We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Nrf2 Signaling: An Adaptive Response Pathway for Neurodegenerative Disorders

Annalisa Lo Gerfo, Lucia Petrozzi, Lucia Chico and
Gabriele Siciliano

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/65300>

Abstract

Oxidative damage contributes to pathogenesis in many neurodegenerative diseases. As the indicator and regulator of oxidative stress, the nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element (ARE) pathway has been shown to have dynamic changes and examined for its neuroprotective role in many cases. Nrf2 is emerging as a regulatory protein in neuronal death, since it helps neuronal cells to meet with oxidative insults. In this chapter, we summarize the role of Nrf2 as a master regulator of oxidative stress. Furthermore, we treat some natural and chemical substances able to modulate the Nrf2 pathway and, therefore, their possible use in the neurodegenerative diseases therapeutic treatment.

Keywords: cell metabolism, oxidative damage, neurodegenerative diseases, neuro-protection, modulators of Nrf2/ARE pathway

1. Introduction

To maintain redox homeostasis is very important for the normal function of the brain. This mechanism is regulated by antioxidant system. With age, genetic, and environmental risk factors, this system becomes imbalanced and oxidative stress (OS) follows through increased levels of reactive oxygen and nitrogen species (ROS/RNS). The accumulation of oxidative damage induces modifications of lipids, proteins, and DNA/RNA, a common feature of many neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). Although it is difficult to impose one if oxidative stress is a cause or epiphenomenon of neuronal death, the nuclear

factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element (ARE) pathway is a primary sensor of oxidative stress and regulates the expression of several genes encoding antioxidant proteins and detoxifying. The Nrf2-ARE pathway activation, in animal models of neurodegeneration, has produced positive effects; these data support the need for studies aimed to develop drugs able to activate Nrf2-ARE pathway in the central and peripheral nervous system. This chapter sums up the role of oxidative damage in neurodegenerative disorders and the protective functions of the Nrf2-ARE pathway.

2. Cell metabolism and oxidative stress

Originally, the primordial eukaryotic cells were unable to use oxygen for metabolic purposes. More than one billion years ago, according to endosymbiosis theory, these eukaryotic cells were colonized by aerobic bacteria, which can change with the host cells, and intracellular organelles became those we now call mitochondria. This alliance has facilitated bacteria to the availability of metabolic substrates, now assigned to the host cell, and at the same time has made a new kind of metabolism, much more efficient, eukaryotes: aerobic or oxidative metabolism [1]. Mitochondria are cytoplasmic organelles ranging from 1 to 10 μ and are often described as “electrical control units of the cell” because they generate most cell supply of adenosine triphosphate (ATP), used precisely as a chemical energy source from cell. Mitochondria not only perform this function but are also involved in other processes, such as signaling, cell differentiation, death, and also in the cell cycle control and cell growth [2]. The number of mitochondria in a cell varies according to the type of tissue and the body; there are cells with a single mitochondrion and cells with many thousands of mitochondria; these organelles are able to move freely in the cytoplasm and tend to thicken in the points where there is a greater demand for energy. The mitochondria, by means of the mitochondrial respiratory chain (OXPHOS), and through the process of oxidative phosphorylation, fulfill the requirements of ATP and therefore of energy of the cell [3]. The respiratory chain consists of a series of electron carriers (complexes), most of which are integral proteins of the inner membrane, containing prosthetic groups associated to proteins able to accept and donate one or two electrons [3]. The electron carrier complexes are four types: complexes I, II, III, and IV, in which two mobile electron carriers are to be added: cytochrome c and coenzyme Q. The respiratory chain is a very efficient mechanism, but during the step of transporting electrons, it may happen that a small percentage of electrons may prematurely reduce oxygen, forming reactive oxygen species (ROS), which are potentially harmful and dangerous for the cell. ROS are ions or very small molecules that include oxygen ions, free radicals and peroxides, organic and inorganic; they are highly reactive due to the presence of unpaired electrons in the orbital outside and are formed as a natural byproduct of oxygen metabolism and play an important role in cell signaling. The main source of ROS in vivo is aerobic respiration precisely, although they are also produced by the fatty acids beta-oxidation, by the xenobiotic components metabolism, after the activation of phagocytosis by pathogens. During periods of environmental stress, the ROS levels can increase dramatically, causing significant damage to cell structures. This increase is identified with the term of oxidative stress (OS) [4]. OS is usually

defined as the altered balance between the production of ROS and their removal by cellular antioxidant mechanisms, such as enzymatic scavengers and low-molecular-weight reductants. Mitochondria use most of available oxygen (85–90%) to produce ATP, but, at the same time, are the major producers of ROS, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) principally originate by loss of electrons from OXPHOS during oxidative phosphorylation with the consequent incomplete reduction of molecular oxygen [5, 6]. Superoxide itself is not greatly dangerous; nevertheless, it can rapidly react with the mild oxidant nitric oxide (NO) to generate peroxynitrite ($ONOO^-$) [7, 8]. Similarly, H_2O_2 is a slight oxidant but bit by bit it decomposes to generate the hydroxyl radical ($\cdot OH$). Both $ONOO^-$ and $\cdot OH$ damage the function of biomolecules inside the cell. Particularly, ROS attack the backbone and the side chains of proteins determining protein misfolding and aggregation. In addition, they attack nucleic acids, leading to alteration of purine and pyrimidine bases. Moreover, ROS cause lipid peroxidation, producing highly dangerous molecules, such as malondialdehyde, 4-hydroxy-2-trans-nonenal (HNE), acrolein, and thiobarbituric acid reactive substances (TBARSs) [9]. Summarizing, OS causes several interdependent mechanisms leading to cell death. All the human body's cells are subjected to oxidative stress, but the neurons are particularly affected by oxidative damage of aerobic metabolism. This susceptibility can be attributed on the one hand to their high oxygen requirement and on the other hand to low expression of antioxidant proteins [10]. Strong production of ROS is associated with deleterious effects on neuronal cell, also exerting crucial roles in regulating specific signaling mechanisms. In particular, ROS are able to activate kinase cascade [11], to regulate the calcium mobilization and signaling [12, 13], to control the expression of antioxidant genes [14, 15], and, finally, the ROS seem to control the differentiation [16] and neurogenesis [17] in neural stem cell. OS is a critical gambler in several diseases, including age-dependent neurodegenerative disorders such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). The involvement of OS in several neurodegenerative conditions has been demonstrated by the identification of pathological mutations in genes performing in antioxidant pathways as well as oxidative stress markers in patients' samples [18–20]. Nevertheless, in many cases it is not clear whether this kind of stress is a primary cause or downstream event associated with the progression of the neurodegeneration. Consequently, a better understanding of ROS involvement in the pathogenesis of neurodegenerative diseases can offer the possibility to identify new targets for neuroprotective therapies.

3. Nrf2/ARE pathway

Several lines of evidence in the literature suggest that the reactive chemical species and electrophilic substances can have an important role in inducing different causative mechanisms of various pathologies such as tumorigenesis, diseases affecting the cardiovascular system, central nervous system, and peripheral nervous system [21, 22]. The human body, in order to neutralize these toxic substances, has developed a plethora of defense mechanisms [23]. Between the several mechanisms, the Nrf2-ARE pathway is now considered the most regulator of cellular defense mechanisms against oxidative stress [24].

Nrf2 belongs to the Cap'n'collar (Cnc) transcription factor family and is considered the leader of the antioxidant response since it regulates the expression of several defensive genes [25, 26]. Nrf2 is a very unstable protein, typically present in association with its negative regulator Kelch-like ECH-associated protein 1 (Keap1), which acts as a molecular sensor of cellular oxidative stress. Under basal condition, Keap1 restrains Nrf2 in the cytoplasm leading to its degradation. Particularly, Keap1 acts as a connection protein between Nrf2 and the Cul3-based E3-ubiquitin ligase complex, promoting Nrf2 ubiquitination and consequent degradation by the 26S proteasome [27, 28]. Activation of Nrf2 involves its cytosolic stabilization; specific cysteine residues (Cys 151, Cys 273, and Cys 288) have been identified as direct sensors for electrophiles and oxidants; chemical modifications in these sensor residues cause a conformational change that produce the dissociation of Nrf2 from Keap1. Nrf2, detached from his repressors, translocates to the nucleus and binds its partner, small Maf protein. The heterodimer Nrf2-sMAF ultimately binds antioxidant response element (ARE) sequences leading to the expression of cytoprotective genes thus allowing cell to efficiently cope with endogenous stress and exogenous toxicants [29]. Nrf2 also is able to modulate the transcription of genes involved in mitochondrial biogenesis [30] (**Figure 1**).

