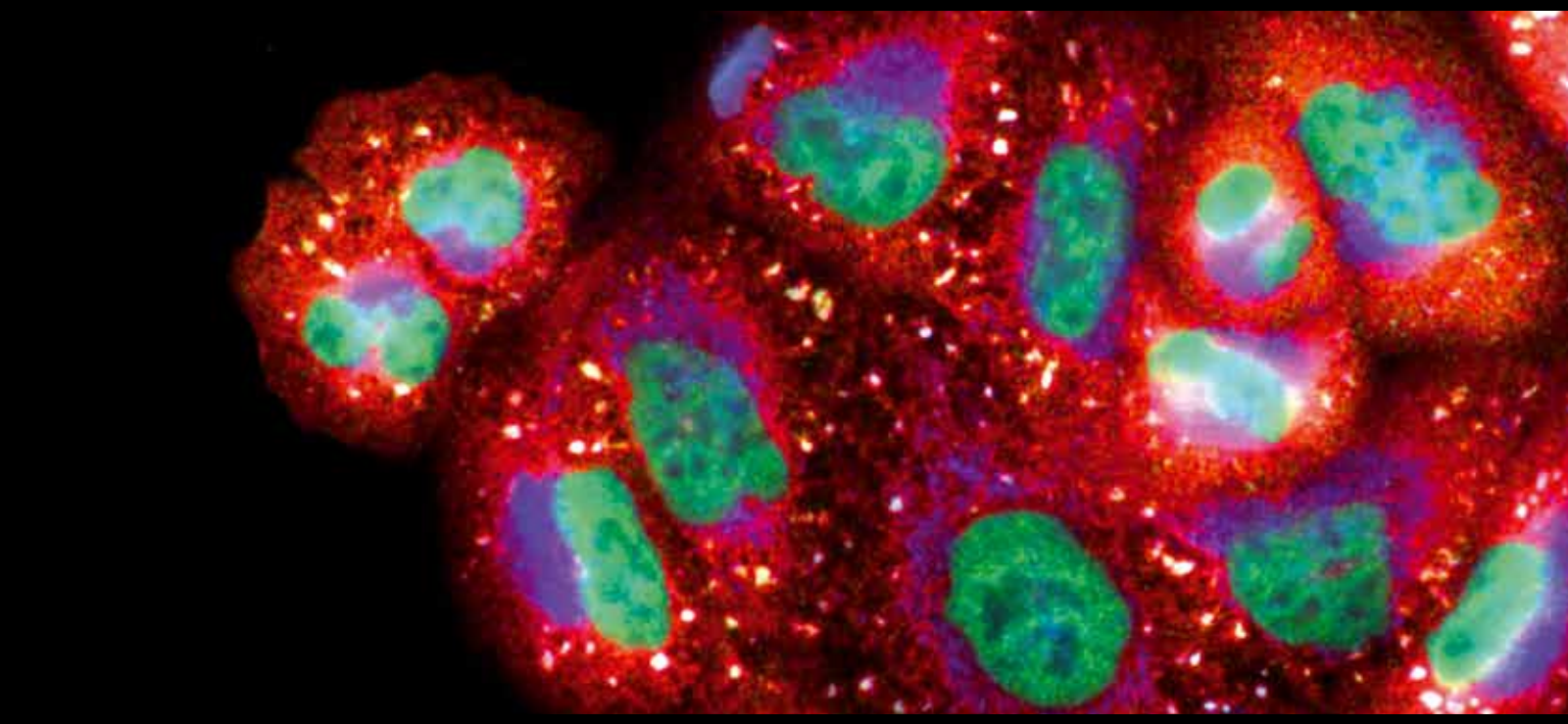
Oxidative Medicine and Cellular Longevity

Neurodegeneration, Neurogenesis, and Oxidative Stress

Guest Editors: Renata Santos, Carmen Ruiz de Almodóvar, Anne-Laure Bulteau, and Cláudio M. Gomes



Neurodegeneration, Neurogenesis, and Oxidative Stress

Oxidative Medicine and Cellular Longevity

Neurodegeneration, Neurogenesis, and Oxidative Stress

Guest Editors: Renata Santos, Carmen Ruiz de Almodóvar, Anne-Laure Bulteau, and Cláudio M. Gomes

Editorial Board

Contents

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 730581, 2 pages
http://dx.doi.org/10.1155/2013/730581

Editorial
Neurodegeneration, Neurogenesis, and Oxidative Stress

Renata Santos,[1] Carmen Ruiz de Almodóvar,[2] Anne-Laure Bulteau,[3] and Cláudio M. Gomes[4]

[1] *Development of the Nervous System, IBENS, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France*
[2] *Biochemistry Center, University of Heidelberg, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany*
[3] *IPREM, University of Pau, 2 rue du President Angot, 64000 Pau, France*
[4] *Instituto Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Avenida da República, EAN, 2784-505 Oeiras, Portugal*

Correspondence should be addressed to Renata Santos; rsantos@biologie.ens.fr

Received 2 October 2013; Accepted 2 October 2013

Oxidative stress is implicated in the pathophysiology of a wide variety of neurodegenerative and neurologic disorders. This special issue includes 11 articles that cover different aspects of the importance of oxidative stress in neurotoxicity, neurodegeneration, and potential therapies.

Neurotoxicity can occur after exposure to natural or artificial molecules that alter the normal function of the nervous system. The review article by R. Lugo-Huitrón et al. focuses on the toxicity of quinolinic acid, an endogenous metabolite of the kynurenine pathway, normally present in the brain and cerebrospinal fluid. This paper discusses the different mechanisms by which quinolinic acid acts as a neurotoxin, such as the activation of N-methyl-D-aspartate (NMDA) receptors, mitochondrial dysfunction, oxidative stress, inflammation, and cell death. B. Wang and Y. Du review the *in vitro* and *in vivo* evidence of the neurotoxic effects of cadmium, a heavy metal, in the central nervous system.

Several original and review articles examine the role of oxidative stress in diverse neurodegenerative diseases. The original article by C. Savino et al. shows the implication of p66Shc protein and the mitochondrial permeability transition pore pathway in neurodegeneration in a mouse model of autoimmune encephalomyelitis. The role of mitochondria in neurodegeneration is further discussed in two review articles by V. García-Escudero et al. and by Y. Zhao and B. Zhao. These authors present data on the role of mitochondrial dysfunction and oxidative stress in the initiation and progression of Alzheimer's disease, respectively. H. Ariga et al. describe the function of DJ-1 in the pathogenesis of Parkinson's disease. Furthermore, P. Milani et al. summarize the recent literature describing the interaction between Nrf2, a master regulator of the antioxidant response, and DJ-1 and SOD1 proteins and its interest in the development of therapeutic strategies for Parkinson's disease and amyotrophic lateral sclerosis. Two review articles focus on Friedreich's ataxia disease. C. M. Gomes and R. Santos overview the data supporting that oxidative stress is a central feature of this disease. In an opinion paper A. Bayot and P. Rustin present arguments in favor of the hypothesis that the reduction in the expression of *PIP5K1B*, the gene adjacent to the frataxin-encoding gene, in Friedreich's ataxia patient cells is implicated in disease onset and progression.

The antioxidant effect of numerous phytochemicals has long been recognized and has been proposed as an alternative form of treatment, since still no drugs are available that prevent the progression of most neurodegenerative diseases. In an original article, H. Mo et al. describe the potential neuroprotective effect of tea polyphenols in an *in vivo* subarachnoid hemorrhage mice model. A. Tarozzi et al. present a review of the antioxidant properties of sulforaphane primarily attributed to its ability to activate the Nrf2/ARE pathway.

We believe that these contributions provide an updated view on this dynamic field and evidence that supports the importance of considering oxidative stress as a player in neurodegenerative diseases as well as neurotoxicity.

Acknowledgment

We would like to thank all the authors and reviewers that participated in this issue.

Renata Santos
Carmen Ruiz de Almodóvar
Anne-Laure Bulteau
Cláudio M. Gomes

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 725635, 7 pages
http://dx.doi.org/10.1155/2013/725635

Research Article

Friedreich's Ataxia, Frataxin, PIP5K1B: Echo of a Distant Fracas

Aurélien Bayot[1,2] and Pierre Rustin[1,2]

[1] *INSERM UMR 676, Bâtiment Ecran, Hôpital Robert Debré, 48 boulevard Sérurier, 75019 Paris, France*
[2] *Université Paris 7, Faculté de Médecine Denis Diderot, Site Robert Debré, 48 boulevard Sérurier, 75019 Paris, France*

Correspondence should be addressed to Pierre Rustin; pierre.rustin@inserm.fr

Received 26 June 2013; Accepted 12 August 2013

Academic Editor: Renata Santos

"*Frataxin fracas*" were the words used when referring to the frataxin-encoding gene (*FXN*) burst in as a motive to disqualify an alternative candidate gene, *PIP5K1B*, as an actor in Friedreich's ataxia (FRDA) (Campuzano et al., 1996; Cossee et al., 1997; Carvajal et al., 1996). The instrumental role in the disease of large triplet expansions in the first intron of *FXN* has been thereafter fully confirmed, and this no longer suffers any dispute (Koeppen, 2011). On the other hand, a recent study suggests that the consequences of these large expansions in *FXN* are wider than previously thought and that the expression of surrounding genes, including *PIP5K1B*, could be concurrently modulated by these large expansions (Bayot et al., 2013). This recent observation raises a number of important and yet unanswered questions for scientists and clinicians working on FRDA; these questions are the substratum of this paper.

