p70) (Koike et al., 2007). IFN-γ predominated when the GSH level was high, but declined when GSH was depleted (Romagnani, 1994). In a murine asthma model, intraperitoneal injection of the GSH precursor enhanced the production of IL-12 and IFN-γ, but reduced the levels of IL-4, IL-5, IL-10 and the chemokines eotaxin and RANTES in BALF (Koike et al., 2007).

**GSH and thioredoxin (TRX)-dependent enzymes.**

GSH and TRX-dependent enzymes (i.e., GSH peroxidase, GSH reductase, GSH S-transferase, peroxiredoxin, and TRX reductase) are coordinately regulated by the Nrf2-ARE signaling (Chen and Kunsch, 2004). GSH and TRX systems constitute the major cellular reducing power and free radical scavenging activity in the body. GSH peroxidase and peroxiredoxin metabolize H₂O₂, generating H₂O and oxidized glutathione (GSSG) and/or oxidized thioredoxin (Fig. 4B). GSH reductase and TRX reductase regenerate GSH and TRX, respectively, for GSH peroxidase and peroxiredoxin to reuse them. The coordinated regulation of genes encoding aforementioned enzymes by Nrf2 may provide a synergistic effect on maintaining the proper levels of GSH and TRX, eliminating ROS, and subsequently attenuating the inflammation processes.

Lack of GSH peroxidase accelerated inflammatory response in brain (Flentjar et al., 2002), diabetes-associated atherosclerosis (Lewis et al., 2007) and gastrointestinal inflammation (Chu et al., 2004). The reduced activity of GSH peroxidase was associated with the pathogenesis and severity of asthma (Misso et al., 1996). Transgenic mice over-expressing GSH peroxidase were able to modulate host response during endotoxemic conditions (Mirochnitchenko et al., 2000), and showed significant reduction of chemokines and IL-6 in experimentally induced stroke (Ishibashi et al., 2002). A GSH peroxidase mimetic inhibited LPS-induced COX-2 protein expression in RAW264.7 macrophage cells (Nakamura et al., 2002). The treatment of endothelial cells with GSH peroxidase mimetic prevented TNF-α production and neutrophil-induced endothelial alterations (Moutet et al., 1998) and suppressed TNF-α-induced VCAM-1 and ICAM-1 gene expression (d’Alessio et al., 1998).

Peroxiredoxin utilizes redox-active cysteines to reduce peroxides, lipid hydroperoxides, and peroxinitrites (Kisucka et al., 2008). It was found that peroxiredoxin I protects against excessive endothelial activation and atherosclerosis (Kisucka et al., 2008). Peroxiredoxin II negatively regulated LPS-induced inflammatory signaling through modulation of ROS synthesis via NADPH oxidase and therefore, prevented excessive host response to microbial products (Yang et al., 2007).

Thioredoxin-1 (TRX-1) reduced cigarette smoke-induced systemic inflammatory responses, lung inflammation and emphysema in mice (Sato et al., 2008). TRX-1 suppressed airway hyper-responsiveness and prevented the development of airway remodeling by inhibiting production of chemokines and Th2 cytokines in an asthma model (Imaoka et al., 2007). Human TRX-1 ameliorated experimental murine colitis in association with reduced macrophage inhibitory factor (MIF) production (Tamaki et al., 2006). Over-expression of TRX-1 prevented the development of chronic pancreatitis via the suppression of oxidative stress and MCP-1-mediated chronic inflammation (Ohashi et al., 2006). TRX also protected against joint destruction in a murine arthritis model (Tsui et al., 2006).

**CD36.**

Nrf2 has been shown to regulate the expression of CD36 in murine macrophages (Ishii et al., 2004). Thus, CD36 gene expression was not inducible in Nrf2 knockout mice (Iizuka et al., 2005). Multiple ARE-like sequences were found in the promoter region of murine CD36-encoding gene (GeneBank accession No. AF434766) (Ishii et al., 2004). Agents that increase CD36 expression have anti-inflammatory activities by inducing anti-inflammatory cytokine IL-10 (Parsons et al., 2008). CD36 mediates uptake of oxidatively modified low-density lipoproteins (oxLDL), which is known to cause atherosclerosis (Steinberg, 1997; Ross, 1999). CD36 also enhances phagocytosis of apoptotic neutrophils (Greaves et al., 1998; Steinbrecher, 1999; Asada et al., 2004). Since Nrf2 knockout mice showed the significantly smaller number of macrophages that engulfed neutrophils (Iizuka et al., 2005), Nrf2 may play an important role in neutrophil clearance via CD36 induction and thereby protect against neutrophilic inflammation.

**Secretory leukocyte protease inhibitor (SLPI).**

Nrf2 has been shown to induce the expression of the SLPI gene in macrophages (Iizuka et al., 2005; Ishii et al., 2005). SLPI is a cationic serine protease inhibitor with anti-microbial and anti-inflammatory properties present in large quantities in mucosal fluids, including saliva (Angelov et al., 2004). SLPI is a pivotal endogenous factor necessary for optimal tissue repair including intra-oral wound healing and limiting neutrophil
elastase-induced pulmonary inflammation (Bingle and Tetley, 1996; Angelov et al., 2004). Adenoviral delivery of SLPI gene attenuated NF-κB-dependent inflammatory signaling in human endothelial cells and macrophages in response to atherogenic stimuli (Henriksen et al., 2004). Mice lacking SlPI are susceptible to LPS-induced endotoxic shock (Nakamura et al., 2003).