Figure 1. Regulation of Nrf2 by Keap1. In basal conditions, Nrf2 is sequestered in the cytoplasm by a Keap1 homodimer that facilitates ubiquitination and degradation of Nrf2 in the proteasome. In the presence of inducers that react with specific cysteine residues of Keap1, you get the release of Nrf2 and its nuclear translocation. In the nucleus, Nrf2 heterodimerizes with small Maf proteins and binds the antioxidant response element (ARE), by activating the expression of a battery of cytoprotective genes.

4. The Nrf2-ARE pathway and neuroprotection

4.1. Modifications of Nrf2-ARE pathway in neurodegenerative diseases

Abnormalities of Nrf2-ARE pathway were observed in several models of disease-aging dependent and in degenerative disorders; changes of Nrf2-ARE pathway cause ROS accumu-

lation and therefore increase of oxidative damage to biological macromolecules. Several evidences in literature have showed that Nrf2 activation is induced in dopaminergic neurons in PD cases, but is decreased in hippocampus of AD patients [31]. In the motor cortex and spinal cord of ALS patients, a reduction of mRNA and protein levels of Nrf2 was observed; conversely, mRNA level of Keap1 is increased in the motor cortex [32]. Free radical scavengers regulated by Nrf2 such as superoxide dismutase 1 (SOD1) and catalase are reduced in patients, whereas other Nrf2-dependent genes are upregulated, for example, NAD(P)H:quinone oxidoreductase 1 (NQO1), an antioxidant enzyme, is upregulated in astrocytes, neurons, and other cell types in human AD [32–34] and PD brain [34]. Also heme oxygenase 1 (HO-1) is overexpressed in astrocytes and neurons of PD [35, 36] and AD patients [37]. HO-1 regulates heme degradation in two highly antioxidant molecules: biliverdin and bilirubin [38, 39]. Peroxiredoxin is able to reduce hydrogen peroxide and is also upregulated in PD [40], AD [41], and HD patients [42].

Pluripotent stem cells (iPSC)-derived neurons, which are generated from PD patients with PARK2 mutations, show an altered redox balance, mitochondrial dysfunction, and increased activity of the Nrf2 pathway [43]. Another study shows a significant alteration of the Nrf2-ARE pathway in neurospheres derived from the olfactory mucosa of PD patients [44]. Similar fluctuations are seen in two transgenic animal models of PD: α Syn-mutant A53T h α SynA53T [45] and MPTP mouse model [46]. In particular, Nrf2 downstream genes related to glutathione synthesis increase at the early stage and decrease at the late-stage disease with corresponding change in the total glutathione levels. Nrf2-regulated genes involved in glutathione synthesis, metabolism and transportation and detoxification of hydrogen peroxide/quinones increase in the SN and striatum of 1-month-old α Syn mice [47]. The Nrf2-ARE system is activated in cell culture systems as well in response to paraquat and maneb [48], and 6-hydroxydopamine [48, 49]. Apparently, the endogenous activation of the Nrf2-ARE pathway seems insufficient to neutralize the accumulation of oxidative damage. Thus, the research is focused in identifying an exogenous substance able to maintain long endogenous Nrf2-ARE activation to help the brain defend itself from oxidative damage [45, 50–53].

4.2. Nrf2-ARE pathway and Parkinson's disease

Parkinson's disease affects more than 1% of the population over 60 years of age and is the second most common neurodegenerative disorder after AD [54]. The 90% of cases are sporadic, whereas about 10% show a family background [55].

PD is caused by the degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNc) and it is known that PD neurons are more susceptible to OS [56]. The selective vulnerability of SNc dopaminergic neurons can be caused by several pathogenic mechanisms that include exposure to genetic and environmental risk factors, altered proteolytic systems, and mitochondrial dysfunctions [57]. In particular, mitochondrial defects lead to impaired energy and ROS production and therefore to altered bioenergetic and redox balance.

Consistent evidence shows that disrupted mitochondrial integrity and OS play a pivotal role in PD pathogenesis and disease progression.

Mutations in several genes, such as PARK2, PARK6, and PARK7, are associated with early-onset familial forms of PD and with mitochondrial alterations leading to neuronal death [58]. Several studies show that some substances, such as MPTP and rotenone, are able to inhibit mitochondrial complex I and increase ROS production with possible loss of dopaminergic neurons in the SNc [59]. Furthermore, peripheral and central markers of oxidative damage are altered in PD patients, indicating that OS is a crucial player in PD pathogenesis [60–62]. PD has also been associated with alterations in the expression of antioxidant molecules such as glutathione and antioxidant enzymes. It was shown that oxidized glutathione is significantly higher, while other antioxidant molecules and catalase activity are decreased in blood cells from PD patients [63]. Furthermore, several studies have shown that the activation of antioxidant genes expression, in particular those under the control of the Nrf2/ARE system, has neuroprotective effects in different models of PD [64, 65].

Activation of the Nrf2-ARE pathway is able to protect against the toxic forms of α Syn. In SK-N-SH neuroblastoma cells, ferrous iron promotes α Syn aggregation through inhibiting Nrf2 pathway [66]. In a PD animal model, it was observed that the transgenic activation of Nrf2 and knockdown of Keap1 could delay the α Syn-mediated dopaminergic neuron loss and motor dysfunction [67]. Conversely, genetic deletion of Nrf2 increases α Syn toxicity and exaggerates α Syn/p- α Syn accumulation in dopaminergic neurites and gliosis. Nrf2 deficiency enhances the inflammatory response and lowers the capability of phagocytosis in primary microglial cells [68].

Recently, studies have identified the importance of astrocytic Nrf2-regulating α Syn proteostasis. Astrocytic overexpression of Nrf2 (GFAP-Nrf2) can reduce α Syn aggregates in the central nervous system of a PD mouse model with neuronal overexpression of human α Syn-mutant A53T [45]. The accumulation of h α SynA53T in the Triton-soluble fraction from the spinal cord decreases 60% in symptomatic mice. This is accompanied by a significant increase in h α SynA53T in the Triton-insoluble/SDS-soluble fraction. This movement of α SynA53T into Triton-insoluble/SDS-soluble aggregates is completely reversed by the overexpression of Nrf2 in astrocytes.

Similar changes are observed for phosphorylated (Ser129) α SynA53T (p-h α SynA53T) in Triton-soluble and Triton-insoluble/SDS-soluble fractions. Fluorescent staining of h α SynA53T also shows a dramatic increase in h α SynA53T aggregates that colocalized with p-h α SynA53T. Again, these changes are completely reversed by GFAP-Nrf2.

The autophagy-lysosome pathway (ALP) is a protein degradation system responsible for the turnover of proteins, aggregate proteins, and damaged organelles. Dysfunctions of autophagic mechanism result in the accumulation of cytoplasmic aggregates composed of misfolded proteins and deformed organelles, leading to neurodegeneration and other diseases [69–71]. A significant dysfunction of autophagic machinery is observed in the α SynA53T mice model [45, 72–74]. Nrf2 prevents chaperone-mediated autophagy dysfunction and increases lifespan, delays onset, and reduces aggregation in α SynA53T mice [45].

4.3. Nrf2-ARE pathway and Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disease, accounting for 60–70% of cases of dementia, and although its etiology is still unclear, it is characterized by the presence of brain amyloid plaques and neurofibrillary tangles whose accumulation ultimately leads to extensive neuronal loss and progressive decline of cognitive function [75–77]. They are aggregates of proteins distributed in the entorhinal cortex, hippocampus, and temporal, frontal, and inferior parietal lobes. Amyloid plaques are composed of aggregates of β -amyloid ($A\beta$) and of other protein aggregates such as hyperphosphorylated Tau, ubiquitin, and presenilins 1 and 2; amyloid plaques are sticky buildup which accumulates outside nerve cells. Neurofibrillary tangles are abnormal collections of twisted protein threads found inside nerve cells; the tangles are aggregates of hyperphosphorylated Tau protein [78]. Some of the major risk factors for AD are unhealthy aging in sporadic AD cases, the presence of ApoE-4 alleles in both sporadic and familial AD [79], and genetic factors, such as mutation in amyloid precursor protein (APP) and presenilin-1 (PS1) in familial AD [80] among others. AD brain is characterized by mitochondrial dysfunction, reactive gliosis, and oxidative damage to lipids and proteins [81–85].

