

## Review

## NRF2-regulation in brain health and disease: Implication of cerebral inflammation

Mats Sandberg<sup>a</sup>, Jaspal Patil<sup>a</sup>, Barbara D'Angelo<sup>b</sup>, Stephen G. Weber<sup>c</sup>, Carina Mallard<sup>b,\*</sup><sup>a</sup> Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Sweden<sup>b</sup> Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden<sup>c</sup> Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, United States

## ARTICLE INFO

## Article history:

Received 21 August 2013

Received in revised form

17 October 2013

Accepted 8 November 2013

## Keywords:

Neuroinflammation

NRF2

Anti-oxidants

## ABSTRACT

The nuclear factor erythroid 2 related factor 2 (NRF2) is a key regulator of endogenous inducible defense systems in the body. Under physiological conditions NRF2 is mainly located in the cytoplasm. However, in response to oxidative stress, NRF2 translocates to the nucleus and binds to specific DNA sites termed "anti-oxidant response elements" or "electrophile response elements" to initiate transcription of cytoprotective genes. Acute oxidative stress to the brain, such as stroke and traumatic brain injury is increased in animals that are deficient in NRF2. Insufficient NRF2 activation in humans has been linked to chronic diseases such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis.

New findings have also linked activation of the NRF2 system to anti-inflammatory effects via interactions with NF- $\kappa$ B. Here we review literature on cellular mechanisms of NRF2 regulation, how to maintain and restore NRF2 function and the relationship between NRF2 regulation and brain damage. We bring forward the hypothesis that inflammation via prolonged activation of key kinases (p38 and GSK-3 $\beta$ ) and activation of histone deacetylases gives rise to dysregulation of the NRF2 system in the brain, which contributes to oxidative stress and injury.

© 2013 Elsevier Ltd. All rights reserved.

## 1. Regulation of the NRF2 system

During evolution, cells have developed inducible defense systems against harmful endogenous and exogenous substances. Several transcription factors are involved in boosting the cell's defenses. The major regulator of the so-called phase II and some phase III genes is the nuclear factor erythroid 2 related factor 2 (NRF2) (Itoh et al., 1997; Moi et al., 1994). The principle of the NRF2 system is to keep NRF2 protein low under normal conditions with the possibility of rapid induction in case of a sudden increase in oxidation status in the cell. This is achieved by constitutive synthesis and degradation of NRF2 with the possibility of rapid redirection of NRF2 to the nucleus.

NRF2 is a member of the cap'n'collar (CNC) family of transcription factors, which also include NRF1, NRF3 and p45 NF-E2.

NRF2 is a basic leucine zipper (bZIP) protein that in the nucleus heterodimerizes with small MAF or JUN proteins followed by binding to specific DNA sites termed anti-oxidant response elements (ARE) or electrophile response elements (EpRE) (Itoh et al., 1997; Venugopal and Jaiswal, 1998). NRF2-ARE binding can initiate transcription of hundreds of cytoprotective genes including enzymes in the glutathione defense system and proteasome subunits (Kwak et al., 2003a). New findings connect NRF2 not only to an elevated anti-oxidant capacity but also to expression of other types of protective proteins such as brain derived neurotrophic factor (Sakata et al., 2012), the anti-apoptotic B-cell lymphoma 2 (Niture and Jaiswal, 2012), the anti-inflammatory interleukin (IL)-10, the mitochondrial transcription (co)-factors NRF-1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 $\alpha$ ) (Piantadosi et al., 2011), the iron exporter ferroportin 1 (Harada et al., 2011), and the autophagic protein p62 (Komatsu et al., 2010).

## 1.1. Regulation of NRF2 by KEAP1

The NRF2 molecule contains six functional domains named NRF2-ECH homologies (Neh1–6). In the unstressed cell NRF2 is

\* Corresponding author. Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Box 432, 40530 Gothenburg, Sweden. Tel.: +46 7863498.

E-mail addresses: [carina.mallard@neuro.gu.se](mailto:carina.mallard@neuro.gu.se), [carina.mallard@fysiologi.gu.se](mailto:carina.mallard@fysiologi.gu.se) (C. Mallard).

bound to Kelch-like ECH associated protein 1 (KEAP1) in the cytoplasm (Itoh et al., 1999) via its KEAP1 binding domain. KEAP1 is a homodimer with three major domains. Each C-terminal in the KEAP1 dimer, the so called DRG domain, binds to NRF2, while the N-terminal part of KEAP1 binds to an E3 ubiquitin ligase complex (Rbx-1) via an adaptor, cullin-3 (Kobayashi et al., 2004). This directs NRF2 to ubiquitination and constitutive degradation by the 26S proteasome (Dhakshinamoorthy and Jaiswal, 2001).

There are several theories on how NRF2 is released from KEAP1 in response to cellular stress. NRF2 binds to the DRG sites of each KEAP1 subunit via two different binding sites, one high affinity (hinge) and one low affinity (latch) (Kobayashi et al., 2006). One hypothesis is that NRF2 can still move freely when bound to the hinge site (high affinity), while the latch site restricts the movement of NRF2 and places the NRF2 lysins in the Neh2 regions for poly-ubiquitination via the cullin-3 E3 ligase. In this model, oxidative/electrophilic molecules modify cysteine residues of KEAP1, which activates NRF2 (Dinkova-Kostova et al., 2002). The KEAP1 protein contains relatively many cysteines, which are in the vicinity of basic amino acids. This configuration lowers the pKa values for many of the thiols and makes them extra reactive to oxidants/electrophiles. Modifications of (some of) these cysteines result in a different conformation of KEAP1, which then can dissociate from the latch (low-affinity) site. The result is that KEAP1 is bound to NRF2 at the hinge site and that “new” non-bound NRF2 can be phosphorylated by protein kinase C (PKC) (Niture et al., 2009) and possibly other kinases (see below), translocated to the nucleus and initiate transcription of cytoprotective genes. Alternative hypotheses on how NRF2 becomes free from KEAP1 include that oxidants/electrophiles can dissociate the NRF2-KEAP1 complex directly (Levonen et al., 2004) or that oxidants/electrophiles dissociate cullin-3 from KEAP1, which leads to blocking of further ubiquitination (Gao et al., 2007) and degradation of KEAP1 (Zhang et al., 2005). Recent studies also show that NRF2 activation can be achieved by autophagic degradation of KEAP1 (see more below).

The abundance of the proteins in the NRF2 system discussed above appears to be regulated by an autoregulatory loop. Thus NRF2 regulates the transcription of KEAP1, cullin-3 and Rbx-1 and, in turn, KEAP1/cullin-3/Rbx-1 degrades NRF2 (Kaspar and Jaiswal, 2010). The KEAP1 complex can also be imported into the nucleus and participate in the degradation of nuclear NRF2 (Niture et al., 2009). Further, feed back systems appear to exist where NRF2 activation induces an increase in proteasome expression and activity. In response to NRF2 activators, increased expression of genes coding for the proteasome subunits 20S and 19S have been observed (Kwak et al., 2003a,b; Kapeta et al., 2010). The NRF2-mediated induction of proteasome genes has been linked to increased resistance to oxidative stress (Kwak et al., 2007; Pickering et al., 2012).

Although KEAP1 is the main regulator of NRF2 activation others exist, including the transcription factor Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B), as discussed below. Another regulator of NRF2 is Broad-complex, tramtrack and bric-a-brac and CNC homology 1 (BACH1). This is a transcription factor that competes with NRF2 for binding to ARE sites and thus functions as a repressor of NRF2 activation (Dhakshinamoorthy et al., 2005). Interestingly BACH1 appears to serve as a negative feedback regulator of NRF2 activation as it is regulated by NRF2-activating agents in an NRF2-dependent manner (Jyrkanen et al., 2011).

### 1.2. Phosphorylation of NRF2

The transport to and from the nucleus is influenced by factors such as the phosphorylation of NRF2. Several kinases have been

shown to affect NRF2 transport differently via specific phosphorylation sites. One of the best characterized include phosphorylation by PKC (Huang et al., 2000), which is necessary for liberating NRF2 from KEAP1 (Bloom and Jaiswal, 2003) and thus promoting transport to the nucleus. Protein kinase CK2 can also phosphorylate NRF2, which promotes transport of NRF2 into the nucleus (Pi et al., 2007). Similar activating effects on NRF2 translocation to the nucleus have been observed for phosphatidylinositol 3-kinases (PI3K) (Nakaso et al., 2003), c-Jun N-terminal kinase (JNK), extracellular regulated kinase (ERK) (Xu et al., 2006) and PERK (Cullinan et al., 2003). However, it has been suggested that mitogen-activated protein kinases (MAPKs)-induced phosphorylation of NRF2 does not directly affect KEAP1-NRF2 protein interaction, but rather acts through indirect mechanisms (Sun et al., 2009a).

Phosphorylation catalyzed by kinases can also increase NRF2 breakdown. The MAPK p38 has been shown to stabilize the interaction between KEAP1 and NRF2 thereby elevating the breakdown of NRF2 (Keum et al., 2006). Activation of glycogen synthase kinase 3-beta (GSK-3 $\beta$ ) can cause degradation of NRF2 by the proteasome that is independent of KEAP1. Phosphorylation of the Neh6 domain in NRF2 by GSK-3 $\beta$  leads to recognition by an E3 ubiquitin ligase (beta-TrCP) (Chowdhry et al., 2013). Binding of beta-TrCP couples NRF2 to the cullin-3/Rbx1 ubiquitination complex (Rada et al., 2012), directing NRF2 to proteasomal degradation. GSK3 $\beta$  activation acts upstream and phosphorylates Src kinases, leading to their nuclear localization and NRF2 phosphorylation (Niture et al., 2011; Jain and Jaiswal, 2007). Ultimately this will lead to export, with or without KEAP1, followed by proteasomal degradation of NRF2. Several Src kinases (FYN, SRC, YES, and FGR) in the nucleus can regulate NRF2 via phosphorylation of NRF2 Tyr568 that triggers nuclear export and degradation of NRF2. Thus, kinase activation is of utmost importance in regulation of the NRF2-system.

