

Review

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Nrf2 activation in the treatment of neurodegenerative diseases: a focus on its role in mitochondrial bioenergetics and function

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Abstract: The nuclear factor erythroid-derived 2 (NF-E2)-related factor 2 (Nrf2) is a transcription factor well-known for its function in controlling the basal and inducible expression of a variety of antioxidant and detoxifying enzymes. As part of its cytoprotective activity, increasing evidence supports its role in metabolism and mitochondrial bioenergetics and function. Neurodegenerative diseases are excellent candidates for Nrf2-targeted treatments. Most neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia and Friedreich's ataxia are characterized by oxidative stress, misfolded protein aggregates, and chronic inflammation, the common targets of Nrf2 therapeutic strategies. Together with them, mitochondrial dysfunction is implicated in the pathogenesis of most neurodegenerative disorders. The recently recognized ability of Nrf2 to regulate intermediary metabolism and mitochondrial function makes Nrf2 activation an attractive and comprehensive strategy for the treatment of neurodegenerative disorders. This review aims to focus on the potential therapeutic role of Nrf2 activation in neurodegeneration, with special emphasis on mitochondrial bioenergetics and function, metabolism and the role of transporters, all of which collectively contribute to the cytoprotective activity of this transcription factor.

Introduction

The nuclear factor erythroid-derived 2 (NF-E2)-related factor 2 (Nrf2) is a well-known transcription factor for its role in controlling the basal and inducible expression of a variety of antioxidant and detoxifying enzymes (Ishii et al., 2000; McMahon et al., 2001). Together with its cytoprotective activity against oxidants and electrophiles, increasing evidence supports its role in metabolism and mitochondrial bioenergetics and function (Hayes and Dinkova-Kostova, 2014; Dinkova-Kostova and Abramov, 2015).

Nrf2 is encoded by the gene *NFE2L2* and belongs to the family of basic leucine zipper (bZIP) transcription factors. It also contains an upstream cap 'n' collar (CNC) domain, which contributes to the DNA-binding specificity of this family (Moi et al., 1994; Chevillard and Blank, 2011). In vertebrates, other members of the CNC-bZIP family of proteins include p45 NF-E2, the NF-E2-related factor 1 (Nrf1) and the NF-E2-related factor 3 (Nrf3) (Andrews et al., 1993; Chan et al., 1993; Kobayashi et al., 1999). p45 NF-E2 is mainly expressed in hematopoietic progenitors and it is essential for megakaryocyte maturation and platelet formation (Shivdasani et al., 1995; Gasiorok and Blank, 2015). In contrast, Nrf1, Nrf2 and Nrf3 are ubiquitously expressed (Sykietis and Bohmann, 2010). In mammals, Nrf1 and Nrf2 are well known for their role in the transcriptional up-regulation of cytoprotective genes in response to redox stress (Venugopal and Jaiswal, 1998; Biswas and Chan, 2010), whereas Nrf3 has been linked to differentiation, inflammation and carcinogenesis (Chevillard and Blank, 2011).

Nrf2 forms a heterodimer with the small musculoaponeurotic fibrosarcoma (sMaf) proteins to bind DNA (Itoh et al., 1997). The sMaf proteins are also bZIP factors

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which form a family of three members, MafF, MafG and MafK, with similar functions but different distribution (Onodera et al., 1999). Heterodimerization with sMaf is essential to give Nrf2 binding specificity to its target DNA sequence (Motohashi et al., 2004), known as ARE (antioxidant response element) (Venugopal and Jaiswal, 1996; Venugopal and Jaiswal, 1998). AREs are upstream regulatory sequences that mediate the transcriptional activation of genes constitutively or in response to an oxidative or electrophilic stress signal (Nguyen et al., 2003). Nrf2 appears to be more potent in activating ARE-regulated genes than Nrf1 (Jaiswal, 2004). Binding of the heterodimer Nrf2/sMaf to the ARE, recruits the general transcriptional machinery for expression of ARE-regulated genes (Nguyen et al., 2003; Kensler et al., 2007). Many of these genes are implicated in xenobiotics detoxification. Drugs and xenobiotics undergo a series of metabolic reactions directed to increase the solubility of the molecules in water and make them easily removable from the cell. In the first step (phase I) drugs are functionalized to make them more reactive. The common reactions in this phase are oxidation, reduction or hydrolysis and are catalyzed by enzymes that can be induced by Nrf2, such as cytochrome P450 (Abu-Bakar et al., 2007; Yokota et al., 2011); aldo-keto reductases (AKR) (Ellis, 2007) or NAD(P)H:quinone oxidoreductase 1 (NQO1) (Venugopal and Jaiswal, 1996), (considered a broad antioxidant and cytoprotector; Dinkova-Kostova and Talalay, 2010). During phase II detoxification, xenobiotics are subjected to conjugation with different polar compounds, such as glutathione (GSH), glucuronic acid or a sulfonic group. Conjugation with GSH is catalyzed by glutathione S-transferases (GST) and can also be induced by Nrf2 (McMahon et al., 2001; Chanas et al., 2002; Aleksunes and Klaassen, 2012). Additionally, Nrf2 can induce UDP glucuronosyltransferases (Enomoto et al., 2001; Kalthoff et al., 2010), which catalyze the conjugation of the xenobiotic with glucuronic acid.

Nrf2 is also able to induce different genes related to direct endogenous antioxidant systems in the body, such as GSH, thioredoxin (TXN) or peroxiredoxin (PRDX) systems, for reviews see Tebay et al. (2015) and Hayes and Dinkova-Kostova (2014). Those systems generally consist of molecules that hold cysteine residues with reactive thiol (-SH) groups. The -SH groups are able to reduce oxidized proteins, getting themselves oxidized and forming a disulphide bridge (-S-S-). The oxidized forms of the antioxidants are then reduced by reductases, which allow regeneration of the pool of the active form of the antioxidant. Generally, the regeneration of the reduced form requires the presence of NADPH as a cofactor. Nrf2 is able to induce both the catalytic and the modifier subunits of

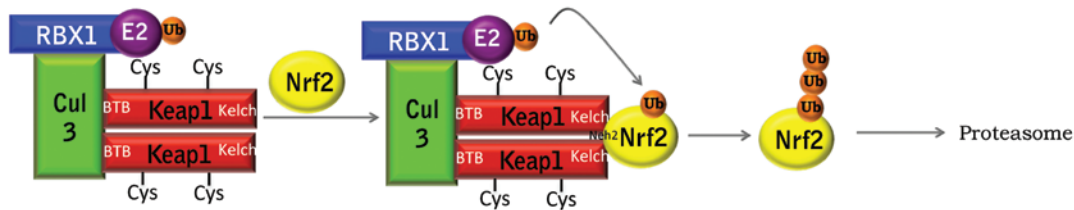
glutamate-cysteine ligase together with glutathione reductase, which are essential for GSH synthesis and regeneration, respectively; thioredoxin and thioredoxin reductase 1 or peroxiredoxin (MacLeod et al., 2009; Aggeman et al., 2012; Chorley et al., 2012; Hirotsu et al., 2012). Moreover, Nrf2 is able to induce the principal enzymes implicated in NADPH generation, namely glucose-6-phosphate dehydrogenase (G6PD), malic enzyme 1 (ME-1), isocitrate dehydrogenase 1 (IDH-1), and 6-phosphogluconate dehydrogenase (PGD) (Thimmulappa et al., 2002; Lee et al., 2003; Wu et al., 2011; Aggeman et al., 2012; Tebay et al., 2015). NADPH regeneration is crucial in the maintenance of redox homeostasis, and is also required for biosynthetic reactions (Ying, 2008). In fact, there is growing evidence indicating that Nrf2-regulated genes are not restricted to redox homeostasis but also implicated in metabolism. It has been described that in cancer cells, Nrf2 activation through PI3K/Akt, contributes to enhance cell proliferation by redirecting glucose and glutamine into anabolic pathways (Mitsuishi et al., 2012b). In addition to carbohydrate metabolism regulation, Nrf2 is also implicated in lipid metabolism, as it promotes β -oxidation of fatty acids (Wu et al., 2011; Ludtmann et al., 2014), and negatively regulates genes associated with lipid biosynthesis (Yates et al., 2009) (reviewed in Hayes and Dinkova-Kostova, 2014). The involvement of Nrf2 in mitochondrial bioenergetics is getting increasing attention and will be discussed later.

Nrf2 structure and regulation

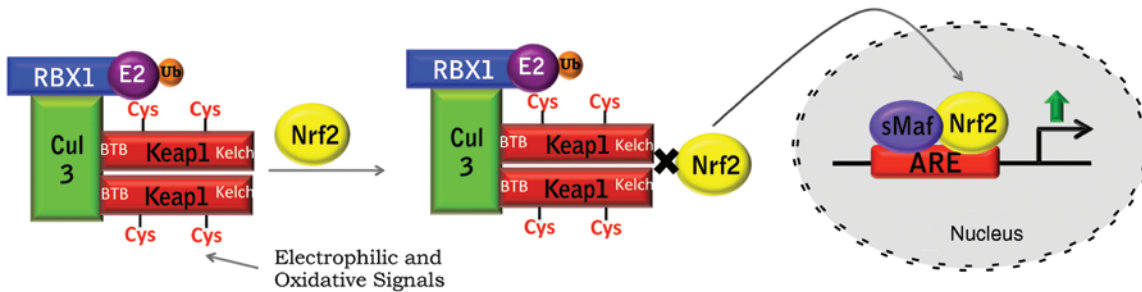
Nrf2 activity is tightly controlled. Under basal conditions, Nrf2 is maintained at low levels, as it is continuously being degraded in the proteasome. There are different ubiquitin ligase systems responsible for targeting Nrf2 for degradation in the proteasome. The best known is the Cullin 3 (Cul3) RING-box 1 (RBX1) E3 ubiquitin ligase complex, which needs the substrate adaptor protein Kelch-like ECH-associated protein 1 (Keap1) to ubiquitinate Nrf2 (Cullinan et al., 2004; Kobayashi et al., 2004; Zhang et al., 2004). Keap1 is a cysteine-rich regulatory protein located in the cytoplasm (Itoh et al., 1999; Holland and Fishbein, 2010). Keap1 dimerizes and binds Cul3 through its BTB domain (Furukawa and Xiong, 2005), while its Kelch domain is able to interact with the Neh2 domain of Nrf2 (Itoh et al., 1999; McMahon et al., 2006). By a cyclical mechanism, Nrf2 is then ubiquitinated and transferred to the proteasome, where it is degraded, while Keap1 is regenerated (Baird et al., 2013; Baird et al., 2014) (Figure 1A). In the

Regulation of Nrf2 by Keap1

A

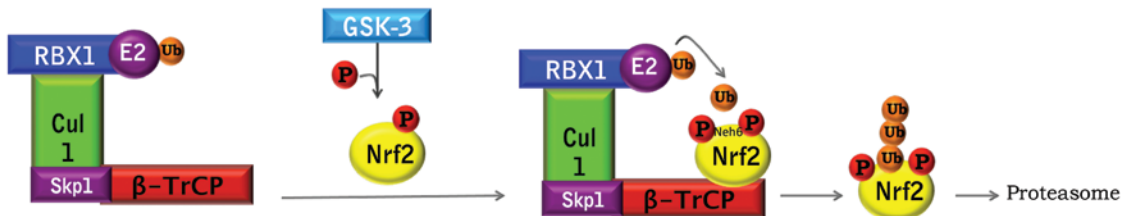


B



Regulation of Nrf2 by GSK-3

C



D

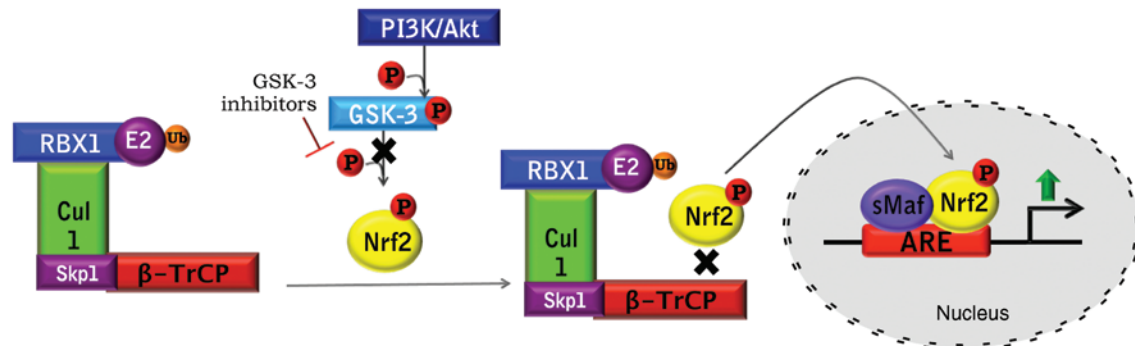


Figure 1: Regulation of Nrf2 activity by Keap1 and GSK-3.