Several studies demonstrate that the AD brain is under oxidative stress attack. A significantly increased HO-1 expression was reported in postmortem AD temporal cortex and hippocampus [82]. Additionally, an increased Nqo1 activity and expression were found in astrocytes and neurons of AD brain [86, 87] and Nrf2 was localized in cytoplasm in AD hippocampal neurons [31]. Furthermore, there is increased protein oxidation [88, 89] and lipid peroxidation [90–92] in AD brain. Recent studies in aged APP/PS1 AD mouse models showed Nrf2 protein levels [93].

Several evidences in literature have shown that Nrf2-ARE pathway is able to mitigate the toxicity mediated by $A\beta$. These studies have confirmed the neuroprotective role of Nrf2 against ROS generation and cell death induced by $A\beta$ in vitro [94–97]. Tert-butylhydroquinone (tBHQ), a prototypical Nrf2 activator, has been reported to reduce $A\beta$ 1-42 secretion in the NT2N cell line with increased cell viability [98]. The sulforaphane, Nrf2 activator, is able to preserve cognitive function in an AD animal model [99]. Strikingly, overexpression of mitochondria catalase in APP (Tg2576) transgenic mice dramatically reduces full-length APP and its c-terminal fragment 99, lowers soluble and insoluble $A\beta$ levels, extends lifespan, and improves working memory [100]. A genetic study has demonstrated that overexpression of Nrf2 in the hippocampus causes increase in mTOR activity; these data suggest that Nrf2 could mediate autophagy and alter processing/clearance of APP and/or $A\beta$.

4.4. Nrf2-ARE pathway and amyotrophic lateral sclerosis

ALS is a progressive disease with fatal outcome, in which the motor cortex and spinal cord motor neurons are selectively affected. The disease in 90% of cases occur sporadically (SALS) while in 10% of cases there is a clear familiarity (FALS) [101]. The etiology and pathogenesis of ALS are currently largely unknown. ALS is considered a degenerative multifactorial disease in which cell death is a consequence of a complex interaction between genetic risk factors and environmental factors. To explain the neuronal death, several hypotheses have been proposed,

among which the most accredited implicates oxidative stress [102–106]. In fact, levels of oxidative stress biomarkers were observed to be altered in SALS patients; these data indicate that most likely a redox imbalance is relevant in the pathogenesis of disease [107–112]. Elevated levels of HNE have been detected also in cerebrospinal fluid (CSF) from ALS patients [113, 114]. Additionally, mitochondrial alterations have been observed in motor neuron of ALS patients [115–118]. These dysfunctions are tightly interrelated with OS cascades, activating overlapping molecular pathways in a vicious cycle of harmful events. Specifically, alterations in mitochondrial morphology and biochemistry have been extensively detected in postmortem tissues [119] and in lymphocytes [120] from SALS patients, in SOD1 transgenic mice, and cellular models [52]. Dynamic and morphological abnormalities, along with metabolic deficits in the activities of the OXPHOS proteins, have also been described in both SALS and FALS patients [121]. Furthermore, impairment in antioxidant mechanisms has also been shown in ALS, including downregulation of members of glutathione S-transferase family [122, 123], peroxiredoxins [124], and Nrf2 [125–129].

The first causative gene associated with genetic ALS form was the Cu-Zn superoxide dismutase 1. In FALS patients with SOD1 gene mutations and in G85R animal model, cytoplasmic inclusions containing modified SOD1 proteins have been observed [130]. In the last decade, genome-wide association (GWA) studies identified two genes associated with sporadic and non-SOD1 familial ALS: RNA/DNA-binding proteins, 43-kDa transactive response (TAR) DNA-binding protein (TDP-43), and fused in sarcoma/translocated in liposarcoma (FUS/TLS) [131–135]. Both TDP-43 and FUS are predominantly nuclear proteins involved in RNA metabolism; however, both are observed as aggregates in the cytosol of ALS neurons [136].

Nrf2 activators have been shown to protect against oxidative stress and cell death induced by SOD1-mutant protein [137, 138]. The Nrf2 overexpression in glial cells directly increases the resistance to oxidative stress and helps indirectly, through the increase secretion of glutathione, the ability of the motor neurons to neutralize the toxic effects caused by SOD1-mutant protein [139]. Also Nrf2 and Keap1 expression analysis showed a reduction of Nrf2 protein in patients than in controls; conversely, there have been no significant differences in the expression of Keap1 levels between patients and controls [140–142].

Recently, NSC34 motor neuronal cell lines expressing TDP-43 mutants exhibit shortened neurites, alteration of oxidative stress markers levels. These effects are reversed by the UPS inhibitor MG132, but not by the Nrf2 activator sulforaphane [143, 144]. This is attributed to an increase in HO-1 following MG132 treatment that appeared to be independent of Nrf2 activation. While the role of Nrf2 in protection against SOD1-mutant neuronal toxicity is clear, its effect on other ALS-associated gene mutations particularly TDP43 and FUS needs to be clarified by future studies.

5. Modulators of Nrf2/ARE pathway

The manipulation of the Nrf2-ARE pathway at the genetic level is being studied through the use of siRNA or antisense oligonucleotides against Keap1 to activate/overexpress Nrf2.

Antisense drugs are being researched to study neurodegenerative disorders, cancer, metabolic disorders, and disorders with inflammatory components among others. Antisense drug fomivirsen, marketed as Vitravene, has been approved by the US Food and Drug Administration (FDA) for the treatment of cytomegalovirus retinitis. Since then, numerous antisense therapies have been tested but have not produced significant clinical result. This has not diminished the potential of gene therapies. Antisense oligonucleotide can bind to the target RNA and disrupt RNA splicing, transcription, translation, and replication, thereby modulating gene expression. Several studies showed that siRNA-mediated knockdown of Keap1-activated Nrf2-ARE pathway in mouse cortical astrocytes and provided partial protection against MPTP-mediated toxicity in mouse, *in vivo* [65, 145]. The overexpression of target gene can also be achieved by viral-mediated gene transduction but it is too early to conclude on efficacy of viral-mediated gene therapy in human neurodegenerative disorder cases. Nrf2 modulation in various neurodegenerative disorders has been previously described in this chapter. Hence, using the antisense oligonucleotide against Keap1, lentiviral-mediated Nrf2 overexpression or siRNA against Keap1-mediated overexpression of Nrf2 treatment can prove beneficial in neurodegenerative disorders.

Among recent patents, Curna, Inc. filed patent for the use of antisense for the treatment of Nrf2-related disorders. The initial study published under International Application for the Patent Cooperation Treaty (PCT) showed that antisense CUR-0330 and CUR 0332 showed two- to threefold increase in Nrf2 mRNA expression compared to control (PCT/US2010/027394). The invention is targeted at the inhibition of natural antisense transcript to Nrf2 as a strategy toward modulation of Nrf2 expression in disease models [145].

The modulation of Nrf2 expression by using several other pharmacological interventions to inhibit Keap1 and Nrf2 interaction is under investigation.

The Nrf2/ARE pathway can be pharmacologically activated also by molecules of both natural derivation (nutraceuticals) and chemical synthesis. Between Nrf2/ARE activators of natural origin, sulforaphane, polyphenols, and curcumin have been included; between chemical synthesis substances, chemical Nrf2/ARE activators include triterpenoids and N-(4-(2-pyridyl)(1,3-thiazol-2-yl))-2-(2,4,6-trimethylphenoxy) acetamide (CPN-9).

SFN, derived from cruciferous vegetables such as broccoli, activates Nrf2 through the modification of reactive cysteine residues of Keap1 [146, 147], and SFN is able to overstep the blood-brain barrier, induce the transcription of Nrf2-dependent gene expression in the basal ganglia, and protect dopaminergic neurons from cell death MPTP induced [64, 148]. Other Nrf2/ARE pathway natural inducers are EGCG and resveratrol, belonging to the family of polyphenols that, for their antioxidant qualities, are considered to be important nutraceuticals. EGCG, a flavonoid polyphenol, for example, showed antioxidant and neuroprotective functions in cultured motoneuron-neuroblastoma hybrid cell line transfected with mutSOD1 [149] and in PC12 cells exposed to paraquat [150]. Furthermore, EGCG was shown to be neuroprotective in mice model of ALS: oral administration to mice expressing mutSOD1 delayed symptoms onset [151–154].