**CONCLUDING REMARKS**

The new aspect of Nrf2 in innate immunity and inflammation has been recently highlighted. Innate immune response is known to be influenced by cellular redox state (Kolls, 2006). Nrf2-mediated transactivation of antioxidant and other cytoprotective genes play an important role in cellular defence against infections by reducing the production of pro-inflammatory mediators. CO, biliverdin and bilirubin produced as by-products of reactions catalyzed by HO-1 have anti-inflammatory effects. NQO1, another antioxidant enzyme up-regulated by Nrf2, inhibits cytokine-induced VCAM-1 activation. In several inflammatory pathogenic conditions, a protective role of GSH formed by GCL was observed. GSH peroxidase and peroxiredoxin can eliminate hydrogen peroxide, which otherwise activates pro-inflammatory signaling. Nrf2 was also found to induce neutrophil clearance and uptake of oxLDLs through CD36 induction. Nrf2 induces the expression of SlPI which inhibits NF-κB activation and protects against protease-mediated damage. Endogenous molecules such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) and KGF were found to cause Nrf2 activation, which accounts for their anti-inflammatory potential. In general, the coordinated induction of genes targeted by Nrf2 is essential in mounting the innate immune responses upon inflammatory insult.

Intracellular ROS have a fundamental role in pro-inflammatory responses through the activation of redox-sensitive protein kinases and transcription factors such as NF-κB and AP-1. It has been suggested that Nrf2 may inactivate NF-κB, AP-1 and p38 MAPK that mediate proinflammatory signaling (Table 1). Nrf2 was shown to inhibit the expression of proinflammatory cytokines, chemokines, cell adhesion molecules, MMPs, iNOS, and COX-2. Furthermore, Nrf2 deficiency exacerbates inflammatory responses and severe tissue injuries. The activity of Nrf2 influences the pathogenesis of lupus-like autoimmune diseases, rheumatoid arthritis, asthma, emphysema, gastritis, colitis, atherosclerosis and neuroinflammation. Nrf2 also plays an important role in balancing the population of CD4+ and CD8+ lymph node cells, and Nrf2 deficiency enhances the production of Th2 (CD4+) cytokine and IgE. An association between polymorphism in the Nrf2 promoter and enhanced susceptibility to acute lung injury and some inflammatory ailments was demonstrated (Reddy et al., 2006).

### Table 1. The relationship between Nrf2 and inflammation-regulating factors such as NF-κB, AP-1 and p38 MAPK

| The evidence that the over-expression of Nrf2 or Nrf2 activity inducers negatively regulates the activation of NF-κB in response to stimulation |
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| Nrf2 knockout cell increased NF-κB activity in response to LPS or TNF-α (Thimmulappa et al., 2006). |
| Dithiolethione showed inhibition of LPS-induced NF-κB activity (Karuri et al., 2006). |
| Benzyl isothiocyanates inhibited LPS/IFN-γ-mediated NF-κB activity (Murakami et al., 2003). |
| Sulforaphane inhibited LPS-induced NF-κB activity (Heiss et al., 2001). |
| Phenolic antioxidant, 1,4-dihydroquinone inhibited LPS-induced NF-κB activation (Ma and Kinneer, 2002). |
| 15d-PGJ2 has been shown to inhibit NF-κB activity (Kim and Surh, 2006). |
| EGCG inhibited NF-κB activity (Gopalakrishnan and Tony Kong, 2008). |
| Curcumin inhibited NF-κB activity (Gopalakrishnan and Tony Kong, 2008). |

| The evidence that the over-expression of Nrf2 or Nrf2 activity inducers negatively regulates the activation of AP-1 in response to stimulation |
|---|
| Transfection with PI3K (the upstream kinase activator of Nrf2) expression plasmid inhibited shear-induced AP-1 (c-Jun) expression (Healy et al., 2005). |
| Resveratrol inhibited TNF-induced activation of AP-1 activity (Manna et al., 2000). |
| 15d-PGJ2 inhibited AP-1 activity (Kim and Surh, 2006). |
| Curcumin inhibited AP-1 activity (Gopalakrishnan and Tony Kong, 2008). |
| Sulforaphane inhibited UVB-induced AP-1 activation (Zhu et al., 2004). |
| Dithiolethiones inhibited shear-induced JNK2 and AP-1 (c-Jun) expression (Healy et al., 2005). |

| The evidence that the over-expression of Nrf2 or Nrf2 activity inducers negatively regulates the activation of p38 MAPK in response to stimulation |
|---|
| The expression of Nrf2, using Nrf2-containing adenovirus, inhibited the activation of p38 MAPK in response to TNF-α (Chen et al., 2006). |
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Agents activating Nrf2 signaling have been shown to protect against rheumatoid arthritis, gastritis, colitis, atherosclerosis and intracerebral hemorrhage. Adeno-viral gene transfer of Nrf2 was also protective against atherosclerosis and kidney inflammation. Overall, Nrf2 is a master transcription regulator of diverse stress response genes whose protein products protect tissues and cells against infection and inflammation-mediated disease development (Fig. 5). Strong inducers for Nrf2 activation or devices which can efficiently deliver Nrf2 gene to target tissues could be developed as effective therapeutics for inflammation-mediated disorders (Kim et al., 2009).

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REFERENCES

Aburaya, M., Tanaka, K., Hoshino, T., Tsutsumi, S., Suzuki, K., Makise, M., Akagi, R. and Mizushima, T. (2006). Heme oxygenase-1 protects gastric mucosal cells against non-steroidal anti-inflammatory drugs. J. Biol. Chem., 281, 33422-33432.

Almolki, A., Guenegou, A., Golda, S., Boyer, L., Benallaoua, M., Amara, N., Bachoual, R., Martin, C., Rannou, F., Lanone, S., Dulak, J., Burgel, P.R., El-Benna, J., Leynaert, A.B., Aubier, M. and Boczkowski, J. (2008). Heme oxygenase-1 prevents airway mucus hypersecretion induced by cigarette smoke in rodents and humans. Am. J. Pathol., 173, 981-992.

Angelov, N., Moutsopoulos, N., Jeong, M.J., Nares, S., Ashcroft, G. and Wahl, S.M. (2004). Aberrant mucosal wound repair in the absence of secretory leukocyte protease inhibitor. Thromb. Haemost., 92, 288-297.