1. Friedreich's Ataxia

With an estimated prevalence of 1 : 50,000 and a carrier frequency of about 1 : 60 to 1 : 90, Friedreich's ataxia (FRDA) is the most commonly inherited ataxia in the Caucasian population [1, 2]. It is a multisystemic, degenerative disease typically associated with dysarthria, muscle weakness, spasticity in the lower limbs, scoliosis, bladder dysfunction, absent lower limb reflexes, loss of position and vibration sense, and speech and listening difficulties [3]. A majority of the affected individuals have hypertrophic cardiomyopathy. Glucose intolerance and diabetes mellitus are observed in a subset (about 30%) of cases. The onset of symptoms is usually between 10 and 15 years of age, but either much earlier or later onset has been infrequently observed. Initial symptoms can be purely neurological, but occasionally, cardiomyopathy can be the presenting symptom. Altogether, atypical presentations represent as much as 25% of the cases [4].

2. The Molecular Mechanism

In more than 98% of the cases, the disease originates from large homozygous GAA repeat expansions (66 to 1700 repeats; normal: 5 to 33, with 85% less than 12) in the first intron of *FXN* encoding a mitochondrial matrix targeted protein, frataxin. In between uninterrupted expansions, 34 to 66 represent premutation, or borderline alleles, at risk for intergenerational expansion. The few residual cases represent compound heterozygous for an expanded allele and a point mutation, most frequently a null mutation [5].

3. Frataxin Depletion: Iron-Sulfur Cluster Deficiency

Simultaneously to the discovery of the molecular basis of FRDA by the European consortium combining the teams of Koenig and Pandolfo [6], shutting down debate on the origin of the disease [7, 8], we studied an endomyocardial biopsy of a young girl, undiagnosed at the moment of the investigation, presenting with massive cardiac hypertrophy but hardly detectable neurological signs [9]. We evidenced a specific and severe deficiency of the activity of all the studied enzymes harbouring iron-sulfur cluster (ISC), namely, mitochondrial respiratory chain complexes I, II, and III and both Krebs cycle mitochondrial and cytosolic aconitase. As this little girl was later on shown to be homozygous for expanded FRDA alleles, we investigated a few more FRDA cases and

established that expansion in *FXN* actually caused an activity defect of ISC-containing proteins (ISPs) in the hypertrophied heart of patients [9]. The generalized loss of ISP activity thereafter found its explanation, when the role of frataxin in the mitochondrial biogenesis of ISC was demonstrated in the yeast *Saccharomyces cerevisiae* [10] and human cells [11, 12] by the group of R. Lill.

4. Low Frataxin, Normal Iron-Sulfur Clusters: What Causes Hypersensitivity to Oxidative Stress?

However, detailed investigation of affected persons showed that ISPs from circulating lymphocytes, lymphoblastoid cell lines, skeletal muscle, and skin fibroblasts were curiously spared despite low frataxin levels. In the absence of ISP deficiency, a hypersensitivity to oxidative stress was nevertheless frequently observed as a result of expansion in the *FXN* gene, associated in some cells with cytoskeleton anomalies [13]. Obviously these later cytosolic anomalies could not be easily reconciled with the putative intramitochondrial location and function of the frataxin in the ISC biogenesis [14], which moreover did not appear significantly altered in these tissues.

To account for this puzzling observation, we focused our attention on frataxin-deficient FRDA patient fibroblasts that displayed normally functional ISP. Studying these cells, we could first ascribe the hypersensitivity to oxidative stress to impaired signaling of antioxidant defenses [15]. We further ascribed this impairment to a mislocation and a decrease of the NRF2 (nuclear factor erythroid 2-related factor 2) transcription factor [16]. This mislocation was concomitant with an abnormal remodeling, already noticed in these cells [17], of the actin fibers known to bind NRF2 through interaction with Keap1 (Kelch-like ECH-associated protein 1). But this only displaced the question, what could be the link between the depletion of the mitochondrial frataxin protein and the abnormal status of the cytosolic actin network [13]? This was an incentive to reexamine the whole series of potential consequences of the GAA expansion from the very first event, the gene modification itself.

5. GAA Expansions in *FXN*: Which Consequences?

Rather than to focus our effort on the *FXN* gene only, the idea came to extend the study to the adjacent genes, inspired by the increasingly recognized importance of epigenetics in nucleotide repeat expansion disorders [18]. To our surprise, in patients' cells (skin fibroblasts and circulating lymphocytes), the expansion was found not only to impair the transcription of the frataxin gene, but also that of the adjacent *PIP5K1B* [19]. The decrease was somewhat less pronounced than what could be observed for the frataxin gene, with rare (1/5) fibroblast cultures where the decrease was weaker than expected from the size of the expansion. Nevertheless, we could establish that it had a major impact on the cell actin network, and this was observed in the absence of any detectable consequence of the frataxin depletion on ISPs. Extensive cell biology studies established that PIP5K1B depletion impaired phosphatidylinositol 4,5-bisphosphate [$PI(4.5)P_2$] synthesis, causing actin fiber disorganization and a number of associated structural anomalies, including significant delay in cell spreading.

These observations raise a number of questions dealing with (1) the mechanism of gene extinction in the disease, (2) the potential consequence(s) of the PIP5K1B depletion in the expression and course of the FRDA disease, and (3) with therapeutic strategies to be adopted.

6. The Mechanisms of *PIP5K1B* Decreased Expression

We previously established that *PIP5K1B* expression decrease was largely correlated with the size of GAA expansion in *FXN*, and that both PIP5K1B mRNA and protein were concomitantly decreased [19]. To date at least twenty neurological diseases are caused by genomic expansions of repeats in protein-coding or noncoding regions of the genome [20]. The noncoding expansions often result in complex disease phenotypes which may be either developmental and/or degenerative. In FRDA, it was first proposed that the expansion positioned two kilobases from the frataxin promoter in the first intron of the gene tends to form triplex DNA structures which may contribute to inhibiting transcriptional elongation [21]. Later on, relatively short GAA expansions of 200 repeats have been shown to confer variegated gene silencing of a linked mouse transgene marker, similar to position-effect variegation [22]. Then, GAA repeat expansions have been associated with a number of epigenetic changes [23], including (1) di- and trimethylation of H3K9, together with hypoacetylation of H3K14, H4K5, H4K8, and H4K12 and (2) an increase in DNA methylation at specific CpG sites in the region of *FXN* intron 1 immediately upstream of the GAA repeat. The direct effect of GAA expansions on the generation of local transcriptionally inactive chromatin was demonstrated by the use of histone deacetylase (HDAC) inhibitors, molecules known to increase global histone acetylation and thereby reactivate epigenetically silenced genes [24]. In FRDA patients' cells, such compounds led to an increased acetylation of several histone proteins at the *FXN* locus, including H3K14, H4K5, and H4K12 and to a significant reversion of *FXN* silencing [25]. Additional testing of selective HDAC inhibitors in appropriate mouse models further reported positive effects, including a reversion of specific repressive histone marks, a significant correction of frataxin levels, and an improvement of some phenotypic features [26–28]. Parallel to the formation of local heterochromatin, further work has pointed out that some heterochromatin marks propagate into the *FXN* promoter, thus supporting the idea that in addition to impairing elongation of gene transcription at the repeat site [29, 30], GAA expansions may also interfere with transcription initiation at the *FXN* locus [31]. In keeping with this, an intriguing finding is the observation that depletion of CTCF, a highly conserved multifunctional transcription regulator and chromatin organizer, in the 5′-UTR of expanded *FXN* alleles may initiate spreading of repressive chromatin from GAA expansions by

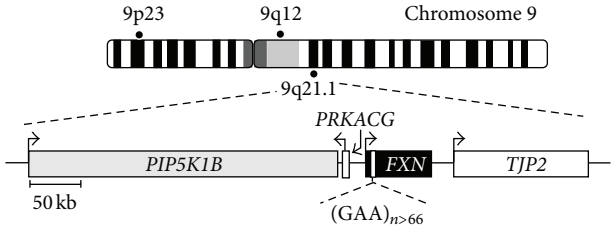


Figure 1: Map of chromosome 9 and the 549.5-kb region containing the human *FXN* and its close neighboring genes. The intronic site of GAA expansions causing FRDA is indicated in *FXN* and gene transcription orientation is shown by black arrows.

increasing the expression of an antisense transcript (FAST-1) [32]. Altogether, these data support the view that a heterochromatinization process at the *FXN* locus, consisting of the formation and local spreading of heterochromatin, compromises optimal access/progress of the transcription machinery and possibly of transcription factors for correct *FXN* gene expression. Both chromatin reorganization and one or more of the abovementioned epigenetic changes might also account for the reduced expression of the adjacent *PIP5K1B* gene. It is too early to decipher a mechanism accounting for *PIP5K1B* decreased expression, and it remains to be shown that the expression of these two genes only is affected, as other genes are present in the vicinity (Figure 1).

7. The Potential Consequence(s) of the *PIP5K1B* Depletion in the FRDA Disease?

The consequences of frataxin depletion have been a matter of discussion since the early days of its discovery. According to authors, frataxin might be or act as mitochondrial iron export carrier [33], a source or a trap for mitochondrial iron acting as a gate keeper for ISC synthesis [34], an instrumental component of the ISC synthesis machinery [10], an ISC chaperone [35], or an antioxidant factor [36]. All of these hypotheses have been indeed supported by more or less convincing experimental data, sometimes hardly reconcilable, recently discussed in details [37]. Adding to these interrogations, the recently described phenotypes of diseases originating from mutations in genes instrumental in ISC synthesis are surprisingly enough, not significantly overlapping with FRDA [13, 38].

