Hirosaki Med. J. 59 (Suppl.) : S167—S171, 2007

# THE ROLE OF NRF2 IN THE PROTECTION AGAINST INFLAMMATION AND INNATE IMMUNITY

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**Abstract** NF-E2-related factor 2 (Nrf2) regulates the coordinate induction of phase 2 detoxifying and antioxidant enzymes in response to xenobiotics and oxidative stress via antioxidant responsive element. Nrf2 knockout mice are highly susceptible to the acute toxicity generated by acetaminophen, butylated hydroxytoluene or hyperoxia and to carcinogenesis induced by benzo[a]pyrene. Recently, it becomes increasingly evident that Nrf2 also plays essential roles in the protection against inflammation. Nrf2 regulates the inflammation during carrageenin-induced pleurisy and lung inflammation, wound healing in skin, and dextran sulfate-induced colitis. Nrf2 knockout mice are also susceptible to endotoxin-induced septic shock and allergen-induced asthma. Using carrageenin-induced pleurisy and porcine neutrophil elastase-induced lung injury models, we proposed that this anti-inflammatory action of Nrf2 relies on the activities in macrophages. In macrophages, Nrf2 modulates the inflammatory response by up-regulating a range of anti-oxidative and anti-inflammatory enzymes, such as CD36 and secretory leukoprotease inhibitor (SLPI). CD36 is a macrophage class B scavenger receptor involved in the uptake of apoptotic neutrophils and microorganisms such as *Staphylococcus aureus*. Thus, Nrf2 may play important roles in the protection of inflammation and innate immune response.

Hirosaki Med. J. **59, Supplement** : S167—S171, 2007

**Key words:** Nrf2; antioxidant responsive element (ARE); macrophage

## Introduction

When organisms encounter electrophiles in food or environmental particles, a battery of genes are induced via antioxidant responsive elements (AREs) or electrophile responsive elements (EpREs) to mobilize a coordinate cytoprotective response[1]. Nrf2 belongs to the Cap'n'collar (CNC) family of transcription factors and regulates this innate stress response via binding to ARE[2]. The Nrf2-ARE system regulates expression of numerous cytoprotective enzymes, including glutathione-S-transferase, NAD (P) H:quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1), and the subunits of $\gamma$-glutamylcysteine synthetase ($\gamma$-GCS). Under non-stimulus condition, Keap1 (Kelch-like ECH-associated protein1) negatively regulates nuclear translocation of Nrf2 and facilitates degradation of Nrf2 via proteasome[3,4]. Upon exposure to xenobiotics, Nrf2 is liberated from Keap1-dependent degradation and accumulates in the nucleus.

Because of the lack of this coordinated cytoprotective response, Nrf2 knockout mice ($Nrf2^{-/-}$ mice) are highly susceptible to the acute toxicity generated by acetaminophen[5], butylated hydroxytoluene[6] or hyperoxia[7] and to carcinogenesis induced by benzo[a]pyrene[1,2,8]. Nrf2 knockout mice are also susceptible to DNA adduct formation provoked by diesel exhaust particle[9], aflatoxin[10] and benzo[a]pyrene[11]. Recent microarray analysis in Nrf2 knockout mice has expanded the range of Nrf2 target genes to include NADPH-generating enzymes such as malic enzymes and glucose 6-phosphate dehydrogenase, phase 3 detoxifying enzymes such as MRP1, and a group of 26S proteasome subunits[12,13]. In

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macrophages, Nrf2 regulates a distinct subset of gene batteries to modulate inflammation. In addition to the classical Nrf2 target genes, Nrf2 appears to regulate the macrophage specific target genes such as SLPI[14] and CD36[15]. In this review article, we discuss the recent advances in the role of Nrf2 in inflammation and innate immunity focusing on the roles of macrophage CD36 in the downstream of Nrf2.

## Nrf2 modulates a variety of inflammatory response

Recent studies have revealed that the Nrf2-ARE pathway plays important roles in the regulation of inflammation and autoimmune diseases, as well as in protection against tumor formation[2]. Nrf2 knockout mice are susceptible to a variety of inflammatory diseases such as carrageenin-induced pleurisy and lung inflammation[16,17], cigarette smoke-induced emphysema[18], elastase-induced lung injury[14], allergen-induced asthma[19], endotoxin- and cecal ligation and punture-induced septic shock[20] and dextran sulfate sodium-induced colitis[21]. On the other hand, natural substances that are known to activate ARE-mediated gene expression, such as curcumin, sulforaphane and resveratrol are known to exert anti-inflammatory activity[22,23].