Arisawa, T., Tahara, T., Shibata, T., Nagasaka, M., Nakamura, M., Kamiya, Y., Fujita, H., Hasegawa, S., Takagi, T., Wang, F.Y., Hirata, I. and Nakano, H. (2007). The relation-

Fig. 5. Proposed molecular events of the genes targeted by Nrf2 and their role in innate immunity and anti-inflammation. Innate immunity is the host's first line of defence against infection. However, the defensive innate immune response against infection can be a double-edged sword. The protective process of innate immunity response can develop deleterious excessive inflammation. A delicate balance between innate immunity and inflammation is therefore required, making it possible to fight pathogens effectively while limiting inflammation that might be damaging to the host. Transcription factor Nrf2 might regulate this delicate balance for the proper innate immune response.
ship between Helicobacter pylori infection and promoter polymorphism of the Nrf2 gene in chronic gastritis. *Int J. Mol Med.*, **19**, 143-148.

Asghar, M., George, L. and Lokhandwala, M.F (2007). Antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. *J. Biol. Chem.*, **278**, 703-711.

Cho, H.Y., Reddy, S.P. and Kleeberger, S.R. (2006). Nrf2 defends the lung from oxidative stress. *Antioxidants & Redox Signaling*, **8**, 76-87.

Cho, H.Y., Jedlicka, A.E., Reddy, S.P., Zhang, L.Y., Kensler, T.W. and Kleeberger, S.R. (2002a). Linkage analysis of susceptibility to hyperoxia. Nrf2 is a candidate gene. *Am. J. Res. Cell Mol. Biol.*, **26**, 42-51.

Cho, H.Y., Jedlicka, A.E., Reddy, S.P., Kensler, T.W., Yamamoto, M., Zhang, L.Y. and Kleeberger, S.R. (2002b). Role of Nrf2 in protection against hyperoxic lung injury in mice. *Am. J. Res. Cell Mol. Biol.*, **26**, 175-182.

Chu, F.F., Esworthy, R.S. and Doroshow, J.H. (2004). Role of Se-dependent glutathione peroxidases in gastrointestinal inflammation and cancer. *Free Radic. Biol. Med.*, **36**, 1481-1495.

Chung, S.W., Liu, X., Macias, A.A., Baron, R.M. and Perrell, M.A. (2008). Heme oxygenase-1-derived carbon monoxide enhances the host defense response to microbial sepsis in mice. *J. Clin. Investig.*, **118**, 239-247.

Cohen, J. (2002). The immunopathogenesis of sepsis. *Nature*, **420**, 885-889.

Criddle, D.N., Gerasimenko, J.V., Baumgartner, H.K., Jaffar, M., Voronina, S., Sutton, R., Petersen, O.H. and Gerasimenko, O.V. (2007). Calcium signalling and pancreatic cell death: apoptosis or necrosis? *Cell Death Differ.*, **14**, 1285-1294.

Cuadrado, A. and Rojo, A.I. (2008). Heme oxygenase-1 as a therapeutic target in neurodegenerative diseases and brain infections. *Current Pharmaceutical Design*, **14**, 429-442.

Cuzzocrea, S., Costantino, G., Zingarelli, B., Mazzen, E., Miceli, A. and Caputi, A.P. (1999). The protective role of endogenous glutathione in carrageenan-induced pleurisy in the rat. *Eur. J. Pharmacol.*, **372**, 187-197.

d’Alessio, P., Moutet, M., Coudrier, E., Darquenne, S. and Chaudriere, J. (1998). ICAM-1 and VCAM-1 expression induced by TNF-alpha are inhibited by a glutathione peroxidase mimic. *Free Radic. Biol. Med.*, **24**, 979-987.

Das, A., Kole, L., Wang, L., Barrios, R., Moorby, B. and Jaiswal, A.K. (2006). BALF development and augmentation of hyperoxic lung injury in mice deficient in NQO1 and NQO2. *Free Radic. Biol. Med.*, **40**, 1843-1856.

Dinkova-Kostova, A.T., Holtzclaw, W.D., Cole, R.N., Itoh, K., Wakabayashi, N., Kato, Y., Yamamoto, M. and Talalay, P. (2002). Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. USA*, **99**, 11908-11913.

Dinkova-Kostova, A.T., Holtzclaw, W.D. and Kensler, T.W. (2005a). The role of Keap1 in cellular protective responses. *Chem. Res. Toxicol.*, **18**, 1779-1791.

Dinkova-Kostova, A.T., Liby, K.T., Stephenson, K.K., Holtzclaw, W.D., Gao, X., Suh, N., Williams, C., Risingsong, R., Honda, T., Gribble, G.W., Sporn, M.B. and Talalay, P. (2005b). Extremely potent interpenoid inducers of the phase 2 response: correlations of protection against oxi-
dant and inflammatory stress. *Proc. Natl. Acad. Sci. USA, 102*, 4584-4589.

Ferrandiz, M.L., Maicas, N., Garcia-Amandis, I., Terencio, M.C., Motterlini, R., Devesa, I., Joosten, L.A., van den Berg, W.B. and Alcaraz, M.J. (2008). Treatment with a CO-releasing molecule (CORM-3) reduces joint inflammation and erosion in murine colon-derived arthritis. *Ann. Rheum. Dis.*, 67, 1211-1217.

Flenjar, N.J., Crack, P.J., Boyd, R., Malin, M., de Haan, J.B., Hertzog, P., Kola, I. and Iannello, R. (2002). Mice lacking glutathione peroxidase-1 activity show increased TUNEL staining and an accelerated inflammatory response in brain following a cold-induced injury. *Exp. Neurol.*, 177, 9-20.

Freitas, A., Alves-Filho, J.C., Secco, D.D., Neto, A.F., Ferrreira, S.H., Barja-Fidalgo, C. and Cunha, F.Q. (2006). Heme oxygenase/carbon monoxide-biliverdin pathway down regulates neutrophil rolling, adhesion and migration in acute inflammation. *Br. J. Pharmacol.*, 149, 345-354.