### 1.3. Autophagic regulation of NRF2

The protein p62, which represents both a selective autophagy substrate and a cargo receptor for autophagic degradation of misfolded proteins, has been reported to activate NRF2 in response to oxidative stress (Komatsu et al., 2010; Bjorkoy et al., 2005; Komatsu et al., 2007; Lamark et al., 2009). The promoter/enhancer region of the p62 gene contains ARE sites (Jain et al., 2010) and autophagy defects induce an excessive accumulation of p62 and oxidative stress (Komatsu et al., 2007; White et al., 2010). An increase in endogenous p62, either due to a defect in autophagy or as a result of its ectopic expression, sequesters KEAP1 into aggregates, hence causing inhibition of KEAP1-mediated NRF2 ubiquitination and degradation (Lau et al., 2010). Thus, when autophagy is impaired, p62 accumulates and activates NRF2 by sequestering KEAP1 in inclusion bodies. In turn NRF2 promotes new p62 products creating a positive loop of NRF2 activation. With regard to the brain, recent findings demonstrate that interactions between p62 and the KEAP1-NRF2 signaling pathway play a key role in preventing oxidative injury and alleviate endoplasmic reticulum stress during cerebral ischemia/reperfusion (Wang et al., 2013).

### 1.4. Epigenetic regulation of NRF2

Histone acetylation and phosphorylation, methylation of CpG islands and synthesis of specific miRNAs are additional means by which cells can regulate the levels of NRF2. This field of NRF2 regulation is just beginning to be explored but it contains interesting targets for future therapeutic activation of the NRF2 system, including in the brain. We have shown that factors released from activated microglia activate histone deacetylases (HDACs), reduce the overall acetylation level of histone 3 and decrease the activation

of NRF2 in cultured astrocytes. Restoring the acetylation level by HDAC inhibitors also repaired the NRF2 system indicating that histone acetylation may be one epigenetic mechanism to regulate NRF2 function (Correa et al., 2011a) (see more below). In support of this idea, the HDAC inhibitor trichostatin A (TSA) increased cell viability in cortical neurons after oxygen-glucose deprivation and reduced infarct volume in wild-type mice subjected to permanent middle cerebral artery occlusion (MCAO) in an NRF2/KEAP1-dependent manner (Wang et al., 2012a). TSA reduced the expression of KEAP1, activated NRF2 nuclear translocation, NRF2–ARE binding and increased expression of the NRF2 regulated proteins heme oxygenase (HO1), NAD(P)H:quinone oxidoreductase 1 (NQO1), and glutamate-cysteine ligase (GCL) catalytic subunit. It should be noted that direct acetylation of NRF2 by p300/CBP has been shown to stimulate NRF2 binding to DNA, an effect that could also contribute to the positive effects of HDAC inhibitors on NRF2 activation (Sun et al., 2009b).

Elevated methylation of CpG islands in the promoter of the *Nrf2* gene was shown to suppress expression of NRF2 in mice with prostate cancer. Interestingly the NRF2 function could be restored by phytochemicals that reduced the methylation level (Su et al., 2013). To our knowledge CpG methylation of the *Nrf2* promoter as a cause of low NRF2 function and oxidative stress in neurodegenerative diseases and aging has not yet been explored (see more below). Similarly, several miRNAs (miR153, miR27a, miR142-5p, miR144) reduce NRF2 activation in neuronal SH-SY5Y cells (Narasimhan et al., 2012), but there is overall very limited knowledge of the effects of miRNA modifications on the NRF2 system in the brain.

## 2. The NRF2 system and CNS disease

The NRF2 system is widely expressed in the CNS and is regulated in response to both acute cerebral insults and in neurodegenerative disease. In addition to its crucial regulatory role in the endogenous defense to various cellular stresses, NRF2 is recognized as an important regulator of inflammation in the brain. Dysregulation of these mechanisms has been suggested to contribute to brain injury.

### 2.1. CNS effects in animals with genetic deletion of *Nrf2*

Suppression of the NRF2 system by genetic deletion in animals leads to elevated neurotoxicity resulting from endogenous and exogenous stresses. However, spontaneous pathological effects in brains from *Nrf2*-deficient mice are less clear, but do exist. The predominant general findings in animals with genetic deletion of *Nrf2*, compared with age-matched control animals, are white matter leukoencephalopathy with widespread astrogliosis (Hubbs et al., 2007), but with no overall difference in microglial activation and overt signs of neurodegeneration. However, a predominant pro-inflammatory microglial phenotype was reported in dopamine metabolizing brain areas (striatum and ventral midbrain) of *Nrf2* deficient animals. Markers characteristic of classical microglial activation, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthases (iNOS), IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were increased, while at the same time anti-inflammatory markers attributable to alternative microglial activation, including Found in inflammatory zone protein (FIZZ-1), YM-1, Arginase-1, and IL-4 were decreased (Rojo et al., 2010). These studies show that *Nrf2* deficient mice indeed demonstrate signs of neurodegeneration, but mainly in areas with high inherent oxidative reactions, i.e. dopamine-metabolizing areas.

On the other hand, different types of cellular stresses superimposed on an *Nrf2*-deficient background have significant detrimental effects on neuronal systems in the brain. Loss of NRF2-

mediated transcription exacerbates vulnerability to the neurotoxin 6-hydroxydopamine (6-OHDA), both in cultured neurons and in *Nrf2* deficient mice *in vivo* (Jakel et al., 2007). In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of Parkinson's disease there is greater loss of the dopamine transporter in the striatum (Burton et al., 2006), and exacerbated gliosis and dopaminergic nigrostriatal degeneration (Innamorato et al., 2010) in *Nrf2* deficient mice compared to wild type controls. Also, in animal models of acute brain injury such as stroke and traumatic brain injury, NRF2 deficient mice show more severe injuries and more intense immunological reactions (Shih et al., 2005; Shah et al., 2007; Jin et al., 2009).

### 2.2. NRF2 and neurodegenerative disease in humans

A dysfunctional NRF2 system has been linked to several chronic diseases in humans, including chronic kidney disease and chronic obstructive pulmonary disease (COPD) although the exact role for the NRF2 decline is yet to be characterized (Kim and Vaziri, 2010). The NRF2 system is compromised by increased age and aging is a strong risk factor for almost all neurological diseases involving oxidative stress. If suppression of NRF2 activity occurs in human brains the implications are elevated vulnerability to acute insults that involve oxidative stress, prolonged and more severe inflammation and likely an elevated risk to develop chronic diseases such as Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). Indeed, brains from AD patients have decreased amounts of NRF2 in astrocytes of the hippocampus, one of the brain areas where neurodegeneration starts in AD (Ramsey et al., 2007). The NRF2 target protein p62 is significantly reduced in the frontal cortex of AD patients (Salminen et al., 2012). As discussed above, p62 has been linked to autophagy and it was recently suggested that interaction between p62 and KEAP1 provides a molecular basis for the formation of cytoplasmic inclusions observed in several neurodegenerative diseases (Tanji et al., 2013). In post mortem brains from ALS patients NRF2 is reduced in the motor neurons in spinal cord and cortex (Sarlette et al., 2008).

Brains from individuals with PD are characterized by a prominent decrease in glutathione in the substantia nigra. In the neurons that survive in substantia nigra, NRF2 is localized to the nucleus indicating that in these cells NRF2 transport is functional. However, it is not known if the NRF2 transcription machinery is deficient even though NRF2 is transported to the nucleus (Ramsey et al., 2007). In agreement with a malfunctioning NRF2 system in PD, olfactory neurosphere-derived cells from patients with sporadic PD had lower glutathione levels, which could be restored by an NRF2 activating agent (Cook et al., 2011). The study indicates that therapy aiming for NRF2 activation could stop neurodegeneration in PD. Further, genetic variation in the *Nrf2* gene has been linked to both AD and PD progression (von Otter et al., 2010a,b). Taken together, although there are only a few studies in humans, these do indicate that the NRF2 system is dysregulated in brains of individuals suffering from neurodegenerative diseases.

The underlying mechanisms behind the apparent poor NRF2 functioning in chronic neurodegenerative diseases are not known. The number of theoretical possibilities behind dysregulation is overwhelming in view of the complex regulation of the NRF2 system described above. However, one possible link between the NRF2 system and neurodegenerative diseases is that many of them are proteinopathies, as described below. It also appears clear, if it is correct to extrapolate from mice to humans, that lack of NRF2 does not induce general neurodegeneration *per se*. Rather it is when cells are stressed that absence of NRF2 will become detrimental to neurons. Thus in the following sections, we will also review data that supports the idea that inflammation and prolonged activation

of key kinases are factors that lead to the paradoxical decrease in NRF2 observed in some neurodegenerative diseases.

### 2.3. Proteinopathies and NRF2

Several of the neurodegenerative diseases are proteinopathies where specific proteins become aggregated in the brain. In view of the role of NRF2 in upregulation of proteasomal enzymes, a link between proteinopathies and NRF2 dysfunction is possible, and potentially upregulation of the NRF2 system could be beneficial under such circumstances. AD is characterized by brain accumulation of amyloid beta-protein (A $\beta$ ) plaques. Activation of NRF2, by tert-butylhydroquinone (TBHQ) treatment or adenoviral *Nrf2* gene transfer, protected against A $\beta$  toxicity in a transgenic mouse model of AD (Kanninen et al., 2008). Recently, it was also shown that the NRF2 activator carnosic acid suppressed the production of A $\beta$  1–42 by in SH-SY5Y human neuroblastoma cells (Meng et al., 2013).

