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Nrf2 in toxicology and pharmacology: the good, the bad and the ugly?

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In the last fifteen years, the understanding of the Nuclear factor erythroid-derived factor 2-related factor (Nrf) family and their involvement in a multitude of physiological processes has been greatly advanced. More than 1000 research articles and 170 reviews regarding Nrf2 have been published since Nrf2 was characterized in 1994 (Moi *et al.*, 1994). In this special issue, we have sought to represent the current frontier of Nrf2-related research and help readers comprehend major progress in the field.

The Nrf proteins belong to the cap “n” collar (CNC) subfamily of basic-region leucine zipper (bZIP) transcription factors, which include Nrf1 (NFE2L1/LCRF1/TCF11) (Chan *et al.*, 1993), Nrf2 (NFE2L2) (Itoh *et al.*, 1995), Nrf3 (NFE2L3) (Kobayashi *et al.*, 1999) and the NF-E2 p45 subunit (Andrews *et al.*, 1993), as well as the more distantly related factors such as BTB and CNC homology 1 (Bach1) and Bach2 proteins (Oyake *et al.*, 1996). Both Nrf1 and Nrf2 are now known to heterodimerize with small Maf or other bZIP proteins, and bind to *cis*-acting element(s) termed antioxidant or electrophile response elements (AREs or EpREs) in the proximal promoters of target genes (Motohashi *et al.*, 2002), leading to activation of transcription (Venugopal and Jaiswal, 1996; Venugopal and Jaiswal, 1998; Biswas and Chan, 2009). Although Nrf3 can heterodimerize with MafK or MafG and bind AREs (Kobayashi *et al.*, 1999; Chenais *et al.*, 2005), the role of Nrf3 in the regulation of ARE-responsive genes remains elusive (Braun *et al.*, 2002; Sankaranarayanan and Jaiswal, 2004; Zhang *et al.*, 2009b). ARE-dependent genes encode for various enzymes, including detoxification enzymes such as glutathione *S*-transferases (GST), NAD(P)H: quinone oxidoreductase 1 (NQO1), and heme oxygenase 1 (HMOX-1), as well as antioxidant enzymes such as catalase (CAT), sulfiredoxin (SRX), γ -glutamylcysteine ligase catalytic subunit (GCLC) and regulatory subunit (GCLM).

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Nrf1 (Wang and Chan, 2006) and Nrf3 (Nouhi *et al.*, 2007; Zhang *et al.*, 2009b) are targeted to the endoplasmic reticulum, whereas Nrf2 is primarily localized to the nucleoplasm and cytoplasm. Supporting the importance of Nrf1 in the developmental process is the finding that loss of Nrf1 function in mice results in late gestational embryonic lethality (Chan *et al.*, 1998). In contrast, Nrf2-deficient mice are viable, but show a higher susceptibility to both oxidative damage and chemical carcinogenesis (Chan and Kan, 1999; Chan *et al.*, 2001; Ramos-Gomez *et al.*, 2001), whereas Nrf3^{-/-} mice develop normally and reveal no obvious phenotype (Derjuga *et al.*, 2004). Fibroblasts derived from Nrf1 or Nrf2 mutant embryos showed decreased glutathione (GSH) levels and enhanced sensitivity to the toxic effects of oxidants (Kwong *et al.*, 1999; Chan and Kwong, 2000), suggesting critical roles for Nrf1 and Nrf2 in cellular oxidative defense.

The first topic relates to Nrf2 evolution, and is titled: “The rise of antioxidant signaling-The evolution and hormetic actions of Nrf2” by Maher and Yamamoto (Maher and Yamamoto, 2010). This manuscript summarizes the origins of Nrf2 and CNC family members in various species, and describes their functional evolution. Emphasis is placed on the hormetic health effects of Nrf2 activation in several disease models. The second manuscript focuses primarily on the Nrf1, and is titled: “Role of Nrf1 in antioxidant response element-mediated gene expression and beyond” by Biswas and Chan (Biswas and Chan, 2009). This paper introduces the overall gene structure, protein isoforms, and downstream targets of Nrf1. They review some of recent literature on the emerging role of Nrf1 and discuss both the regulation and function of this transcription factor. They also highlight major challenges we are facing in defining the role and regulation of Nrf1 in the oxidative stress response and in other physiological processes.