Two main ubiquitin ligase systems are responsible for targeting Nrf2 for degradation in the proteasome. (A) Cullin 3 (Cul3) RING-box 1 (RBX1) E3 ubiquitin ligase complex, binds the adaptor protein Keap1 dimer through its BTB domain. Keap1 is then able to interact with the Neh2 domain of Nrf2, allowing the ubiquitination of Nrf2 and its degradation in the proteasome. (B) Electrophiles and oxidants (termed inducers) chemically modify specific Cys sensor residues of Keap1, leading to conformational changes that prevent Nrf2 ubiquitination. Nrf2 then accumulates and translocates to the nucleus, where it forms a dimer with the small musculoaponeurotic fibrosarcoma (sMaf) proteins to bind ARE regions in the DNA and mediate the up-regulation of its target genes. (C) The adaptor protein β-TrCP binds the SCF ubiquitin ligase complex (formed by the Skp1 adaptor, Cullin 1 and Rbx1, where the E2-ubiquitin conjugate binds). Glycogen synthase kinase-3 (GSK-3) phosphorylates Nrf2 in the Neh6 domain. Phosphorylated Nrf2 is then recognized by β-TrCP, targeting the protein for degradation through this system. (D) Certain signaling pathways, such as PI3K/Akt, are able to phosphorylate GSK-3 and inactivate it, therefore allowing Nrf2 accumulation.

absence of Keap1, Nrf2 is no longer sequestered in the cytoplasm and degraded, and is able to translocate to the nucleus and allow the transcription of its target genes.

Keap1 contains reactive cysteine residues, some of which act as sensors of electrophilic and/or oxidative signals (Dinkova-Kostova et al., 2002; Eggler et al., 2005; Fourquet et al., 2010; McMahon et al., 2010; Saito et al., 2015). Under stress conditions, certain Keap1 cysteine residues are chemically modified, which prevents Nrf2 ubiquitination and degradation, allowing its stabilization and the transcription of its target genes (Holland and Fishbein, 2010) (Figure 1B). It has also been described that some electrophilic lipids, such as the cyclopentenone prostaglandin, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J_2 (which serves as a model to study the effects of lipid peroxidation products derived by the reaction of reactive oxygen/nitrogen species with unsaturated fatty acids), are able to induce Nrf2 by modifying thiol groups of Keap1 (Levonen et al., 2004). Another mechanism by which modification of certain cysteines in Keap1 leads to diminished degradation of Nrf2 is by decreasing the affinity of Keap1 for Cul3, and subsequently diminishing Nrf2 ubiquitination (Gao et al., 2007; Eggler et al., 2009).

The p62-dependent autophagic degradation of Keap1 is another mechanism of Nrf2 activation and cytoprotection (Taguchi et al., 2012). This mechanism has been shown to occur in response to lipotoxicity (Park et al., 2015) or to be mediated by sestrins (Bae et al., 2013). p62 has also been reported to interact with the Nrf2-binding site on Keap1, competing with Nrf2, and thus preventing its degradation (Jain et al., 2010; Komatsu et al., 2010; Lau et al., 2010). This is especially prominent under conditions when p62 is phosphorylated (Ichimura et al., 2013).

Although Keap1 is the most studied regulator of Nrf2 activity, more recently the role of another E3-ubiquitin ligase adaptor, β -TrCP, was described, together with the SCF ubiquitin ligase complex. This mechanism, which is independent of Keap1, is regulated by glycogen synthase kinase-3 (GSK-3), which phosphorylates Nrf2 in the Neh6 domain, targeting the protein for degradation by the proteasome through SCF/ β -TrCP and therefore inhibiting Nrf2 activity (Rada et al., 2011; Chowdhry et al., 2013) (Figure 1C). It has been proposed that while Keap1 acts as a major sensor of electrophiles and oxidants, GSK-3/ β -TrCP system participates in receptor-mediated signal transduction, modulating the Nrf2 levels in response to transient metabolic demands (Cuadrado, 2015). This mechanism could involve pathways that can participate in GSK-3 regulation such as PI3K/Akt or WNT signaling (Figure 1D).

Nrf2 activators

A wide range of compounds are able to react with cysteine sensors of Keap1, inducing conformational changes that prevent Nrf2 ubiquitination, leading to its accumulation. The majority of the small molecule pharmacological activators of Nrf2 are electrophilic and structurally varied, and include cyano enones, such as TBE-31, a tricyclic compound with two highly reactive Michael acceptor groups (Liby et al., 2008). TBE-31 binds to its target in a reversible covalent mode, and represents one of the most potent Nrf2 activators known to date. Sulforaphane (SFN), an isothiocyanate obtained from cruciferous vegetables such as broccoli, is one of the most potent naturally occurring Nrf2 activators (Zhang et al., 1992). The fumaric acid ester dimethyl fumarate is also able to stabilize Nrf2 (Spencer et al., 1990), and it has been successfully used for the treatment of psoriasis (Kolbach and Nieboer, 1992). Its oral formulation BG-12 has been recently approved for the treatment of multiple sclerosis (Linker et al., 2011). Other molecules able to stabilize Nrf2 are the quinone compound *tert*-butylhydroquinone (tBHQ) (Li et al., 2005) and the triterpenoids bardoxolone methyl (Dinkova-Kostova et al., 2005) and RTA 408 (Probst et al., 2015), both of which are currently in clinical trials.

All the Nrf2 activators presented above exemplify electrophilic activators of Nrf2, but there is also an increasing interest in developing non-electrophilic molecules that can activate this pathway (see Richardson et al., 2015, for a review).

Activating Nrf2 can represent an excellent pharmacological strategy for diseases in which oxidative stress or mitochondrial dysfunction are key features of the pathology. However, it is also important to examine the potential toxicity that these molecules may exhibit (de Zeeuw et al., 2013), specially regarding to the dosing regimen. It should also be considered that in certain context, Nrf2 activation could be detrimental. One of the major concerns is related to the association between Nrf2 activation and cancer progression. It has been suggested that the increase in cytoprotective activity in the cell and the decrease in ROS levels may promote the survival of tumor cells in certain situations. Gain-of-function *NFE2L2* mutations or loss-of-function *KEAP1* mutations have been found in different carcinomas, and decreased expression of Keap1 or increased expression of Nrf2 have been associated with poor prognosis (Solis et al., 2010). For those reasons, taking into account the context is essential in designing Nrf2 therapeutic approaches (Sporn and Liby, 2012).

Nrf2 in metabolism and mitochondrial function

Although most of the studies up to date have been focused on the antioxidant properties of Nrf2 activation, there is increasing evidence pointing at the role of Nrf2 in metabolism and mitochondrial function. There are several mechanisms by which Nrf2 may affect metabolism. One of them is by directly activating the transcription of enzymes containing ARE-sequences in their gene promoters. The presence of ARE-sequences has been described in the promoters of the genes encoding the Peroxisome Proliferator-Activated Receptor γ (PPAR γ) and the Retinoid X Receptor α (RXR α) (Pi et al., 2010; Chorley et al., 2012). RXR α forms part of the retinoic acid receptors (RARs), which are nuclear receptors that mediate the biological effects of retinoids. These receptors function as transcription factors by binding as homodimers or heterodimers with other nuclear receptors such as PPAR γ to specific sequences in the promoters of target genes. PPAR γ is a nuclear receptor that regulates adipocyte differentiation and adipogenesis. For this reason, it was suggested that Nrf2 regulation of both RXR α and PPAR γ has implications for response to retinoid treatments and adipogenesis (Chorley et al., 2012).

Recent studies have used chromatin immunoprecipitation-sequencing (ChIP-Seq) to identify human Nrf2-regulated genes in lymphoid cells treated with sulforaphane (Chorley et al., 2012) or in mouse embryonic fibroblasts (MEF) with constitutive accumulation of Nrf2 (*Keap1*^{-/-}) or Nrf2 depletion (*Nrf2*^{-/-}) (Malhotra et al., 2010). Together with known Nrf2 targets, such as *HMOX1*, which participates in the heme degradation pathway, or ferritin (*FTL* and *FTH1*), implicated in iron homeostasis, or those implicated in antioxidant signaling and detoxification (e.g. *TXNRD1*, *GSTM*), these authors have identified several novel Nrf2 targets. An example of an enzyme linked to metabolism and positively regulated by Nrf2 is transaldolase 1, whose gene *TALDO1* contains an ARE sequence (Chorley et al., 2012). Transaldolase 1 is a key enzyme in the nonoxidative pentose phosphate pathway, providing ribose-5-phosphatase and NADPH for nucleic acid and lipid biosynthesis. Among others, they found that several genes involved in mitochondrial function are upregulated by Nrf2, such as *ABCB6*, which encodes a member of a family of ATP-binding cassette transporters (Chorley et al., 2012).

Also, it is important to note that together with the direct binding of Nrf2 to the ARE sequences in the DNA, Nrf2 can indirectly regulate many other proteins. For example, Nrf2 directly controls the expression of MafG,

one of the transcriptional coactivators with which it forms a heterodimer to bind DNA. It was found that Nrf2-MafG heterodimer directly regulates numerous genes involved in glucose metabolism through AREs. Among the pathways found in this study, there was a notable contribution of genes regulating pyruvate metabolism, glycolysis and gluconeogenesis (Hirotsu et al., 2012). But, independently of the Nrf2-MafG mediated transcription, MafG can also form homodimers and/or heterodimers with other members of the CNC family, thus activating different genes than Nrf2-MafG (Kimura et al., 2007).

Another important mechanism by which Nrf2 can alter metabolism or mitochondrial function is by preventing the oxidative thiol modifications that can modulate the function of proteins implicated in metabolic pathways. It has been described that mitochondrial ROS can reversibly modify thiol groups present in several enzymes implicated in carbohydrate and lipid metabolism, affecting their activity (Hurd et al., 2007). Most of the enzymes found in the cited study are involved in fatty acid oxidation (FAO) (carnitine acetyltransferase, very long chain acyl-CoA dehydrogenase, propionyl-CoA carboxylase); and also in the regulation of pyruvate dehydrogenase (PDH) by pyruvate dehydrogenase kinase 2, which inhibits PDH and prevents the entry of pyruvate from glycolysis into the Krebs cycle (Hurd et al., 2007). It has also been well documented that glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme that catalyzes the sixth step of glycolysis, possesses a cysteine in its active site, which can be oxidized by reactive oxygen and nitrogen species (Ishii et al., 1999). This oxidative modification is able to inhibit its dehydrogenase activity, not only affecting the glycolytic metabolism, but also promoting apoptosis, which has important implications in neurodegenerative disorders such as Alzheimer's disease (AD) (Butterfield et al., 2010). As it has been reviewed in (Brandes et al., 2009), there are other metabolic enzymes that can be modified by thiol:disulphide exchange, such as creatine kinase, glycogen synthase or protein phosphatase-I. In this context, one of the mechanisms by which Nrf2 may affect lipid and carbohydrate metabolism is by preventing the redox modifications of these sensitive thiol groups by mitochondrial ROS.

Nrf2 in brain and neurodegenerative diseases

Neurodegenerative diseases are excellent candidates for Nrf2-targeted treatments. Most neurodegenerative

disorders such as AD, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia, Friedreich's ataxia or Huntington disease are characterized by oxidative stress and misfolded protein aggregates, so targeting both alterations has always been considered an important therapeutic approach for these diseases (Burchell et al., 2010). The ability of the Nrf2 pathway to regulate genes associated with antioxidant defense, autophagy and proteasome activation has been attracting attention to the use of Nrf2 activators as therapeutic strategies for neurodegenerative diseases (reviewed in (Hensley and Harris-White, 2015; Johnson and Johnson, 2015)).