In, PD the protein  $\alpha$ -synuclein aggregates to form so called “Lewy bodies”, and dopaminergic neurons in the substantia nigra pars compacta degenerate. Several studies indicate a relationship between NRF2 and  $\alpha$ -synuclein. Mutation and deletion of the protein DJ-1 can cause a variant of autosomal recessive familial PD. Overexpression of DJ-1 stabilizes NRF2 and reduces aggregation of  $\alpha$ -synuclein (Clements et al., 2006; Liu et al., 2008a). The NRF2 activator triterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) methyl amide reduces neurodegeneration of dopamine neurons, diminishes  $\alpha$ -synuclein accumulation and oxidative stress in an experimental model of PD (Yang et al., 2009). It has also been shown that mice that lack NRF2 and have increased expression of human  $\alpha$ -synuclein, have aggravated dopaminergic neuron loss, increased neuroinflammation and elevated protein aggregation (Lastres-Becker et al., 2012). Interestingly, in a mutant  $\alpha$ -synuclein transgenic mouse model, it appears sufficient to increase NRF2 expression in astrocytes to achieve protection against neurotoxicity. It was suggested that the protection was mediated via elevated degradation of mutant  $\alpha$ -synuclein through the autophagy-lysosome pathway (Gan et al., 2012).

Huntington disease (HD) is an inherited neurodegenerative disease caused by an expansion of polyglutamine in the protein huntingtin. Expression of mutant huntingtin caused reduced activity of NRF2, and lowered the activation of the NRF2 pathway by the oxidant TBHQ in cultured cells (Jin et al., 2013). In a transgenic mouse model of HD triterpenoid derivatives in the diet upregulated NRF2/ARE induced genes in the brain, reduced oxidative stress, improved motor impairment and increased longevity (Stack et al., 2010).

In familial ALS, 20% of the cases are due to mutations in the Cu/Zn superoxide dismutase gene (SOD1). In a cellular model of this disease it was found that NRF2 and activation of NRF2 driven genes were down-regulated by mutated SOD1 (Kirby et al., 2005). In support, over-expression of NRF2, specifically in astrocytes, delayed onset and extended survival in a mouse model of familial ALS (Vargas et al., 2008).

## 3. Neuroinflammation and the NRF2 system

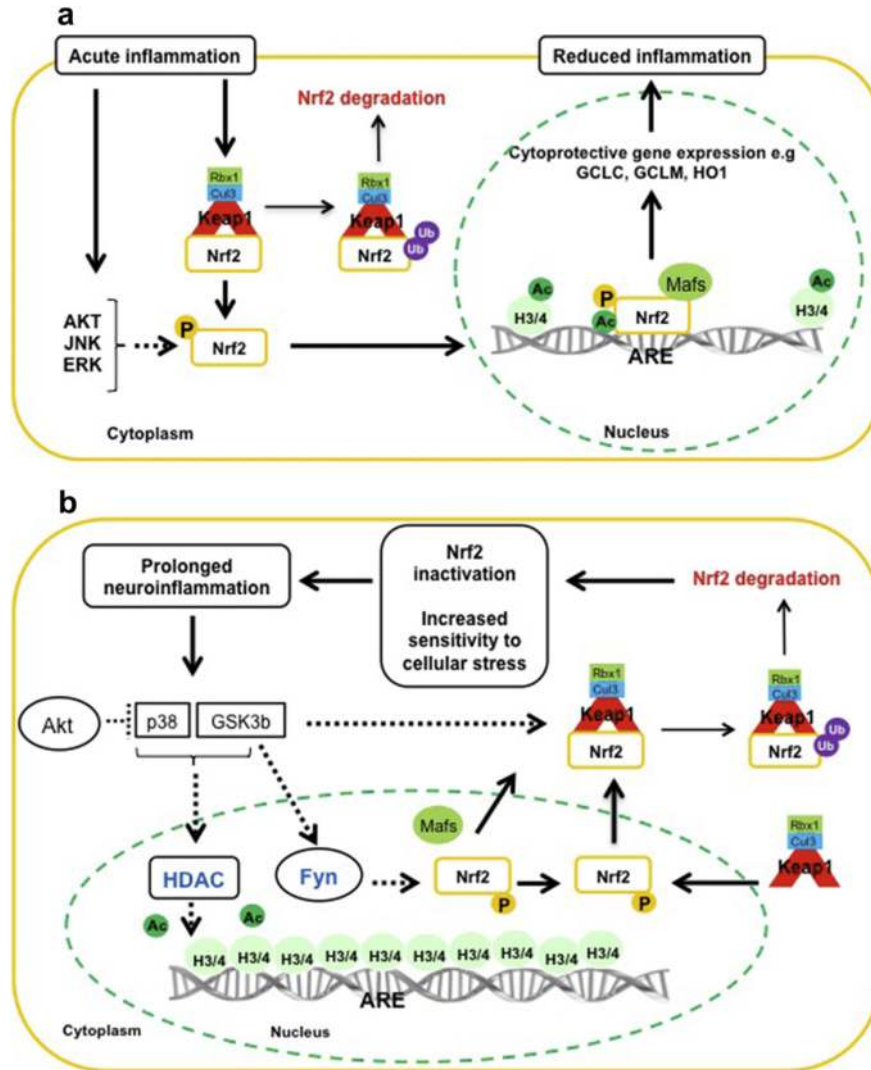
The transcription factor NF- $\kappa$ B is a key regulator of inflammation, which also suppresses the transcription of ARE-dependent genes (Nair et al., 2008). Under physiological conditions, NF- $\kappa$ B is inactive and sequestered in the cytoplasm by binding to the inhibitory kinase, Inhibitor of nuclear factor kappa-B kinase subunit beta (IKK- $\beta$ ) protein. The relation between NRF2 and NF- $\kappa$ B is not well characterized but the identification of NF- $\kappa$ B binding sites in the promoter region of the *Nrf2* gene suggests cross-talk between these two regulators of inflammatory processes (Nair et al., 2008).

The NF- $\kappa$ B subunit p65 has been shown to function as a negative regulator of NRF2 activation either by depriving CREB binding protein (CBP) from NRF2 or by recruitment of histone deacetylase 3 (HDAC3), a corepressor, to ARE via interaction of and CBP or MafK, causing local histone hypoacetylation and down-regulation of NRF2-ARE signaling (Liu et al., 2008b). In another study it was shown that p65 decreased NRF2 binding to its cognate DNA sequences and enhanced NRF2 ubiquitination. Nuclear translocation of KEAP1 was augmented by p65 and the studies suggested that NF- $\kappa$ B signaling inhibits the NRF2-ARE pathway through the interaction of p65 and KEAP1 (Yu et al., 2011). It has also been shown that KEAP1 is a negative regulator of NF- $\kappa$ B signaling via inhibition of IKK- $\beta$  phosphorylation and by mediating IKK- $\beta$  degradation by autophagocytosis (Kim et al., 2010). Further, activation of NRF2 in response to lipopolysaccharide (LPS) has been suggested to be dependent on the key innate immunity-regulating adaptor protein Myeloid differentiation primary response gene 88 (MyD88) (Kim et al., 2011). In NRF2-deficient mice, the inflammatory response in the brain and the microglia activation is much more pronounced in response to LPS when compared to normal animals (Innamorato et al., 2008). Thus a functional NRF2-system is important to regulate neuroinflammation in response to oxidative stress in the brain. However, emerging evidence indicates that although neuroinflammation, i.e. activation of microglia, is implicated in both acute and chronic brain diseases, it is not fully clear under which circumstances inflammation may be beneficial, deleterious or both (Block et al., 2007).

### 3.1. Context dependent NRF2 regulation

We have shown that LPS-induced systemic inflammation can both reduce and aggravate neuronal damage induced by postnatal hypoxia-ischemia (Wang et al., 2007). Neuropathological outcome was assessed in neonatal rats that were subjected to LPS prior to an episode of hypoxia-ischemia. It was found that the neuropathological effects were time dependent so that an interval of 24 h between the LPS injection and hypoxia-ischemia protected the brain, whereas a 72 h interval caused enhanced brain damage. The deleterious effect was inhibited by the anti-oxidant and glutathione precursor N-acetylcysteine. Our interpretation of this study was that neonatal infection can reduce the anti-oxidant capacity via pro-inflammatory cytokines, a hypothesis which we have further explored mechanistically *in vitro*. Treatment of astrocytes with the pro-inflammatory cytokine TNF- $\alpha$  (10 ng/ml) or medium from microglia exposed to LPS (10 ng/ml) activated the NRF2-system after 24 h. This protective up-regulation of the NRF2-system was mediated by activation of several kinases including AKT, ERK and JNK (Correa et al., 2011a,b, 2012) (Fig 1a). In contrast, treatment of astrocytes with medium from activated microglia or TNF- $\alpha$  for 72 h down-regulated proteins in the NRF2-system and prevented induction of glutathione synthesis via NRF2 (Fig 1b). Inhibitors of GSK-3 $\beta$  and p38 kinases blocked the detrimental effects induced by prolonged inflammation. Also HDAC activity was elevated after 72 h and the level of acetylated histone 3 was decreased in astrocytes treated with medium from activated microglia for 72 h (Correa et al., 2011a). The mechanisms behind these epigenetic effects were also related to activation of GSK-3 $\beta$  and p38 as inhibitors of each of these kinases blocked histone deacetylation. The negative effects on NRF2 activation by these two kinases is well established by earlier work in which it was shown that the HDAC blocker TSA and the less selective blocker valproate reversed the acetylation levels, restored the inducibility of the NRF2-system and protected astrocytes from oxidative stress (Naidu et al., 2009; Salazar et al., 2006) The data clearly demonstrate that prolonged inflammation can induce HDAC activation, which in turn down-regulates the