Gopalaratnam, A. and Kong, A.N.T. (2008). Anticarcinogenesis by dietary phytochemicals: cytoprotection by Nrf2 in normal cells and cytotoxicity by modulation of transcription factors NF-kappa B and AP-1 in abnormal cancer cells. *Food Chem. Toxicol.*, 46, 1257-1270.

Greaves, D.R., Gough, P.J. and Gordon, S. (1998). Recent progress in defining the role of scavenger receptors in lipid transport, atherosclerosis and host defence. *Curr. Opin. Lipidol.*, 9, 425-432.

Hollweil, B. and Gutteridge, J.M.C. (2007). *Free Radicals in Biology and Medicine*. Oxford University Press, New York.

Hartl, D., Starosta, V., Maier, K., Beck-Speier, E., Huber, R.M., Rietschel, E., Krauss-Etschmann, S. and Griese, M. (2005). Inhaled glutathione decreases PGE2 and increases lymphocytes in cystic fibrosis lungs. *Free Radic. Biol. Med.*, 39, 463-472.

Hashimoto, S., Gon, Y., Matsumoto, K., Takeshita, I., Machino, T. and Horie, T. (2001). Intracellular glutathione regulates tumour necrosis factor-alpha-induced p38 MAP kinase activation and RANTES production by human bronchial epithelial cells. *Clin. Exp. Allergy, 31*, 144-151.

Hassan, M.Q., Hadi, R.A., Al-Rawi, Z.S., Padron, V.A. and Stohs, S.J. (2001). The glutathione defense system in the pathogenesis of rheumatoid arthritis. *J. Appl. Toxicol.*, 21, 69-73.

Healy, Z.R., Lee, N.H., Gao, X., Goldring, M.B., Talay, P., Kensler, T.W. and Konstantopoulos, K. (2005). Divergent responses of chondrocytes and endothelial cells to shear stress: cross-talk among COX-2, the phase 2 response, and apoptosis. *Proc. Natl. Acad. Sci. USA, 102*, 14010-14015.

Heiss, E., Herhaus, C., Klimo, K., Bartsch, H. and Gerhauser, C. (2001). Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J. Biol. Chem.*, 276, 32006-32015.

Henriksen, P.A., Hitt, M., Xing, Z., Wang, J., Haslett, C., Riemersma, R.A., Webb, D.J., Kotelevtsev, Y.V. and Salenave, J.M. (2004). Adenoviral gene delivery of elafin and secretory leukocyte protease inhibitor attenuates NF-kappa B-dependent inflammatory responses of human endothelial cells and macrophages to atherogenic stimuli. *J. Immunol.*, 172, 4535-4544.

Hong, F., Freeman, M.L. and Liebler, D.C. (2005). Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. *Chem. Res. Toxicol.*, 18, 1917-1926.

Horvath, K., Varga, C., Berko, A., Posa, A., Laszlo, F. and Whittle, B.J. (2008). The involvement of heme oxygenase-1 activity in the therapeutic actions of 5-aminosalicylic acid in rat colitis. *Eur. J. Pharmacol.*, 581, 315-323.

Hubbs, A.F., Benkovic, S.A., Miller, D.B., O’Callaghan, J.P., Battelli, L., Schwegler-Berry, D. and Ma, Q. (2007). Vacular leukoencephalopathy with widespread astrogliosis in mice lacking transcription factor Nrf2. *Am. J. Pathol.*, 170, 2068-2076.

Iizuka, T., Ishii, Y., Itoh, K., Kiwamoto, T., Kimura, T., Matsuno, Y., Morishima, Y., Hegab, A.E., Homma, S., Nomura, A., Sakamoto, T., Shimura, M., Yoshida, A., Yamamoto, M. and Sekizawa, K. (2005). Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. *Genes Cells, 10*, 1113-1125.

Iomaoka, H., Hoshino, T., Takei, S., Sakazaki, Y., Kinoshita, T., Okamoto, M., Kawayama, Y., Yodoi, J., Kato, S., Iwana­ga, T. and Aizawa, H. (2007). Effects of thiorodoxin on established airway remodeling in a chronic antigen exposure asthma model. *Biochem. Biophys. Res. Commun.*, 360, 525-530.

Innamorato, N.G., Rojo, A.I., Garcia-Yague, A.J., Yamamoto, M., de Ceballos, M.L. and Cuadrado, A. (2008). The transcription factor Nrf2 is a therapeutic target against brain inflammation. *J. Immunol.*, 181, 680-689.

Ishibashi, N., Prokopenko, O., Reuhl, K.R. and Mirochnichenko, O. (2002). Inflammatory response and glutathione peroxidase in a model of stroke. *J. Immunol.*, 168, 1926-1933.

Ishii, T., Itoh, K., Ruiz, E., Leake, D.S., Unoki, H., Yamamoto, M. and Mann, G.E. (2004). Role of Nrf2 in the regulation of CD36 and stress protein expression in murine macrophages: activation by oxidatively modified LDL and 4-hydroxynonenal. *Circulation Res.*, 94, 609-616.

Ishii, Y., Itoh, K., Morishima, Y., Kimura, T., Kiwamoto, T., Iizuka, T., Hegab, A.E., Hosoya, T., Nomura, A., Sakamoto, T., Yamamoto, M. and Sekizawa, K. (2005). Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. *J. Immunol.*, 175, 6968-6975.

Iskander, K., Li, J., Han, S., Zheng, B. and Jaiswal, A.K. (2006). NQO1 and NQO2 regulation of humoral immunity and autoimmunity. *J. Biol. Chem.*, 281, 30917-30924.

Jaiswal, A.K. (2000). Regulation of genes encoding NAD(P)H:quinone oxidoreductases. *Free Radic. Biol. Med.*, 29, 254-262.

Jeannin, P., Delineste, Y., Lecanozet-Henchoz, S., Gauchat, J.F., Life, P., Holmes, D. and Bonefroy, J.Y. (1995). Thiol- decrease human interleukin (IL) 4 production and IL-4-induced immunoglobulin synthesis. *J. Exp. Med.*, 182, 1785-1792.