The importance and potential of Nrf2 activation for the treatment of neurodegenerative disorders has been demonstrated with the approval of BG-12, the oral formulation of the Nrf2 inducer dimethyl fumarate, for the treatment of multiple sclerosis (Linker et al., 2011; Kawalec et al., 2014). Currently, REATA Pharmaceuticals has initiated a clinical trial with a novel Nrf2 activator, named RTA 408, for the treatment of Friedreich's ataxia (ClinicalTrials.gov, NCT02255435). This neurodegenerative disease is caused by deficiency of the protein frataxin, which causes the disruption of iron-sulfur cluster biosynthesis, mitochondrial iron overload and an increased sensitivity to oxidative stress, mainly due to a decrease in the expression of Nrf2 (Shan et al., 2013).

Although most studied, oxidative stress and protein aggregation are not the only Nrf2-related targets for the treatment of neurodegenerative diseases. Beyond them, metabolism and mitochondrial dysfunction are common alterations found in these disorders, and can benefit from Nrf2 activation.

Mitochondria are involved in essential cellular processes. They are the main source of energy in the cell, by providing ATP through oxidative phosphorylation and harboring several metabolic pathways such as FAO and the TCA cycle. In addition, they are fundamental regulators of calcium homeostasis and signaling. Mitochondria also represent a major source of reactive oxygen species in the cell (mainly as a result of the electron transport chain activity) and play a key role in apoptosis.

As a result of their constant activity and signaling, cells in the central nervous system have a high requirement for energy, and are especially vulnerable to mitochondrial dysfunction. Decreased ATP production is a common hallmark of neurodegenerative diseases and can be caused by various mechanisms, such as impaired activity of any of the complexes of the respiratory chain, alterations in glucose uptake, glycolysis, TCA cycle or uncoupling (Burchell et al., 2010).

Nrf2, mitochondrial bioenergetics and neurodegenerative disorders

Work from our laboratory has shown the role of Nrf2 in mitochondrial bioenergetics using primary neuronal cultures, MEFs and isolated mitochondria from wild-type and mice where Nrf2 has been genetically induced by knockdown of *Keap1* (Keap1 KD) or disrupted (Nrf2 KO) (Holmstrom et al., 2013). In cells where Nrf2 was inactivated, mitochondrial function was impaired as demonstrated by the decrease in the mitochondrial membrane potential (MMP), an indicator of the mitochondrial health. In Nrf2 KO cells, MMP is maintained by F_1F_0 - ATPase working in reverse rather than by respiration. This was not caused by a decrease in the activity of the respiratory complexes, but instead by a lower availability of substrates for complexes I and II, as it was demonstrated by the strongly decreased NADH and $FADH_2$ pools in Nrf2 KO, compared to WT (Holmstrom et al., 2013). In parallel, ATP production was reduced due to a less efficient oxidative phosphorylation, and was maintained mainly through activation of glycolysis (Holmstrom et al., 2013). Conversely, constitutive Nrf2 activation was able to increase the availability of substrates for respiration, enhancing ATP production and increasing the mitochondrial membrane potential (Holmstrom et al., 2013).

Reduced ATP production and O_2 consumption were also found in a different model of Nrf2 deficiency (Kim et al., 2011). In cells with a high-energy demand such as neurons, the dysfunction in oxidative phosphorylation and the decrease in ATP levels may be detrimental, especially because of their low glycolytic capacity. Also, the diversion of glucose from the pentose phosphate pathway to glycolysis in neurons can affect the regeneration of glutathione and result in oxidative stress and apoptosis (Herrero-Mendez et al., 2009).

These results suggest that activation of Nrf2 can lead to an improvement in the bioenergetics features of cells with impaired substrates availability, and this may be very important in cells with high-energy demand as neurons. Inhibition of mitochondrial respiration due to reduced substrates from the TCA cycle has been previously shown in the PINK1 model of PD, leading to dopamine-induced cell death (Gandhi et al., 2009; Abramov et al., 2011). This can be prevented either by providing the cells directly with mitochondrial substrates, and also by treating them with the Nrf2 activators sulforaphane or RTA 408, which are able to revert the bioenergetic alterations caused by the lack of substrates for the TCA cycle and prevent the dopamine-induced cell death in

this model (Dinkova-Kostova et al., 2015). Mitochondrial complex I dysfunction is a well-known pathophysiological characteristic of PD. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an inhibitor of complex I, is able to induce the clinical and pathological hallmarks of PD, and for this reason, is used as a model of the disease. Nrf2 activators have been found to be neuroprotective in the MPTP model of PD (Burton et al., 2006; Chen et al., 2009; Jazwa et al., 2011). The authors found that the deficiency in Nrf2 increased the sensitivity to the complex I inhibitor, while its activation in astrocytes was able to protect against MPTP-mediated toxicity. The protective role of Nrf2 in PD is also supported by a multicentre study which discovered that variations in the gene encoding Nrf2 are associated with risk and age at onset of idiopathic PD, finding a protective haplotype of the gene with decreased risk and delayed onset (von Otter et al., 2010).

It is not yet well known by which mechanism Nrf2 activation is able to increase the availability of substrates for the TCA cycle, but Nrf2 is able to regulate several enzymes implicated in glucose metabolism (Hirotsu et al., 2012; Singh et al., 2013). The latter study shows a role for Nrf2 in the regulation of the carbon flux through the TCA cycle (Singh et al., 2013). Pyruvate, the final product of glycolysis, is able to enter the TCA cycle as acetyl-CoA, in a reaction catalyzed by the pyruvate dehydrogenase complex, but also as oxaloacetate (OAA), in a reaction catalyzed by pyruvate carboxylase. These authors show that in the absence of Nrf2, there is a dramatic reduction in both substrates entry into the TCA cycle, together with a decrease in glucose oxidation (Singh et al., 2013). Conversely, increased Nrf2 activity led to enhanced glucose flux through the TCA cycle.

As discussed previously in this review, a possible mechanism would be the potential role of Nrf2 in preventing the oxidation of thiol groups present in key enzymes in these pathways, such as GAPDH or pyruvate dehydrogenase kinase 2. It has been also proposed that in cancer cells, Nrf2 plays a role in increasing glucose uptake and glycolytic activity, finally leading to an increase in the supply of glycolytic intermediates (Mitsuishi et al., 2012a).

Nrf2 is also able to up-regulate *ME1* (Thimmulappa et al., 2002; Lee et al., 2003; Chorley et al., 2012), which encodes the cytosolic NADP⁺-dependent malic enzyme 1 (ME1) that generates NADPH. The activity of this enzyme, the reversible oxidative decarboxylation of malate to pyruvate, links the glycolytic and citric acid cycles. Malate represents one of the main Krebs cycle intermediates exported from the mitochondria to the cytosol, where cytosolic ME1 can regenerate pyruvate that can cycle back to the mitochondria. In this reaction NADP⁺ is converted to NADPH,

making ME1 one of the main cytosolic sources of NADPH production, which can be then utilized for both fatty acid synthesis and antioxidant protection. Malic enzyme 1 is considered an anaplerotic enzyme for the TCA cycle. For the normal function of the Krebs cycle, the levels of TCA intermediates must remain constant. As many reactions need some of these intermediates as substrates, they have to be extracted from the TCA in the mitochondria through cataplerotic reactions. In the opposite direction, anaplerotic reactions replenish the TCA cycle intermediates to maintain the function of the mitochondrial cycle. Cytosolic ME1 activity has an essential role in replenishing the TCA cycle in astrocytes, sustaining the pyruvate recycling activity (McKenna et al., 1995; Calvetti and Somersalo, 2012), and Nrf2-dependent activation of ME1 may be important to maintain the TCA cycle in astrocytes enabling neurotransmitter synthesis and recycling.

It is also noteworthy that the impaired substrate availability caused by the lack of Nrf2 in glioneuronal cultures from Nrf2 KO mice was responsible for the increased mitochondrial ROS production found in this model (Kovac et al., 2015). In basal conditions, these cells presented an increased rate of mitochondrial ROS production compared to WT cells. While inhibition of complex I by rotenone did not increase further the rate of ROS, suggesting that complex I activity was already impaired at basal levels; application of pyruvate, a substrate for the TCA cycle, diminished the rate of ROS production (Kovac et al., 2015). This finding supports the link between metabolic pathways and redox homeostasis within the cell.

Nrf2 and lipid metabolism

As reviewed in (Hayes and Dinkova-Kostova, 2014), Nrf2 positively regulates lipases involved in phospholipids and triglycerides degradation and enzymes involved in FAO. Conversely, it is also able to negatively regulate genes implicated in lipid biosynthesis, fatty acid desaturation and fatty acid transport. Work from our laboratory shows an additional role for Nrf2 in mitochondrial bioenergetics by controlling the efficiency of FAO. In the absence of glucose, Nrf2 deficiency induced a much stronger loss of MMP suggesting that FAO, which would provide substrates for the TCA cycle during glucose deprivation, is altered in the absence of Nrf2 (Ludtmann et al., 2014). Addition of fatty acids stimulated respiration and increased the ATP production both in WT and Keap1 KO MEFs, with ATP levels increasing faster in the latter. In contrast, in the absence of Nrf2, application of palmitoylcarnitine did

not affect the ATP levels, indicating that in the absence of Nrf2, the efficiency of FAO is significantly reduced, altering mitochondrial metabolism (Ludtmann et al., 2014). These results are in agreement with another report that shows that resveratrol, an inducer of Nrf2, is able to increase fatty acid utilization in FAO disorders (Bastin et al., 2011).

Although the existing knowledge points at glucose as the main source of energy in the brain (either as glucose or lactate), it has been also shown that fatty acids can be used by the brain and contribute to the energy production in astrocytes (Ebert et al., 2003). Moreover, some reports found alterations in FAO in neurodegenerative disorders and also neuroprotective effects associated to the activation of FAO, which suggest that Nrf2-induced FAO can be a potential therapeutic target. For example, it was shown that the levels of two of the main enzymes for β -oxidation, acyl-coenzyme A dehydrogenase and mitochondrial trifunctional enzyme subunit α are reduced in AD patients (Choi et al., 2014). In a different study, 3-hydroxyacyl-CoA dehydrogenase, which catalyzes the oxidation of 3-hydroxyacyl-CoAs in the mitochondrial FAO, was shown to protect against the MPTP-induced impairment of oxidative phosphorylation and ATP production in this model of PD (Tieu et al., 2004).

There has also been described a relationship between Nrf2 activation, phospholipid metabolism and endocannabinoids. Nrf2 up-regulates *ABHD4*, which has been described as a main regulator of N-acyl phospholipid metabolism in the mammalian nervous system (Lee et al., 2015). N-acyl phospholipids are considered atypical lipids implicated in the biosynthesis of lipid mediators such as endocannabinoids. In parallel, it has been shown that phenolic endocannabinoids like N-acyl 5-HT, present in the brain exert a cytoprotective action against glutamate-induced oxidative damage of HT-22 cells possibly by an Nrf2-mediated mechanism (Jin et al., 2014).

As discussed before, Nrf2 is able to induce both PPAR γ and RXR α . These receptors are not only implicated in adipocyte differentiation, but also regulate ApoE (Yue and Mazzone, 2009). ApoE is the main apolipoprotein in the brain, which facilitates the trafficking of lipids in the central nervous system, and has been also shown to modulate A β deposition and clearance in an isoform-dependent manner (Castellano et al., 2011). Possession of an *APOE* ϵ 4 allele is the strongest risk factor for sporadic AD, and has been linked to decreased efficiency of A β clearance (Castellano et al., 2011). The production of ApoE is regulated by liver X receptors (LXR) that function as cholesterol sensors and form heterodimers with retinoid receptors RXRs, promoting the transcription of *APOE* and other genes involved in cholesterol metabolism such

as the ones that encode the ABC transporters ABCA1 and ABCG1 (Hong and Tontonoz, 2014). It has been shown that ligands of PPAR γ are able to induce the expression of LXR, and there is strong evidence that activation of either PPAR γ , LXR or RXR ameliorate A β pathology and behaviour in different animal models of AD (Yamanaka et al., 2012; Skerrett et al., 2015). In fact, the PPAR γ agonist pioglitazone is currently undergoing clinical trials for the treatment of AD (Geldmacher et al., 2011). In this context, Nrf2 activation would potentially have beneficial effects, as it is able to induce not only PPAR γ but also RXR α and, moreover, ABC transporters, as will be discussed in the next section. A summary of the role of Nrf2 activation in mitochondrial function and metabolism in neurodegenerative diseases can be found in Figure 2.

