Chapter 8

Nrf2 Signaling: An Adaptive Response Pathway for Neurodegenerative Disorders

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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, neuroprotection, 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

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defined as the altered balance between the production of ROS and their removal by cellular antioxidant mechanisms, such us 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 (·OH). Both ONOO− and ·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 be up 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**).

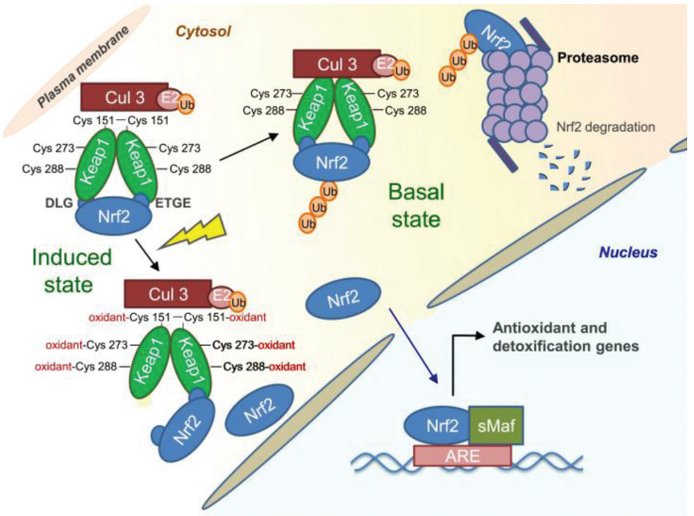

Figure 1. Regulation of Nrf2 by Keap1. In basal conditions, Nrf2 is sequestered in the cytoplasm by a Keap1 homodimer that facilitates ubiquitination and degradation of Nrf2 in the proteasome. In the presence of inducers that react with specific cysteine residues of Keap1, you get the release of Nrf2 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-

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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: αSyn-mutant A53T hαSynA53T [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 αSyn 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, alterated 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 αSyn. In SK-N-SH neuroblastoma cells, ferrous iron promotes αSyn 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 αSyn-mediated dopaminergic neuron loss and motor dysfunction [67]. Conversely, genetic deletion of Nrf2 increases αSyn toxicity and exaggerates αSyn/p-αSyn 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 αSyn proteostasis. Astrocytic overexpression of Nrf2 (GFAP-Nrf2) can reduce αSyn aggregates in the central nervous system of a PD mouse model with neuronal overexpression of human αSyn-mutant A53T [45]. The accumulation of hαSynA53T in the Triton-soluble fraction from the spinal cord decreases 60% in symptomatic mice. This is accompanied by a significant increase in hαSynA53T in the Triton-insoluble/SDS-soluble fraction. This movement of αSynA53T into Triton-insoluble/SDS-soluble aggregates is completely reversed by the overexpression of Nrf2 in astrocytes.

Similar changes are observed for phosphorylated (Ser129) αSynA53T (p-hαSynA53T) in Triton-soluble and Triton-insoluble/SDS-soluble fractions. Fluorescent staining of hαSynA53T also shows a dramatic increase in hαSynA53T aggregates that colocalized with phαSynA53T. 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 αSynA53T mice model [45, 72–74]. Nrf2 prevents chaperone-mediated autophagy dysfunction and increases lifespan, delays onset, and reduces aggregation in αSynA53T 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 (Aβ) 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 Aβ. These studies have confirmed the neuroprotective role of Nrf2 against ROS generation and cell death induced by Aβ in vitro [94–97]. Tert-butylhydroquinone (tBHQ), a prototypical Nrf2 activator, has been reported to reduce Aβ1-42 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 Aβ 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 Aβ.

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 increate 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.

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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 H_2O_2 [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 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.

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