Kadl, A., Pontiller, J., Exner, M. and Leitinger, N. (2007). Sin­
gle bolus injection of bilirubin improves the clinical outcome in a mouse model of endotoxemia. Shock, 28, 582-588.

Karuri, A.R., Huang, Y., Bodreddigari, S., Sutter, C.H., Roebuck, B.D., Kensler, T.W. and Sutter, T.R. (2006). H.12-dithiole-3-thione targets nuclear factor kappaB to block expression of inducible nitric-oxide synthase, prevents hypotension, and improves survival in endotoxemic rats. J. Pharmacol. Exp. Ther., 317, 61-67.

Kataoka, K., Handa, H. and Nishizawa, M. (2001). Induction of cellular antioxidant stress genes through heterodimeric transcription factor Nrf2/small Maf by antirheumatic gold(I) compounds. J. Biol. Chem., 276, 34074-34081.

Kawamura, K., Ishikawa, K., Wada, Y., Kimura, S., Matsumoto, H., Kohro, M., Itabe, H., Kodama, T. and Maruyama, Y. (2005). Bilirubin from heme oxygenase-1 attenuates vascular endothelial activation and dysfunction. Arteriosclerosis, Thrombosis, and Vascular Biology, 25, 155-160.

Kensler, T., Waakabayashi, N. and Biswal, S. (2007). Cell survival response to environmental stresses via Keap1-Nrf2-ARE pathway. Annu. Rev. Pharmacol. Toxicol., 47, 89-116.

Keshavan, P., Deem, T.L., Schwemberger, S.J., Babcock, Q.F., Cook-Mills, J.M. and Zucker, S.D. (2005). Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. J. Immunol., 174, 3708-3718.

Khor, L.O., Huang, M.T., Kwon, K.H., Chan, J.Y., Reddy, B.S., Keshavan, P., Deem, T.L., Schwemberger, S.J., Babcock, Q.F., Cook-Mills, J.M. and Zucker, S.D. (2005). Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. J. Immunol., 174, 3708-3718.

Lee, J.M., Kan, Y.W. and Johnson, J.A. (2006). Neuronal sensitivity to kainic acid is dependent on the Nrf2-mediated actions of the antioxidant response element. J. Neurochem., 98, 1852-1865.

Lanone, S., Bloc, S., Foresti, R., Almokli, A., Taille, C., Callebert, J., Conti, M., Goven, D., Aubier, M., Dureuil, B., El-Benna, J., Motterlini, R. and Boczowski, J. (2005). Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implications for protection against endotoxic shock in rats. FASEB J., 19, 1890-1892.

Lee, J.M., Chan, K., Kan, Y.W. and Johnson, J.A. (2004). Targeted disruption of Nrf2 causes regenerative immune-mediated hemolytic anemia. Proc. Natl. Acad. Sci USA, 101, 9751-9756.

Lee, J.M. and Johnson, J.A. (2004). An important role of Nrf2-ARE pathway in the cellular defence mechanism. J. Biochem. Mol. Biol., 37, 139-143.

Lee, S.H., Sohn, D.H., Jin, X.Y., Kim, S.W., Choi, S.C. and Seo, G.S. (2007). 2',4',6'-tris(methoxymethoxy) chalcone protects against trinitrobenzene sulfonic acid-induced colitis and blocks tumor necrosis factor-alpha-induced intestinal epithelial inflammation via heme oxygenase-1-dependent and independent pathways. Biochem. Pharmacol., 74, 870-880.

Levonen, A.L., Inkala, M., Heikura, T., Jauhiainen, S., Jyrkkonen, H.K., Kansanen, E., Maatta, K., Romppanen, E., Turunen, P., Rutanen, J. and Yla-Herttuala, S. (2007). Nrf2 gene transfer induces antioxidant enzymes and suppresses smooth muscle cell growth in vitro and reduces oxidative stress in rabbit aorta in vivo. Arteriosclerosis, Thrombosis, and Vascular Biology, 27, 741-747.

Lewis, P., Stefanovic, N., Pete, J., Calkin, A.C., Giunti, S., Thallas-Bonke, V., Jandelet-Dahm, K.A., Allen, T.J., Kola, I., Cooper, M.E. and de Haan, J.B. (2007). Lack of the antioxidant enzyme glutathione peroxidase-1 accelerates atherosclerosis in diabetic apolipoprotein E-deficient mice. Circulation, 115, 2178-2187.

Li, N. and Nel, A.E. (2006). Role of the Nrf2-mediated signaling pathway as a negative regulator of inflammation: implications for the impact of particulate pollutants on asthma. Antioxid. Redox Signal., 8, 88-98.

Lin, C.C., Liu, X.M., Peyton, K., Wang, H., Yang, W.C., Lin, S.J. and Durante, W. (2008). Far infrared therapy inhibits vascular endothelial inflammation via the induction of heme oxygenase-1. Arteriosclerosis, Thrombosis, and Vascular Biology, 28, 739-745.

Liu, X.M., Peyton, K.J., Ensenat, D., Wang, H., Hannink, M., Alam, J. and Durante, W. (2007). Nitric oxide stimulates heme oxygenase-1 gene transcription via the Nrf2/ARE complex to promote vascular smooth muscle cell survival. Cardiovasc Res., 75, 381-389.

Liu, Y., Zhu, B., Wang, X., Luo, L., Li, P., Paty, D.W. and Cynader, M.S. (2003). Bilirubin as a potent antioxidant suppresses experimental autoimmune encephalomyelitis: implications for the role of oxidative stress in the development of multiple sclerosis. J. Neuroimmunol., 139, 27-35.

Ma, Q. and Kinneer, K. (2002). Chemoprotection by phenolic antioxidants. Inhibition of tumor necrosis factor alpha induction in macrophages. J. Biol. Chem., 277, 2477-2484.

Ma, Q., Battelli, L. and Hubbs, A.F. (2005). Multifactor autoim-
mune inflammation, enhanced lymphoproliferation, and impaired homeostasis of reactive oxygen species in mice lacking the antioxidant-activated transcription factor Nrf2. *Am. J. Pathol.*, 168, 1960-1974.