Nrf2 and drug transporters

Nrf2 activation is related with the up-regulation of two different transporter families in the cell: ATP-binding cassette (ABC) transporters and solute carrier (SLC) transporters (Table 1).

ABC transporters

ABC transporters are divided into different subfamilies (from ABCA to ABCG) and are expressed in every cell type of the brain mediating the transport of a variety of substances. Its role in the brain and in neurodegenerative diseases is mainly attributed to their function or dysfunction at the blood brain barrier (BBB) (Pahnke et al., 2014), where they are determining elements for the delivery of drugs to the central nervous system. ABC transporters function to pump substrates outside the cell by hydrolyzing ATP, and together with a wide spectrum of drugs and xenobiotics, they can transport many different metabolites and signaling molecules, and thus have a very important function for neuroprotection and cellular communication.

Nrf2 plays a role in the up-regulation of the major ABC transporters in the brain (Table 1), most of which have been implicated in neurodegenerative diseases. *ABCC1* encodes the protein MRP1, member of the multidrug resistance protein family. It works as a multispecific organic anion transporter whose substrates are oxidized glutathione, glucuronides and sulfate conjugates of steroid hormones and bile salts, among others. It was shown both in a mouse model of AD and in a cellular model, that *ABCC1*/MRP1 expression is induced in the

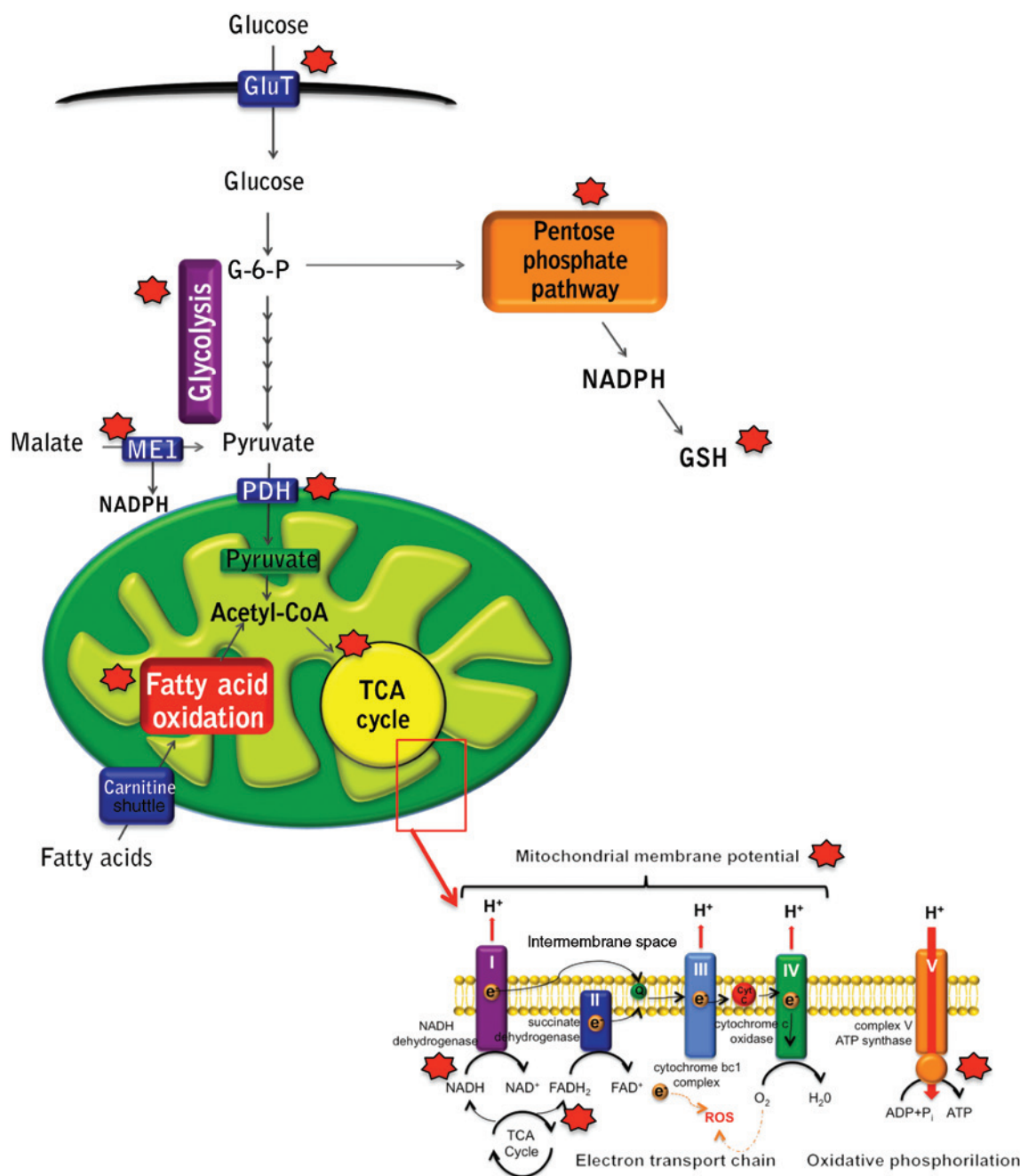


Figure 2: Nrf2 activation: its role in mitochondrial function and metabolism in neurodegenerative diseases.

Nrf2 activation is a potential therapeutic strategy for neurodegenerative diseases, not only for its role in antioxidant defense, autophagy and proteasome activation; metabolism and mitochondrial dysfunction are common alterations in these disorders, that can also benefit from Nrf2 activation. Nrf2 activation is able to increase the mitochondrial membrane potential (MMP), the availability of substrates for respiration and the ATP production in the mitochondria, improving the bioenergetics features of cells, which is especially important in cells with high-energy demands such as neurons; its deregulation is a hallmark of several neurodegenerative disorders. Nrf2 activation has been demonstrated to be beneficial in the PINK1 and MPTP models of Parkinson's disease, which present substrates availability impairment and complex I dysfunction. Although the mechanism has not been fully elucidated, Nrf2 is able to regulate enzymes implicated in glucose metabolism, flux of glucose through the TCA cycle, and has a potential role in regulating the pyruvate dehydrogenase complex (PDH) (which controls the entrance of pyruvate into the mitochondria) and glucose uptake. Nrf2 regulates malic-enzyme 1 (ME-1), an anaplerotic enzyme that generates NADPH and regenerates pyruvate that can cycle back to the mitochondria. Its activation is important in astrocytes, enabling neurotransmitter synthesis and recycling. Nrf2 controls the efficiency of mitochondrial fatty acid oxidation, which can be used in the brain and contribute to energy production in astrocytes. Alterations in fatty acid oxidation have been shown in Alzheimer's and Parkinson's diseases. Nrf2 enhances the activity of enzymes of the pentose phosphate pathway that generate NADPH, which can be used for glutathione (GSH) and other antioxidants regeneration.

Table 1: Family members of the ABC and SLC transporters regulated by Nrf2.

Gene	Protein	Function	Refs
<i>ABCC1</i> (MRP subfamily of ABC transporters)	MRP1 Multidrug resistance protein 1	– Multispecific organic anion transporter (oxidized glutathione, glucuronides, sulfate conjugates of steroid hormones and bile salts)	Hayashi et al., 2003; Udasin et al., 2015
<i>ABCC2</i> (MRP subfamily of ABC transporters)	MRP2 Multidrug resistance protein 2	– Multidrug resistance	Maher et al., 2007; Wang et al., 2014
<i>ABCC3</i> (MRP subfamily of ABC transporters)	MRP3 Multidrug resistance protein 3	– Multidrug resistance – Excretion of organic anions	Maher et al., 2007; Shelton and Jaiswal, 2013
<i>ABCC4</i> (MRP subfamily of ABC transporters)	MRP4 Multidrug resistance protein 3	– Multidrug resistance – Pump organic anions – Prostaglandin-mediated cAMP signaling	Maher et al., 2007; Cheng et al., 2011; Hirotsu et al., 2012; Shelton and Jaiswal, 2013
<i>ABCA8B</i> (ABC-1 subfamily of ABC transporters)	ABCA8B	– Transport of lipophilic compounds – Digoxin metabolism – Lipid metabolism	Hirotsu et al., 2012
<i>ABCG2</i> (White subfamily of ABC transporters)	BRCP Breast cancer resistance protein	– Multidrug resistance – Xenobiotic transporter (mitoxantrone, anthracycline)	Shelton and Jaiswal, 2013; Wang et al., 2014
<i>ABCB1</i> (MDR/TAP subfamily of ABC transporters)	MDR1 P-glycoprotein	– Transporter in the BBB – Multidrug resistance – Efflux pump for xenobiotic compounds	Wang et al., 2014
<i>SLC7A11</i>	xCT, CCB1 solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	– Light chain of the Xc(-) transporter system – Transport system specific for cysteine (in its anionic form) in exchange for glutamate	Chorley et al., 2012; Hirotsu et al., 2012
<i>SLC1A4</i>	ASCT1 solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	– High-affinity glutamate and neutral amino acid transporter	Hirotsu et al., 2012
<i>SLC6A9</i>	GlyT1 solute carrier family 6 (neurotransmitter transporter, glycine), member 9	– Glycine transporter	Hirotsu et al., 2012
<i>SLC48A1</i>	HRG1 solute carrier family 48 (heme transporter), member 1	– Heme transporter	Chorley et al., 2012; Hirotsu et al., 2012
<i>SLC22A23</i> <i>SLC19A2</i>	THTR1 solute carrier family 19 (thiamine transporter), member 2	– Organic cation/anion/zwitterion transporter – Thiamine transporter protein	Hirotsu et al., 2012 Hirotsu et al., 2012
<i>SLC14A1</i>	UT1 solute carrier family 14 (urea transporter), member 1 (Kidd blood group)	– Urea transport in erythrocytes Basis for the Kidd blood group system	Hirotsu et al., 2012
<i>SLC16A6</i>	MCT6 solute carrier family 16, member 6	– Monocarboxylate transporter family	Chorley et al., 2012
<i>SLC3A2</i>	MDU1 solute carrier family 3 (amino acid transporter heavy chain), member 2	– Heavy chain of an heterodimer covalently bound through di-sulfide bonds to one or several light chains – Calcium levels	Chorley et al., 2012
<i>SLC12A8</i>	CCC9 Solute carrier family 12, member 8	– L-type amino acids transport – Electroneutral cation/Cl ⁻ co-transporter family – Psoriasis susceptibility	Chorley et al., 2012

early stages of AD pathogenesis by less aggregated A β , promoting the GSH release from astrocytes, which protects the cells temporarily from oxidative stress (Ye et al., 2015). In contrast, later in the disease, or with prolonged incubation with aggregated A β , the levels of MRP1 are reduced (Ye et al., 2015). The repercussion of this transporter in AD is much stronger. MRP1, together with MDR1, or P-glycoprotein (encoded by the *ABCB1* gene) have been critically implicated in the clearance of endogenous proteins, as amyloid- β (A β), from the brain, being discovered as major A β exporting molecules at the BBB (reviewed in Pahnke et al., 2014). The alteration of the expression or function of these transporters in the brain may contribute to the aggregation of A β , and its accumulation in the blood vessels, which is known as cerebral amyloid angiopathy (CAA). CAA is associated with cognitive decline, and represents a hallmark of AD and an important cause of vascular dementia. It was found in different mice models and human tissue, that *ABCB1* expression is decreased in patients with capillary CAA (capCAA), a disease characterized by A β -deposits in the cerebral capillaries (Carrano et al., 2014). The decline of the levels of the transporter at the BBB occurs during normal aging, and is positively correlated with the accumulation of A β (Silverberg et al., 2010). Due the contribution of ABC transporters on A β clearance in AD, they have been proposed as potential therapeutic targets (Krohn et al., 2011). Moreover, *ABCB1* dysfunction has been also associated with other neurodegenerative disorders such as PD, progressive supranuclear palsy (PSP) or genetically associated with depressive disorders (Bernstein et al., 2014). As most of these efflux pumps are up-regulated by Nrf2, using Nrf2 activators may provide an important tool to increase the activity of ABC transporters.