Considering that the triplet expansion in *FXN* can also hamper *PIP5K1B* expression, it might as well be that part of the biochemical phenotypes reported in human cells/samples are not caused by the frataxin depletion only. PIP5K1B participates in the biosynthesis of PI(4,5)P_2 and is expressed, essentially after childhood, in a number of tissues in human, highest expression being reported in the brain (Figure 2). PIP5K1B is at the crossroad of different signaling pathways, mediating RAC1-dependent reorganization of actin filaments and contributing to the activation of phospholipase D2 [39, 40]. Considering the pleiotropic role in the cell of PIP5K1B, it is not easy to predict what would be, presumably dependent on cell type, the consequences of a partial depletion of the protein, not necessarily restricted to actin network remodeling. Moreover, the consequences of PIP5K1B depletion might well be mixed with the consequences of frataxin deficiency. Indeed, when occurring, impairment of ISC synthesis resulting from frataxin depletion predictably has also a number of various consequences. In addition to affecting the activity of the respiratory chain and of the Krebs cycle enzyme, aconitase, this will impair the overall ISC homeostasis in the cell, impacting on a number of cytosolic enzymes containing ISC, and in turn this might ultimately affect organic acid balance, iron homeostasis, and oxidative stress sensitivity. Obviously, each of these factors can have unpredicted effect on cellular metabolism. For example, discrete metabolic disturbance increasing electrophile acids may modify cysteine residues of Keap1 that links NRF2 and actin [41, 42]. The result will be a constitutive activation of NRF2 that could on a long term exhaust the antioxidant capacities of the cell and results in hypersensitivity to oxidative stress. Similar impairment can possibly result from PIP5K1B depletion since this latter has been shown to trigger abnormal remodeling of the actin network known to control NRF2-ARE activation [43]. Either frataxin or PIP5K1B depletion, or both, might thus contribute to the oxidative-stress hypersensitivity observed in FRDA [44].

Timing (after birth) and territories of *PIP5K1B* expression are compatible with the course and presentation of FRDA (Figure 2). Predicted cellular consequences of PIP5K1B depletion, especially actin network remodeling, are sufficiently severe as to be instrumental in the pathology. However it would be premature to speculate on the potential role of PIP5K1B depletion in the disease, as depletion is yet to be demonstrated in affected tissues (especially brain and heart).

8. Impact on Present and Future Therapeutic Strategies

Strategies targeting different steps of the disease have been proposed/tested to counteract FRDA (Figure 3). A very promising approach is to target the GAA expansion using effective reagents for deacetylation of histones "wrapping" the expansion as to facilitate gene reading [24]. Obviously, the hypothesis that the expansion hampers more than frataxin expression does not modify the rationale of this approach.

Then a series of approaches aim at restoring the level of frataxin, either by bringing the gene or by targeting posttranscriptional, or even post translational steps, in both frataxin synthesis or activity [45]. Depending on the actual impact of frataxin depletion versus that of PIP5K1B, the importance to restore frataxin level/activity might be more or less crucial. However for the time being, we have all reasons to believe that, even if variable according to tissues, and possibly time, the impact of frataxin depletion is instrumental in the disease, for example, severe ISC deficiency evidenced in the heart of affected persons. Restoring frataxin level thus remains a major issue, even if other actors might be shown to play a role in the disease. For example, the hypersensitivity to oxidative insult might result from the concurrent depletion of frataxin and PIP5K1B, and increasing frataxin level might as

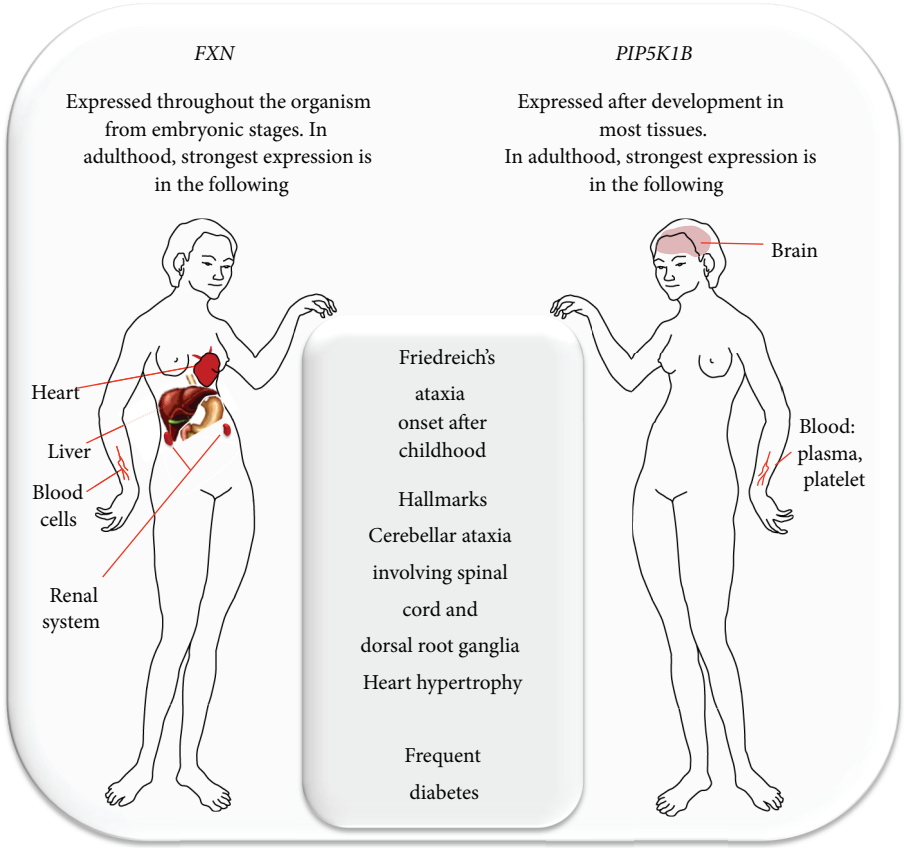


Figure 2: Territories of the strongest expression of *FXN* and *PIP5KB* in human. Data for human healthy tissues from MOPED (Model Organism Protein Expression Database) and PaxDb (Protein Abundance Across Organisms).

well be sufficient to restore the proper capacity of antioxidant defenses.

Last, strategies targeting one of the supposedly major downstream effects of frataxin depletion, that is, hypersensitivity to oxidative insult, have been proposed [46–48] and, for some of them, tested since nearly 15 years [49]. So far, clinical trials (mostly with idebenone) have resulted in unsatisfactory, partial, and contradictory results. We are left with the conclusion that was reached in our early study: the antioxidant treatment, having no spectacular effect on the ataxia, decreases the heart mass, improving fatigability, thin movements, and speech fluency, although in a subset of the affected persons only [50, 51]. As discussed above, the hypersensitivity to oxidative stress may possibly stand from the PIP5K1B depletion as well, not weakening in any sense the rational to use antioxidants in FRDA.

Interestingly enough a number of strategies have been devised based on experiments attempting to modulate the phenotype (sensitivity to oxidative stress) of FRDA patients' fibroblasts [52–54]. We now know that the phenotype of these cells, variably severe from individual to individual, does not result from a putative ISC depletion induced by decreased frataxin, but is most probably caused by PIP5K1B depletion. Obviously, the question of the model to be used to screen for therapeutic strategies will become a central one if PIP5K1B depletion is shown instrumental in the disease. By definition, the numerous organisms—flies, worms, and mice—or cells—HeLa cells, induced pluripotent stem cells, etc.—that have been engineered by specifically targeting frataxin, cannot pretend to represent the complexity that can be envisioned if more than frataxin is affected in the disease.

9. Concluding Remarks

The identification of PIP5K1B as a potential actor in the pathological process underlying FRDA obviously raises a number of questions that urgently need to be answered. The very first of these is the actual implication of the PIP5K1B depletion in the disease onset and course. To date, decreased PIP5K1B has been shown in circulating blood cells and cultured skin fibroblasts in FRDA [19]. To further consider an implication in the disease, a decreased expression of the gene should be established in at least one affected tissue. The mechanism of decreased *PIP5K1B* expression is a second important question to answer, especially as depending on the mechanism involved, expression of additional genes in the region might be modulated as well. It is noticeable that because we are dealing with subtle epigenetic modulations, the phenomenon might be affected by a number of interfering factors possibly variable among individuals and conditions. In any case, these epigenetic modulations might possibly account in part for the poor correlation between size of the expansions

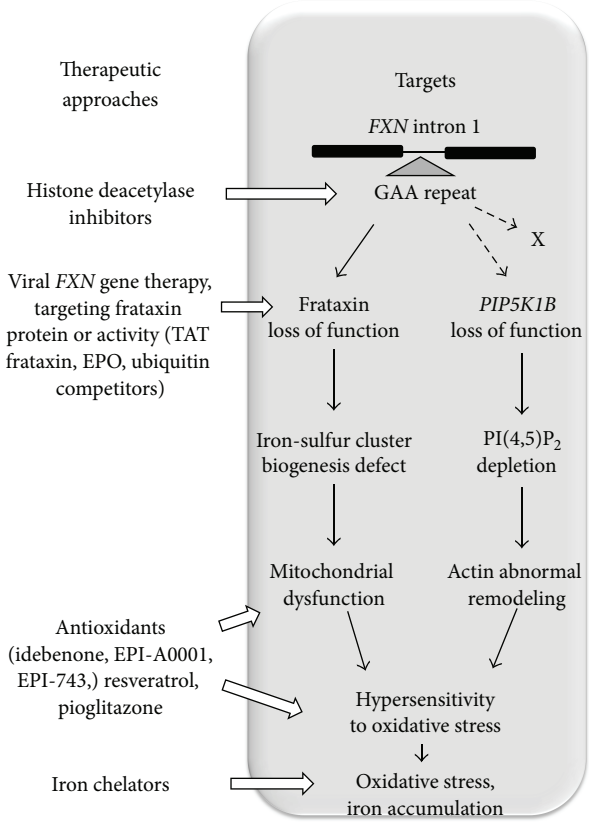


Figure 3: The therapeutic strategies envisioned to fight Friedreich's ataxia and their targets.

and severity of the disease. Many other factors may explain the variability observed in the disease. To mention only one, in FRDA as in most degenerative diseases which are primarily systemic bioenergetics diseases, mitochondria and their confined genetic material, prone to mutations, presumably participate in the clinical heterogeneity [55].