## The mechanisms of anti-inflammatory activity of macrophage Nrf2 gene battery

Using carrageenin-induced pleurisy and lung injury models, we previously demonstrated that Nrf2 is activated by cyclooxygenase 2/15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ pathway in macrophages during inflammation[16,17]. We also demonstrated using porcine neutrophil elasetase-induced lung injury model that wild-type bone marrow transplantation to Nrf2 knockout mice significantly ameliorates the exacerbated inflammation in Nrf2 knockout mice[14]. It is thus plausible that it might be macrophages through which Nrf2 exerts its anti-inflammatory function during inflammation. In these disease models, the increased numbers and/or deleterious effect of neutrophils (ex. increased level of neutrophil elastase activity) were one of the hallmarks in the exacerbation of inflammation in Nrf2 knockout mice[16-19]. Therefore, proinflammatory cytokines, such as IL-1$\beta$ or possibly leukotriene B4 (LTB4) might be increased and contribute to the exacerbated inflammation in Nrf2 knockout mice[24,25]. Interestingly, it has been previously shown that Nrf2 regulates the expression of dithiolethione-inducible gene-1 (DIG1) gene that degrades LTB4[24]. In cigarette smoke-induced emphysema, the neutrophil elastase is known to damage the alveolar wall and lead to alveolar wall destruction. We demonstrated that Nrf2 activated in macrophage induces the production of SLPI that inhibits neutrophil elastase[16]. Therefore, the defense mechanism against the neutrophil-induced tissue damage might be one of the Nrf2-mediated anti-inflammatory responses.

## The role of CD36 in the inflammation and innate immunity

Macrophages and dendritic cells are the main 'professional phagocytes' in the body and play an important role in the first line host defense. Scavenger receptors are integral membrane proteins that bind to a wide variety of ligands including modified or oxidized low-density lipoproteins (oxLDL), apoptotic cells and pathogens. CD36, one of the class B scavenger receptors, has been reported to recognize thrombospondin, fatty acids, oxLDL, apoptotic neutrophils[26]. We previously demonstrated that oxLDLs cause Nrf2 nuclear accumulation in murine peritoneal macrophages and regulates the activation of CD36[15]. In cigarette smoke-induced emphysema model, CD36 gene expression is induced in the lung in an Nrf2-dependent manner[18]. Furthermore, the uptake of apoptotic neutrophils in the macrophage was reduced,

leading to the increase of neutrophil number in the lungs of Nrf2 knockout mice[17]. Recently, it has been shown that CD36 acts as a co-receptor molecule against Toll like receptor (TLR) 2 and 6 [27,28]. Also, it has been reported that CD36 plays an important role in the regulation of dendritic cell activation[29]. Apoptotic cells and thrombospondin 1 transduce anti-inflammatory signals through CD36 in dendritic cells[30,31]. Thus, CD36 is one of the important anti-inflammatory factors downstream of Nrf2 in macrophages.

## Inhibition of ROS formation contributes to the anti-inflammatory mechanism of Nrf2

In Nrf2 knockout mice, the increased formation of reactive oxygen species (ROS) appears to induce a variety cytokines that exacerbate inflammation. Thus, Nrf2 play critical roles in preventing the excessive formation of ROS during inflammation[32]. In endotoxin-induced sepsis, Thimmulappa et al demonstrated that both Myd88-dependent NF-$\kappa$B activation and Myd88-independent IRF3 activation were increased in Nrf2 knockout mice[20,32]. As this increased response of TLR pathways is blocked by *N*-acetylcysteine, increased formation of ROS may contribute to the increased inflammatory reactions in Nrf2 knockout mice. In endothelial cells, it is known that the overexpression of either Nrf2 or NQO1 inhibits the induction of VCAM-1 (vascular cell adhesion molecule 1) promoter activity by tumor necrosis factor-$\alpha$ (TNF-$\alpha$) in human microvascular endothelial cells[33]. Since TNF-$\alpha$ induction of VCAM-1 involves the generation of ROS, the effect of Nrf2 or NQO1 overexpression might be also attributable to the neutralization of the ROS [34].

## Perspectives

Accumulating evidences demonstrate that the Nrf2-mediated gene battery is important in the protection against inflammation. Chronic inflammation underlies the pathogenesis of diseases such as chronic obstructive pulmonary disease or chronic inflammatory bowel disease. Since such diseases are worldwide causes of morbidity and mortality, an effective treatment is in great want. Thus, activation of Nrf2 by dietary natural food contents might be a useful therapeutic approach for the prevention and the treatment of inflammation-based diseases.

## References

1) Kensler TW, Wakabayashi N, Biswal S. Cell Survival Responses to Environmental Stresses Via the Keap1-Nrf2-ARE Pathway. Annu Rev Pharmacol Toxicol 2007;47:89-116.

2) Tong KI, Kobayashi A, Katsuoka F, Yamamoto M. Two-site substrate recognition model for the Keap1-Nrf2 system: a hinge and latch mechanism. Biol Chem 2006;387:1311-20.

3) Kobayashi M, Yamamoto M. Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. Adv Enzyme Regul 2006;46:113-40.

4) Itoh K, Tong KI, Yamamoto M. Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. Free Radic Biol Med 2004;36:1208-13.

5) Enomoto A, Itoh K, Nagayoshi E, et al. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicol Sci 2001;59:169-77.