**Fig. 1.** Regulation of NRF2 activation following acute (A) and prolonged (B) inflammation. (A) Based on our studies (Correa et al., 2011a; Correa et al., 2011b; Correa et al., 2012; Correa et al., 2013) we suggest that acute inflammation can oxidize/modify certain thiols in the KEAP1 dimer and activate kinases such as AKT, ERK and JNK. This will lead to that NRF2 escapes from being ubiquitinated by Cullin-3 (Cul3) and then transported to the nucleus, a process that is promoted by phosphorylation. Inside the nucleus, NRF2 combines with proteins such as MAFs. The complex binds to certain DNA motifs in promoters, so called anti-oxidant response elements (ARE), of hundreds of protective genes. The NRF2-mediated transcription is enhanced by acetylation of histones (H3/4). The effect of acute inflammation on NRF2 activation is reduced oxidative stress by elevated levels of cytoprotective proteins and anti-oxidants such as glutathione, creating a cellular environment that will reduce inflammation. For a more complete description of established (solid arrows) and putative (dashed arrows) kinases involved in NRF2 activation during acute inflammation see main text. (B) Prolonged neuroinflammation, i.e. overactivation of microglia and elevated levels of proinflammatory cytokines, leads to sustained activation of GSK-3 $\beta$  and p38 (Correa et al., 2011a; Correa et al., 2011b; Correa et al., 2012; Correa et al., 2013). We propose that the activation of GSK-3 $\beta$  and p38 leads to phosphorylation of NRF2 at sites that enhance the binding to KEAP1, which will elevate the ubiquitination of NRF2 and thereby enhance degradation. GSK-3 $\beta$  can also phosphorylate FYN, which is transported to the nucleus. Inside the nucleus phosphorylation of NRF2 by FYN will lead to export of NRF2 out of the nucleus. Intracellular KEAP1 can compete with binding of NRF2 to ARE, and the complex NRF2-KEAP1 can be exported to the cytosol where NRF2 is degraded. Further, decreased histone acetylation caused by elevated HDAC activity will reduce NRF2 mediated transcription. The increase in HDAC activity can be partly caused by the prolonged activation of GSK-3 $\beta$  and p38. The inactivation of the NRF2 system will lead to sustained and elevated activation of microglia and increased vulnerability to oxidative stress. Note the key importance of activation of AKT as a mean to reduce GSK-3 $\beta$  activation. For a more complete description of established (solid arrows) and putative (dashed arrows) kinases involved in NRF2 activation during prolonged inflammation see main text.

NRF2-activation likely via the kinases p38 and GSK3- $\beta$ . There are few previous reports on such a link, but a recent study suggests that GSK3- $\beta$  can directly phosphorylate and activate one HDAC subtype, which then exerts neurotoxic effects (Bardai and D'Mello, 2011). Similar, but less pronounced, effects on the NRF2 system in brains were observed *in vivo* in LPS injected 8 day old rats (Correa et al., 2013).

### 3.2. Effect of peripheral inflammation on NRF2 regulation in CNS

There is evidence to suggest that inflammation around birth may have long-term detrimental effects on the NRF2 system. A

dysfunctional NRF2 system in adulthood after prenatal inflammation was indicated by a decrease in the activity of GCL at four and seventeen months after prenatal injection of LPS (Zhu et al., 2007). A lower level of the modulatory subunit of GCL paralleled the decrease in glutathione. As this subunit is a target of NRF2 activation it is possible that the reason for the low glutathione levels is related to poor NRF2 function. However, further studies are needed to support this as several other enzymes in the glutathione system were unchanged or elevated. The work of Cai and coworkers show that systemic neonatal infection, induced by LPS, leads to prolonged changes in neuroinflammation and enhanced vulnerability of the substantia nigra to environmental toxins such as rotenone

(Cai et al., 2013). The mechanisms are yet to be resolved but one possibility is that the NRF2 system is down-regulated after post-natal inflammation similar to that observed with prenatal inflammation. In support of this idea, we have found that neonatal LPS, at least in the short term, suppresses NRF2 target genes in cells at the blood-cerebral spinal fluid barrier (D'Angelo et al., 2013).

Systemic inflammation is often observed in AD patients and increased serum TNF- $\alpha$  levels are associated with a two-fold increase in the rate of cognitive decline (Holmes et al., 2009). Other studies that support the concept that peripheral inflammation causes increased vulnerability to the brain include observations that patients with PD have inflammatory reactions within the enteric nervous system (Devos et al., 2013) and in models of PD increased vulnerability of the substantia nigra is observed in rats with ulcerative colitis (Villaran et al., 2010). Thus, there are indications that peripheral inflammation can sensitize the brain to become vulnerable and increase the risk of development of neurodegenerative disease.

#### 4. Prophyllactic activation of the NRF2 system

For humans it appears that adopting a diet rich in phytochemicals, such as the Mediterranean diet, decreases the risk of developing stroke, dementia and AD (Lourida et al., 2013; Psaltopoulou et al., 2013). A diet rich in strawberries, blueberries and spirulina has also been shown to reduce inflammation and oxidative stress (Chen et al., 2013; Gemma et al., 2002). Whether this is due to increased activation of NRF2 in the brain is not known. It is unclear whether it is even necessary that the phytochemicals enter the brain. As described above, brain inflammation may be partly driven from peripheral inflammation and reducing peripheral inflammation by dietary means (Konstantinidou et al., 2013) may reduce inflammation also in brain. It should be noted also that moderate exercise can activate NRF2, for example in the aging heart (Gounder et al., 2012). This type of endogenous activation of the NRF2 system is attractive. It is interesting to speculate that such endogenous activation could be used in combination with an NRF2 promoting diet to reduce inflammatory reactions. In brain, activation of NRF2 could also be achieved via the increase in peripheral IGF-1 that enters the brain after exercise (Cotman et al., 2007). IGF-1 may be one beneficial mediator of exercise on brain health, via activation of NRF2 by promoting increased AKT (Sequea et al., 2012) and decreased GSK-3 $\beta$  activity (Salazar et al., 2006). Another more direct link between neuronal activity and NRF2 is that activation of the muscarinic receptor M1 activates NRF2 partly by inhibition of GSK-3 $\beta$  (Espada et al., 2009).

#### 5. Restoring decreased NRF2 function

Designing therapies that aim to elevate NRF2 function are likely to be more beneficial if the underlying mechanisms for dysregulation can be deciphered. There is even a potential risk that NRF2 activators can be harmful if used in high concentrations in tissues that are deficient in NRF2. The basis for such detrimental effects is hormesis in which a low concentration of a substance such as phytochemicals present in fruit and vegetables results in activation and protection but a high dose can result in toxicity (Son et al., 2008). The dose response curve could be shifted in diseased tissue so that a normally beneficial concentration of phytochemical becomes toxic. An example of this is the effect of the NRF2 activator sulforaphane that has been described for naïve and TNF- $\alpha$  treated synoviocytes. The naïve cells were protected, but the TNF- $\alpha$  exposed cells underwent apoptosis (Fragoulis et al., 2012). A similar type of argument concerning context biology of NRF2 in cancer was recently discussed (Sporn and Liby, 2012). Activation of NRF2 can

reduce tumorigenesis in its early stages but in later stages of tumorigenesis NRF2 activation can develop malignant cancer cells resistant to treatment.

From the discussions above one reason for a decrease in the NRF2 system by inflammation in brain appears to be related to prolonged activation of p38 and GSK-3 $\beta$ . These kinases are activated in chronic neurodegenerative diseases and have been suggested earlier as potential targets in AD and PD (Yasuda et al., 2011; Kanninen et al., 2011). Therefore, a therapy that is directed to increase activation of NRF2 via release from KEAP1 only may be inadequate if GSK-3 $\beta$  is still activated. We speculate that under such circumstances NRF2 could be exported out of the nucleus via GSK-3 $\beta$ , may not enter the nucleus due to p38 activity or may not be able to activate/bind to promoter sites because of deacetylated histones. Thus a combination of HDAC, p38 and/or GSK-3 $\beta$  inhibition in combination with NRF2 activation may be the most effective way to restore a dormant/inadequate NRF2 system and reduce inflammation in neurodegenerative diseases. As GSK-3 $\beta$  is inhibited by AKT phosphorylation it follows that therapies/diets/conditions that activate PI3K (upstream of AKT) may be as effective as inhibitors of GSK-3 $\beta$ . Interestingly, several established NRF2 activators such as curcumin, sulforaphane, spirulina and ketonic diet, appear to have multiple positive effects on the regulatory machinery of NRF2. For example, the turmeric ingredient curcumin has been shown to inactivate GSK-3 $\beta$ , inhibit DNA methyltransferase, modulate HDACs and miRNAs (Huang et al., 2012; Teiten et al., 2013). Likewise, the broccoli component sulforaphane is an HDAC inhibitor (Myzak et al., 2004) and a spirulina extract reduces the expression of HDACs (Ku et al., 2013). A neuroprotective ketogenic diet activates NRF2 nuclear accumulation in both the hippocampus and liver (Milder et al., 2010) and produces ketone bodies that act as HDACs (Shimazu et al., 2013). This is in stark contrast to a high lard diet, which reduces NRF2 in hippocampus (Morrison et al., 2010). Some formulas used in traditional medicine activate NRF2-mediated antioxidant signaling pathways and thus contain potential substances for more refined NRF2 activation (Wang et al., 2010). Further research on the active substances, their safety, ability to cross the blood brain barrier and action on the key kinases and epigenetic regulators in NRF2 activation is warranted.