Our last concluding remark deals with the consequence in term of therapeutic strategies. As detailed above, none of the actual therapeutic approaches should be invalidated. Indeed, if PIP5K1B depletion proves to be instrumental, then it might provide additional targets to fight FRDA and allow devising new therapeutic strategies.

Acknowledgment

Our work on Friedreich's ataxia would not have been possible without the support of organizations of Friedreich's ataxia patients (BabelFAmily, Ataxia UK, ACHAF, AISA, APAHE, FEDAES, FASI, FARA, RevaMoto, and AFAF) and of CNRS, Inserm and ANR.

References

[1] A. E. Harding, "Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features," *Brain*, vol. 104, no. 3, pp. 589–620, 1981.

[2] A. H. Koeppen, "Friedreich's ataxia: pathology, pathogenesis, and molecular genetics," *Journal of the Neurological Sciences*, vol. 303, no. 1-2, pp. 1–12, 2011.

[3] A. Dürr, M. Cossee, Y. Agid et al., "Clinical and genetic abnormalities in patients with Friedreich's ataxia," *The New England Journal of Medicine*, vol. 335, no. 16, pp. 1169–1175, 1996.

[4] S. I. Bidichandani and M. B. Delatycki, "Friedreich ataxia," in *GeneReviews*, R. A. Pagon, M. P. Adam, T. D. Bird, C. R. Dolan, C. T. Fong, and K. Stephens, Eds., vol. 1993–2012, University of Washington, Seattle, Wash, USA, 1998.

[5] M. de Castro, J. García-Planells, E. Monrós et al., "Genotype and phenotype analysis of Friedreich's ataxia compound heterozygous patients," *Human Genetics*, vol. 106, no. 1, pp. 86–92, 2000.

[6] V. Campuzano, L. Montermini, M. D. Moltò et al., "Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion," *Science*, vol. 271, no. 5254, pp. 1423–1427, 1996.

[7] M. Cossée, V. Campuzano, H. Koutnikova et al., "Frataxin fracas," *Nature Genetics*, vol. 15, no. 4, pp. 337–338, 1997.

[8] J. J. Carvajal, M. A. Pook, M. dos Santos et al., "The Friedreich's ataxia gene encodes a novel phosphatidylinositol-4-phosphate 5-kinase," *Nature Genetics*, vol. 14, no. 2, pp. 157–162, 1996.

[9] A. Rötig, P. de Lonlay, D. Chretien et al., "Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia," *Nature Genetics*, vol. 17, no. 2, pp. 215–217, 1997.

[10] U. Mühlenhoff, N. Richhardt, M. Ristow, G. Kispal, and R. Lill, "The yeast frataxin homolog Yfh1p plays a specific role in the maturation of cellular Fe/S proteins," *Human Molecular Genetics*, vol. 11, no. 17, pp. 2025–2036, 2002.

[11] O. Stehling, H.-P. Elsässer, B. Brückel, U. Mühlenhoff, and R. Lill, "Iron-sulfur protein maturation in human cells: evidence for a function of frataxin," *Human Molecular Genetics*, vol. 13, no. 23, pp. 3007–3015, 2004.

[12] O. Stehling and R. Lill, "The role of mitochondria in cellular iron-sulfur protein biogenesis: mechanisms, connected processes, and diseases," *Cold Spring Harbor Perspectives in Biology*, vol. 5, no. 8, 2013.

[13] A. Bayot, R. Santos, J.-M. Camadro, and P. Rustin, "Friedreich's ataxia: the vicious circle hypothesis revisited," *BMC Medicine*, vol. 9, article no. 112, 2011.

[14] A. Martelli, M. Wattenhofer-Donzé, S. Schmucker, S. Bouvet, L. Reutenauer, and H. Puccio, "Frataxin is essential for extramitochondrial Fe-S cluster proteins in mammalian tissues," *Human Molecular Genetics*, vol. 16, no. 22, pp. 2651–2658, 2007.

[15] K. Chantrel-Groussard, V. Geromel, H. Puccio et al., "Disabled early recruitment of antioxidant defenses in Friedreich's ataxia," *Human Molecular Genetics*, vol. 10, no. 19, pp. 2061–2067, 2001.

[16] V. Paupe, E. P. Dassa, S. Goncalves et al., "Impaired nuclear Nrf2 translocation undermines the oxidative stress response in Friedreich ataxia," *PLoS ONE*, vol. 4, no. 1, article e4253, 2009.

[17] A. Pastore, G. Tozzi, L. M. Gaeta et al., "Actin glutathionylation increases in fibroblasts of patients with Friedreich's ataxia: a potential role in the pathogenesis of the disease," *Journal of Biological Chemistry*, vol. 278, no. 43, pp. 42588–42595, 2003.

[18] F. He and P. K. Todd, "Epigenetics in nucleotide repeat expansion disorders," *Seminars in Neurology*, vol. 31, no. 5, pp. 470–483, 2011.

[19] A. Bayot, S. Reichman, S. Lebon et al., "Cis-silencing of PIP5K1B evidenced in Friedreich's ataxia patient cells results in cytoskeleton anomalies," *Human Molecular Genetics*, vol. 22, pp. 2894–2904, 2013.

[20] M. Nakamori and C. Thornton, "Epigenetic changes and non-coding expanded repeats," *Neurobiology of Disease*, vol. 39, no. 1, pp. 21–27, 2010.

[21] S. I. Bidichandani, T. Ashizawa, and P. I. Patel, "The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure," *American Journal of Human Genetics*, vol. 62, no. 1, pp. 111–121, 1998.

[22] A. Saveliev, C. Everett, T. Sharpe, Z. Webster, and R. Festenstein, "DNA triplet repeats mediate heterochromatin-protein-1-sensitive variegated gene silencing," *Nature*, vol. 422, no. 6934, pp. 909–913, 2003.

[23] C. Sandi, S. Al-Mahdawi, and M. A. Pook, "Epigenetics in Friedreich's ataxia: challenges and opportunities for therapy," *Genetics Research International*, vol. 2013, Article ID 852080, 12 pages, 2013.

[24] J. M. Gottesfeld, "Small molecules affecting transcription in Friedreich ataxia," *Pharmacology & Therapeutics*, vol. 116, no. 2, pp. 236–248, 2007.

[25] D. Herman, K. Jenssen, R. Burnett, E. Soragni, S. L. Perlman, and J. M. Gottesfeld, "Histone deacetylase inhibitors reverse gene silencing in Friedreich's ataxia ," *Nature Chemical Biology*, vol. 2, no. 10, pp. 551–558, 2006.

[26] M. Rai, E. Soragni, C. J. Chou et al., "Two new pimelic diphenylamide HDAC inhibitors induce sustained frataxin upregulation in cells from Friedreich's ataxia patients and in a mouse model," *PloS ONE*, vol. 5, no. 1, article e8825, 2010.

[27] M. Rai, E. Soragni, K. Jenssen et al., "HDAC inihibitors correct frataxin deficiency in a Friedreich ataxia mouse model," *PLoS ONE*, vol. 3, no. 4, article e1958, 2008.

[28] C. Sandi, R. M. Pinto, S. Al-Mahdawi et al., "Prolonged treatment with pimelic o-aminobenzamide HDAC inhibitors ameliorates the disease phenotype of a Friedreich ataxia mouse model," *Neurobiology of Disease*, vol. 42, no. 3, pp. 496–505, 2011.

[29] E. Kim, M. Napierala, and S. Y. R. Dent, "Hyperexpansion of GAA repeats affects post-initiation steps of FXN transcription in Friedreich's ataxia," *Nucleic Acids Research*, vol. 39, no. 19, pp. 8366–8377, 2011.

[30] T. Punga and M. Bühler, "Long intronic GAA repeats causing Friedreich ataxia impede transcription elongation," *EMBO Molecular Medicine*, vol. 2, no. 4, pp. 120–129, 2010.

[31] D. Kumari, R. E. Biacsi, and K. Usdin, "Repeat expansion affects both transcription initiation and elongation in Friedreich ataxia cells," *Journal of Biological Chemistry*, vol. 286, no. 6, pp. 4209–4215, 2011.

[32] I. de Biase, Y. K. Chutake, P. M. Rindler, and S. I. Bidichandani, "Epigenetic silencing in Friedreich ataxia is associated with depletion of CTCF (CCCTC-binding factor) and antisense transcription," *PLoS ONE*, vol. 4, no. 11, article e7914, 2009.

[33] M. Babcock, D. de Silva, R. Oaks et al., "Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin," *Science*, vol. 276, no. 5319, pp. 1709–1712, 1997.

[34] S. Adinolfi, C. Iannuzzi, F. Prischi et al., "Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS," *Nature Structural & Molecular Biology*, vol. 16, no. 4, pp. 390–396, 2009.

[35] A.-L. Bulteau, H. A. O'Neill, M. C. Kennedy, M. Ikeda-Saito, G. Isaya, and L. I. Szweda, "Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity," *Science*, vol. 305, no. 5681, pp. 242–245, 2004.