MRP4, the multidrug resistance-associated protein 4, encoded by gene *ABCC4*, is also up-regulated by Nrf2 (Chan et al., 1993; Maher et al., 2007; Yates et al., 2009; Kalra et al., 2011; Kalra et al., 2012) and is able to transport not only drugs, but many different signaling molecules such as cAMP, cGMP, ADP or prostaglandins (PG), playing a very important role in cellular communication and signaling (reviewed in Wen et al., 2015). The clearance of PGs (which are anionic) from the brain to either the blood or the cerebrospinal fluid is mediated by efflux transport processes, with MRP4 playing a central role (Tachikawa et al., 2014). A recent study depicts that microglial beneficial functions combating the toxic effect of A β in the synapses are inhibited by PGE2 signaling, suggesting the inhibition of this activity as a promising strategy to prevent the progression of AD (Johansson et al., 2015). Increasing PGE2 clearance by MRP4 may be considered as

a tool to achieve it, and Nrf2 inducers have been shown to up-regulate this ABC transporter.

It is also important to point out that the activity of ABC transporters is dependent on ATP production and availability. It is widely known the essential role of mitochondria in ATP production (among other vital functions in the cell), and that mitochondrial dysfunction, very often leading to diminished ATP production, is a central hallmark in several neurodegenerative diseases. It has been demonstrated that Nrf2 induction is able to enhance ATP levels by increasing substrates for respiration (Holmstrom et al., 2013), and thus induction of Nrf2 could confer both the beneficial effect of increasing ATP production and the expression of ATP-dependent transporters in the treatment of neurodegenerative diseases.

However, it is also important to note that the increased expression of some of these transporters may be linked to reduced drug availability in the brain, as they prevent the entrance of xenobiotics through the BBB. There are findings pointing at the increase in the expression and activity of ABC proteins in the brain in amyotrophic lateral sclerosis as the main cause for the lack of effectiveness *in vivo* of those promising drugs *in vitro* (Jablonski et al., 2015).

SLC transporters

Solute-carrier transporters (SLC) are a family of more than 300 proteins that mediate the transport of many different substrates across membranes. Their activity does not depend directly on ATP hydrolysis and they are considered uptake transporters, as generally are implicated in the uptake of molecules into the cell. Nrf2 has been shown to positively regulate many of them (Table 1).

Nrf2 up-regulates *SLC7A11* and *SLC3A2* genes, which encode the light and heavy chains subunits of the cysteine/glutamate antiporter system x $_c^-$ (Chorley et al., 2012; Hirotsu et al., 2012). This antiporter mediates the uptake of cystine, the anionic form of cysteine, into cells in exchange for glutamate, so it plays a critical role in both the biosynthesis of intracellular GSH and in the regulation of glutamate release. It was found that the expression of the two subunits of this antiporter is reduced in patients with schizophrenia, which agrees with the hypo-glutamatergic neurotransmission hypothesis in the pathogenesis of this disease (Lin et al., 2015). However, an exacerbated activity of this antiporter may lead to glutamate excitotoxicity and neuronal death in ischemia (Soria et al., 2014). Also, higher expression of this subunit was found in glioma patients, and was associated with glutamate excitotoxicity, induced seizures and worse prognosis

(Robert et al., 2015). Interestingly, there is a positive correlation between overexpression of Nrf2, tumor grade and reduced survival in patients with glioma (Zhao et al., 2015). It has been proposed that in basal conditions, Nrf1 negatively regulates cysteine/glutamate antiporter system x_c^- , but in the presence of oxidative stress, Nrf2 is recruited to the ARE sequence in the promoter of the gene and activates its transcription (Tsujita et al., 2014).

SLC1A4 is also positively regulated by Nrf2 and encodes ASCT1, a transporter for neutral amino acids, being their main substrates L-Ala, L-Ser, L-Cys and L-Thr. It is expressed in astrocytes and neurons and represents the main uptake of L-Ser in neurons (Kanai et al., 2013). Missense mutations in the gene lead to neurological disorders with intellectual disability (Heimer et al., 2015) likely due to the alterations in the serine transport.

SLC19A2 gene is up-regulated by Nrf2 and encodes the thiamine transporter THTR-1. Thiamine is a cofactor of important enzymes in energy metabolism. Mutations in this gene leading to decreased activity of the transporter have been associated with hematological diseases such as thiamine-responsive megaloblastic anemia. Interestingly, thiamine deficiency linked to mutations in *SLC19A2* was shown to affect mitochondrial complex I activity, causing severe complex I deficiency (Scharfe et al., 2000). Intracellular deficiency of THTR-1 leads to decreased activity of enzymes dependent of thiamine diphosphate (TPP) as cofactor, such as the pentose phosphate pathway enzyme transketolase, the pyruvate dehydrogenase complex, alpha-ketoglutarate dehydrogenase and branched chain ketoacid dehydrogenase (Scharfe et al., 2000). There is also increasing evidence that suggest alterations in thiamine neurochemistry in AD, as thiamine levels and activity of the thiamine-dependent enzymes shown above are decreased in the brain of patients (Lu'o'ng and Nguyen, 2011). In this context, Nrf2 activation would potentially have positive effects by two different ways: up-regulating the thiamine transporter *SLC19A2*, and directly up-regulating the expression of some thiamine-dependent enzymes as transketolase (Xue et al., 2008; Hirotsu et al., 2012).

GSK-3 inhibitors as Nrf2 activators in neurodegenerative disorders

GSK-3 is a well-known therapeutic target for AD, as it has been shown to hyperphosphorylate tau protein leading to its aggregation, and also to mediate A β production through its precursor APP. GSK-3 inhibitors have beneficial

effects by reducing the amount of A β and the hyperphosphorylation of tau in different models of AD (Maqbool et al., 2015). But beyond these effects, GSK-3 inhibitors are also able to increase Nrf2 activation, so they can exert additional therapeutic effects through this mechanism. As it was described earlier in this review, GSK-3 phosphorylates Nrf2 in the Neh6 domain, targeting the protein for degradation in the proteasome through SCF/ β -TrCP and therefore inhibiting Nrf2 activity (Rada et al., 2011). Consequently, GSK-3 inhibitors are able to prevent β -TrCP-dependent degradation of Nrf2, increasing its availability and its translocation to the nucleus.

Exacerbated GSK-3 β activity has been reported not only in AD, but also in other neurodegenerative diseases such as PD, and it has been speculated that consequent Nrf2 deficiency can be partially responsible for oxidative stress and energy-deficits that accompanies these conditions (Rada et al., 2011). Under these pathological conditions GSK-3 inhibitors could also cooperate to increase Nrf2 levels.

In this context, it was recently shown that the phytoestrogen β -ecdysterone has a protective effect against oxidative stress and cell death in the MPTP model of PD. The authors show that the phytoestrogen is able to increase Nrf2 activity by enhancing Akt signaling pathways, and therefore inactivating GSK-3 β (Zou et al., 2015). Under these conditions, cells showed decreased oxidative stress and apoptosis. When Akt was blocked by specific inhibitors, the antioxidative properties were lost.

In vivo experiments in SAMP8 mice, a model of AD, show that the inhibition of GSK-3 β in the brain with antisense oligonucleotides leads to an improvement in learning and memory. The authors show that GSK-3 suppression was responsible for an increase in the nuclear localization of Nrf2 and the levels of its target enzyme glutathione S-transferase, which led to decreased oxidative stress. Together with this, tau phosphorylation was reduced and there was an improvement in learning and memory, suggesting that antisense nucleotide directed at GSK-3 β may be considered as possible treatment for AD (Farr et al., 2014).

A protective role of GSK-3 inhibition-dependent Nrf2 activation against glutamate excitotoxicity has also been shown in the kainate model of epilepsy (Rojo et al., 2008). The authors of this study describe that just after an intraperitoneal injection with kainate, mice show a rapid activation of Akt, followed by GSK inhibition and translocation of Nrf2 to the nucleus. However, later on, Akt was no longer active, leading to GSK-3 activation and Nrf2 exclusion from the nucleus, which was associated with oxidative-stress and cell death. Treatment of the mice with

lithium, a GSK-3 inhibitor, together with sulforaphane, led to a decrease in oxidative stress and cell death in kainate-treated hippocampal slices of wild type, but not in Nrf2 KO mice. These results suggest that GSK-3/Nrf2 constitutes a pharmacological target for the prevention of excitotoxic neuronal death.

Conclusions

Up to now, most of the Nrf2-mediated therapeutic strategies for neurodegenerative disorders have been focused on the prevention of oxidative stress and misfolded protein aggregation. There is growing evidence of the role of Nrf2 in metabolism and mitochondrial bioenergetics and function, which are commonly altered in neurodegenerative disorders. For this reason, pharmacological activation of Nrf2 to restore mitochondrial alterations is an interesting therapeutic target that needs to be explored for the treatment of neurodegenerative disorders. The ability of Nrf2 activation to increase the substrates availability for the mitochondrial TCA cycle, enhance the mitochondrial membrane potential and ATP production is specially important in neurons, because of their high energy demand and their low glycolytic capacity, which can be detrimental. As bioenergetics alterations have been found in several neurodegenerative conditions such as PD, the ability of Nrf2 activators to restore the mitochondrial bioenergetics and prevent cell death needs to be further explored as a therapeutic target not only for PD but also for other neurodegenerative disorders where bioenergetic alterations are present.

The Nrf2-dependent regulation of PPAR γ and the different ABC and SLC transporters also points at the potential role of this activation to increase the amyloid clearance from the brain, being of special interest for the treatment of AD.

It is also worthy to note that the activation of Nrf2 may be achieved not only with classical Keap1-targeting compounds, but also through GSK-3 inhibition (and related pathways), which increases the range of drug candidates for the prevention and treatment of neurodegenerative diseases.