#### 6. NRF2 as a pharmaceutical target

There is an increasing clinical interest in using NRF2 activators for therapeutic purposes. One approach to activate/restore the NRF2 system is to use drugs that are already used clinically and have been found to activate the NRF2 system. Examples of this group include melatonin which activates NRF2 (Wang et al., 2012b) and is used in the clinic against sleep disorders in children and adolescents (Cummings, 2012), valproate, an anti-epileptic which restores NRF2 via HDAC inhibition, and celecoxib, a COX-2 inhibitor used in arthritic patients (Wang et al., 2011). To our knowledge, none of these have been used as therapeutics with the aim to restore NRF2 levels in brain disorders. However, there are a number of NRF2 inducers that have been tested clinically. Sulforaphane-rich broccoli sprout extract is undergoing clinical evaluation in schizophrenia and autism disorders ([www.ClinicalTrials.gov](http://www.ClinicalTrials.gov); NCT01716858; NCT01474993). Experimental studies have shown that the fumaric acid ester Dimethyl fumarate (DMF) protects the brain from oxidative stress via NRF2-dependent mechanisms (Scannevin et al., 2012). The use of DMF is approved for psoriasis and was found to have beneficial effects in relapsing-remitting multiple sclerosis in recent placebo-controlled phase II and III studies (Kappos et al., 2008; Gold et al., 2012). Further, clinical trials in the treatment of MS using DMF are ongoing ([www.ClinicalTrials.gov](http://www.ClinicalTrials.gov); NCT01930708). Triterpenoids are strong inducers of NRF2-

regulated genes (Yates et al., 2007), and new synthetic triterpenoids penetrate the blood–brain barrier well and have been shown to reduce both the number of CD11b positive microglia and the transcription of proinflammatory cytokines in an MPTP-model of PD, suggesting beneficial effects on the brain (Kaidery et al., 2013). Previous phase II clinical trials reported beneficial effects of the triterpenoid bardoxolone methyl (CDDO-Me) in ameliorating kidney function in patients with less severe clinical conditions (Pergola et al., 2011). However, lately questions have arisen about the therapeutic use of CDDO-Me and a phase III clinical trial in patients at the late stage of chronic kidney disease, associated with severe renal dysfunction or cardiovascular failure, has been terminated due to safety concerns ([www.ClinicalTrials.gov](http://www.ClinicalTrials.gov); NCT01351675; 99).

NRF2-based therapy is still an open field of discussion, however, the failure of CDDO-Me highlights important issues concerning the clinical use of NRF2 activators. So far results indicate that activation of NRF2 may be more successful in preventing oxidation-related pathologies than in reversing advanced pathological conditions. Thus introduction of novel NRF2-based therapies should perhaps consider a more preventive approach in the future (Zhang, 2013).

## 7. Concluding remarks

NRF2 has a well-established role as the regulator of key endogenous defenses against oxidative stress in the body. Emerging evidence suggests that NRF2, in addition to its antioxidant functions, may also play an important role in regulating inflammation in the brain. Thus, human neurodegenerative diseases have been associated with inflammation and dysregulation of the NRF2 system, in particular sensitizing vulnerable brain regions to additional stresses. We propose that prolonged inflammation induces activation of specific kinases and epigenetic modifications, which block the NRF2 system and thereby contribute to neurological injury. Diets or behavioral changes (e.g., exercise) that boost the NRF2 system may be important avenues to reduce the risk of neurodegenerative disease.

## Acknowledgments

This research received financial assistance from the Swedish Research Council (VR2009-2630, 2012-2992), a Government grant to a researcher in Public Health Service at the Sahlgrenska University Hospital (ALFGBG-142881), European Union grant FP7, (Neurobid, HEALTHF2-2009-241778), the Leducq foundation (DSRR\_P34404), Åhlén-stiftelsen, Wilhelm and Martina Lundgren Foundation, Swedish Brain Foundation (FO2013-095) and the National Institutes of Health (R01 GM044842). Barbara D'Angelo was supported by Foundation Olle Engkvist Byggmästare.

## References

- Bardai, F.H., D'Mello, S.R., 2011. Selective toxicity by HDAC3 in neurons: regulation by Akt and GSK3beta. *J. Neurosci.* 31, 1746–1751.
- Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., Stenmark, H., Johansen, T., 2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J. Cell Biol.* 171, 603–614.
- Block, M.L., Zecca, L., Hong, J.S., 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69.
- Bloom, D.A., Jaiswal, A.K., 2003. Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from Irf2, 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, 44675–44682.
- Burton, N.C., Kensler, T.W., Guilarte, T.R., 2006. In vivo modulation of the Parkinsonian phenotype by Nrf2. *Neurotoxicology* 27, 1094–1100.
- Cai, Z., Fan, L.W., Kaizaki, A., Tien, L.T., Ma, T., Pang, Y., Lin, S., Lin, R.C., Simpson, K.L., 2013. Neonatal systemic exposure to lipopolysaccharide enhances susceptibility of nigrostriatal dopaminergic neurons to rotenone neurotoxicity in later life. *Dev. Neurosci.* 35 (2–3), 155–171.
- Chen, L., Xin, X., Yuan, Q., Su, D., Liu, W., 2013 May 7. Phytochemical properties and antioxidant capacities of various colored berries. *J. Sci. Food Agric.* <http://dx.doi.org/10.1002/jsfa.6216> [Epub ahead of print].
- Chowdhry, S., Zhang, Y., McMahon, M., Sutherland, C., Cuadrado, A., Hayes, J.D., 2013. Nrf2 is controlled by two distinct beta-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. *Oncogene* 32, 3765–3781.
- Clements, C.M., McNally, R.S., Conti, B.J., Mak, T.W., Ting, J.P., 2006. DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. *Proc. Natl. Acad. Sci. U. S. A.* 103, 15091–15096.
- Cook, A.L., Vitale, A.M., Ravishankar, S., Matigian, N., Sutherland, G.T., Shan, J., Sutharsan, R., Perry, C., Silburn, P.A., Mellick, G.D., et al., 2011. NRF2 activation restores disease related metabolic deficiencies in olfactory neurosphere-derived cells from patients with sporadic Parkinson's disease. *PLoS One* 6, e21907.
- Correa, F., Mallard, C., Nilsson, M., Sandberg, M., 2011a. Activated microglia decrease histone acetylation and Nrf2-inducible anti-oxidant defence in astrocytes: restoring effects of inhibitors of HDACs, p38 MAPK and GSK3beta. *Neurobiol. Dis.* 44, 142–151.
- Correa, F., Ljunggren, E., Mallard, C., Nilsson, M., Weber, S.G., Sandberg, M., 2011b. The Nrf2-inducible antioxidant defense in astrocytes can be both up- and down-regulated by activated microglia: involvement of p38 MAPK. *Glia* 59, 785–799.
- Correa, F., Mallard, C., Nilsson, M., Sandberg, M., 2012. Dual TNFalpha-induced effects on NRF2 mediated antioxidant defence in astrocyte-rich cultures: role of protein kinase activation. *Neurochem. Res.* 37, 2842–2855.
- Correa, F., Ljunggren, E., Patil, J., Wang, X., Hagberg, H., Mallard, C., Sandberg, M., 2013. Time-dependent effects of systemic lipopolysaccharide injection on regulators of antioxidant defence Nrf2 and PGC-1alpha in the neonatal rat brain. *Neuroimmunomodulation* 20, 185–193.
- Cotman, C.W., Berchtold, N.C., Christie, L.A., 2007. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci.* 30, 464–472.
- Cullinan, S.B., Zhang, D., Hannink, M., Arvisais, E., Kaufman, R.J., Diehl, J.A., 2003. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol. Cell Biol.* 23, 7198–7209.
- Cummings, C., 2012. Melatonin for the management of sleep disorders in children and adolescents. *Paediatr. Child Health* 17, 331–336.
- D'Angelo, B., Ek, C.J., Sandberg, M., Mallard, C., 2013. Expression of the Nrf2-system at the blood-CSF barrier is modulated by neonatal inflammation and hypoxia-ischemia. *J. Inherit. Metab. Dis.* 36, 479–490.
- Devos, D., Leboviev, T., Lardeux, B., Biraud, M., Rouaud, T., Poulet, H., Coron, E., Bruley des Varannes, S., Naveilhan, P., Nguyen, J.M., et al., 2013. Colonic inflammation in Parkinson's disease. *Neurobiol. Dis.* 50, 42–48.
- Dhakshinamoorthy, S., Jaiswal, A.K., 2001. Functional characterization and role of Nrf2 in antioxidant response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase 1 gene. *Oncogene* 20, 3906–3917.
- Dhakshinamoorthy, S., Jain, A.K., Bloom, D.A., Jaiswal, A.K., 2005. Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. *J. Biol. Chem.* 280, 16891–16900.
- Dinkova-Kostova, A.T., Holtzclaw, W.D., Cole, R.N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M., 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. U. S. A.* 99, 11908–11913.
- Espada, S., Rojo, A.I., Salinas, M., Cuadrado, A., 2009. The muscarinic M1 receptor activates Nrf2 through a signaling cascade that involves protein kinase C and inhibition of GSK-3beta: connecting neurotransmission with neuroprotection. *J. Neurochem.* 110, 1107–1119.
- Fragoulis, A., Laufs, J., Muller, S., Soppa, U., Siegl, S., Reiss, L.K., Tohidnezhad, M., Rosen, C., Tenbrock, K., Varoga, D., et al., 2012. Sulforaphane has opposing effects on TNF-alpha stimulated and unstimulated synoviocytes. *Arthritis Res. Ther.* 14, R220.
- Gan, L., Vargas, M.R., Johnson, D.A., Johnson, J.A., 2012. Astrocyte-specific overexpression of Nrf2 delays motor pathology and synuclein aggregation throughout the CNS in the alpha-synuclein mutant (A53T) mouse model. *J. Neurosci.* 32, 17775–17787.
- Gao, L., Wang, J., Sekhar, K.R., Yin, H., Yared, N.F., Schneider, S.N., Sasi, S., Dalton, T.P., Anderson, M.E., Chan, J.Y., et al., 2007. Novel n-3 fatty acid oxidation products activate Nrf2 by destabilizing the association between Keap1 and Cullin3. *J. Biol. Chem.* 282, 2529–2537.
- Gemma, C., Mesches, M.H., Sepesi, B., Choo, K., Holmes, D.B., Bickford, P.C., 2002. Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. *J. Neurosci.* 22, 6114–6120.
- Gold, R., Kappos, L., Arnold, D.L., Bar-Or, A., Giovannoni, G., Selmaj, K., Tornatore, C., Sweetser, M.T., Yang, M., Sheikh, S.I., et al., 2012. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. *N. Engl. J. Med.* 367, 1098–1107.
- Gounder, S.S., Kannan, S., Devadoss, D., Miller, C.J., Whitehead, K.S., Odelberg, S.J., Firpo, M.A., Paine 3rd, R., Hoidal, J.R., Abel, E.D., et al., 2012. Impaired transcriptional activity of Nrf2 in age-related myocardial oxidative stress is reversible by moderate exercise training. *PLoS One* 7, e45697.