[36] S. Lefevre, C. Brossas, F. Auchere, N. Boggetto, J. M. Camadro, and R. Santos, "Apn1 AP-endonuclease is essential for the repair of oxidatively damaged DNA bases in yeast frataxin-deficient cells," *Human Molecular Genetics*, vol. 21, pp. 4060–4072, 2012.

[37] R. A. Vaubel and G. Isaya, "Iron-sulfur cluster synthesis, iron homeostasis and oxidative stress in Friedreich ataxia," *Molecular and Cellular Neuroscience*, vol. 55, pp. 50–61, 2013.

[38] N. Ajit Bolar, A. V. Vanlander, C. Wilbrecht et al., "Mutation of the iron-sulfur cluster assembly gene IBA57 causes severe myopathy and encephalopathy," *Human Molecular Genetics*, vol. 22, pp. 2590–2602, 2013.

[39] M. Vidal-Quadras, M. Gelabert-Baldrich, D. Soriano-Castell et al., "Rac1 and calmodulin interactions modulate dynamics of ARF6-dependent endocytosis," *Traffic*, vol. 12, no. 12, pp. 1879–1896, 2011.

[40] H.-J. Peng, K. M. Henkels, M. Mahankali et al., "The dual effect of Rac2 on phospholipase D2 regulation that explains both the onset and termination of chemotaxis," *Molecular and Cellular Biology*, vol. 31, no. 11, pp. 2227–2240, 2011.

[41] A. Ooi and K. A. Furge, "Fumarate hydratase inactivation in renal tumors: HIF1alpha, NRF2, and, "cryptic targets" of transcription factors," *Chinese Journal of Cancer*, vol. 31, pp. 413–420, 2012.

[42] J. Adam, E. Hatipoglu, L. O'Flaherty et al., "Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling," *Cancer Cell*, vol. 20, no. 4, pp. 524–537, 2011.

[43] S. Papaiahgari, A. Yerrapureddy, P. M. Hassoun, J. C. N. Garcia, K. G. Birukov, and S. P. Reddy, "EGFR-activated signaling and actin remodeling regulate cyclic stretch-induced NRF2-ARE activation," *American Journal of Respiratory Cell and Molecular Biology*, vol. 36, no. 3, pp. 304–312, 2007.

[44] S. Jiralerspong, B. Ge, T. J. Hudson, and M. Pandolfo, "Manganese superoxide dismutase induction by iron is impaired in Friedreich ataxia cells," *FEBS Letters*, vol. 509, no. 1, pp. 101–105, 2001.

[45] R. B. Wilson, "Therapeutic developments in Friedreich ataxia," *Journal of Child Neurology*, vol. 27, pp. 1212–1216, 2012.

[46] P. Rustin, A. Munnich, and A. Rötig, "Quinone analogs prevent enzymes targeted in Friedreich ataxia from iron-induced injury in vitro," *BioFactors*, vol. 9, no. 2-4, pp. 247–251, 1999.

[47] T. E. Richardson, A. E. Yu, Y. Wen, S.-H. Yang, and J. W. Simpkins, "Estrogen prevents oxidative damage to the mitochondria in Friedreich's ataxia skin fibroblasts," *PLoS ONE*, vol. 7, no. 4, Article ID e34600, 2012.

[48] G. M. Enns, S. L. Kinsman, S. L. Perlman et al., "Initial experience in the treatment of inherited mitochondrial disease with EPI-743," *Molecular Genetics and Metabolism*, vol. 105, no. 1, pp. 91–102, 2012.

[49] P. Rustin, J.-C. von Kleist-Retzow, K. Chantrel-Groussard, D. Sidi, A. Munnich, and A. Rötig, "Effect of idebenone on cardiomyopathy in Friedreich's ataxia: a preliminary study," *The Lancet*, vol. 354, no. 9177, pp. 477–479, 1999.

[50] P. Rustin, "The use of antioxidants in Friedreich's ataxia treatment," *Expert Opinion on Investigational Drugs*, vol. 12, no. 4, pp. 569–575, 2003.

[51] P. Rustin, D. Bonnet, A. Rotig, A. Munnich, and D. Sidi, "Idebenone restores mitochondrial respatory chain enzyme activities in the cardiac muscle in Friedreich's ataxia," *Neurology*, vol. 62, pp. 524–525, 2004.

[52] M. L. Jauslin, T. Wirth, T. Meier, and F. Schoumacher, "A cellular model for Friedreich ataxia revelas small-molecule glutathione peroxidase mimetics as novel treatment strategy," *Human Molecular Genetics*, vol. 11, no. 24, pp. 3055–3063, 2002.

[53] T. E. Richardson, S.-H. Yang, Y. Wen, and J. W. Simpkins, "Estrogen protection in Friedreich's ataxia skin fibroblasts," *Endocrinology*, vol. 152, no. 7, pp. 2742–2749, 2011.

[54] D. Marmolino, F. Acquaviva, M. Pinelli et al., "PPAR-γ agonist azelaoyl PAF increases frataxin protein and mRNA expression: new implications for the Friedreich's ataxia therapy," *Cerebellum*, vol. 8, no. 2, pp. 98–103, 2009.

[55] D. C. Wallace, "A mitochondrial bioenergetic etiology of disease," *The Journal of Clinical Investigation*, vol. 123, pp. 1405–1412, 2013.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 104024, 14 pages
http://dx.doi.org/10.1155/2013/104024

Review Article

Quinolinic Acid: An Endogenous Neurotoxin with Multiple Targets

Rafael Lugo-Huitrón,[1] Perla Ugalde Muñiz,[1] Benjamin Pineda,[2] José Pedraza-Chaverrí,[3] Camilo Ríos,[1] and Verónica Pérez-de la Cruz[1]

[1] *Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, Insurgentes Sur 3877, S.S.A., 14269 México, DF, Mexico*
[2] *Laboratorio de Neuroinmunología, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, Insurgentes Sur 3877, S.S.A., 14269 México, DF, Mexico*
[3] *Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México, 04510 México, DF, Mexico*

Correspondence should be addressed to Verónica Pérez-de la Cruz; veped@yahoo.com.mx

Received 14 May 2013; Revised 23 July 2013; Accepted 1 August 2013

Academic Editor: Renata Santos

Quinolinic acid (QUIN), a neuroactive metabolite of the kynurenine pathway, is normally presented in nanomolar concentrations in human brain and cerebrospinal fluid (CSF) and is often implicated in the pathogenesis of a variety of human neurological diseases. QUIN is an agonist of N-methyl-D-aspartate (NMDA) receptor, and it has a high *in vivo* potency as an excitotoxin. In fact, although QUIN has an uptake system, its neuronal degradation enzyme is rapidly saturated, and the rest of extracellular QUIN can continue stimulating the NMDA receptor. However, its toxicity cannot be fully explained by its activation of NMDA receptors it is likely that additional mechanisms may also be involved. In this review we describe some of the most relevant targets of QUIN neurotoxicity which involves presynaptic receptors, energetic dysfunction, oxidative stress, transcription factors, cytoskeletal disruption, behavior alterations, and cell death.

1. Biosynthesis of Quinolinic Acid (QUIN)

Tryptophan (TRP) is an essential amino acid that has various important biological functions. In mammals, about 90% of dietary TRP is metabolized along the kynurenine pathway (KP) (Figure 1) [1, 2], which represents the major catabolic route of TRP and a source of nicotinamide adenine nucleotide (NAD^+), a cofactor in cellular respiration and energy production that plays an important role in the DNA repair and transcriptional regulation [3, 4]. In recent years, the KP has been studied given that it contains metabolites with neuroactive and redox properties. An imbalance in the levels of some metabolites of this pathway has been involved in different pathologies.

The first regulatory step of the KP is the oxidative cleavage of the TRP by tryptophan 2,3-dioxygenase and indolamine 2,3-dioxygenases 1 and 2 (IDO-1 and IDO-2). The product of this cleavage is formylkynurenine, which is hydrolyzed by a formamidase enzyme to give kynurenine (KYN). This metabolite is at a branch point in the pathway and can be further metabolized by three different enzymes: (1) kynureninase, which catalyzes the conversion of KYN to anthranilic acid (AA), (2) kynurenine aminotransferases I, II and III, which catalyze the transamination of KYN to form kynurenic acid (KYNA), and (3) kynurenine 3-hydroxylase, which produces 3-hydroxykynurenine (3-HK) from L-KYN. This branch is the most important route for QUIN synthesis, and it is known that this enzyme has the highest affinity for L-KYN, suggesting that under normal conditions, it metabolizes most of the available kynurenine [5]. At this point, kynureninase cleaves the 3-HK to give 3-hydroxyanthranilic acid (3-HA). The 3-hydroxyanthranilic acid oxygenase (3-HAO) catalyzes the conversion of 3-HA acid to an unstable intermediate, aminocarboxymuconic semialdehyde, which then preferentially converts to QUIN by a nonenzymatic cyclisation [6]. This intermediate compound can also produce picolinic acid