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References

- Abramov, A.Y., Gegg, M., Grunewald, A., Wood, N.W., Klein, C., and Schapira, A.H. (2011). Bioenergetic consequences of PINK1 mutations in Parkinson disease. *PLoS One* 6, e25622.
- Abu-Bakar, A., Lamsa, V., Arpiainen, S., Moore, M.R., Lang, M.A., and Hakkola, J. (2007). Regulation of *CYP2A5* gene by the transcription factor nuclear factor (erythroid-derived 2)-like 2. *Drug Metab. Dispos.* 35, 787–794.
- Agyeman, A.S., Chaerkady, R., Shaw, P.G., Davidson, N.E., Visvanathan, K., Pandey, A., and Kensler, T.W. (2012). Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles. *Breast Cancer Res. Treat* 132, 175–187.
- Aleksunes, L.M. and Klaassen, C.D. (2012). Coordinated regulation of hepatic phase I and II drug-metabolizing genes and transporters using AhR-, CAR-, PXR-, PPAR α -, and Nrf2-null mice. *Drug Metab. Dispos.* 40, 1366–1379.
- Andrews, N.C., Erdjument-Bromage, H., Davidson, M.B., Tempst, P., and Orkin, S.H. (1993). Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. *Nature* 362, 722–728.
- Bae, S.H., Sung, S.H., Oh, S.Y., Lim, J.M., Lee, S.K., Park, Y.N., Lee, H.E., Kang, D., and Rhee, S.G. (2013). Sestrins activate Nrf2 by promoting p62-dependent autophagic degradation of Keap1 and prevent oxidative liver damage. *Cell Metab.* 17, 73–84.
- Baird, L., Lleres, D., Swift, S., and Dinkova-Kostova, A.T. (2013). Regulatory flexibility in the Nrf2-mediated stress response is conferred by conformational cycling of the Keap1-Nrf2 protein complex. *Proc. Natl. Acad. Sci. USA* 110, 15259–15264.
- Baird, L., Swift, S., Lleres, D., and Dinkova-Kostova, A.T. (2014). Monitoring Keap1-Nrf2 interactions in single live cells. *Biotechnol. Adv* 32, 1133–1144.
- Bastin, J., Lopes-Costa, A., and Djouadi, F. (2011). Exposure to resveratrol triggers pharmacological correction of fatty acid utilization in human fatty acid oxidation-deficient fibroblasts. *Hum. Mol. Genet.* 20, 2048–2057.
- Bernstein, H.G., Holz, G., Dobrowolny, H., Hildebrandt, J., Trubner, K., Krohn, M., Bogerts, B., and Pahnke, J. (2014). Vascular and extravascular distribution of the ATP-binding cassette transporters ABCB1 and ABCC1 in aged human brain and pituitary. *Mech. Ageing Dev.* 141–142, 12–21.
- Biswas, M. and Chan, J.Y. (2010). Role of Nrf1 in antioxidant response element-mediated gene expression and beyond. *Toxicol. Appl. Pharmacol.* 244, 16–20.
- Brandes, N., Schmitt, S., and Jakob, U. (2009). Thiol-based redox switches in eukaryotic proteins. *Antioxid. Redox. Signal.* 11, 997–1014.
- Burchell, V.S., Gandhi, S., Deas, E., Wood, N.W., Abramov, A.Y., and Plun-Favreau, H. (2010). Targeting mitochondrial dysfunction in

- neurodegenerative disease: Part I. *Expert Opin. Ther. Targets* 14, 369–385.
- Burton, N.C., Kensler, T.W., and Guilarte, T.R. (2006). *In vivo* modulation of the Parkinsonian phenotype by Nrf2. *Neurotoxicology* 27, 1094–1100.
- Butterfield, D.A., Hardas, S.S., and Lange, M.L. (2010). Oxidatively modified glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Alzheimer's disease: many pathways to neurodegeneration. *J. Alzheimers Dis.* 20, 369–393.
- Calvetti, D. and Somersalo, E. (2012). Menage a trois: the role of neurotransmitters in the energy metabolism of astrocytes, glutamatergic, and GABAergic neurons. *J. Cereb. Blood Flow Metab.* 32, 1472–1483.
- Carrano, A., Snkhchyan, H., Kooij, G., van der Pol, S., van Horssen, J., Veerhuis, R., Hoozemans, J., Rozemuller, A., and de Vries, H.E. (2014). ATP-binding cassette transporters P-glycoprotein and breast cancer related protein are reduced in capillary cerebral amyloid angiopathy. *Neurobiol. Aging* 35, 565–575.
- Castellano, J.M., Kim, J., Stewart, F.R., Jiang, H., DeMattos, R.B., Patterson, B.W., Fagan, A.M., Morris, J.C., Mawuenyega, K.G., Cruchaga, C., et al. (2011). Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. *Sci. Transl. Med.* 3, 89ra57.
- Chan, J.Y., Han, X.L., and Kan, Y.W. (1993). Cloning of Nrf1, an NF-E2-related transcription factor, by genetic selection in yeast. *Proc. Natl. Acad. Sci. USA* 90, 11371–11375.
- Chanas, S.A., Jiang, Q., McMahon, M., McWalter, G.K., McLellan, L.I., Elcombe, C.R., Henderson, C.J., Wolf, C.R., Moffat, G.J., Itoh, K., et al. (2002). Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice. *Biochem J.* 365, 405–416.
- Chen, P.C., Vargas, M.R., Pani, A.K., Smeyne, R.J., Johnson, D.A., Kan, Y.W., and Johnson, J.A. (2009). Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proc. Natl. Acad. Sci. USA* 106, 2933–2938.
- Cheng, Q., Taguchi, K., Aleksunes, L.M., Manautou, J.E., Cherrington, N.J., Yamamoto, M., and Slitt, A.L. (2011). Constitutive activation of nuclear factor-E2-related factor 2 induces biotransformation enzyme and transporter expression in livers of mice with hepatocyte-specific deletion of Kelch-like ECH-associated protein 1. *J. Biochem. Mol. Toxicol.* 25, 320–329.
- Chevillard, G. and Blank, V. (2011). NFE2L3 (NRF3): the Cinderella of the Cap'n'Collar transcription factors. *Cell. Mol. Life Sci.* 68, 3337–3348.
- Choi, J., Ravipati, A., Nimmagadda, V., Schubert, M., Castellani, R.J., and Russell, J.W. (2014). Potential roles of PINK1 for increased PGC-1 α -mediated mitochondrial fatty acid oxidation and their associations with Alzheimer disease and diabetes. *Mitochondrion* 18, 41–48.
- Chorley, B.N., Campbell, M.R., Wang, X., Karaca, M., Sambandan, D., Bangura, F., Xue, P., Pi, J., Kleeberger, S.R., and Bell, D.A. (2012). Identification of novel NRF2-regulated genes by ChIP-Seq: influence on retinoid X receptor α . *Nucleic Acids Res.* 40, 7416–7429.
- Chowdhry, S., Zhang, Y., McMahon, M., Sutherland, C., Cuadrado, A., and Hayes, J.D. (2013). Nrf2 is controlled by two distinct β -TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. *Oncogene* 32, 3765–3781.
- Cuadrado, A. (2015). Structural and functional characterization of Nrf2 degradation by glycogen synthase kinase 3/ β -TrCP. *Free Radic. Biol. Med.* 88, 147–157.
- Cullinan, S.B., Gordan, J.D., Jin, J., Harper, J.W., and Diehl, J.A. (2004). The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. *Mol. Cell. Biol.* 24, 8477–8486.
- de Zeeuw, D., Akizawa, T., Audhya, P., Bakris, G.L., Chin, M., Christ-Schmidt, H., Goldsberry, A., Houser, M., Krauth, M., Lambers Heerspink, H.J., et al. (2013). Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *N. Engl. J. Med.* 369, 2492–2503.
- Dinkova-Kostova, A.T. and Abramov, A.Y. (2015). The emerging role of Nrf2 in mitochondrial function. *Free Radic. Biol. Med.* 88, 179–188.
- Dinkova-Kostova, A.T., Holtzclaw, W.D., Cole, R.N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M., and Talalay, P. (2002). Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. USA* 99, 11908–11913.
- Dinkova-Kostova, A.T., Liby, K.T., Stephenson, K.K., Holtzclaw, W.D., Gao, X., Suh, N., Williams, C., Risingsong, R., Honda, T., Gribble, G.W., et al. (2005). Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc. Natl. Acad. Sci. USA* 102, 4584–4589.
- Dinkova-Kostova, A.T. and Talalay, P. (2010). NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. *Arch. Biochem. Biophys.* 501, 116–123.
- Dinkova-Kostova, A.T., Baird, L., Holmstrom, K.M., Meyer, C.J., and Abramov, A.Y. (2015). The spatiotemporal regulation of the Keap1-Nrf2 pathway and its importance in cellular bioenergetics. *Biochem. Soc. Trans.* 43, 602–610.
- Ebert, D., Haller, R.G., and Walton, M.E. (2003). Energy contribution of octanoate to intact rat brain metabolism measured by ^{13}C nuclear magnetic resonance spectroscopy. *J. Neurosci.* 23, 5928–5935.
- Eggler, A.L., Liu, G., Pezzuto, J.M., van Breemen, R.B., and Mesecar, A.D. (2005). Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. *Proc. Natl. Acad. Sci. USA* 102, 10070–10075.
- Eggler, A.L., Small, E., Hannink, M., and Mesecar, A.D. (2009). Cul3-mediated Nrf2 ubiquitination and antioxidant response element (ARE) activation are dependent on the partial molar volume at position 151 of Keap1. *Biochem. J.* 422, 171–180.
- Ellis, E.M. (2007). Reactive carbonyls and oxidative stress: potential for therapeutic intervention. *Pharmacol. Ther.* 115, 13–24.
- Enomoto, A., Itoh, K., Nagayoshi, E., Haruta, J., Kimura, T., O'Connor, T., Harada, T., and Yamamoto, M. (2001). High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. *Toxicol. Sci.* 59, 169–177.

- Farr, S.A., Ripley, J.L., Sultana, R., Zhang, Z., Niehoff, M.L., Platt, T.L., Murphy, M.P., Morley, J.E., Kumar, V., and Butterfield, D.A. (2014). Antisense oligonucleotide against GSK-3 β in brain of SAMP8 mice improves learning and memory and decreases oxidative stress: Involvement of transcription factor Nrf2 and implications for Alzheimer disease. *Free Radic. Biol. Med.* 67, 387–395.
- Fourquet, S., Guerois, R., Biard, D., and Toledano, M.B. (2010). Activation of NRF2 by nitrosative agents and H₂O₂ involves KEAP1 disulfide formation. *J. Biol. Chem.* 285, 8463–8471.
- Furukawa, M. and Xiong, Y. (2005). BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. *Mol. Cell. Biol.* 25, 162–171.
- Gandhi, S., Wood-Kaczmar, A., Yao, Z., Plun-Favreau, H., Deas, E., Klupsch, K., Downward, J., Latchman, D.S., Tabrizi, S.J., Wood, N.W., Duchen, M.R., and Abramov, A.Y. (2009). PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol. Cell.* 33, 627–638.
- 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.
- Gasiorek, J.J. and Blank, V. (2015). Regulation and function of the NFE2 transcription factor in hematopoietic and non-hematopoietic cells. *Cell. Mol. Life Sci.* 72, 2323–2335.
- Geldmacher, D.S., Fritsch, T., McClendon, M.J., and Landreth, G. (2011). A randomized pilot clinical trial of the safety of pioglitazone in treatment of patients with Alzheimer disease. *Arch. Neurol.* 68, 45–50.
- Hayashi, A., Suzuki, H., Itoh, K., Yamamoto, M., and Sugiyama, Y. (2003). 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.* 310, 824–829.
- Hayes, J.D. and Dinkova-Kostova, A.T. (2014). The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem. Sci.* 39, 199–218.
- Heimer, G., Marek-Yagel, D., Eyal, E., Barel, O., Oz Levi, D., Hoffmann, C., Ruzzo, E.K., Ganelin-Cohen, E., Lancet, D., Pras, E., et al. (2015). SLC1A4 mutations cause a novel disorder of intellectual disability, progressive microcephaly, spasticity and thin corpus callosum. *Clin. Genet.* 88, 327–335.
- Hensley, K. and Harris-White, M.E. (2015). Redox regulation of autophagy in healthy brain and neurodegeneration. *Neurobiol. Dis.* 84, 50–59.
- Herrero-Mendez, A., Almeida, A., Fernandez, E., Maestre, C., Moncada, S., and Bolanos, J.P. (2009). The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat. Cell. Biol.* 11, 747–752.
- Hirotsu, Y., Katsuoka, F., Funayama, R., Nagashima, T., Nishida, Y., Nakayama, K., Engel, J.D., and Yamamoto, M. (2012). Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. *Nuc. Acids Res.* 40, 10228–10239.
- Holland, R. and Fishbein, J.C. (2010). Chemistry of the cysteine sensors in Kelch-like ECH-associated protein 1. *Antioxid. Redox Signal.* 13, 1749–1761.
- Holmstrom, K.M., Baird, L., Zhang, Y., Hargreaves, I., Chalasani, A., Land, J.M., Stanyer, L., Yamamoto, M., Dinkova-Kostova, A.T., and Abramov, A.Y. (2013). Nrf2 impacts cellular bioenergetics by controlling substrate availability for mitochondrial respiration. *Biol. Open* 2, 761–770.
- Hong, C. and Tontonoz, P. (2014). Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nat. Rev. Drug Discov.* 13, 433–444.
- Hurd, T.R., Prime, T.A., Harbour, M.E., Lilley, K.S., and Murphy, M.P. (2007). Detection of reactive oxygen species-sensitive thiol proteins by redox difference gel electrophoresis: implications for mitochondrial redox signaling. *J. Biol. Chem.* 282, 22040–22051.
- Ichimura, Y., Waguri, S., Sou, Y.S., Kageyama, S., Hasegawa, J., Ishimura, R., Saito, T., Yang, Y., Kouno, T., Fukutomi, T., et al. (2013). Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. *Mol. Cell* 51, 618–631.
- Ishii, T., Sunami, O., Nakajima, H., Nishio, H., Takeuchi, T., and Hata, F. (1999). Critical role of sulfenic acid formation of thiols in the inactivation of glyceraldehyde-3-phosphate dehydrogenase by nitric oxide. *Biochem. Pharmacol.* 58, 133–143.
- Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S., and Yamamoto, M. (2000). Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J. Biol. Chem.* 275, 16023–16029.
- 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., and Yamamoto, M. (1999). Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Gene Dev.* 13, 76–86.
- Jablonski, M., Miller, D.S., Pasinelli, P., and Trotti, D. (2015). ABC transporter-driven pharmacoresistance in amyotrophic lateral sclerosis. *Brain Res.* 1607, 1–14.
- Jain, A., Lamark, T., Sjøttem, E., Larsen, K.B., Awuh, J.A., Overvatn, A., McMahon, M., Hayes, J.D., and 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.
- Jaiswal, A.K. (2004). Nrf2 signaling in coordinated activation of antioxidant gene expression. *Free Radic. Biol. Med.* 36, 1199–1207.
- Jazwa, A., Rojo, A.I., Innamorato, N.G., Hesse, M., Fernandez-Ruiz, J., and Cuadrado, A. (2011). Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism. *Antioxid. Redox Signal.* 14, 2347–2360.
- Jin, M.C., Yoo, J.M., Sok, D.E., and Kim, M.R. (2014). Neuroprotective effect of N-acyl 5-hydroxytryptamines on glutamate-induced cytotoxicity in HT-22 cells. *Neurochem. Res.* 39, 2440–2451.
- Johansson, J.U., Woodling, N.S., Wang, Q., Panchal, M., Liang, X., Trueba-Saiz, A., Brown, H.D., Mhatre, S.D., Loui, T., and Andreasson, K.I. (2015). Prostaglandin signaling suppresses beneficial microglial function in Alzheimer's disease models. *J. Clin. Invest.* 125, 350–364.
- Johnson, D.A. and Johnson, J.A. (2015). Nrf2-a therapeutic target for the treatment of neurodegenerative diseases. *Free Radic. Biol. Med.* 88, 253–267.