- Harada, N., Kanayama, M., Maruyama, A., Yoshida, A., Tazumi, K., Hosoya, T., Mimura, J., Toki, T., Maher, J.M., Yamamoto, M., et al., 2011. Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages. *Arch. Biochem. Biophys.* 508, 101–109.
- Holmes, C., Cunningham, C., Zotova, E., Woolford, J., Dean, C., Kerr, S., Culliford, D., Perry, V.H., 2009. Systemic inflammation and disease progression in Alzheimer disease. *Neurology* 73, 768–774.
- Huang, H.C., Nguyen, T., Pickett, C.B., 2000. Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12475–12480.
- Huang, H.C., Xu, K., Jiang, Z.F., 2012. Curcumin-mediated neuroprotection against amyloid-beta-induced mitochondrial dysfunction involves the inhibition of GSK-3beta. *J. Alzheimers Dis.* 32, 981–996.
- Hubbs, A.F., Benkovic, S.A., Miller, D.B., O'Callaghan, J.P., Battelli, L., Schwegler-Berry, D., Ma, Q., 2007. Vacuolar leukoencephalopathy with widespread astrogliosis in mice lacking transcription factor Nrf2. *Am. J. Pathol.* 170, 2068–2076.
- Innamorato, N.G., Rojo, A.I., Garcia-Yague, A.J., Yamamoto, M., de Ceballos, M.L., Cuadrado, A., 2008. The transcription factor Nrf2 is a therapeutic target against brain inflammation. *J. Immunol.* 181, 680–689.
- Innamorato, N.G., Jazwa, A., Rojo, A.I., Garcia, C., Fernandez-Ruiz, J., Grochot-Przeczek, A., Stachurska, A., Jozkowicz, A., Dulak, J., Cuadrado, A., 2010. Different susceptibility to the Parkinson's toxin MPTP in mice lacking the redox master regulator Nrf2 or its target gene heme oxygenase-1. *PLoS One* 5, e11838.
- Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., et al., 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* 236, 313–322.
- Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D., Yamamoto, M., 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev.* 13, 76–86.
- Jain, A.K., Jaiswal, A.K., 2007. GSK-3beta acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. *J. Biol. Chem.* 282, 16502–16510.
- Jain, A., Lamark, T., Sjøttem, E., Larsen, K.B., Awuh, J.A., Overvatn, A., McMahon, M., Hayes, J.D., Johansen, T., 2010. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J. Biol. Chem.* 285, 22576–22591.
- Jakel, R.J., Townsend, J.A., Kraft, A.D., Johnson, J.A., 2007. Nrf2-mediated protection against 6-hydroxydopamine. *Brain Res.* 1144, 192–201.
- Jin, W., Wang, H., Yan, W., Zhu, L., Hu, Z., Ding, Y., Tang, K., 2009. Role of Nrf2 in protection against traumatic brain injury in mice. *J. Neurotrauma* 26, 131–139.
- Jin, Y.N., Yu, Y.V., Gundemir, S., Jo, C., Cui, M., Tieu, K., Johnson, G.V., 2013. Impaired mitochondrial dynamics and Nrf2 signaling contribute to compromised responses to oxidative stress in striatal cells expressing full-length mutant huntingtin. *PLoS One* 8, e57932.
- Jyrkkänen, H.K., Kuosmanen, S., Heinaniemi, M., Laitinen, H., Kansanen, E., Mella-Aho, E., Leinonen, H., Ylä-Herttuala, S., Levenon, A.L., 2011. Novel insights into the regulation of antioxidant-response-element-mediated gene expression by electrophiles: induction of the transcriptional repressor BACH1 by Nrf2. *Biochem. J.* 440, 167–174.
- Kaidery, N.A., Banerjee, R., Yang, L., Smirnova, N.A., Hushpulia, D.M., Liby, K.T., Williams, C.R., Yamamoto, M., Kensler, T.W., Ratan, R.R., et al., 2013. Targeting Nrf2-mediated gene transcription by extremely potent synthetic triterpenoids attenuate dopaminergic neurotoxicity in the MPTP mouse model of Parkinson's disease. *Antioxid. Redox Signal* 18, 139–157.
- Kanninen, K., Malm, T.M., Jyrkkänen, H.K., Goldsteins, G., Keksa-Goldsteine, V., Tanila, H., Yamamoto, M., Ylä-Herttuala, S., Levenon, A.L., Koistinaho, J., 2008. Nuclear factor erythroid 2-related factor 2 protects against beta amyloid. *Mol. Cell. Neurosci.* 39, 302–313.
- Kanninen, K., White, A.R., Koistinaho, J., Malm, T., 2011. Targeting glycogen synthase Kinase-3beta for therapeutic benefit against oxidative stress in Alzheimer's disease: involvement of the Nrf2-ARE pathway. *Int. J. Alzheimers Dis.* 2011, 985085.
- Kapeta, S., Chondrogianni, N., Gonos, E.S., 2010. Nuclear erythroid factor 2-mediated proteasome activation delays senescence in human fibroblasts. *J. Biol. Chem.* 285, 8171–8184.
- Kappos, L., Gold, R., Miller, D.H., Macmanus, D.G., Havrdova, E., Limmroth, V., Polman, C.H., Schmierer, K., Youstry, T.A., Yang, M., et al., 2008. Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. *Lancet* 372, 1463–1472.
- Kaspar, J.W., Jaiswal, A.K., 2010. An autoregulatory loop between Nrf2 and Cul3-Rbx1 controls their cellular abundance. *J. Biol. Chem.* 285, 21349–21358.
- Keum, Y.S., Yu, S., Chang, P.P., Yuan, X., Kim, J.H., Xu, C., Han, J., Agarwal, A., Kong, A.N., 2006. Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. *Cancer Res.* 66, 8804–8813.
- Kim, H.J., Vaziri, N.D., 2010. Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure. *Am. J. Physiol. Ren. Physiol.* 298, F662–F671.
- Kim, J.E., You, D.J., Lee, C., Ahn, C., Seong, J.Y., Hwang, J.I., 2010. Suppression of NF-kappaB signaling by KEAP1 regulation of IKKbeta activity through autophagic degradation and inhibition of phosphorylation. *Cell Signal* 22, 1645–1654.
- Kim, K.H., Lyu, J.H., Koo, S.T., Oh, S.R., Lee, H.K., Ahn, K.S., Sadikot, R.T., Joo, M., 2011. MyD88 is a mediator for the activation of Nrf2. *Biochem. Biophys. Res. Commun.* 404, 46–51.
- Kirby, J., Halligan, E., Baptista, M.J., Allen, S., Heath, P.R., Holden, H., Barber, S.C., Loynes, C.A., Wood-Allum, C.A., Lunec, J., et al., 2005. Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS. *Brain* 128, 1686–1706.
- Kobayashi, A., Kang, M.I., Okawa, H., Ohtsujii, M., Zenke, Y., Chiba, T., Igarashi, K., Yamamoto, M., 2004. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol. Cell Biol.* 24, 7130–7139.
- Kobayashi, A., Kang, M.I., Watai, Y., Tong, K.I., Shibata, T., Uchida, K., Yamamoto, M., 2006. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. *Mol. Cell Biol.* 26, 221–229.
- Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., et al., 2007. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell* 131, 1149–1163.
- Komatsu, M., Kurokawa, H., Waguri, S., Taguchi, K., Kobayashi, A., Ichimura, Y., Sou, Y.S., Ueno, I., Sakamoto, A., Tong, K.I., et al., 2010. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat. Cell Biol.* 12, 213–223.
- Konstantinidou, V., Covas, M.I., Sola, R., Fito, M., 2013. Up-to date knowledge on the in vivo transcriptomic effect of the Mediterranean diet in humans. *Mol. Nutr. Food Res.* 57, 772–783.
- Ku, C.S., Pham, T.X., Park, Y., Kim, B., Shin, M.S., Kang, I., Lee, J., 2013. Edible blue-green algae reduce the production of pro-inflammatory cytokines by inhibiting NF-kappaB pathway in macrophages and splenocytes. *Biochim. Biophys. Acta* 1830, 2981–2988.
- Kwak, M.K., Wakabayashi, N., Greenlaw, J.L., Yamamoto, M., Kensler, T.W., 2003a. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol. Cell Biol.* 23, 8786–8794.
- Kwak, M.K., Wakabayashi, N., Itoh, K., Motohashi, H., Yamamoto, M., Kensler, T.W., 2003b. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. *J. Biol. Chem.* 278, 8135–8145.
- Kwak, M.K., Cho, J.M., Huang, B., Shin, S., Kensler, T.W., 2007. Role of increased expression of the proteasome in the protective effects of sulforaphane against hydrogen peroxide-mediated cytotoxicity in murine neuroblastoma cells. *Free Radic. Biol. Med.* 43, 809–817.
- Lamark, T., Kirkin, V., Dikic, I., Johansen, T., 2009. NBR1 and p62 as cargo receptors for selective autophagy of ubiquitinated targets. *Cell Cycle* 8, 1986–1990.
- Lastres-Becker, I., Ulusoy, A., Innamorato, N.G., Sahin, G., Rabano, A., Kirik, D., Cuadrado, A., 2012. alpha-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson's disease. *Hum. Mol. Genet.* 21, 3173–3192.
- Lau, A., Wang, X.J., Zhao, F., Villeneuve, N.F., Wu, T., Jiang, T., Sun, Z., White, E., Zhang, D.D., 2010. A noncanonical mechanism of Nrf2 activation by autophagy deficiency: direct interaction between Keap1 and p62. *Mol. Cell Biol.* 30, 3275–3285.
- Levenon, A.L., Landar, A., Ramachandran, A., Ceaser, E.K., Dickinson, D.A., Zononi, G., Morrow, J.D., Darley-Usmar, V.M., 2004. Cellular mechanisms of redox cell signalling: role of cysteine modification in controlling antioxidant defences in response to electrophilic lipid oxidation products. *Biochem. J.* 378, 373–382.
- Liu, F., Nguyen, J.L., Hulleman, J.D., Li, L., Rochet, J.C., 2008a. Mechanisms of DJ-1 neuroprotection in a cellular model of Parkinson's disease. *J. Neurochem.* 105, 2435–2453.
- Liu, G.H., Qu, J., Shen, X., 2008b. NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. *Biochim. Biophys. Acta* 1783, 713–727.
- Lourida, I., Soni, M., Thompson-Coon, J., Purandare, N., Lang, I.A., Ukoumunne, O.C., Llewellyn, D.J., 2013. Mediterranean diet, cognitive function, and dementia: a systematic review. *Epidemiology* 24, 479–489.
- Meng, P., Yoshida, H., Matsumiya, T., Imaizumi, T., Tanji, K., Xing, F., Hayakari, R., Dempoya, J., Tatsuta, T., Aizawa-Yashiro, T., et al., 2013. Carnosic acid suppresses the production of amyloid-beta 1–42 by inducing the metalloprotease gene TACE/ADAM17 in SH-SY5Y human neuroblastoma cells. *Neurosci. Res.* 75, 94–102.
- Milder, J.B., Liang, L.P., Patel, M., 2010. Acute oxidative stress and systemic Nrf2 activation by the ketogenic diet. *Neurobiol. Dis.* 40, 238–244.
- Moi, P., Chan, K., Asunis, I., Cao, A., Kan, Y.W., 1994. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc. Natl. Acad. Sci. U. S. A.* 91, 9926–9930.
- Morrison, C.D., Pistell, P.J., Ingram, D.K., Johnson, W.D., Liu, Y., Fernandez-Him, S.O., White, C.L., Purpera, M.N., Uranga, R.M., Bruce-Keller, A.J., et al., 2010. High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased Nrf2 signaling. *J. Neurochem.* 114, 1581–1589.
- Myzak, M.C., Karplus, P.A., Chung, F.L., Dashwood, R.H., 2004. A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase. *Cancer Res.* 64, 5767–5774.
- Naidu, S., Vijayan, V., Santoso, S., Kietzmann, T., Immenschuh, S., 2009. Inhibition and genetic deficiency of p38 MAPK up-regulates heme oxygenase-1 gene expression via Nrf2. *J. Immunol.* 182, 7048–7057.
- Nair, S., Doh, S.T., Chan, J.Y., Kong, A.N., Cai, L., 2008. Regulatory potential for concerted modulation of Nrf2- and Nfkb1-mediated gene expression in inflammation and carcinogenesis. *Br. J. Cancer* 99, 2070–2082.
- Nakaso, K., Yano, H., Fukuhara, Y., Takeshima, T., Wada-Isoe, K., Nakashima, K., 2003. PI3K is a key molecule in the Nrf2-mediated regulation of