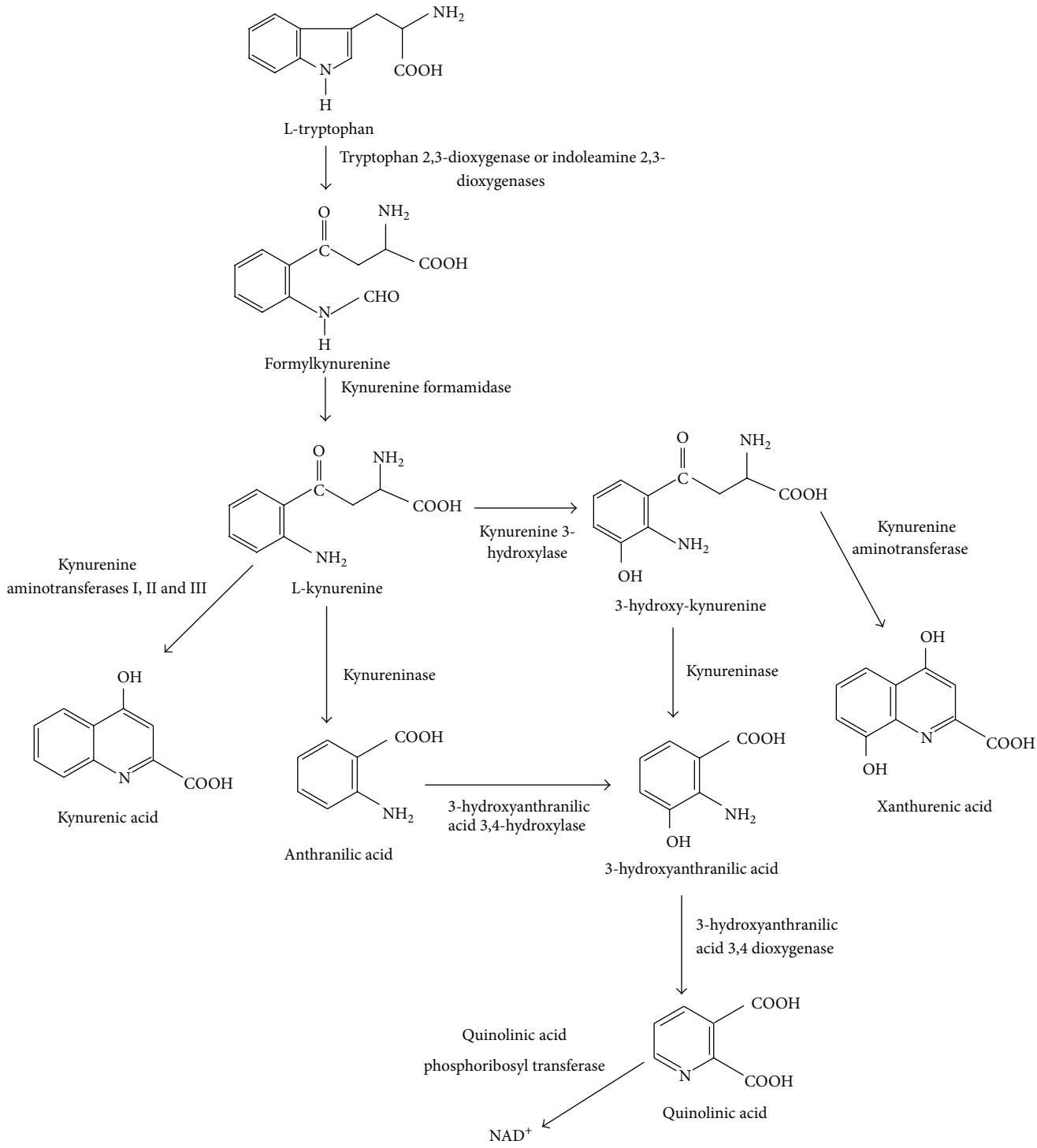


Figure 1: Kynurenine pathway. NAD^+ = nicotinamide adenine dinucleotide.

instead of QUIN [7]. 3-HAO is an iron dependent enzyme requiring Fe^{2+} ions and sulfhydryl groups for its activity and is presented in the mitochondrial membrane [8] and in the excitatory synapses [9]. Finally, QUIN is catabolized to NAD^+ and carbon dioxide by the action of quinolinate phosphoribosyl transferase (QPRT). This enzyme has been identified in rat and human CNS tissue [10]. Magnesium ions are required for QPRT activity and there is evidence that a cysteine residue at the active site is required for catalysis [11]. Interestingly, a different brain localization of 3-HAO and QPRT has been observed, while 3-HAO is in the soluble fraction of brain homogenate, QPRT is in a P2 synaptosomal fractions particulate component [12]. For this reason, QUIN is produced by microglia [13–15] and must exit those cells to

be metabolized by QPRT in a separate population of QPRT-containing astrocytes and neurons [15].

The concentration of QUIN varies among different brain regions, with the cerebral cortex containing approximately 1.8 nmol/g wet weight; almost 2-fold than that found in the hippocampus (1 nmol/g wet weight) [16–18].

2. Metabolism of QUIN

Intraarterial administration of either micromolar or millimolar concentrations of QUIN resulted in only negligible accumulations of this metabolite in the brain, suggesting that the central nervous system (CNS) appears to be well protected by the blood brain barrier (BBB) from peripheral QUIN [19].

Many factors come into play to render QUIN a potent neurotoxin. One of such factors is the performance of the two enzymes involved in QUIN synthesis and metabolism, respectively. There are substantially fewer cells containing QPRT than those that contain 3-HAO [20]. The brain area with the highest QPRT activity is the olfactory bulb, and among the regions with the lowest activity are the frontal cortex, striatum, hippocampus, and retina [11]. A detailed analysis of the properties of 3-HAO and QPRT indicates that both have similar Km values, but 3-HAO reaction velocity was 80-fold higher than QPRT [21]. Consequently, the production of QUIN occurs at a much faster rate within the brain than the conversion to NAD^+. This has implications for the accumulation of QUIN in the brain under certain pathological conditions. Furthermore, 3-HAO activity may normally be restrained by factors such as the availability of Fe^{2+} ions [8, 22, 23]. Stachowski and Schwarcz [23], showed that Fe is a cofactor of 3-HAO and the addition of Fe^{2+} (2–40 μM) stimulated 3-HAO activity 4- to 6-fold in striatal homogenates of mouse, rat, and human; this effect was prevented by ferritin. Thus, if in neuronal damage occurs releasing of Fe^{2+} ions, which is normally the case, then the production of QUIN would be elevated, thereby causing more damage and so the vicious circle would continue.

Some years ago, studies with [^{3}H]QUIN in hippocampus showed that this region as well as the striatum does not appear to possess mechanisms either for the rapid removal of QUIN or for its metabolic degradation in the extracellular space by QPRT [19]. Recently, it was shown that human-mixed brain cells (neurons, astrocytes, and microglia) can take large amounts of QUIN and saturate the neuronal QPRT; however, the precise mechanism by which QUIN is taken up by neurons and astrocytes is unclear [24–26]. All these factors promote QUIN's ability to cause cellular damage.

Additionally, the concentration and metabolism of QUIN appears to depend on the age of the rat. In fact, Moroni and coworkers [17] found that the administration of TRP was able to increase QUIN levels in adult rats but not in newborn rats. Furthermore, a progressive QUIN increase was found in rats of 3 days and 3, 9, and 30-months of age. In rats of 30 month old, half of them were found to have QUIN concentrations approaching to those that cause neurotoxicity [27].

On the other hand, the administration of a TRP-free diet to rats for 15 days resulted in a doubling of QUIN concentrations in the cortex. One explanation for this may be that QUIN can also be synthesized by a different pathway, particularly as some bacteria and plants are able to synthesize QUIN from the condensation of aspartic acid and dihydroxyacetate [8].

3. Excitotoxicity Produced by QUIN

The first evidence that kynurenines may have a role in brain function was given by Lapin [28], who observed convulsions in mice after an intracerebroventricular QUIN injection. In 1981, Stone and Perkins discovered that QUIN was a potent excitant of neurons in the CNS, by acting as an agonist at the *N*-methyl-*D*-aspartate (NMDA) sensitive population glutamate receptors [29], and Schwarcz and coworkers (1983) were the first who demonstrated that QUIN causes selective neuronal lesions, and they also found that focal injections of QUIN into the striatum resulted in neurochemical, behavioral and pathological changes [30].

In the 80's, it was demonstrated that QUIN is about one-quarter as active as NMDA and approximately as active as glutamate and aspartate at stimulating NMDA receptors [29]. It must however be remembered that the latter compounds have an rapid, high-affinity uptake system for their removal from the synapse, while QUIN has a uptake system, but the neuronal QPRT is rapidly saturated by this metabolite (~300 nM) [25]. Although part of QUIN can be removed from the synaptic cleft, the rest of QUIN will continue stimulating the NMDA receptor causing extensive damage. QUIN acts selectively at NMDA receptors, specifically with NMDA receptor subtypes containing the NR2A and NR2B subunits [31], with massive calcium entry into neurons and astrocytes. Therefore, QUIN exerts the greatest damage to neurons where these receptor subtypes are present. Areas of the brain most sensitive to QUIN neurotoxicity are the hippocampus and striatum [32] in which the NMDA receptors are widely distributed [33]. Within these brain areas, some neuronal cell types are more sensitive than others, with cholinergic neuronal death in the striatum observed following QUIN injection [34] and preferential susceptibility of pyramidal cells in the hippocampus [32]. Striatal spiny neurons containing the neurotransmitter γ-aminobutyric acid (GABA) and substance P are also sensitive to QUIN toxicity, with the subclass of striatal spiny neurons containing somatostatin and neuropeptide Y being preserved [35].

QUIN can also increase glutamate release and inhibit its reuptake by astrocytes, thus increasing its concentration in the microenvironments, causing neurotoxicity [36, 37] and also limiting glutamate to glutamine recycling in astrocytes by decreasing glutamine synthetase activity [38, 39]. As shown previously in cortical neurons, dopaminergic neurons do not produce QUIN but take it up from the microenvironment [15, 40]. On the other hand, QUIN (10 μM) prevents of glutamate-induced excitotoxicity in primary cultures of rat cerebellar granule neurons [41], nevertheless mature organotypic cultures of rat corticostriatal system or caudate nucleus chronically exposed to 100 nM QUIN for up to 7 weeks show focal degeneration characterized by the presence

of vacuoles in neuropil, swollen dendrites, occasional swollen post-synaptic elements, and degenerated neurons [42, 43].