The third theme focuses on regulation and activation of Nrf2 via Keap1. “Cysteine-based regulation of CUL3 adaptor protein Keap1” by Sekhar and coauthors (Sekhar *et al.*, 2009) describes the signaling role of Keap1 in mediating the Nrf2-mediated antioxidant response. During unstressed conditions, association of Nrf2 with Keap1 results in rapid Nrf2 ubiquitination and proteasome-dependent degradation. When cells are exposed to electrophiles or oxidants, Nrf2 is liberated from Keap1-mediated degradation, and subsequently translocates and accumulates in the nucleus, leading to activation of ARE-mediated gene transcription. Modification of specific Keap1 sulfhydryl groups appears to mediate the liberation of Nrf2 from Keap1-mediated ubiquitination. In “Activation of the Nrf2/ARE pathway via S-alkylation of cysteine 151 in the chemopreventive agent-sensor Keap1 protein by falcariindiol, a conjugated diacetylene compound” Ohnuma *et al.* (Ohnuma *et al.*, 2009) verifies previous *in vitro* (Zhang and Hannink, 2003) and *in vivo* (Yamamoto *et al.*, 2008) studies in demonstrating which modified Keap1 cysteines are critical for Nrf2 activation using a cell model challenged with falcariindiol. “Nrf2 signaling and cell survival” by Niture *et al.* (Niture *et al.*, 2009) also summarizes the regulatory mechanisms of Nrf2 activation and proposes a hypothetical model illustrating the role of Nrf2, Keap1 and other ARE-binding factors in activation of antioxidant genes by xenobiotics.

Another primary theme is the protective role of Nrf2 in two tissues critical for detoxification, the lung and liver. “Nrf2 protects against airway disorders” is a manuscript by Cho and Kleeberger (Cho and Kleeberger, 2009) that addresses the protective role of Nrf2 in a variety of respiratory disorders based on emerging evidence from experimental oxidative disease models and human studies. In “Nrf2 the rescue: effects of the antioxidative/electrophilic response on the liver” Klaassen and Reisman (Klaassen and Reisman, 2010) highlight the research that has contributed to the understanding of the importance of Nrf2 in toxicodynamics and toxicokinetics, especially in studies that pertain to the liver. In “Targeting Nrf2 signaling for cancer chemoprevention” Kwak and Kensler (Kwak and Kensler, 2009) describes the efforts made in stimulating Nrf2 activation to initiate cancer chemoprevention and promote overall health. They summarize the chemopreventive efficacies of various Nrf2 activators, such

as dithiolethiones, sulforaphane and oltipraz, in a variety of animal models and human populations. In addition, the authors discuss a concern regarding a deleterious role of persistent activation of Nrf2 in cancer cell biology.

In the last topic on Nrf2 and redox signaling, “ROS signaling, oxidative stress and Nrf2 in pancreatic beta-cell function” by Pi et al. (Pi *et al.*, 2009) focuses on emerging evidence that ROS derived from glucose metabolism acts as metabolic signaling molecules in glucose-stimulated insulin secretion (GSIS) in pancreatic beta-cells. Particular emphasis is placed on the potential inhibitory role of endogenous antioxidants, which rise in response to oxidative stress, in glucose-triggered ROS and GSIS. Pi *et al.* proposes that the cellular adaptive response to oxidative stress, such as Nrf2-mediated antioxidant induction, plays a paradoxical role in pancreatic beta-cell function. On one hand, induction of antioxidant enzymes protects beta-cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment of insulin secretion. On the other hand, the induction of antioxidant enzymes by Nrf2 activation blunts glucose-triggered ROS signaling, thus resulting in reduced GSIS. In “A systems biology perspective on Nrf2-mediated antioxidant response” Zhang et al. (Zhang *et al.*, 2009a) examines the transcriptional regulatory network from a systems biology perspective using engineering approaches. Given that endogenously generated ROS also serve as regulatory signaling molecules, this analysis suggests a novel mode of action to explain oxidative stress-induced pathological conditions and diseases. Specifically, by adaptively upregulating antioxidant enzymes, oxidative stress may inadvertently attenuate ROS signals that mediate physiological processes, resulting in aberrations of cellular function and adverse consequences. Lastly, by simultaneously considering the two competing cellular tasks—adaptive antioxidant defense and ROS signaling they comprehensively re-examine the premise that dietary antioxidant supplements are beneficial to overall human health. Their analysis highlights some possible adverse effects that could occur with overconsumption of antioxidants.

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