- antioxidative proteins by hemin in human neuroblastoma cells. *FEBS Lett.* 546, 181–184.
- Narasimhan, M., Patel, D., Vedpathak, D., Rathinam, M., Henderson, G., Mahimainathan, L., 2012. Identification of novel microRNAs in post-transcriptional control of Nrf2 expression and redox homeostasis in neuronal, SH-SY5Y cells. *PLoS One* 7, e51111.
- Niture, S.K., Jaiswal, A.K., 2012. Nrf2 protein up-regulates antiapoptotic protein Bcl-2 and prevents cellular apoptosis. *J. Biol. Chem.* 287, 9873–9886.
- Niture, S.K., Jain, A.K., Jaiswal, A.K., 2009. Antioxidant-induced modification of INrf2 cysteine 151 and PKC-delta-mediated phosphorylation of Nrf2 serine 40 are both required for stabilization and nuclear translocation of Nrf2 and increased drug resistance. *J. Cell Sci.* 122, 4452–4464.
- Niture, S.K., Jain, A.K., Shelton, P.M., Jaiswal, A.K., 2011. Src subfamily kinases regulate nuclear export and degradation of transcription factor Nrf2 to switch off Nrf2-mediated antioxidant activation of cytoprotective gene expression. *J. Biol. Chem.* 286, 28821–28832.
- Pergola, P.E., Raskin, P., Toto, R.D., Meyer, C.J., Huff, J.W., Grossman, E.B., Krauth, M., Ruiz, S., Audhya, P., Christ-Schmidt, H., et al., 2011. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *N. Engl. J. Med.* 365, 327–336.
- Pi, J., Bai, Y., Reece, J.M., Williams, J., Liu, D., Freeman, M.L., Fahl, W.E., Shugar, D., Liu, J., Qu, W., et al., 2007. Molecular mechanism of human Nrf2 activation and degradation: role of sequential phosphorylation by protein kinase CK2. *Free Radic. Biol. Med.* 42, 1797–1806.
- Piantadosi, C.A., Withers, C.M., Bartz, R.R., MacGarvey, N.C., Fu, P., Sweeney, T.E., Welty-Wolf, K.E., Suliman, H.B., 2011. Heme oxygenase-1 couples activation of mitochondrial biogenesis to anti-inflammatory cytokine expression. *J. Biol. Chem.* 286, 16374–16385.
- Pickering, A.M., Linder, R.A., Zhang, H., Forman, H.J., Davies, K.J., 2012. Nrf2-dependent induction of proteasome and Pa28alpha regulator are required for adaptation to oxidative stress. *J. Biol. Chem.* 287, 10021–10031.
- Psaltopoulou, T., Sergentanis, T.N., Panagiotakos, D.B., Sergentanis, I.N., Kosti, R., Scarmeas, N., 2013 May 30. Mediterranean diet and stroke, cognitive impairment, depression: a meta-analysis. *Ann. Neurol.* Psaltopoulou et al: *Ann Neurol.* <http://dx.doi.org/10.1002/ana.23944> [Epub ahead of print].
- Rada, P., Rojo, A.I., Evrard-Todeschi, N., Innamorato, N.G., Cotte, A., Jaworski, T., Tobon-Velasco, J.C., Devijver, H., Garcia-Mayoral, M.F., Van Leuven, F., et al., 2012. Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/beta-TrCP axis. *Mol. Cell. Biol.* 32, 3486–3499.
- Ramsey, C.P., Glass, C.A., Montgomery, M.B., Lindl, K.A., Ritson, G.P., Chia, L.A., Hamilton, R.L., Chu, C.T., Jordan-Sciutto, K.L., 2007. Expression of Nrf2 in neurodegenerative diseases. *J. Neurochem.* 101, 75–85.
- Rojo, A.I., Innamorato, N.G., Martin-Moreno, A.M., De Ceballos, M.L., Yamamoto, M., Cuadrado, A., 2010. Nrf2 regulates microglial dynamics and neuroinflammation in experimental Parkinson's disease. *Glia* 58, 588–598.
- Sakata, H., Niizuma, K., Yoshioka, H., Kim, G.S., Jung, J.E., Katsu, M., Narasimhan, P., Maier, C.M., Nishiyama, Y., Chan, P.H., 2012. Minocycline-preconditioned neural stem cells enhance neuroprotection after ischemic stroke in rats. *J. Neurosci.* 32, 3462–3473.
- Salazar, M., Rojo, A.I., Velasco, D., de Sagarra, R.M., Cuadrado, A., 2006. Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. *J. Biol. Chem.* 281, 14841–14851.
- Salminen, A., Kaarniranta, K., Haapasalo, A., Hiltunen, M., Soininen, H., Alafuzoff, I., 2012. Emerging role of p62/sequestosome-1 in the pathogenesis of Alzheimer's disease. *Prog. Neurobiol.* 96, 87–95.
- Sarlette, A., Krampfl, K., Grothe, C., Neuheff, N., Dengler, R., Petri, S., 2008. Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. *J. Neurochem.* 106, 1055–1062.
- Scannevin, R.H., Chollate, S., Jung, M.Y., Shackett, M., Patel, H., Bista, P., Zeng, W., Ryan, S., Yamamoto, M., Lukashev, M., et al., 2012. Fumarates promote cytoprotection of central nervous system cells against oxidative stress via the nuclear factor (erythroid-derived 2)-like 2 pathway. *J. Pharmacol. Exp. Ther.* 341, 274–284.
- Sequea, D.A., Sharma, N., Arias, E.B., Cartee, G.D., 2012. Calorie restriction enhances insulin-stimulated glucose uptake and Akt phosphorylation in both fast-twitch and slow-twitch skeletal muscle of 24-month-old rats. *J. Gerontol. A Biol. Sci. Med. Sci.* 67, 1279–1285.
- Shah, Z.A., Li, R.C., Thimmulappa, R.K., Kensler, T.W., Yamamoto, M., Biswal, S., Dore, S., 2007. Role of reactive oxygen species in modulation of Nrf2 following ischemic reperfusion injury. *Neuroscience* 147, 53–59.
- Shih, A.Y., Li, P., Murphy, T.H., 2005. A small-molecule-inducible Nrf2-mediated antioxidant response provides effective prophylaxis against cerebral ischemia in vivo. *J. Neurosci.* 25, 10321–10335.
- Shimazu, T., Hirscheby, M.D., Newman, J., He, W., Shirakawa, K., Le Moan, N., Grueter, C.A., Lim, H., Saunders, L.R., Stevens, R.D., et al., 2013. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* 339, 211–214.
- Son, T.G., Camandola, S., Mattson, M.P., 2008. Hormetic dietary phytochemicals. *Neuromolecular Med.* 10, 236–246.
- Sporn, M.B., Liby, K.T., 2012. NRF2 and cancer: the good, the bad and the importance of context. *Nat. Rev. Cancer* 12, 564–571.
- Stack, C., Ho, D., Wille, E., Calingasan, N.Y., Williams, C., Liby, K., Sporn, M., Dumont, M., Beal, M.F., 2010. Triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improve the behavioral phenotype and brain pathology in a transgenic mouse model of Huntington's disease. *Free Radic. Biol. Med.* 49, 147–158.