Additionally, chronic exposure of human neurons to QUIN causes significant structural changes including dendritic beading, microtubular disruption, and a decrease in organelles. Rahman and coworkers show that the *in vitro* QUIN treatment of human primary foetal neurons led to a substantial increase of tau phosphorylation at multiple positions. The observed increase in QUIN-induced phosphorylation of tau was attributed to a decrease in the expression and activity of the major tau phosphatases [25].

Recently, Pierozan and coworkers (2010) described that acute intrastriatal administration of QUIN targets the phosphorylating system associated with the cytoskeleton of neural striatal cells, causing intermediate filament hyperphosphorylation; this effect was mediated by Ca^{2+} influx through NMDA channels and by oxidative stress [44]. Additionally, alterations in the homeostasis of the cytoskeleton of astrocytes and neurons were found in rat striatal slices treated with 100 μM of QUIN. These events were secondary to the following specific mechanism: (a) in astrocytes, the effect by QUIN was mediated by increased Ca^{2+} influx through NMDA receptors and L-type voltage-dependent Ca^{2+} channels (L-VDCC) and (b) in neurons, QUIN actions involving metabotropic glutamate receptors and the Ca^{2+} from intracellular stores besides Ca^{2+} influx through NMDA receptor and L-VDCC. In both cases the increase in the intracellular Ca^{2+} levels set off a cascade of events including activation of the second messengers-dependent protein kinases, which phosphorylate head domain sites on GFAP and neurofilaments subunits and potentially misregulating intermediate filament assembly in both glia and neuronal cells [45]. Additionally, the *in vivo* overstimulation of NMDA receptors by QUIN causes an early impairment of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump which may result in important disturbances in intracellular Ca^{2+} signaling [46].

4. Alterations Energetic and QUIN

Recent evidence shows that metabolic impairment is an important mechanism by which QUIN can exert its toxicity. In this context, it was found that QUIN can inhibit B monoamine oxidase (MAO-B) in human brain synaptosomal mitochondria [47] and also can be a potent inhibitor of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) from rat liver cytoplasm, an important enzyme in the gluconeogenesis pathway that converts oxaloacetate to phosphoenolpyruvate [12]. QUIN can potentiate its own toxicity and that of other excitotoxins, like NMDA and glutamate, producing progressive mitochondrial dysfunction [48].

Different studies have shown that intrastriatal injection of QUIN provokes a decrease of cellular respiration and ATP levels [48, 49]; however, these findings may be due to a primary activation of glutamate receptors and a secondary effect of QUIN on energy production via free radicals [50–52]. However, Ribeiro and coworkers [53] observed that QUIN injection also inhibited creatine kinase activity, an important enzyme involved in intracellular energy transfer. QUIN also provoked significant reductions of the activities of complexes II (50%), II–III (35%), and III (46%) of the respiratory chain in the striatum of young rats, and this impairment of striatum bioenergetics induced by QUIN injection was partially mediated by generation of reactive species.

Recently, Schuck and coworkers [54] have shown that QUIN inhibits the $^{14}CO_2$ production and increased glucose uptake in cerebral cortex homogenates of young rats indicating that this kynurenine stimulated the transport and/or utilization of this substrate by the brain. QUIN also inhibits around 35% succinate dehydrogenase (SDH), an enzyme involved in the citric acid cycle and in the respiratory chain. Moreover, this effect was not dependent of the NMDA receptor since MK-801 and kynurenic acid (two NMDA receptor antagonists) and L-NG-nitroarginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, did not prevent the inhibition, but the preincubation with superoxide dismutase and catalase can do it. In this context, our group has been shown that QUIN interacts in the SDH-binding site with the arginine 297 residue (R290 of the sequence numbering of SDH-QUIN by docking), whose positive charge is important for the binding affinity of negatively charged inhibitors, and in *in vitro* assays QUIN can inhibit SDH and ATP levels, and the effect in the enzyme is depending on each brain region in which mitochondria were isolated [55]. All this evidence suggests that QUIN has different targets that could be independent of its agonist activity under NMDA receptor, and the mitochondrial impairment represent other mechanism of the QUIN toxicity.

5. Oxidative Stress and QUIN

Free radical generation and oxidative stress are involved in the QUIN-induced toxicity; however, we need to take in mind that these mechanisms can be dependent and/or independent of its activity on NMDA receptors. In this line, it has been shown that QUIN can produce oxidative damage independent of its activity under NMDA receptor; this mechanism involves a complex between QUIN and Fe^{2+}. Studies by Stipêk and coworkers [62] showed that the lipid peroxidation induced by QUIN was modulated by its interaction with Fe^{2+} to form QUIN-Fe^{2+} complexes that mediate reactive oxygen species (ROS) generation. In phosphate buffer, the QUIN-Fe^{2+} enhanced the formation of the hydroxyl radical via the Fenton reaction [105], and it was also observed that QUIN inhibits the autooxidation of Fe^{2+} by the complex formation. The QUIN-Fe^{2+} complex was shown to be responsible for the *in vitro* DNA chain breakage and lipid peroxidation mediated by hydroxyl radicals [106].

Moreover, there is evidence showing that QUIN can increase free radical production by inducing NOS activity in astrocytes and neurons, leading to oxidative stress, increasing both poly(ADP-ribose) polymerase (PARP) activity and extracellular lactate dehydrogenase (LDH) activity [90]. In concordance, striatal slices exposed to QUIN show an increase in both lipid peroxidation and LDH activity and a decrease in mitochondrial function [107]; these alterations were related with proteases activation.

Furthermore, it has been shown the QUIN capacity to modify the profiles of some endogenous antioxidants in rat brain such as the content of reduced glutathione and copper and zinc-dependent superoxide dismutase activity (Cu, Zn-SOD) [108] and its ability to generate during early stages of toxicity $^{\bullet}$OH radical [109] and peroxynitrite [86] and to increase lipid peroxidation [108, 110]. In rat brain, intracerebral injection of QUIN resulted in significant neuronal loss and a markedly increased level of SOD1 expression in a time-dependent manner [111]; this increase in SOD1 expression was thought to be a neuroprotective response to limit the oxidative damage caused by QUIN. In support of these results, it was found that QUIN infusion induced cell damage an increase in ROS levels in mice hippocampus, while the second one became normal after 24 hours, the first one persisted for 72 hours. Therefore, the delayed and persistent increase in the antioxidant capacity after QUIN insult may be a cellular adaptive response, probably contributing to the decrease in ROS levels [52]. Additionally, it was observed in synaptosomal fractions exposed to QUIN and 3-nitropropionic acid at nontoxic concentration, a synergic effect in oxidative markers which was just partially prevented by MK-801 [79].

Recently, Tronel and coworkers [112] showed that the HO-1 inducer hemin had a deleterious effect in QUIN *in vivo* model and enhanced tissue loss and microglia activation and showed that this effect is probably linked to a hyperproduction of ROS and iron accumulation.

Different ROS scavengers, molecules with antioxidant properties, inducers of activity of antioxidant enzymes, and others drugs have been tested successfully against QUIN toxicity (Table 1), indicating the importance of oxidative damage in the neurodegeneration induced by QUIN.

Based on this evidence and considering that oxidative stress results from an unbalance between the antioxidant defense and the reactive species formed, this phenomenon should be considered as one of the many mechanism by which QUIN exerts its toxic effect since free radicals also can activate more signaling cascades that can contribute and maximize its neurotoxic effect.

6. Inflammation and QUIN

Inflammatory events are also implicated in the QUIN toxicity. It is known that microglia is responsible for inflammatory responses in the CNS and takes the major role in altered levels of QUIN, since it has been shown that IFN-γ and bacterial lipopolysaccharide (LPS) induce IDO and increase QUIN production [15, 113, 114]. This effect could be potentiated by astrocytes since QUIN induces astrogliosis and in consequence the expression and release of cytokines enhancing the inflammatory response that could compromise cell viability [39, 115, 116]. In this regard, in the brain, large amounts of QUIN are produced and secreted by activated microglia [93]. During CNS inflammation, QUIN levels increase in brain homogenate (246-fold) and extracellular fluid (66-fold), mostly due the increase in local QUIN's synthesis rate [117].

On the other hand, it has been reported the influence of QUIN on inflammatory response. The intrastriatal QUIN administration induces a marked expression of tumor necrosis alpha (TNF-α) [118] and interleukin-6 (IL-6) [119] that can be attenuated by inhibiting the cyclooxygenase 2 (COX-2) [120]. QUIN and TNF-α cause oligodendrocyte death by apoptotic process [121, 122]. Although TNF-α is not neurotoxic itself, this proinflammatory cytokine can contribute to neuronal damage through a variety of effects, such as stimulation of free radical formation, induction of cellular adhesion molecule expression, or potentiation of glutamate-mediate neurotoxicity [123, 124]. Besides, QUIN was able to increase MCP-1 production [116] in human fetal astrocytes, and this effect is likely to be biologically significant. In fact, within the brain, MCP-1 is probably one of the most powerful chemoattractants for monocytes and is mainly produced by astrocytes [125]. During pathological events, macrophages in blood stream also contribute to QUIN formation after an inflammatory response, and this may break blood brain barrier and release QUIN into the brain [126]. In this context, it has been reported that macrophages have the ability to produce approximately 20- to 30-fold more QUIN than microglia [14]. This fact suggests the prevalence of positive feedback in which inflammatory (local or systemic) responses given by microglia or macrophages, respectively, increase QUIN synthesis, and this effect could induce expression of cytokines where both factors may converge resulting in cell death. In this line, Erhardt and coworkers [99] found significantly increased levels of QUIN in the CSF of suicide attempters, and there was a significant correlation between CSF levels of QUIN and the proinflammatory cytokine IL-6.