Manna, S.K., Kuo, M.T. and Aggarwal, B.B. (1999). Overexpression of gamma-glutamylcysteine synthetase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappa B and activator protein-1. *Oncogene*, 18, 4371-4382.

Manna, S.K., Mukhopadhyay, A. and Aggarwal, B.B. (2000). Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J. Immunol.*, 164, 6509-6519.

Marzec, J.M., Christie, J.D., Reddy, S.P., Jedlicka, A.E., Vuong, H., Lanken, P.N., Aplenc, R., Yamamoto, T., Yamamoto, M., Cho, H.Y. and Kleeberger, S.R. (2007). Functional polymorphisms in the transcription factor NRF2 in humans increase the risk of acute lung injury. *FASEB J.*, 21, 2237-2246.

Matthews, G.M. and Butler, R.N. (2005). Cellular mucosal defense during Helicobacter pylori infection: a review of the role of glutathione and the oxidative pentose pathway. *Helicobacter*, 10, 298-306.

Mirochnitchenko, O., Prokopenko, O., Palnitkar, U., Kister, I., Powers, K.S. and Inouye, M. (2000). Endotoxemia in mice overexpressing human glutathione peroxidases. *Circulation Res.*, 87, 289-295.

Misso, N.L., Powers, K.A., Gillon, R.L., Stewart, G.A. and Thompson, P.J. (1995). Reduced platelet glutathione peroxidase activity and serum selenium concentration in atopic asthmatic patients. *Clin. Exp. Allergy*, 26, 838-847.

Mochizuki, M., Ishii, Y., Itoh, K., Iizuka, T., Morishima, Y., Kimura, T., Kiwamoto, T., Matsuno, Y., Hegab, A.E., Nomura, A., Sakamoto, T., Uchida, K., Yamamoto, M. and Sekizawa, K. (2005). Role of 15-deoxy-D12,14-prostaglandin J2 and Nrf2 pathways in protection against acute lung injury. *Am. J. Respir. Critical Care Medicine*, 171, 1260-1266.

Moutet, M., d’Alessio, P., Malette, P., Devaux, V. and Chaudiere, J. (1998). Glutathione peroxidase mimics prevent TNF-alpha- and neutrophil-induced endothelial alterations. *Free Radic. Biol. Med.*, 25, 270-281.

Murakami, A., Matsumoto, K., Koshimizu, K. and Ohigashi, H. (2003). Effects of selected food factors with chemopreventive properties on combined lipopolysaccharide- and interferon-gamma-induced IkappaB degradation in RAW264.7 macrophages. *Cancer Letters*, 195, 17-25.

Murata, Y., Shimamara, T. and Hamuro, J. (2002a). The polarization of TH1/TH2 balance is dependent on the intracellular thiol redox status of macrophages due to the distinctive cytokine production. *Int. Immunol.*, 14, 201-212.

Murata, Y., Amao, M., Yoneda, J. and Hamuro, J. (2002b). Intracellular thiol redox status of macrophages directs the Th1 skewing in thioredoxin transgenic mice during aging. *Mol. Immunol.*, 38, 747-757.

Na, H.-K. and Surh, Y.-J. (2006). Transcriptional regulation via cysteine thiol modification: a novel molecular strategy for chemoprevention and cytoprotection. *Mol. Carcinog.*, 45, 368-380.

Nakamura, A., Mori, Y., Hagiwara, K., Suzuki, T., Sakakibara, T., Kikuchi, T., Igarashi, T., Ebina, M., Abe, T., Miyazaki, J., Takai, T. and Nukiwa, T. (2003). Increased susceptibility to LPS-induced endotoxin shock in secretory leukoprotease inhibitor (SLPI)-deficient mice. *J. Exp. Med.*, 197, 669-674.

Nagai, N., Thimmulappa, R.K., Cano, M., Fujihara, M., Izumi-Nagai, K., Kong, X., Sporn, M.B., Kensler, T.W., Biswal, S. and Handa, J.T. (2009). Nrf2 is a critical modulator of the innate immune response in a model of uveitis. *Free Radic. Biol. Med.*, 47, 300-306.

Nakamura, Y., Feng, Q., Kumagai, T., Tonikai, K., Ohigashi, H., Osawa, T., Noguchi, N., Niki, E. and Uchida, K. (2002). Ebselen, a glutathione peroxidase mimetic seleno-organic compound, as a multifunctional antioxidant. Implication for inflammation-associated carcinogenesis. *J. Biol. Chem.*, 277, 2687-2694.

Niki, E. (1997). Mechanisms and dynamics of antioxidant action of ubiquinol. *Molecular Aspects of Medicine*, 18, Suppl., S83-70.

Ohashi, S., Nishio, A., Nakamura, H., Asada, M., Tamaki, H., Kawasaki, K., Fukui, T., Yodoi, J. and Chiba, T. (2006). Overexpression of redox-active protein thioredoxin-1 prevents development of chronic pancreatitis in mice. *Antioxidants & Redox Signaling*, 8, 1835-1845.

Ortolani, O., Conti, A., De Gaudio, A.R., Moraldi, E. and Novelli, G.P. (2002). [Glutathione and N-acetylcysteine in the prevention of free-radical damage in the initial phase of septic shock] *Recent Prog. Med.*, 93, 125-129.

Osburn, W.O., Karim, B., Dolan, P.M., Liu, G., Yamamoto M., Huso, D.L. and Kensler, T.W. (2007). Increased colonic inflammatory injury and formation of aberrant crypt foci in Nrf2-deficient mice upon dextran sulfate treatment. *Int. J. Cancer*, 121, 1883-1891.

Overhaus, M., Moore, B.A., Barbato, J.E., Behrendt, F.F., Doering, J.G. and Bauer, A.J. (2006). Biliverdin protects against polymicrobial sepsis by modulating inflammatory mediators. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 290, G695-703.