Resveratrol, a polyphenolic compound present in red wine, demonstrated protective effects against hypoxic injury in rat spinal cord dorsal column by activating Nrf2 pathway [155, 156].

Curcumin, a member of the curcuminoid family isolated from plant *Curcuma longa*, showed Nrf2-dependent antioxidant properties in primary spinal cord astrocytes exposed to H₂O₂ [157] and in ischemic brain injury models [158]. Other nutraceuticals, such as naphthazarin, genistein, and carnosic acid, showed positive effects in several models of neurodegenerative and cardiovascular diseases implicating OS as a pathogenic factor [148, 159–164].

Furthermore, several synthetic Nrf2/ARE activators were recently developed. Recently, triterpenoids emerged as a potent class of Nrf2/ARE inducers. Triterpenoids are very powerful inducer of Nrf2 pathway: they are able to protect dopaminergic neurodegeneration in MPTP mouse model of PD [165], and increase the lifespan in ALS mouse models [166]. Another chemical activator of Nrf2/ARE pathway is CPN-9 which selectively suppresses cell death triggered by OS in a cell-type-independent manner. SH-SY5Y cells pretreated with CPN-9 were more resistant to cytokine-induced apoptosis. CPN-9 is able to decrease the ROS levels through the induction of several antioxidant genes [137]. Finally, we know that some drugs such as bromocriptine [167] and azathioprine [168] were capable to induce the Nrf2/ARE pathway, therefore providing insight into a possible development of new synthetic molecules Nrf2 activators.

6. Conclusion

Oxidative stress and misfolded proteins are two mechanisms that act together to the pathogenesis of several inflammatory and degenerative diseases. The detailed mechanism by which Nrf2-ARE pathway carries out its action is still unclear. Current data suggest that Nrf2 affects both primary protein degradation pathways, the UPS and ALP, which are both altered in neurodegenerative diseases.

Despite the progress made in understanding the importance of Nrf2/ARE pathway, it remains to clarify the exact mechanism by which it exerts its function so that it may lead to discovery of new targets for the treatment of neurodegenerative diseases. In the past decade, Nrf2-ARE pathway activation has shown promising results for the treatment of many disorders including neurodegenerative disease. Several of these Nrf2 activators or their brain accessible synthetically modified compounds have passed phase II and III clinical trials. BG-12, an oral formulation of DMF (Biogen Idec, Inc.), is in phase III clinical trials for the treatment of multiple sclerosis (MS). Bardoxolone methyl, an oral formulation of CDDO-MA (Reata Pharmaceuticals, Inc.), is currently in phase III clinical trials for chronic kidney disease in type II diabetes mellitus patients, but there are no existing clinical trials in the pipeline for neurodegenerative disorders. EGCG, resveratrol, and curcumin are in various phases of clinical trial for treatment and efficacy in neurodegenerative disorders such as AD, PD, and ALS. The knowledge gained from these studies will further help in identifying clinically relevant approaches for the activation of Nrf2 in CNS and potentially lead to finding treatments for these devastating neurological disorders.

Acknowledgements

This chapter was supported by the CariLucca Foundation grant (539999_2014_Siciliano_SLAF-CARILUCCA).

Conflict of Interest and Sources of Funding Statement: The authors declare that they do not have conflicts of interest.

Author details

Annalisa Lo Gerfo*, Lucia Petrozzi, Lucia Chico and Gabriele Siciliano

*Address all correspondence to: annalisalogerfo2@virgilio.it

Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa, Pisa, Italy

References

- [1] L. Ernster and G. Schatz, "*Mitochondria: a historical review*", J Cell Biol, 91: 227–255, 1981.
- [2] G. T. Babcock and M. Wikstrom, "*Oxygen activation and the conservation of energy in cell respiration*", Nature, 356: 301–309, 1992.
- [3] Y. Hatefi "*The mitochondrial electron transport and oxidative phosphorylation system*", Ann Rev Biochem, 54: 1015–1969, 1985.
- [4] K. Maiese, Z. Z. Chong, J. Hou, Y. C. Shang, "*Erythropoietin and oxidative stress*", Curr Neurovasc Res, 5: 125–142, 2008.
- [5] M. S. Hernandez and L. R. Britto, "*NADPH oxidase and neurodegeneration*", Curr Neuroparmacol, 10: 321–327, 2012.
- [6] F. Orsini, M. Moroni, C. Contursi et al., "*Regulatory effects of the mitochondrial energetic status on mitochondrial p66Shc*", Biol Chem, 387: 1405–1410, 2006.
- [7] W. A. Pryor and G. L. Squadrito, "*The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide*", Am J Physiol, 268: L699–L722, 1995.
- [8] M. C. Martinez and R. Andriantsitohaina, "*Reactive nitrogen species: molecular mechanisms and potential significance in health and disease*", Antioxid Redox Signal, 11: 669–702, 2009.
- [9] D. Trachootham, W. Lu, M. A. Ogasawara, R. D. Nilsa, P. Huang, "*Redox regulation of cell survival*", Antioxid Redox Signal, 10: 1343–1374, 2008.

- [10] B. Halliwell, "Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment", *Drugs Aging*, 18: 685–716, 2001.
- [11] Y. Son, Y. K. Cheong, N. H. Kim, H. T. Chung, D. G. Kang, H. O. Pae, "Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways?", *J Signal Transduct*, 2011: 792639, 2011.
- [12] Y. Yan, C. L. Wei, W. R. Zhang, H. P. Cheng, J. Liu, "Crosstalk between calcium and reactive oxygen species signaling", *Acta Pharmacol Sin*, 27: 821–826, 2006.
- [13] R. F. Feissner, J. Skalska, W. E. Gaum, S. S. Sheu, "Crosstalk signaling between mitochondrial Ca^{2+} and ROS", *Front Biosci*, 14: 1197–1218, 2009.
- [14] Q. Ma, "Transcriptional responses to oxidative stress: pathological and toxicological implications", *Pharmacol Ther*, 125: 376–393, 2010.
- [15] R. G. Allen and M. Tresini, "Oxidative stress and gene regulation", *Free Radical Biol Med*, 28: 463–499, 2000.
- [16] H. L. A. Vieira, P. M. Alves, A. Vercelli, "Modulation of neuronal stem cell differentiation by hypoxia and reactive oxygen species", *Prog Neurobiol*, 93: 444–455, 2011.
- [17] K. A. M. Kennedy, S. D. E. Sandiford, I. S. Skerjanc, S. S. C. Li, "Reactive oxygen species and the neuronal fate", *Cell Mol Life Sci*, 69: 215–221, 2012.
- [18] B. Halliwell, "Oxidative stress and neurodegeneration: where are we now?", *J Neurochem*, 97: 1634–1658, 2006.
- [19] A. Melo, L. Monteiro, R. M. F. Lima, D. M. de Oliveira, M. D. de Cerqueira, R. S. El-Bachà, "Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives", *Oxid Med Cell Longev*, 2011: 467180, 2011.
- [20] S. Gandhi and A. Y. Abramov, "Mechanism of oxidative stress in neurodegeneration", *Oxid Med Cell Longev*, 2012: 428010, 2012.
- [21] R. Kohen and A. Nyska, "Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification", *Toxicol Pathol*, 30: 620–650, 2002.
- [22] T. Nguyen, P. J. Sherratt, H. C. Huang, C. S. Yang, C. B. Pickett, "Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome", *J Biol Chem*, 278: 4536–4541, 2003.
- [23] G. P. Sykiotis and D. Bohmann, "Stress-activated cap'n'collar transcription factors in aging and human disease", *Sci Signal*, 3(112): re3, 2010.
- [24] D. A. Johnson and J. A. Johnson, "Nrf2-a therapeutic target for the treatment of neurodegenerative diseases", *Free Radic Biol Med*, 88: 253–267, 2015.