- Kalra, S., Zhang, Y., Knatko, E.V., Finlayson, S., Yamamoto, M., and Dinkova-Kostova A.T. (2011). Oral azathioprine leads to higher incorporation of 6-thioguanine in DNA of skin than liver: the protective role of the Keap1/Nrf2/ARE pathway. *Cancer Prev. Res. (Phila)* 4, 1665–1674.
- Kalra, S., Knatko, E.V., Zhang, Y., Honda, T., Yamamoto, M., and Dinkova-Kostova, A.T. (2012). Highly potent activation of Nrf2 by topical tricyclic bis(cyano enone): implications for protection against UV radiation during thiopurine therapy. *Cancer Prev. Res. (Phila)* 5, 973–981.
- Kalthoff, S., Ehmer, U., Freiberg, N., Manns, M.P., and Strassburg, C.P. (2010). Interaction between oxidative stress sensor Nrf2 and xenobiotic-activated aryl hydrocarbon receptor in the regulation of the human phase II detoxifying UDP-glucuronosyltransferase 1A10. *J. Biol. Chem.* 285, 5993–6002.
- Kanai, Y., Clemencon, B., Simonin, A., Leuenberger, M., Lochner, M., Weisstanner, M., and Hediger, M.A. (2013). The SLC1 high-affinity glutamate and neutral amino acid transporter family. *Mol. Aspects Med.* 34, 108–120.
- Kawalec, P., Mikrut, A., Wisniewska, N., and Pilc, A. (2014). The effectiveness of dimethyl fumarate monotherapy in the treatment of relapsing-remitting multiple sclerosis: a systematic review and meta-analysis. *Curr. Neuroparmacol.* 12, 256–268.
- Kensler, T.W., Wakabayashi, N., and Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu. Rev. Pharmacol. Toxicol.* 47, 89–116.
- Kim, T.H., Hur, E.G., Kang, S.J., Kim, J.A., Thapa, D., Lee, Y.M., Ku, S.K., Jung, Y., and Kwak, M.K. (2011). NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1 α . *Cancer Res.* 71, 2260–2275.
- Kimura, M., Yamamoto, T., Zhang, J., Itoh, K., Kyo, M., Kamiya, T., Aburatani, H., Katsuoka, F., Kurokawa, H., Tanaka, T., et al. (2007). Molecular basis distinguishing the DNA binding profile of Nrf2-Maf heterodimer from that of Maf homodimer. *J. Biol. Chem.* 282, 33681–33690.
- Kobayashi, A., Ito, E., Toki, T., Kogame, K., Takahashi, S., Igarashi, K., Hayashi, N., and Yamamoto, M. (1999). Molecular cloning and functional characterization of a new Cap'n'collar family transcription factor Nrf3. *J. Biol. Chem.* 274, 6443–6452.
- Kobayashi, A., Kang, M.I., Okawa, H., Ohtsui, M., Zenke, Y., Chiba, T., Igarashi, K., and 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.
- Kolbach, D.N. and Nieboer, C. (1992). Fumaric acid therapy in psoriasis: results and side effects of 2 years of treatment. *J. Am. Acad. Dermatol.* 27, 769–771.
- 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.
- Kovac, S., Angelova, P.R., Holmstrom, K.M., Zhang, Y., Dinkova-Kostova, A.T., and Abramov, A.Y. (2015). Nrf2 regulates ROS production by mitochondria and NADPH oxidase. *Biochim. Biophys. Acta* 1850, 794–801.
- Krohn, M., Lange, C., Hofrichter, J., Scheffler, K., Stenzel, J., Steffen, J., Schumacher, T., Bruning, T., Plath, A.S., Alfen, F., et al. (2011). Cerebral amyloid-beta proteostasis is regulated by the membrane transport protein ABCC1 in mice. *J. Clin. Invest.* 121, 3924–3931.
- Lau, A., Wang, X.J., Zhao, F., Villeneuve, N.F., Wu, T., Jiang, T., Sun, Z., White, E., and 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.
- Lee, J.M., Calkins, M.J., Chan, K., Kan, Y.W., and Johnson, J.A. (2003). 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.* 278, 12029–12038.
- Lee, H.C., Simon, G.M., and Cravatt, B.F. (2015). ABHD4 regulates multiple classes of N-acyl phospholipids in the mammalian central nervous system. *Biochemistry* 54, 2539–2549.
- Levonen, A.L., Landar, A., Ramachandran, A., Ceaser, E.K., Dickinson, D.A., Zandoni, G., Morrow, J.D., and Darley-Usmar, V.M. (2004). Cellular mechanisms of redox cell signaling: role of cysteine modification in controlling antioxidant defences in response to electrophilic lipid oxidation products. *Biochem. J.* 378, 373–382.
- Li, J., Johnson, D., Calkins, M., Wright, L., Svendsen, C., and Johnson, J. (2005). Stabilization of Nrf2 by tBHQ confers protection against oxidative stress-induced cell death in human neural stem cells. *Toxicol. Sci.* 83, 313–328.
- Liby, K., Yore, M.M., Roebuck, B.D., Baumgartner, K.J., Honda, T., Sundararajan, C., Yoshizawa, H., Gribble, G.W., Williams, C.R., Risingsong, R., et al. (2008). A novel acetylenic tricyclic bis(cyano enone) potently induces phase 2 cytoprotective pathways and blocks liver carcinogenesis induced by aflatoxin. *Cancer Res.* 68, 6727–6733.
- Lin, C.H., Lin, P.P., Lin, C.Y., Huang, C.H., Huang, Y.J., and Lane, H.Y. (2015). Decreased mRNA expression for the two subunits of system x, SLC3A2 and SLC7A11, in WBC in patients with schizophrenia: evidence in support of the hypo-glutamatergic hypothesis of schizophrenia. *J. Psychiatr. Res.* 72, 58–63.
- Linker, R.A., Lee, D.H., Ryan, S., van Dam, A.M., Conrad, R., Bista, P., Zeng, W., Hronowsky, X., Buko, A., Chollate, S., et al. (2011). Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* 134, 678–692.
- Lu'o'ng, K. and Nguyen, L.T. (2011). Role of thiamine in Alzheimer's disease. *Am. J. Alzheimers Dis. Other Dement.* 26, 588–598.
- Ludtmann, M.H., Angelova, P.R., Zhang, Y., Abramov, A.Y., and Dinkova-Kostova, A.T. (2014). Nrf2 affects the efficiency of mitochondrial fatty acid oxidation. *Biochem. J.* 457, 415–424.
- MacLeod, A.K., McMahon, M., Plummer, S.M., Higgins, L.G., Penning, T.M., Igarashi, K., and Hayes, J.D. (2009). Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. *Carcinogenesis* 30, 1571–1580.
- Maher, J.M., Dieter, M.Z., Aleksunes, L.M., Slitt, A.L., Guo, G., Tanaka, Y., Scheffer, G.L., Chan, J.Y., Manautou, J.E., Chen, Y., et al. (2007). Oxidative and electrophilic stress induces multidrug resistance-associated protein transporters via the nuclear factor-E2-related factor-2 transcriptional pathway. *Hepatology* 46, 1597–1610.