Pae, H.O., Ae Ha, Y., Chai, K.Y. and Chung, H.T. (2008). Heme oxygenase-1 attenuates contact hypersensitivity induced by 2,4-dinitrofluorobenzene in mice. *Immunopharmacol. Immunotoxicol.*, 30, 207-216.

Pae, H.O., Oh, G.S., Lee, B.S., Rim, J.S., Kim, Y.M. and Chung, H.T. (2006). 3-Hydroxyanthranilic acid, one of L-tryptophan metabolites, inhibits monocyte chemotactic protein-1 secretion and vascular cell adhesion molecule-1 expression via heme oxygenase-1 induction in human umbilical vein endothelial cells. *Atherosclerosis*, 187, 274-284.

Pang, R., Zhang, S.L., Zhao, L., Liu, S.L., Dong, J.H. and Tao, J.Y. (2008). Effect of petroleum ether extract from Melilotus suaveolens Ledeb on the expression of NF-kappaB and Heme oxygenase-1. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*, 24, 861-863.

Parsons, M.S., Barrett, L., Little, C. and Grant, M.D. (2008). Harnessing CD36 to rein in inflammation. *Endocr. Metab.
Rangasamy, I., Guo, J., Mitzner, WA, Roman, J., Singh, A, Roum, J.H., Borok, Z., McElvaney, N.G, Grimes, GJ., Rahman, I. (2005). Regulation of glutathione in inflammation.

Romagnani, S. (1994). Lymphokine production by human T cells in disease states.

Rahman, I. and MacNee, W (2000). Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches.

Roth, M. and Black, J.L. (2006). Transcription factors in asthma: are transcription factors a new target for asthma therapy? Current Drug Targets, 7, 589-595.

Roum, J.H., Borok, Z., McElvaney, N.G., Grimes, G.J., Bokser, A.D., Buhl, R. and Crystal, R.G. (1999). Glutathione aerosol suppresses lung epithelial surface inflammatory cell-derived oxidants in cystic fibrosis. J. Appl. Physiol., 87, 438-443.

Sarady-Andrews, J.K., Liu, F., Gallo, D., Nakao, A., Overhaus, M., Ollinger, R., Choi, A.M. and Otterbein, L.E. (2005). Biliverdin administration protects against endotoxin-induced acute lung injury in rats. Am. J. Physiol. Lung Cell. Mol. Physiol., 289, L1131-1137.

Sato, A., Hoshino, Y., Hara, T., Muro, S., Nakamura, H., Mishima, M. and Yodoi, J. (2008). Thioredoxin-1 ameliorates cigarette smoke-induced lung inflammation and emphysema in mice. J. Pharmacol. Exp. Ther., 325, 380-388.

Sawada, K., Ohnishi, K., Kosaka, T., Chikano, S., Egashira, A., Okui, M., Shintani, S., Wada, M., Nakasho K. and Shimoyma, T. (1997). Exacerbated autoimmune hepatitis successfully treated with leukocytepheresis and bilirubin adsorption therapy. J. Gastroenterol., 32, 689-695.

Seldon, M.P., Silva, G., Pejanovic, N., Larse, R., Gregoire, I.P., Filipe, J., Arrander, J. and Soares, M.P. (2007). Heme oxygenase-1 inhibits the expression of adhesion molecules associated with endothelial cell activation via inhibition of NF-kappaB RelA phosphorylation at serine 276. J. Immunol., 179, 7840-7851.

Shen, X.D., Ke, B., Zhai, Y., Gao, F., Busuttill, R.W., Cheng, G. and Kupiec-Weglinski, J.W. (2005). Toll-like receptor and heme oxygenase-1 signaling in hepatic ischemia/reperfusion injury. Am. J. Transplant., 5, 1793-1800.

Srisook, K., Kim, C. and Cha, Y.N. (2005). Molecular mechanisms involved in enhancing HO-1 expression: de-repression by heme and activation by Nrf2, the "one-two" punch. Antioxidants & Redox Signaling, 7, 1674-1687.

Steinberg, D. (1997). Low density lipoprotein oxidation and its pathobiological significance. J. Biol. Chem., 272, 20693-20966.

Steinbrecher, U.P. (1999). Receptors for oxidized low density lipoprotein. Biochim. Biophys. Acta, 1436, 279-298.

Sun, B., Zou, X., Chen, Y., Zhang, P. and Shi, G. (2008a). Preconditioning of carbon monoxide releasing molecule-derived CO attenuates LPS-induced activation of HUVEC. Int. J. Biol. Sci., 4, 270-278.

Sun, B.W., Sun, Y., Sun, Z.W. and Chen, X. (2008b). CO liberated from CORM-2 modulates the inflammatory response in the liver of thermally injured mice. World J. Gastroenterol., 14, 547-553.

Sun, J., Hoshino, H., Takaku, K., Nakajima, O., Muto, A., Suzuki, H., Tashiro, S., Takahashi, S., Shibahara, S., Alam, J., Taketo, M.M., Yamamoto, M. and Igarashi, K. (2002). Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. EMBO J., 21, 5216-5224.

Sun, S., Zhang, H., Xue, B., Wu, Y., Wang, J., Yin, Z. and Luo, L. (2006). Protective effect of glutathione against lipopolysaccharide-induced inflammation and mortality in rats. Inflamm. Res., 55, 504-510.

Syapin, P.J. (2008). Regulation of haeme oxygenase-1 for treatment of neuroinflammation and brain disorders. Br. J. Pharmacol., 155, 623-640.

Tamaki, H., Nakamura, H., Nishio, A., Nakase, H., Ueno, S., Uza, N., Kido, M., Inoue, S., Mikami, S., Asada, M., Kiriy, K., Kitamura, H., Ohashi, S., Fukui, T., Kawasaki, K., Matsuura, M., Ishii, Y., Okazaki, K., Yodoi, J. and Chiba,
T. (2006). Human thioredoxin-1 ameliorates experimental murine colitis in association with suppressed macrophage inhibitory factor production. Gastroenterology, 131, 1110-1121.