- [25] S. Petri, S. Körner, M. Kiaei, “*Nrf2/ARE signaling pathway: key mediator in oxidative stress and potential therapeutic target in ALS*”, *Neurol Res Int*, 2012: 878030, 2012.
- [26] K. A. Jung and M. K. Kwak, “*The Nrf2 system as a potential target for the development of indirect antioxidants*”, *Molecules*, 15: 7266–7291, 2010.
- [27] M. Kobayashi and M. Yamamoto, “*Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation*”, *Antioxid Redox Signal*, 7: 385–394, 2005.
- [28] N. F. Villeneuve, A. Lau, D. D. Zhang, “*Regulation of the Nrf2-keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases*”, *Antioxid Redox Signal*, 13: 1699–1712, 2010.
- [29] P. Milani, S. Gagliardi, E. Cova, C. Cereda, “*SOD1 transcriptional and posttranscriptional regulation and its potential implications in ALS*”, *Neurol Res Int*, 2011: 458427, 2011.
- [30] P. Yenki, F. Khodagholi, F. Shaerzadeh, “*Inhibition of phosphorylation of JNK suppresses Abeta-induced ER stress and up regulates pro survival mitochondrial proteins in rat hippocampus*”, *J Mol Neurosci*, 49: 262–269, 2013.
- [31] C. P. Ramsey, C. A. Glass, M. B. Montgomery et al., “*Expression of Nrf2 in neurodegenerative diseases*”, *J Neuropathol Exp Neurol*, 66: 75–85, 2007.
- [32] G. Joshi and J. A. Johnson, “*The Nrf2-ARE pathway: a valuable therapeutic target for the treatment of neurodegenerative diseases*”, *Recent Pat CNS Drug Discov*, 7: 218–229, 2012.
- [33] A. Wagenfeld, J. Gromoll, T. G. Cooper, “*Molecular cloning and expression of rat contraception associated protein 1 (CAP1), a protein putatively involved in fertilization*”, *Biochem Biophys Res Commun* 51: 545–549, 1998.
- [34] V. Bonifati, P. Rizzu, M. J. van Baren et al., “*Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism*”, *Science*, 299: 256–259, 2003.
- [35] K. Honbou, N. N. Suzuki, M. Horiuchi et al., “*The crystal structure of DJ-1, a protein related to male fertility and Parkinson’s disease*”, *J Biol Chem*, 278: 31380–31384, 2003.
- [36] Q. Huai, Y. Sun, H. Wang et al., “*Crystal structure of DJ-1/RS and implication on familial Parkinson’s disease*”, *FEBS Lett*, 549: 171–175, 2003.
- [37] X. Tao and L. Tong, “*Crystal structure of human DJ-1, a protein associated with early onset Parkinson’s disease*”, *J Biol Chem*, 278: 31372–31379, 2003.
- [38] M. A. Wilson, J. L. Collins, Y. Hod, D. Ringe, G. A. Petsko, “*The 1.1- Å resolution crystal structure of DJ-1, the protein mutated in autosomal recessive early onset Parkinson’s disease*”, *Proc Natl Acad Sci U S A*, 100: 9256–9261, 2003.
- [39] M. Lakshminarasimhan, M. T. Maldonado, W. Zhou, A. L. Fink, M. A. Wilson, “*Structural impact of three Parkinsonism associated missense mutations on human DJ-1*”, *Biochemistry*, 47: 1381–1392, 2008.

- [40] I. Irrcher, H. Aleyasin, E. L. Seifert et al., "Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics", *Hum Mol Genet*, 19: 3734–3746, 2010.
- [41] N. J. Larsen, G. Ambrosi, S. J. Mullett, S. B. Berman, D. A. Hinkle, "DJ-1 knock-down impairs astrocyte mitochondrial function", *Neuroscience*, 196: 251–264, 2011.
- [42] S. J. Kim, Y. J. Park, I. Y. Hwang, M. B. Youdim, K. S. Park, Y. J. Oh, "Nuclear translocation of DJ-1 during oxidative stress induced neuronal cell death", *Free Radic Biol Med*, 53: 936–950, 2012.
- [43] L. Gu, T. Cui, C. Fan et al., "Involvement of ERK1/2 signaling pathway in DJ-1-induced neuroprotection against oxidative stress", *Biochem Biophys Res Commun*, 383: 469–474, 2009.
- [44] E. Andres-Mateos, C. Perier, L. Zhang et al., "DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase", *Proc Natl Acad Sci U S A*, 104: 14807–14812, 2007.
- [45] L. Gan, M. R. Vargas, D. A. Johnson, J. A. Johnson, "Astrocyte-specific overexpression of Nrf2 delays motor pathology and synuclein aggregation throughout the CNS in the alpha-synuclein mutant (A53T) mouse model", *J Neurosci*, 32: 17775–17787, 2012.
- [46] R. M. Canet-Avilés, M. A. Wilson, D. W. Miller et al., "The Parkinson's disease DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization", *Proc Natl Acad Sci U S A*, 101: 9103–9108, 2004.
- [47] J. Waak, S. S. Weber, K. Gorner et al., "Oxidizable residues mediating protein stability and cytoprotective interaction of DJ-1 with apoptosis signal-regulating kinase 1", *J Biol Chem*, 284: 14245–14257, 2009.
- [48] M. A. Wilson, "The role of cysteine oxidation in DJ-1 function and dysfunction", *Antioxid Redox Signal*, 15: 111–122, 2011.
- [49] N. Zhong and J. Xu, "Synergistic activation of the human MnSOD promoter by DJ-1 and PGC-1 α : regulation by SUMOylation and oxidation", *Hum Mol Genet*, 17: 3357–3367, 2008.
- [50] J. Y. Im, K. W. Lee, J. M. Woo, E. Junn, M. M. Mouradian, "DJ-1 induces thioredoxin 1 expression through the Nrf2 pathway", *Hum Mol Genet*, 21: 3013–3024, 2012.
- [51] H. Ren, K. Fu, D. Wang, C. Mu, G. Wang, "Oxidized DJ-1 interacts with the mitochondrial protein BCL-XL", *J Biol Chem*, 286: 35308–35317, 2011.
- [52] W. Zhou, M. Zhu, M. A. Wilson, G. A. Petsko, A. L. Fink, "The oxidation state of DJ-1 regulates its chaperone activity toward α -synuclein", *J Mol Biol*, 356: 1036–1048, 2006.
- [53] M. K. McCoy and M. R. Cookson, "DJ-1 regulation of mitochondrial function and autophagy through oxidative stress", *Autophagy*, 7: 531–532, 2011.
- [54] L. M. de Lau and M. M. Breteler, "Epidemiology of Parkinson's disease", *Lancet Neurol*, 5: 525–535, 2006.

- [55] G. Xiromerisiou, E. Dardiotis, V. Tsimourtou et al., “Genetic basis of Parkinson disease”, *Neurosurg Focus*, 28:E7.1–E7.7, 2010.
- [56] H. Kumar, H.W. Lim, S. V. More et al., “The role of free radicals in the aging brain and Parkinson’s disease: convergence and parallelism”, *Int J Mol Sci*, 13: 10478–10504, 2012.
- [57] J. T. Greenamyre and T. G. Hastings, “Parkinsons-divergent causes convergent mechanisms”, *Science*, 304: 1120–1122, 2004.
- [58] R. K. Chaturvedi and M. F. Beal, “Mitochondrial diseases of the brain”, *Free Radic Biol Med*, 63: 1–29, 2013.
- [59] A. H. V. Schapira, A. Hartley, M. W. J. Cleeter, J. M. Cooper, “Free radicals and mitochondrial dysfunction in Parkinson’s disease”, *Biochem Soc Trans*, 21: 367–370, 1993.
- [60] A. Navarro, A. Boveris, M. J. Bández et al., “Human brain cortex: mitochondrial oxidative damage and adaptive response in Parkinson disease and in dementia with Lewy bodies”, *Free Radic Biol Med*, 46: 1574–1580, 2009.
- [61] C. Buhmann, S. Arlt, A. Kontush et al., “Plasma and CSF markers of oxidative stress are increased in Parkinson’s disease and influenced by antiparkinsonian medication”, *Neurobiol Dis*, 15: 160–170, 2004.
- [62] C. Isobe, T. Abe, Y. Terayama, “Levels of reduced and oxidized coenzyme Q-10 and 8-hydroxy-2'-deoxyguanosine in the cerebrospinal fluid of patients with living Parkinson’s disease demonstrate that mitochondrial oxidative damage and/or oxidative DNA damage contributes to the neurodegenerative process”, *Neurosci Lett*, 469: 159–163, 2010.
- [63] S. Nikam, P. Nikam, S. K. Ahaley, A. V. Sontakke, “Oxidative stress in Parkinson’s disease”, *Indian J Clin Biochem*, 24: 98–101, 2009.
- [64] A. Jazwa, A. I. Rojo, N. G. Innamorato, M. Hesse, J. Fernández-Ruiz, A. Cuadrado, “Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism”, *Antioxid Redox Signal*, 14: 2347–2360, 2011.
- [65] T. P. Williamson, D. A. Johnson, J. A. Johnson, “Activation of the Nrf2-ARE pathway by siRNA knockdown of Keap1 reduces oxidative stress and provides partial protection from MPTP-mediated neurotoxicity”, *Neurotoxicology*, 33: 272–279, 2012.
- [66] Q. He, N. Song, F. Jia, H. Xu, X. Yu, J. Xie, H. Jiang, “Role of alpha-synuclein aggregation and the nuclear factor E2-related factor 2/heme oxygenase-1 pathway in iron-induced neurotoxicity”, *Int J Biochem Cell Biol*, 45: 1019–1030, 2013.
- [67] M. C. Barone, G. P. Sykiotis, D. Bohmann, “Genetic activation of Nrf2 signaling is sufficient to ameliorate neurodegenerative phenotypes in a *Drosophila* model of Parkinson’s disease”, *Dis Model Mech*, 4: 701–707, 2011.
- [68] I. Lastres-Becker, A. Ulusoy, N. G. Innamorato, G. Sahin, A. Rabano, D. Kirik, A. Cuadrado, “Alpha-synuclein expression and Nrf2 deficiency cooperate to aggravate protein