6) Chan K, Kan YW. Nrf2 is essential for protection against acute pulmonary injury in mice. Proc Natl Acad Sci U S A 1999;96:12731-6.

7) Cho HY, Jedlicka AE, Reddy SP, et al. Role of NRF2 in protection against hyperoxic lung injury in mice. Am J Respir Cell Mol Biol 2002;26:175-82.

8) Ramos-Gomez M, Kwak MK, Dolan PM, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc Natl Acad Sci U S A 2001;98:3410-5.

9) Aoki Y, Sato H, Nishimura N, Takahashi S, Itoh K, Yamamoto M. Accelerated DNA adduct formation in the lung of the Nrf2 knockout mouse exposed to diesel exhaust. Toxicol Appl Pharmacol 2001;173:154-60.

10) Kwak MK, Egner PA, Dolan PM, et al. Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. Mutat Res 2001;480-481:305-15.

11) Ramos-Gomez M, Dolan PM, Itoh K, Yamamoto M, Kensler TW. Interactive effects of nrf2 genotype and oltipraz on benzo[a]pyrene-DNA adducts and tumor yield in mice. Carcinogenesis 2003;24:461-7.

12) Lee JM, Calkins MJ, Chan K, Kan YW, Johnson JA. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J Biol Chem 2003;278:12029-38.

13) Hayashi A, Suzuki H, Itoh K, Yamamoto M, Sugiyama Y. Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. Biochem Biophys Res Commun 2003;310:824-9.

14) Ishii Y, Itoh K, Morishima Y, et al. Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. J Immunol 2005;175:6968-75.

15) Ishii T, Itoh K, Ruiz E, et al. Role of Nrf2 in the regulation of CD36 and stress protein expression in murine macrophages: activation by oxidatively modified LDL and 4-hydroxynonenal. Circ Res 2004;94:609-16.

16) Itoh K, Mochizuki M, Ishii Y, et al. Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-$\Delta^{12,14)}$-prostaglandin $J_2$. Mol Cell Biol 2004;24:36-45.

17) Mochizuki M, Ishii Y, Itoh K, et al. Role of 15-deoxy-$\Delta^{12,14)}$-prostaglandin $J_2$ and Nrf2 pathways in protection against acute lung injury. Am J Respir Crit Care Med 2005;171:1260-6.

18) Iizuka T, Ishii Y, Itoh K, et al. Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. Genes Cells 2005;10:1113-25.

19) Rangasamy T, Guo J, Mitzner WA, et al. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. J Exp Med 2005; 202:47-59.

20) Thimmulappa RK, Lee H, Rangasamy T, et al. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest 2006;116:984-95.

21) Khor TO, Huang MT, Kwon KH, Chan JY, Reddy BS, Kong AN. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. Cancer Res 2006;66:11580-4.

22) Mattson MP, Cheng A. Neurohormetic phytochemicals: Low-dose toxins that induce adaptive neuronal stress responses. Trends Neurosci 2006;29:632-9.

23) Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. Biochem Pharmacol 2006;72:1439-52.

24) Braun S, Hanselmann C, Gassmann MG, et al. Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. Mol Cell Biol 2002;22:5492-505.

25) Primiano T, Li Y, Kensler TW, Trush MA, Sutter TR. Identification of dithiolethione-inducible gene-1 as a leukotriene B4 12-hydroxydehydrogenase: implications for chemoprevention. Carcinogenesis 1998;19:999-1005.

26) Ge Y, Elghetany MT. CD36: a multiligand molecule. Lab Hematol 2005;11:31-7.

27) Hoebe K, Georgel P, Rutschmann S, et al. CD36 is a sensor of diacylglycerides. Nature 2005;433:523-7.

28) Philips JA, Rubin EJ, Perrimon N. Drosophila RNAi screen reveals CD36 family member required for mycobacterial infection. Science 2005;309:1251-3.

29) Urban BC, Willcox N, Roberts DJ. A role for CD36 in the regulation of dendritic cell function. Proc Natl Acad Sci USA 2001;98:8750-5.

30) Doyen V, Rubio M, Braun D, et al. Thrombospondin 1 is an autocrine negative regulator of human dendritic cell activation. J Exp Med 2003;198:1277-83.

31) Chen X, Doffek K, Sugg SL, Shilyansky J.

Phosphatidylserine regulates the maturation of human dendritic cells. J immunol 2004;173:2985-94.

32) Kolls JK. Oxidative stress in sepsis: a redox redux. J Clin Invest 2006;116:860-3.

33) Chen XL, Dodd G, Thomas S, et al. Activation of Nrf2/ARE pathway protects endothelial cells from oxidant injury and inhibits inflammatory gene expression. Am J Physiol Heart Circ Physiol 2006;290:H1862-70.

34) Kim HJ, Park KG, Yoo EK, et al. Effects of PGC-1alpha on TNF-alpha-Induced MCP-1 and VCAM-1 Expression and NF-kappaB Activation in Human Aortic Smooth Muscle and Endothelial Cells. Antioxid Redox Signal 2007;9:301-7.