Tamion, F., Richard, V., Renet, S. and Thuillez, C. (2007). Intestinal preconditioning prevents inflammatory response by modulating heme oxygenase-1 expression in endotoxin shock model. Am. J. Physiol. Gastrointest. Liver Physiol., 293, G1308-1314.

Thimmulappa, R.K., Lee, H., Rangasamy, T., Reddy, S.P., Yamamoto, M., Kessler, T.W. and Biswal, S. (2006). Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J. Clin. Investig., 116, 984-995.

Tirouvanziam, R., Conrad, C.K., Bottiglieri, T., Herzenberg, L.A. and Moss, R.B. (2005). High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. Proc. Natl. Acad. Science USA, 103, 4628-4633.

Tsuchihashi, S., Zhai, Y., Bo, Q., Busuttil, R.W. and Kupiec-Weglinski, J.W. (2007). Heme oxygenase-1mediated cytoprotection against liver ischemia and reperfusion injury: inhibition of type-1 interferon signaling. Transplantation, 83, 1628-1634.

Tsujii, G., Koshiba, M., Nakamura, H., Kosaka, H., Hatachi, S., Kurimoto, C., Kurosaka, M., Hayashi, Y., Yodoi, J. and Kumagai, S. (2006). Thioredoxin protects against joint destruction in a murine arthritis model. Free Radic. Biol. Med., 40, 1721-1731.

Venugopal, R. and Jaiswal, A.K. (1996). Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quione oxidoreductase 1 gene. Proc. Natl. Acad. Sci. USA, 93, 14960-14965.

Villa, P., Saccani, A., Sica, A. and Ghezzi, P. (2002). Glutathione protects mice from lethal sepsis by limiting inflammation and potentiating host defense. J Infect. Dis., 185, 1115-1120.

Wagener, F.A., Volk, H.D., Willis, D., Abraham, N.G., Soares, M.P., Adema, G.J. and Figdor, C.G. (2003). Different faces of the heme-heme oxygenase system in inflammation. Pharmacol. Rev., 55, 551-571.

Wang, J., Fields, J., Zhao, C., Langer, J., Thimmulappa, R.K., Kessler, T.W., Yamamoto, M., Biswal, S. and Dore, S. (2007). Role of Nrf2 in protection against intracerebral hemorrhage injury in mice. Free Radic. Biol. Med., 43, 408-414.

Wang, W. and Jaiswal, A.K. (2006). Nuclear factor Nrf2 and antioxidant response element regulate NRH:quinone oxidoreductase 2 (NQO2) gene expression and antioxidant induction. Free Radic. Biol. Med., 40, 1119-1130.

Wang, W.W., Smith, D.L. and Zucker, S.D. (2004). Bilirubin inhibits iNOS expression and NO production in response to endotoxin in rats. Hepatology, 40, 424-433.

Xia, Z.W., Xu, L.Q., Zhong, W.W., Wei, J.J., Li, N.L., Shao, J., Li, Y.Z., Yu, S.C. and Zhang, Z.L. (2007). Heme oxygenase-1 attenuates ovalbumin-induced airway inflammation by up-regulation of foxp3 T-regulatory cells, interleukin-10, and membrane-bound transforming growth factor-1. Am. J. Pathol., 171, 1904-1914.

Yachie, A., Toma, T., Mizuno, K., Okamoto, H., Shimura, S., Ohta, K., Kasahara, Y. and Koizumi, S. (2003). Heme oxygenase-1 production by peripheral blood mononcytes during acute inflammatory illnesses of children. Exp. Biol. Med. (Maywood), 228, 550-556.

Yang, C.S., Lee, D.S., Song, C.H., An, S.J., Li, S., Kim, J.M., Kim, C.S., Yoo, D.G., Jeon, B.H., Yang, H.Y., Lee, T.H., Lee, Z.W., El-Benna, J., Yu, D.Y. and Jo, E.K. (2007). Roles of peroxiredoxin II in the regulation of proinflammatory responses to LPS and protection against endotoxin-induced lethal shock. J. Exp. Med., 204, 583-594.

Yoh, K., Itoh, K., Enomoto, A., Hirayama, A., Yamaguchi, N., Kobayashi, M., Morito, N., Koyama, A., Yamamoto, M. and Takahashi, S. (2001). Nrf2-deficient female mice develop lupus-like autoimmune nephritis. Kidney International, 60, 1343-1353.

Zabalgoitia, M., Colston, J.T., Reddy, S.V., Holt, J.W., Regan, R.F., Stoc, D.E., Rimoldi, J.M., Valente, A.J. and Chandrasekar, B. (2008). Carbon monoxide donors or heme oxygenase-1 (HO-1) overexpression blocks interleukin-15-mediated NF-kappaB-PTEN-dependent human cardiac endothelial cell death. Free Radic. Biol. Med., 44, 284-298.

Zhang, F., Wang, X., Wang, W., Li, N. and Li, J. (2008). Glutamine reduces tnf-alpha by enhancing glutathione synthesis in lipopolysaccharide-stimulated alveolar epithelial cells of rats. Inflammation, 31, 344-350.

Zhang, X., Lu, L., Dixon, C., Wilmer, W., Song, H., Chen, X. and Rovin, B.H. (2004). Stress protein activation by the cyclopentenone prostaglandin 15-deoxy-delta12,14-prostaglandin J2 in human mesangial cells. Kidney International, 65, 798-810.

Zhao, X., Sun, G., Zhang, J., Strong, R., Dash, P.K., Kan, Y.W., Grotta, J.C. and Aronowski, J. (2007). Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. Stroke, 38, 3280-3286.

Zhu, M., Zhang, Y., Cooper, S., Sikorski, E., Rohwer, J. and Bowden, G.T. (2004). Phase II enzyme inducer, sulforaphane, inhibits UVB-induced AP-1 activation in human keratinocytes by a novel mechanism. Mol. Carcinog., 41, 179-186.