- aggregation, neuronal death and inflammation in early-stage Parkinson's disease*", Hum Mol Genet, 21: 3173–3192, 2012.
- [69] N. Mizushima and M. Komatsu, "Autophagy: renovation of cells and tissues", Cell, 147: 728–741, 2011.
- [70] B. Ravikumar, S. Sarkar, J. E. Davies et al., "Regulation of mammalian autophagy in physiology and pathophysiology", Physiol Rev, 90: 1383–1435, 2010.
- [71] R. A. Nixon, "The role of autophagy in neurodegenerative disease", Nat Med, 19: 983–997, 2013.
- [72] K. A. Malkus and H. Ischiropoulos, "Regional deficiencies in chaperone-mediated autophagy underlie alpha-synuclein aggregation and neurodegeneration", Neurobiol Dis, 46: 732–744, 2012.
- [73] X. Lin, L. Parisiadou, C. Sgobio, G. Liu et al., "Conditional expression of Parkinson's disease-related mutant alpha-synuclein in the midbrain dopaminergic neurons causes progressive neurodegeneration and degradation of transcription factor nuclear receptor related 1", J Neurosci, 32: 9248–9264, 2012.
- [74] S. J. Chinta, J. K. Mallajosyula, A. Rane, J. K. Andersen, "Mitochondrial alpha-synuclein accumulation impairs complex I function in dopaminergic neurons and results in increased mitophagy in vivo", Neurosci Lett, 486: 235–239, 2010.
- [75] M. Y. Aksenov, M. V. Aksenova, D. A. Butterfield, J. W. Geddes, W. R. Markesbery, "Protein oxidation in the brain in Alzheimer's disease", Neuroscience, 103: 373–383, 2001.
- [76] G. Benzi and A. Moretti, "Age- and peroxidative stress-related modifications of the cerebral enzymatic activities linked to mitochondria and the glutathione system", Free Radic Biol Med, 19: 77–101, 1995.
- [77] J. Y. Chan and M. Kwong, "Impaired expression of glutathione synthetic enzyme genes in mice with targeted deletion of the Nrf2 basic-leucine zipper protein", Biochim Biophys Acta, 1517: 19–26, 2000.
- [78] C. J. Harvey, R. K. Thimmulappa, A. Singh et al., "Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress", Free Radic Biol Med, 46: 443–453, 2009.
- [79] Y. Namba, M. Tomonaga, H. Kawasaki et al., "Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease", Brain Res, 541: 163–166, 1991.
- [80] M. K. Lee, D. R. Borchelt, G. Kim et al., "Hyperaccumulation of FAD linked presenilin 1 variants in vivo", Nat Med, 3: 756–760, 1997.
- [81] D. R. Marshak, S. A. Pesce, L. C. Stanley, W. S. Griffin, "Increased S100 beta neurotrophic activity in Alzheimer's disease temporal lobe", Neurobiol Aging, 13: 1–7, 1992.

- [82] H. M. Schipper, D. A. Bennett, A. Liberman et al., "*Glial heme oxygenase-1 expression in Alzheimer disease and mild cognitive impairment*". *Neurobiol Aging*, 27: 252–261, 2006.
- [83] S. S. Shafteel, W. S. Griffin, M. K. O'Banion, "*The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective*", *J Neuroinflammation*, 5: 7, 2008.
- [84] M. A. Smith, P. L. Richey Harris, L. M. Sayre, J. S. Beckman, G. Perry, "*Widespread peroxynitrite mediated damage in Alzheimer's disease*", *J Neurosci*, 17: 2653–2657, 1997.
- [85] X. Wang, B. Su, G. Perry, M. A. Smith, X. Zhu, "*Insights into amyloid-beta-induced mitochondrial dysfunction in Alzheimer disease*", *Free Radic Biol Med*, 43: 1569–1573, 2007.
- [86] Y. Wang, K. Santa-Cruz, C. De Carli, J. A. Johnson, "*NAD(P)H:quinone oxidoreductase activity is increased in hippocampal pyramidal neurons of patients with Alzheimer's disease*", *Neurobiol Aging*, 21: 525–531, 2000.
- [87] A. K. Raina, D. J. Templeton, J. C. Deak, G. Perry, M. A. Smith, "*Quinone reductase (NQO1), a sensitive redox indicator, is increased in Alzheimer's disease*", *Redox Rep*, 4: 23–27, 1999.
- [88] D. A. Butterfield, J. Drake, C. Pocernich, A. Castegna, "*Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide*", *Trends Mol Med*, 7: 548–554, 2001.
- [89] W. R. Markesbery, "*Oxidative stress hypothesis in Alzheimer's disease*", *Free Radic Biol Med*, 23: 134–147, 1997.
- [90] M. A. Lovell, C. Xie, W. R. Markesbery, "*Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures*", *Neurobiol Aging*, 22: 187–194, 2001.
- [91] W. R. Markesbery and M. A. Lovell, "*Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease*", *Neurobiol Aging*, 19: 33–36, 1998.
- [92] C. M. Lauderback, J. M. Hackett, F. F. Huang et al., "*The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Abeta1-42*", *J Neurochem*, 78: 413–416, 2001.
- [93] K. Kanninen, T. M. Malm, H. K. Jyrkkanen et al., "*Nuclear factor erythroid 2-related factor 2 protects against beta amyloid*", *Mol Cell Neurosci*, 39: 302–313, 2008.
- [94] R. Resende, P. I. Moreira, T. Proença, A. Deshpande et al., "*Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease*", *Free Radic Biol Med*, 44: 2051–2057, 2008.
- [95] C. Lee, G. H. Park, S. R. Lee, J. H. Jang, "*Attenuation of beta-amyloid-induced oxidative cell death by sulforaphane via activation of NF-E2-related factor 2*", *Oxid Med Cell Longev*, 2013: 313510, 2013.