- Malhotra, D., Portales-Casamar, E., Singh, A., Srivastava, S., Arenillas, D., Happel, C., Shyr, C., Wakabayashi, N., Kensler, T.W., Wasserman, W.W., and Biswal, S. (2010). Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nuc. Acids Res.* 38, 5718–5734.
- Maqbool, M., Mobashir, M., and Hoda, N. (2015). Pivotal role of glycogen synthase kinase-3: a therapeutic target for Alzheimer's disease. *Eur. J. Med. Chem.* 107, 63–81.
- McKenna, M.C., Tildon, J.T., Stevenson, J.H., Huang, X., and Kingwell, K.G. (1995). Regulation of mitochondrial and cytosolic malic enzymes from cultured rat brain astrocytes. *Neurochem. Res.* 20, 1491–1501.
- McMahon, M., Itoh, K., Yamamoto, M., Chanas, S.A., Henderson, C.J., McLellan, L.I., Wolf, C.R., Cavin, C., and Hayes, J.D. (2001). The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res.* 61, 3299–3307.
- McMahon, M., Thomas, N., Itoh, K., Yamamoto, M., and Hayes, J.D. (2006). Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a “tethering” mechanism: a two-site interaction model for the Nrf2-Keap1 complex. *J. Biol. Chem.* 281, 24756–24768.
- McMahon, M., Lamont, D.J., Beattie, K.A., and Hayes, J.D. (2010). Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. *Proc. Natl. Acad. Sci. USA* 107, 18838–18843.
- Mitsuishi, Y., Motohashi, H., and Yamamoto, M. (2012a). The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. *Front Oncol.* 2, 200.
- Mitsuishi, Y., Taguchi, K., Kawatani, Y., Shibata, T., Nukiwa, T., Aburatani, H., Yamamoto, M., and Motohashi, H. (2012b). Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell* 22, 66–79.
- Moi, P., Chan, K., Asunis, I., Cao, A., and 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. USA* 91, 9926–9930.
- Motohashi, H., Katsuoka, F., Engel, J.D., and Yamamoto, M. (2004). Small Maf proteins serve as transcriptional cofactors for keratinocyte differentiation in the Keap1-Nrf2 regulatory pathway. *Proc. Natl. Acad. Sci. USA* 101, 6379–6384.
- Nguyen, T., Sherratt, P.J., and Pickett, C.B. (2003). Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu. Rev. Pharmacol. Toxicol.* 43, 233–260.
- Onodera, K., Shavit, J.A., Motohashi, H., Katsuoka, F., Akasaka, J.E., Engel, J.D., and Yamamoto, M. (1999). Characterization of the murine maff gene. *J. Biol. Chem.* 274, 21162–21169.
- Pahnke, J., Langer, O., and Krohn, M. (2014). Alzheimer's and ABC transporters – new opportunities for diagnostics and treatment. *Neurobiol. Dis.* 72, 54–60.
- Park, J.S., Kang, D.H., Lee, D.H., and Bae, S.H. (2015). Concerted action of p62 and Nrf2 protects cells from palmitic acid-induced lipotoxicity. *Biochem. Biophys. Res. Commun.* 466, 131–137.
- Pi, J., Leung, L., Xue, P., Wang, W., Hou, Y., Liu, D., Yehuda-Shnaidman, E., Lee, C., Lau, J., Kurtz, T.W. et al. (2010). Deficiency in the nuclear factor E2-related factor-2 transcription factor results in impaired adipogenesis and protects against diet-induced obesity. *J. Biol. Chem.* 285, 9292–9300.
- Probst, B.L., Trevino, I., McCauley, L., Bumeister, R., Dulubova, I., Wigley, W.C., and Ferguson, D.A. (2015). RTA 408, a novel synthetic triterpenoid with broad anticancer and anti-inflammatory activity. *PLoS One* 10, e0122942.
- Rada, P., Rojo, A.I., Chowdhry, S., McMahon, M., Hayes, J.D., and Cuadrado, A. (2011). SCF/ β -TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. *Mol. Cell. Biol.* 31, 1121–1133.
- Richardson, B.G., Jain, A.D., Speltz, T.E., and Moore, T.W. (2015). Non-electrophilic modulators of the canonical Keap1/Nrf2 pathway. *Bioorg. Med. Chem. Lett.* 25, 2261–2268.
- Robert, S.M., Buckingham, S.C., Campbell, S.L., Robel, S., Holt, K.T., Ogunrinu-Babarinde, T., Warren, P.P., White, D.M., Reid, M.A., Eschbacher, J.M., et al. (2015). SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. *Sci. Transl. Med.* 7, 289ra286.
- Rojo, A.I., Rada, P., Egea, J., Rosa, A.O., Lopez, M.G., and Cuadrado, A. (2008). Functional interference between glycogen synthase kinase-3 beta and the transcription factor Nrf2 in protection against kainate-induced hippocampal cell death. *Mol. Cell Neurosci.* 39, 125–132.
- Saito, R., Suzuki, T., Hiramoto, K., Asami, S., Naganuma, E., Suda, H., Iso, T., Yamamoto, H., Morita, M., Furusawa, Y., et al. (2015). Characterizations of three major cysteine sensors of Keap1 in stress response. *Mol. Cell. Biol.* 36, 271–284.
- Scharfe, C., Hauschild, M., Klopstock, T., Janssen, A.J., Heidemann, P.H., Meitinger, T., and Jaksch, M. (2000). A novel mutation in the thiamine responsive megaloblastic anaemia gene SLC19A2 in a patient with deficiency of respiratory chain complex I. *J. Med. Genet.* 37, 669–673.
- Shan, Y., Schoenfeld, R.A., Hayashi, G., Napoli, E., Akiyama, T., Iodi Carstens, M., Carstens, E.E., Pook, M.A., and Cortopassi, G.A. (2013). Frataxin deficiency leads to defects in expression of antioxidants and Nrf2 expression in dorsal root ganglia of the Friedreich's ataxia YG8R mouse model. *Antioxid. Redox Signal.* 19, 1481–1493.
- Shelton, P. and Jaiswal, A.K. (2013). The transcription factor NF-E2-related factor 2 (Nrf2): a protooncogene? *FASEB J.* 27, 414–423.
- Shivdasani, R.A., Rosenblatt, M.F., Zucker-Franklin, D., Jackson, C.W., Hunt, P., Saris, C.J., and Orkin, S.H. (1995). Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. *Cell* 81, 695–704.
- Silverberg, G.D., Messier, A.A., Miller, M.C., Machan, J.T., Majmudar, S.S., Stopa, E.G., Donahue, J.E., and Johanson, C.E. (2010). Amyloid efflux transporter expression at the blood-brain barrier declines in normal aging. *J. Neuropathol. Exp. Neurol.* 69, 1034–1043.
- Singh, A., Happel, C., Manna, S.K., Acquaah-Mensah, G., Carrerero, J., Kumar, S., Nasipuri, P., Krausz, K.W., Wakabayashi, N., Dewi, R., et al. (2013). Transcription factor NRF2 regulates miR-1 and miR-206 to drive tumorigenesis. *J. Clin. Invest.* 123, 2921–2934.
- Skerrett, R., Pellegrino, M.P., Casali, B.T., Taraboanta, L., and Landreth, G.E. (2015). Combined liver X receptor/ peroxisome

- proliferator-activated receptor γ agonist treatment reduces amyloid β levels and improves behavior in amyloid precursor protein/presenilin 1 mice. *J. Biol. Chem.* **290**, 21591–21602.
- Solis, L.M., Behrens, C., Dong, W., Suraokar, M., Ozburn, N.C., Moran, C.A., Corvalan, A.H., Biswal, S., Swisher, S.G., Bekele, B.N., et al. (2010). Nrf2 and Keap1 abnormalities in non-small cell lung carcinoma and association with clinico-pathologic features. *Clin. Cancer Res.* **16**, 3743–3753.
- Soria, F.N., Perez-Samartin, A., Martin, A., Gona, K.B., Llop, J., Szczupak, B., Chara, J.C., Matute, C., and Domercq, M. (2014). Extrasynaptic glutamate release through cystine/glutamate antiporter contributes to ischemic damage. *J. Clin. Invest.* **124**, 3645–3655.
- Spencer, S.R., Wilczak, C.A., and Talalay, P. (1990). Induction of glutathione transferases and NAD(P)H:quinone reductase by fumaric acid derivatives in rodent cells and tissues. *Cancer Res.* **50**, 7871–7875.
- Sporn, M.B. and Liby, K.T. (2012). NRF2 and cancer: the good, the bad and the importance of context. *Nat. Rev. Cancer* **12**, 564–571.
- Sykietis, G.P. and Bohmann, D. (2010). Stress-activated cap'n'collar transcription factors in aging and human disease. *Sci. Signal.* **3**, re3.
- Tachikawa, M., Hosoya, K., and Terasaki, T. (2014). Pharmacological significance of prostaglandin E2 and D2 transport at the brain barriers. *Adv. Pharmacol.* **71**, 337–360.
- Taguchi, K., Fujikawa, N., Komatsu, M., Ishii, T., Unno, M., Akaike, T., Motohashi, H., and Yamamoto, M. (2012). Keap1 degradation by autophagy for the maintenance of redox homeostasis. *Proc. Natl. Acad. Sci. USA* **109**, 13561–13566.
- Tebay, L.E., Robertson, H., Durant, S.T., Vitale, S.R., Penning, T.M., Dinkova-Kostova, A.T., and Hayes, J.D. (2015). Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radic. Biol. Med.*, FRBMD1500379.
- Thimmulappa, R.K., Mai, K.H., Srisuma, S., Kensler, T.W., Yamamoto, M., and Biswal, S. (2002). Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res.* **62**, 5196–5203.
- Tieu, K., Perier, C., Vila, M., Caspersen, C., Zhang, H.P., Teismann, P., Jackson-Lewis, V., Stern, D.M., Yan, S.D., and Przedborski, S. (2004). L-3-hydroxyacyl-CoA dehydrogenase II protects in a model of Parkinson's disease. *Ann. Neurol.* **56**, 51–60.
- Tsujita, T., Peirce, V., Baird, L., Matsuyama, Y., Takaku, M., Walsh, S.V., Griffin, J.L., Uruno, A., Yamamoto, M., and Hayes, J.D. (2014). Transcription factor Nrf1 negatively regulates the cystine/glutamate transporter and lipid-metabolizing enzymes. *Mol. Cell Biol.* **34**, 3800–3816.
- Udasin, R.G., Wen, X., Bircsak, K.M., Aleksunes, L.M., Shakarjian, M.P., Kong, A.T., Heck, D.E., Laskin, D.L., and Laskin, J.D. (2015). Nrf2 regulates the sensitivity of mouse keratinocytes to nitrogen mustard via multidrug resistance-associated protein 1 (Mrp1). *Toxicol. Sci.* **149**, 202–212.
- Venugopal, R. and Jaiswal, A.K. (1996). Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc. Natl. Acad. Sci. USA* **93**, 14960–14965.
- Venugopal, R. and 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.
- von Otter, M., Landgren, S., Nilsson, S., Celojovic, D., Bergstrom, P., Hakansson, A., Nissbrandt, H., Drozdik, M., Bialecka, M., Kurzawski, M., et al. (2010). Association of Nrf2-encoding NFE2L2 haplotypes with Parkinson's disease. *BMC Med. Genet.* **11**, 36.
- Wang, X., Campos, C.R., Peart, J.C., Smith, L.K., Boni, J.L., Cannon, R.E., and Miller, D.S. (2014). Nrf2 upregulates ATP binding cassette transporter expression and activity at the blood-brain and blood-spinal cord barriers. *J. Neurosci.* **34**, 8585–8593.
- Wen, J., Luo, J., Huang, W., Tang, J., Zhou, H., and Zhang, W. (2015). The pharmacological and physiological role of multidrug-resistant protein 4. *J. Pharmacol. Exp. Ther.* **354**, 358–375.
- Wu, K.C., Cui, J.Y., and Klaassen, C.D. (2011). Beneficial role of Nrf2 in regulating NADPH generation and consumption. *Toxicol. Sci.* **123**, 590–600.
- Xue, M., Qian, Q., Adaikalakoteswari, A., Rabbani, N., Babaei-Jadidi, R., and Thornalley, P.J. (2008). Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. *Diabetes* **57**, 2809–2817.
- Yamanaka, M., Ishikawa, T., Griep, A., Axt, D., Kummer, M.P., and Heneka, M.T. (2012). PPAR γ /RXR α -induced and CD36-mediated microglial amyloid- β phagocytosis results in cognitive improvement in amyloid precursor protein/presenilin 1 mice. *J. Neurosci.* **32**, 17321–17331.
- Yates, M.S., Tran, Q.T., Dolan, P.M., Osburn, W.O., Shin, S., McCulloch, C.C., Silkworth, J.B., Taguchi, K., Yamamoto, M., Williams, C.R., et al. (2009). Genetic versus chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression profiles between Keap1 knockout and triterpenoid-treated mice. *Carcinogenesis* **30**, 1024–1031.
- Ye, B., Shen, H., Zhang, J., Zhu, Y.G., Ransom, B.R., Chen, X.C., and Ye, Z.C. (2015). Dual pathways mediate beta-amyloid stimulated glutathione release from astrocytes. *Glia* **63**, 2208–2219.
- Ying, W. (2008). NAD $^{+}$ /NADH and NADP $^{+}$ /NADPH in cellular functions and cell death: regulation and biological consequences. *Antioxid. Redox Signal.* **10**, 179–206.
- Yokota, S., Higashi, E., Fukami, T., Yokoi, T., and Nakajima, M. (2011). Human CYP2A6 is regulated by nuclear factor-erythroid 2 related factor 2. *Biochem. Pharmacol.* **81**, 289–294.
- Yue, L. and Mazzone, T. (2009). Peroxisome proliferator-activated receptor γ stimulation of adipocyte ApoE gene transcription mediated by the liver receptor X pathway. *J. Biol. Chem.* **284**, 10453–10461.
- Zhang, Y., Talalay, P., Cho, C.G., and Posner, G.H. (1992). A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. USA* **89**, 2399–2403.
- Zhang, D.D., Lo, S.C., Cross, J.V., Templeton, D.J., and Hannink, M. (2004). Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol. Cell Biol.* **24**, 10941–10953.
- Zhao, M., Xu, H., Zhang, B., Hong, B., Yan, W., and Zhang, J. (2015). Impact of nuclear factor erythroid-derived 2-like 2 and p62/sequestosome expression on prognosis of patients with gliomas. *Hum. Pathol.* **46**, 843–849.
- Zou, Y., Wang, R., Guo, H., and Dong, M. (2015). Phytoestrogen β -ecdysterone protects PC12 cells against MPP $^{+}$ -induced neurotoxicity *in vitro*: involvement of PI3K-Nrf2-regulated pathway. *Toxicol. Sci.* **147**, 28–38.