- Su, Z.Y., Khor, T.O., Shu, L., Lee, J.H., Saw, C.L., Wu, T.Y., Huang, Y., Suh, N., Yang, C.S., Conney, A.H., et al., 2013. Epigenetic reactivation of Nrf2 in murine prostate cancer TRAMP C1 cells by natural phytochemicals Z-ligustilide and Radix angelica sinensis via promoter CpG demethylation. *Chem. Res. Toxicol.* 26, 477–485.
- Sun, Z., Huang, Z., Zhang, D.D., 2009a. Phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. *PLoS One* 4, e6588.
- Sun, Z., Chin, Y.E., Zhang, D.D., 2009b. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. *Mol. Cell. Biol.* 29, 2658–2672.
- Tanji, K., Maruyama, A., Odagiri, S., Mori, F., Itoh, K., Kakita, A., Takahashi, H., Wakabayashi, K., 2013. Keap1 is localized in neuronal and glial cytoplasmic inclusions in various neurodegenerative diseases. *J. Neurochem.* 126, 18–28.
- Teiten, M.H., Dicato, M., Diederich, M., 2013. Curcumin as a regulator of epigenetic events. *Mol. Nutr. Food Res.* 7 (9), 1619–1629.
- Vargas, M.R., Johnson, D.A., Sirkis, D.W., Messing, A., Johnson, J.A., 2008. Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *J. Neurosci.* 28, 13574–13581.
- Venugopal, R., Jaiswal, A.K., 1998. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 17, 3145–3156.
- Villaran, R.F., Espinosa-Oliva, A.M., Sarmiento, M., De Pablos, R.M., Arguelles, S., Delgado-Cortes, M.J., Sobrino, V., Van Rooijen, N., Venero, J.L., Herrera, A.J., et al., 2010. Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson's disease. *J. Neurochem.* 114, 1687–1700.
- von Otter, M., Landgren, S., Nilsson, S., Zetterberg, M., Celojovic, D., Bergstrom, P., Minthon, L., Bogdanovic, N., Andreassen, N., Gustafson, D.R., et al., 2010a. Nrf2-encoding NFE2L2 haplotypes influence disease progression but not risk in Alzheimer's disease and age-related cataract. *Mech. Ageing Dev.* 131, 105–110.
- von Otter, M., Landgren, S., Nilsson, S., Celojovic, D., Bergstrom, P., Hakansson, A., Nissbrandt, H., Drozdziak, M., Bialecka, M., Kurzawski, M., et al., 2010b. Association of Nrf2-encoding NFE2L2 haplotypes with Parkinson's disease. *BMC Med. Genet.* 11, 36.
- Wang, X., Svedin, P., Nie, C., Lapatto, R., Zhu, C., Gustavsson, M., Sandberg, M., Karlsson, J.O., Romero, R., Hagberg, H., et al., 2007. N-acetylcysteine reduces lipopolysaccharide-sensitized hypoxic-ischemic brain injury. *Ann. Neurol.* 61, 263–271.
- Wang, S., Penchala, S., Prabhu, S., Wang, J., Huang, Y., 2010. Molecular basis of traditional Chinese medicine in cancer chemoprevention. *Curr. Drug Discov. Technol.* 7, 67–75.
- Wang, J.S., Ho, F.M., Kang, H.C., Lin, W.W., Huang, K.C., 2011. Celecoxib induces heme oxygenase-1 expression in macrophages and vascular smooth muscle cells via ROS-dependent signaling pathway. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 383, 159–168.
- Wang, B., Zhu, X., Kim, Y., Li, J., Huang, S., Saleem, S., Li, R.C., Xu, Y., Dore, S., Cao, W., 2012a. Histone deacetylase inhibition activates transcription factor Nrf2 and protects against cerebral ischemic damage. *Free Radic. Biol. Med.* 52, 928–936.
- Wang, Z., Ma, C., Meng, C.J., Zhu, G.Q., Sun, X.B., Huo, L., Zhang, J., Liu, H.X., He, W.C., Shen, X.M., et al., 2012b. Melatonin activates the Nrf2-ARE pathway when it protects against early brain injury in a subarachnoid hemorrhage model. *J. Pineal Res.* 53, 129–137.
- Wang, W., Kang, J., Li, H., Su, J., Wu, J., Xu, Y., Yu, H., Xiang, X., Yi, H., Lu, Y., et al., 2013. Regulation of endoplasmic reticulum stress in rat cortex by p62/ZIP through the Keap1-Nrf2-ARE signalling pathway after transient focal cerebral ischaemia. *Brain Inj.* 27, 924–933.
- White, E., Karp, C., Strohecker, A.M., Guo, Y., Mathew, R., 2010. Role of autophagy in suppression of inflammation and cancer. *Curr. Opin. Cell Biol.* 22, 212–217.
- Xu, C., Yuan, X., Pan, Z., Shen, G., Kim, J.H., Yu, S., Khor, T.O., Li, W., Ma, J., Kong, A.N., 2006. Mechanism of action of isothiocyanates: the induction of ARE-regulated genes is associated with activation of ERK and JNK and the phosphorylation and nuclear translocation of Nrf2. *Mol. Cancer Ther.* 5, 1918–1926.
- Yang, L., Calingasan, N.Y., Thomas, B., Chaturvedi, R.K., Kiaei, M., Wille, E.J., Liby, K.T., Williams, C., Royce, D., Risingsong, R., et al., 2009. Neuroprotective effects of the triterpenoid, CDDO methyl amide, a potent inducer of Nrf2-mediated transcription. *PLoS One* 4, e5757.
- Yasuda, S., Sugiura, H., Tanaka, H., Takigami, S., Yamagata, K., 2011. p38 MAP kinase inhibitors as potential therapeutic drugs for neural diseases. *Cent. Nerv. Syst. Agents Med. Chem.* 11, 45–59.
- Yates, M.S., Tauchi, M., Katsuoka, F., Flanders, K.C., Liby, K.T., Honda, T., Gribble, G.W., Johnson, D.A., Johnson, J.A., Burton, N.C., et al., 2007. Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes. *Mol. Cancer Ther.* 6, 154–162.
- Yu, M., Li, H., Liu, Q., Liu, F., Tang, L., Li, C., Yuan, Y., Zhan, Y., Xu, W., Li, W., et al., 2011. Nuclear factor p65 interacts with Keap1 to repress the Nrf2-ARE pathway. *Cell Signal* 23, 883–892.
- Zhang, D.D., 2013. Bardoxolone brings Nrf2-based therapies to light. *Antioxid. Redox Signal* 19, 517–518.
- Zhang, D.D., Lo, S.C., Sun, Z., Habib, G.M., Lieberman, M.W., Hannink, M., 2005. Ubiquitination of Keap1, a BTB-Kelch substrate adaptor protein for Cul3, targets Keap1 for degradation by a proteasome-independent pathway. *J. Biol. Chem.* 280, 30091–30099.
- Zhu, Y., Carvey, P.M., Ling, Z., 2007. Altered glutathione homeostasis in animals prenatally exposed to lipopolysaccharide. *Neurochem. Int.* 50, 671–680.