- [96] X. H. Li, C. Y. Li, J. M. Lu, R. B. Tian, J. Wei, "Allicin ameliorates cognitive deficits ageing-induced learning and memory deficits through enhancing of Nrf2 antioxidant signaling pathways", *Neurosci Lett*, 514: 46–50, 2012.
- [97] C. J. Wruck, M. E. Gotz, T. Herdegen, D. Varoga, L. O. Brandenburg, T. Pufe, "Kavalactones protect neural cells against amyloid beta peptide-induced neurotoxicity via extracellular signal-regulated kinase 1/2-dependent nuclear factor erythroid 2-related factor 2 activation", *Mol Pharmacol*, 73: 1785–1795, 2008.
- [98] B. Eftekharzadeh, N. Maghsoudi, F. Khodaghali, "Stabilization of transcription factor Nrf2 by tBHQ prevents oxidative stress-induced amyloid beta formation in NT2N neurons", *Biochimie*, 92: 245–253, 2010.
- [99] H. V. Kim, H. Y. Kim, H. Y. Ehrlich, S. Y. Choi, D. J. Kim, Y. Kim, "Amelioration of Alzheimer's disease by neuroprotective effect of sulforaphane in animal model", *Amyloid*, 20: 7–12, 2013.
- [100] P. Mao, M. Manczak, M. J. Calkins et al., "Mitochondria-targeted catalase reduces abnormal APP processing, amyloid beta production and BACE1 in a mouse model of Alzheimer's disease: implications for neuroprotection and lifespan extension", *Hum Mol Genet*, 21: 2973–2990, 2012.
- [101] A. LoGerfo, L. Chico, L. Borgia, L. Petrozzi et al., "Lack of association between nuclear factor erythroid-derived 2-like 2 promoter gene polymorphisms and oxidative stress biomarkers in amyotrophic lateral sclerosis patients", *Oxid Med Cell Longev*, 2014: 432626, 2014.
- [102] P. Pasinelli and R. H. Brown, "Molecular biology of amyotrophic lateral sclerosis: insights from genetics", *Nat Rev Neurosci*, 7: 710–723, 2006.
- [103] M. Cozzolino, M. G. Pesaresi, V. Gerbino, J. Grosskreutz, M. T. Carri, "Amyotrophic lateral sclerosis: new insights into underlying molecular mechanisms and opportunities for therapeutic intervention", *Antioxid Redox Signal*, 17: 1277–1330, 2012.
- [104] S. Gagliardi, P. Milani, V. Sardone, O. Pansarasa, C. Cereda, "From transcriptome to noncoding RNAs: implications in ALS mechanism", *Neurol Res Int*, 2012: 278725, 2012.
- [105] L. Rossi, C. Valle, M. T. Carri, "Altered gene expression, mitochondrial damage and oxidative stress: converging routes in motor neuron degeneration", *Int J Cell Biol*, 2012: 908724, 2012.
- [106] M. J. Strong, "The evidence for altered RNA metabolism in amyotrophic lateral sclerosis (ALS)", *J Neurol Sci*, 288: 1–12, 2010.
- [107] R. J. Ferrante, S. E. Browne, L. A. Shinobu et al., "Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis", *J Neurochem*, 69: 2064–2074, 1997.
- [108] S. C. Barber, R. J. Mead, P. J. Shaw, "Oxidative stress in ALS: a mechanism of neurodegeneration and a therapeutic target", *Biochim Biophys Acta*, 1762: 1051–1067, 2006.

- [109] S. C. Barber and P. J. Shaw, "*Oxidative stress in ALS: key role in motor neuron injury and therapeutic target*", *Free Radic Biol Med*, 48: 629–641, 2010.
- [110] K. Abe, L. H. Pan, M. Watanabe, T. Kato, Y. Itoyama, "*Induction of nitrotyrosine-like immunoreactivity in the lower motor neuron of amyotrophic lateral sclerosis*", *Neurosci Lett*, 199: 152–154, 1995.
- [111] M. F. Beal, R. J. Ferrante, S. E. Browne, R. T. Mathews et al., "*Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis*", *Ann Neurol*, 42: 644–654, 1997.
- [112] P. J. Shaw, P. G. Ince, G. Falkous, D. Mantle, "*Oxidative damage to protein in sporadic motor neuron disease spinal cord*", *Ann Neurol*, 38: 691–695, 1995.
- [113] R. G. Cutler, W. A. Pedersen, S. Camandola, J. D. Rothstein, M. P. Mattson, "*Evidence that accumulation of ceramides and cholesterol esters mediates oxidative stress—induced death of motor neurons in amyotrophic lateral sclerosis*", *Ann Neurol*, 52: 448–457, 2002.
- [114] W. A. Pedersen, W. Fu, J. N. Keller et al., "*Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients*", *Ann Neurol*, 44: 819–824, 1998.
- [115] R. G. Smith, Y. K. Henry, M. P. Mattson, S. H. Appel, "*Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis*", *Ann Neurol*, 44: 696–699, 1998.
- [116] E. P. Simpson, Y. K. Henry, J. S. Henkel, R. G. Smith, S. H. Appel, "*Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden*", *Neurology*, 62: 1758–1765, 2004.
- [117] M. T. Carri and M. Cozzolino, "*SOD1 and mitochondria in ALS: a dangerous liaison*", *J Bioenerg Biomembr*, 43: 593–599, 2011.
- [118] L. J. Martin, "*Mitochondrial pathobiology in ALS*", *J Bioenerg Biomembr*, 43: 569–579, 2011.
- [119] G. Manfredi and Z. Xu, "*Mitochondrial dysfunction and its role in motor neuron degeneration in ALS*", *Mitochondrion*, 5: 77–87, 2005.
- [120] D. Curti, A. Malaspina, G. Facchetti et al., "*Amyotrophic lateral sclerosis: oxidative energy metabolism and calcium homeostasis in peripheral blood lymphocytes*", *Neurology*, 47: 1060–1064, 1996.
- [121] J. Kong and Z. Xu, "*Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1*", *J Neurosci*, 18: 3241–3250, 1998.
- [122] F. R. Wiedemann, G. Manfredi, C. Mawrin, M. F. Beal, E. A. Schon, "*Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients*", *J Neurochem*, 80: 616–625, 2002.

- [123] E. Usarek, B. Gajewska, B. Kaźmierczak et al., "A study of glutathione S-transferase pi expression in central nervous system of subjects with amyotrophic lateral sclerosis using RNA extraction from formalin-fixed, paraffin-embedded material", *Neurochem Res*, 30: 1003–1007, 2005.
- [124] M. Kuźma, Z. Jamrozik, A. Barańczyk-Kuźma, "Activity and expression of glutathione S-transferase pi in patients with amyotrophic lateral sclerosis", *Clin Chim Acta*, 364: 217–221, 2006.
- [125] S. Kato, M. Kato, Y. Abe et al., "Redox system expression in the motor neurons in amyotrophic lateral sclerosis (ALS): immunohistochemical studies on sporadic ALS, superoxide dismutase 1 (SOD1)-mutated familial ALS, and SOD1-mutated ALS animal models", *Acta Neuropathol*, 110: 101–112, 2005.
- [126] J. Kirby, E. Halligan, M. J. Baptista et al., "Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS", *Brain*, 128: 1686–1706, 2005.
- [127] A. Sarlette, K. Krampfl, C. Grothe, N. V. Neuhoff, R. Dengler, S. Petri, "Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis", *J Neuropathol Exp Neurol*, 67: 1055–1062, 2008.
- [128] T. Mimoto, K. Miyazaki, N. Morimoto et al., "Impaired antioxydative Keap1/Nrf2 system and the downstream stress protein responses in the motor neuron of ALS model mice", *Brain Res*, 1446: 109–118, 2012.
- [129] P. Milani, G. Ambrosi, O. Gammoh, F. Blandini, C. Cereda, "SOD1 and DJ-1 converge at Nrf2 pathway: a clue for antioxidant therapeutic potential in neurodegeneration", *Oxid Med Cell Longev*, 2013: 836760, 2013.
- [130] S. Kato, S. Horiuchi, J. Liu et al., "Advanced glycation end product-modified superoxide dismutase-1 (SOD1)-positive inclusions are common to familial amyotrophic lateral sclerosis patients with SOD1 gene mutations and transgenic mice expressing human SOD1 with a G85R mutation", *Acta Neuropathol*, 100: 490–505, 2000.
- [131] M. Neumann, D. M. Sampathu, L. K. Kwong et al., "Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis", *Science*, 314: 130–133, 2006.
- [132] I. R. Mackenzie, E. H. Bigio, P. G. Ince et al., "Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations", *Ann Neurol*, 61: 427–434, 2007.
- [133] T. J. Kwiatkowski Jr., D. A. Bosco, A. L. Leclerc et al., "Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis", *Science*, 323: 1205–1208, 2009.
- [134] C. Vance, B. Rogelj, T. Hortobagyi et al., "Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6", *Science*, 323: 1208–1211, 2009.

- [135] M. A. Gitcho, R. H. Baloh, S. Chakraverty et al., “TDP-43 A315T mutation in familial motor neuron disease”, *Ann. Neurol*, 63: 535–538, 2008.
- [136] C. Lagier-Tourenne, M. Polymenidou, D. W. Cleveland, “TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration”, *Hum Mol Genet*, 19: 46–64, 2010.
- [137] T. Kanno, K. Tanaka, Y. Yanagisawa et al., “A novel small molecule, N-(4-(2-pyridyl)(1,3-thiazol-2-yl))-2-(2,4,6-trimethylphenoxy) acetamide, selectively protects against oxidative stress-induced cell death by activating the Nrf2-ARE pathway: therapeutic implications for ALS”, *Free Radic Biol Med*, 53: 2028–2042, 2012.
- [138] R. J. Mead, A. Higginbottom, S. P. Allen et al., “S[+] Apomorphine is a CNS penetrating activator of the Nrf2-ARE pathway with activity in mouse and patient fibroblast models of amyotrophic lateral sclerosis”, *Free Radic Biol Med*, 61: 438–452, 2013.
- [139] M. R. Vargas, D. A. Johnson, D. W. Sirkis et al., “Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis”, *J Neurosci*, 28: 13574–13581, 2008.
- [140] A. Nanou, A. Higginbottom, C. F. Valori et al., “Viral delivery of antioxidant genes as a therapeutic strategy in experimental models of amyotrophic lateral sclerosis”, *Mol. Ther*, 21: 1486–1496, 2013.
- [141] M. R. Vargas, N. C. Burton, J. Kutzke et al., “Absence of Nrf2 or its selective overexpression in neurons and muscle does not affect survival in ALS-linked mutant hSOD1 mouse models”, *PLoS One*, 8: e56625, 2013.
- [142] Y. Guo, Y. Zhang, D. Wen, W. Duan et al., “The modest impact of transcription factor Nrf2 on the course of disease in an ALS animal model”, *Lab Invest*, 93: 825–833, 2013.
- [143] W. Duan, Y. Guo, H. Jiang, X. Yu, C. Li, “MG132 enhances neurite outgrowth in neurons overexpressing mutant TAR DNA-binding protein-43 via increase of HO-1”, *Brain Res*, 1397: 1–9, 2011.
- [144] W. Duan, X. Li, J. Shi, Y. Guo, Z. Li, C. Li, “Mutant TAR DNA-binding protein-43 induces oxidative injury in motor neuron-like cell”, *Neuroscience* 169: 1621–1629, 2010.
- [145] C. Stack, D. Ho, E. Wille, N. Y. Calingasan, C. Williams et al., “Triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improve the behavioral phenotype and brain pathology in a transgenic mouse model of Huntington’s disease”, *Free Radic Biol Med*, 49: 147–158, 2010.
- [146] A. T. Dinkova-Kostova, W. D. Holtzclaw, R. N. Cole et al., “Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants”, *Proc Natl Acad Sci U S A*, 99: 11908–11913, 2002.
- [147] F. Hong, M. L. Freeman, D. C. Liebler, “Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane”, *Chem Res Toxicol*, 18: 1917–1926, 2005.

- [148] J. H. Chen, H. P. Ou, C. Y. Lin, F. J. Lin et al., "*Carnosic acid prevents 6-hydroxydopamine-induced cell death in SH-SY5Y cells via mediation of glutathione synthesis*" *Chem Res Toxicol*, 25: 1893–1901, 2012.
- [149] S. H. Koh, H. Kwon, K. S. Kim et al., "*Epigallocatechin gallate prevents oxidative-stress-induced death of mutant Cu/Znsuperoxide dismutase (G93A) motoneuron cells by alteration of cell survival and death signals*", *Toxicology*, 202: 213–225, 2004.
- [150] R. R. Hou, J. Z. Chen, H. Chen, X. G. Kang, M. G. Li, B. R. Wang, "*Neuroprotective effects of (-)-epigallocatechin-3-gallate (EGCG) on paraquat-induced apoptosis in PC12 cells*", *Cell Biol Int*, 32: 22–30, 2008.
- [151] S. H. Koh, S. M. Lee, H. Y. Kim et al., "*The effect of epigallocatechin gallate on suppressing disease progression of ALS model mice*", *Neurosci Lett*, 395: 103–107, 2006.
- [152] Z. Xu, S. Chen, X. Li, G. Luo, L. Li, W. Le, "*Neuroprotective effects of (-)-epigallocatechin-3-gallate in a transgenic mouse model of amyotrophic lateral sclerosis*", *Neurochem Res*, 31: 1263–1269, 2006.
- [153] H. K. Na and Y. J. Surh, "*Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG*", *Food Chem Toxicol*, 46: 1271–1278, 2008.
- [154] L. Romeo, M. Intrieri, V. D'Agata et al., "*The major green tea polyphenol, (-)-epigallocatechin-3-gallate, induces heme oxygenase in rat neurons and acts as an effective neuroprotective agent against oxidative stress*", *J Am Coll Nutr*, 28: 492–499, 2009.
- [155] V. Kesharwani, F. Atif, S. Yousuf, S. K. Agrawal, "*Resveratrol protects spinal cord dorsal column from hypoxic injury by activating Nrf-2*", *Neuroscience*, 241: 80–88, 2013.
- [156] A. M. Vincent, K. Kato, L. L. McLean, M. E. Soules, E. L. Feldman, "*Sensory neurons and Schwann cells respond to oxidative stress by increasing antioxidant defense mechanisms*", *Antioxid Redox Signal*, 11: 425–438, 2009.
- [157] H. Jiang, X. Tian, Y. Guo, W. Duan, H. Bu, C. Li, "*Activation of nuclear factor erythroid 2-related factor 2 cytoprotective signaling by curcumin protect primary spinal cord astrocytes against oxidative toxicity*", *Biol Pharm Bull*, 34: 1194–1197, 2011.
- [158] J. Wu, Q. Li, X. Wang et al., "*Neuroprotection by curcumin in ischemic brain injury involves the akt/nrf2 pathway*", *PLoS One*, 8: e59843, 2013.
- [159] T. G. Son, E. M. Kawamoto, Q. S. Yu, N. H. Greig, M. P. Mattson, S. Camandola, "*Naphthazarin protects against glutamate induced neuronal death via activation of the Nrf2/ARE pathway*", *Biochem Biophys Res Commun*, 433: 602–606, 2013.
- [160] R. Wang, J. Tu, Q. Zhang et al., "*Genistein attenuates ischemic oxidative damage and behavioral deficits via eNOS/Nrf2/HO-1 signaling*", *Hippocampus*, 23: 634–647, 2013.
- [161] T. Satoh, K. Kosaka, K. Itoh et al., "*Carnosic acid, a catechol type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1*", *J Neurochem*, 104: 1116–1131, 2008.

- [162] N. A. Kelsey, H. M. Wilkins, D. A. Linseman, *"Nutraceutical antioxidants as novel neuroprotective agents"*, *Molecules*, 15: 7792–7814, 2010.
- [163] W. Ma, L. Yuan, H. Yu et al., *"Genistein as a neuroprotective antioxidant attenuates redox imbalance induced by β -amyloid peptides 25-35 in PC12 cells"*, *Int J Dev Neurosci*, 28: 289–295, 2010.
- [164] Y. D. Xi, H. L. Yu, J. Ding et al., *"Flavonoids protect cerebrovascular endothelial cells through Nrf2 and PI3K from β - amyloid peptide-induced oxidative damage"*, *Curr Neurovasc Res*, 9: 32–41, 2012.
- [165] N. A. Kaidery, R. Banerjee, L. Yang et al., *"Targeting Nrf2- mediated gene transcription by extremely potent synthetic triterpenoids attenuate dopaminergic neurotoxicity in the MPTP mouse model of Parkinson's disease"*, *Antioxid Redox Signal*, 18: 139–157, 2013.
- [166] A. Neymotin, N. Y. Calingasan, E. Wille et al., *"Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis"*, *Free Radic Biol Med*, 51: 88–96, 2011.
- [167] J. H. Lim, K. M. Kim, S. W. Kim, O. Hwang, H. J. Choi, *"Bromocriptine activates NQO1 via Nrf2-PI3K/Akt signaling: novel cytoprotective mechanism against oxidative damage"*, *Pharmacol Res*, 57: 325–331, 2008.
- [168] S. Kalra, Y. Zhang, E. V. Knatko et al., *"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*, 4: 1665–1674, 2011.