7. Behavioral, Morphological Alterations and Death Induced by QUIN

Several reports have been shown that the intrastriatal administration of QUIN in rats produced significant behavioral changes. Intrastriatal rat injection of QUIN resulted in an initial period of involuntary movements and intrahippocampal injection triggering convulsions [34]. Sanberg and coworkers (1989) showed that after 4 weeks after lesion with QUIN (150 and 225 nmol), the rats display significantly increased levels in locomotion, and there was a persistent hyperactivity throughout the nocturnal period [127]. However the bilateral intrastriatal injections with QUIN (120 nmoles per side) produce significant motor/kinetic deficits. The motor alterations were seen at both early (24 h after lesion) and late (7 days after lesion) and comprised total distance walked/traveled—which is probably the most accurate index of motility among all tested here—and vertical activity—likely indicating exploratory behavior [128].

On the other hand, the few studies in which it has been investigated the cognitive deficits of rats with QUIN lesions have indicated that this metabolite causes deficits in spatial reference memory. QUIN disrupted the performance of rats on the radial arm water maze, balance-beam, and open-field tasks [129]. In rodents, QUIN unilateral lesion with asymmetrical rotation behavior occurs stimulated by apomorphine, a widely known dopamine agonist [130]. The rotation behavior results from an imbalance of dopaminergic signaling between the injured and the intact hemisphere.

Table 1: Effect of various molecules on the toxicity induced by QUIN.

Compound	Mechanism of action	Reference
Melatonin	(i) Attenuates the convulsant effect of quinolinate (ii) Partially protects against the increase of circling behavior (iii) Protects against the increase in ROS and protein carbonyl levels as well as the inhibition of superoxide dismutase activity	[56–58]
Denepryl	(i) Acts as a potent-free radical scavenger (ii) Increases the activity of endogenous antioxidant enzymes	[59]
α-phenyl-t-butyl nitrone	(i) Presents cytoprotective effects	[60]
Polyamines such as spermine and spermidine	(i) Inhibit QUIN-induced TBARS production and have antioxidant properties	[61]
Deferoxamine (iron chelator)	(i) Reduces lipid peroxidation	[62]
Reduced glutathione	(i) Decreases lipid peroxidation and ROS formation in brain synaptosomes	[63]
Selenium	(i) Attenuates the QUIN-induced early reactive oxygen species formation and lipid peroxidation (ii) Prevents loss of mitochondrial reductive capacity and morphological alterations in the striatum (iii) Induces stimulation of striatal GPx activity (iv) Prevents IκB-α degradation (v) Reduces the nuclear translocation of NF-κB and inhibits the activity of caspase-3, resulting in internucleosomal DNA preservation (vi) Induces an early stimulation of TrxR activity	[64–66]
Selenocompounds such as ebselen	(i) Inhibit TBARS production	[67]
Ksheerabala	(i) Decreases de lipid peroxidation and protein peroxidation (ii) Increases the activity of antioxidant enzymes	[68]
Licofelone, Montelukast, and Pioglitazone	(i) Significantly improve body weight, locomotor activity, oxidative defense, activity of mitochondrial enzyme complex, rotarod performance, and balance beam walk	[69–71]
Dizocilpine (MK-801)	(i) Improves body weight, behavioral alterations (locomotor activity and rotarod performance) and attenuates oxidative damage and mitochondrial enzymes complexes dysfunction (ii) Improves learning task in rats receiving chronic i.c.v, infusion of QUIN (iii) Decreases release of lactate dehydrogenase (LDH) in NSC-34 cells after 48 h of QUIN	[72–74]
Nimesulide, rofecoxib, and caffeic acid	(i) Restore mitochondrial enzyme complex activities in striatum	[75, 76]
Memantine	(i) Significantly attenuates QUIN-mediated PARP activation, NAD^+ depletion, and LDH release in both neurons and astrocytes as well as decreases LDH release in NSC-34 cells induced by QUIN	[74, 77]
2-amino-5-phosphonopentanoic acid (APV)	(i) Decreases QUIN-induced LDH release in NSC-34 cell (ii) Abolishes the release of aspartate and glutamate	[74, 78]
L-carnitine and acetyl L-carnitine	(i) Reduce lipid peroxidation (ii) Prevent mitochondrial dysfunction in brain synaptosomes (iii) Attenuate the behavioral alterations and striatal degeneration	[79–81]
Tolmetin and sulindac	(i) Reduce the generation of superoxide anions (ii) Reduce the lipid peroxidation after an intrahippocampal injection of QUIN (iii) Reduce the spatial memory deficit	[82, 83]
Acyclovir	(i) Inhibits the lipid peroxidation after *in vitro* and *in vivo* exposure to QUIN (ii) Reduces necrosis of hippocampal neurons	[84]
Nitroarginine and L-arginine	(i) Prevent lipid peroxidation induced by QUIN	[85]
Iron metalloporphyrins such as Fe(TPFPP) and Fe(TPPS)	(i) Decrease 3-nitrotyrosine levels (ii) Prevent lipid peroxidation and mitochondrial dysfunction (iii) Reduce the DNA fragmentation and decreases caspase-3-like activation (iv) Abolish the circling behavior (v) Partially recover GABA levels (vi) Reduce the immunochemical expression of IL-6 and iNOS	[86–88]

Table 1: Continued.

Compound	Mechanism of action	Reference
Safranal	(i) Inhibits lipid peroxidation (ii) Inhibits oxidative DNA damage (iii) Improves hippocampal antioxidant and thiol redox status	[89]
Polyphenols (epigallocatechin gallate, curcumin)	(i) Inhibit QUIN-induced nNOS activity and subsequent nitrite production (ii) Reduce 3-nitrotyrosine production (iii) Prevent DNA damage and PARP-1 activation (iv) Attenuate QUIN-induced Ca^{2+} influx	[90]

Thiobarbituric acid reactive species: TBARS; reactive oxygen species: ROS; thioredoxin reductase: TrxR; glutathione peroxidase: GPx; nuclear factor-kappaB: NF-κB; quinolinic acid: QUIN; deoxyribonucleic acid: DNA; γ-Aminobutyric acid: GABA; interleukin 6: IL-6; inducible nitric oxide synthase: iNOS.

Table 2: Alterations in QUIN levels presented in different neurodegenerative diseases and experimental models. Key references are shown in the quarter column.

Disease/model	QUIN levels	Associated alterations	Reference
Alzheimer	↑ in demented patient ↑ in senile plaques	(i) IDO overexpression (ii) ↓ KYNA (iii) QUIN colocalizes with tau in cortical sections (iv) ↑ QUIN/3-HK quotient in plasma	[25, 26, 91, 92]
Aβ 1-42	↑	(i) IDO over-expression in microglia and macrophages	[93]
Huntington	↑	(i) ↑ 3-HK	[94]
Huntingtin transgenic mice	↑ in YAC128 mice, Hdh^{Q92}/Hdh^{Q111} knock-in mice	(i) ↑ 3-HK	[95]
Human immunodeficiency virus (HIV)	↑ in CSF and serum of patients	(i) Cytokines release (ii) ↑ IDO (iii) QUIN levels enhanced independent blood brain barrier breakdown	[96–98]
Suicide attempters	↑ in CSF of suicide attempters	(i) An increased QUIN/KYNA quotient	[99]
Depression	↑ QUIN expression in human brain during acute depressive episodes	(i) Abnormal NMDA receptor function	[100]
Autism	↑ in CFS of patients	(i) ↑ biopterin (ii) ↓ neopterin	[101]
Amyotrophic lateral sclerosis	↑ in CSF and serum of patients	(i) ↑ TRP and L-KYN, human leukocyte antigen-DR (ii) IDO over-expression	[102]
Experimental allergic encephalomyelitis, a model of multiple sclerosis	↑ in the spinal cords of rats	(i) ↑ in KMO activity and 3-HK levels	[103, 104]

IDO: indolamine 2,3-dioxigenase, KYNA: kynurenic acid, QUIN: quinolinic acid, L-KYN: L-kynurenine, 3-HK: 3-hydroxykynurenine, TRP: tryptophan, and CSF: cerebrospinal fluid.

Administration of QUIN directly into the rat striatum produced "axon-sparing" lesions, with marked swelling of dendrites and loss of cell structure in postsynaptic sites, but generally good preservation of axons and presynaptic terminals [30, 131]. Infusion of 120 nmol QUIN into several regions of the rat's brain revealed differences in vulnerability to its neurotoxic effects. The striatum, the pallidal formation, and the hippocampus were the most susceptive brain areas whereas cerebellum, substantia nigra, amygdala, medial septum, and hypothalamus were more resistant [32]. Within these brain areas, some neuronal cell types are more sensitive than others, with cholinergic neuronal death in the striatum observed following QUIN and preferential susceptibility of pyramidal cells in the hippocampus [32]. Striatal spiny neurons containing the neurotransmitter γ-aminobutyric acid (GABA) and substance P are also sensitive to QUIN toxicity, with the subclass of striatal spiny neurons containing somatostatin and neuropeptide Y being preserved [35]. Moreover, intrastriatal injections of QUIN cause significant striatal atrophy, ventricular dilation, metabolic depression, and loss of neurons in the striatum. Histological evaluation of cytochrome-oxidase-stained tissue indicated that intrastriatal injections of QUIN caused widespread metabolic depression and QUIN (200 nmoles) results in relatively extensive loss of NADPH-diaphorase-containing neurons [129]. QUIN induces not only cell death, but also damage to axons and dendrites [43, 132]; in this regard, recent studies demonstrated that QUIN toxicity could lead

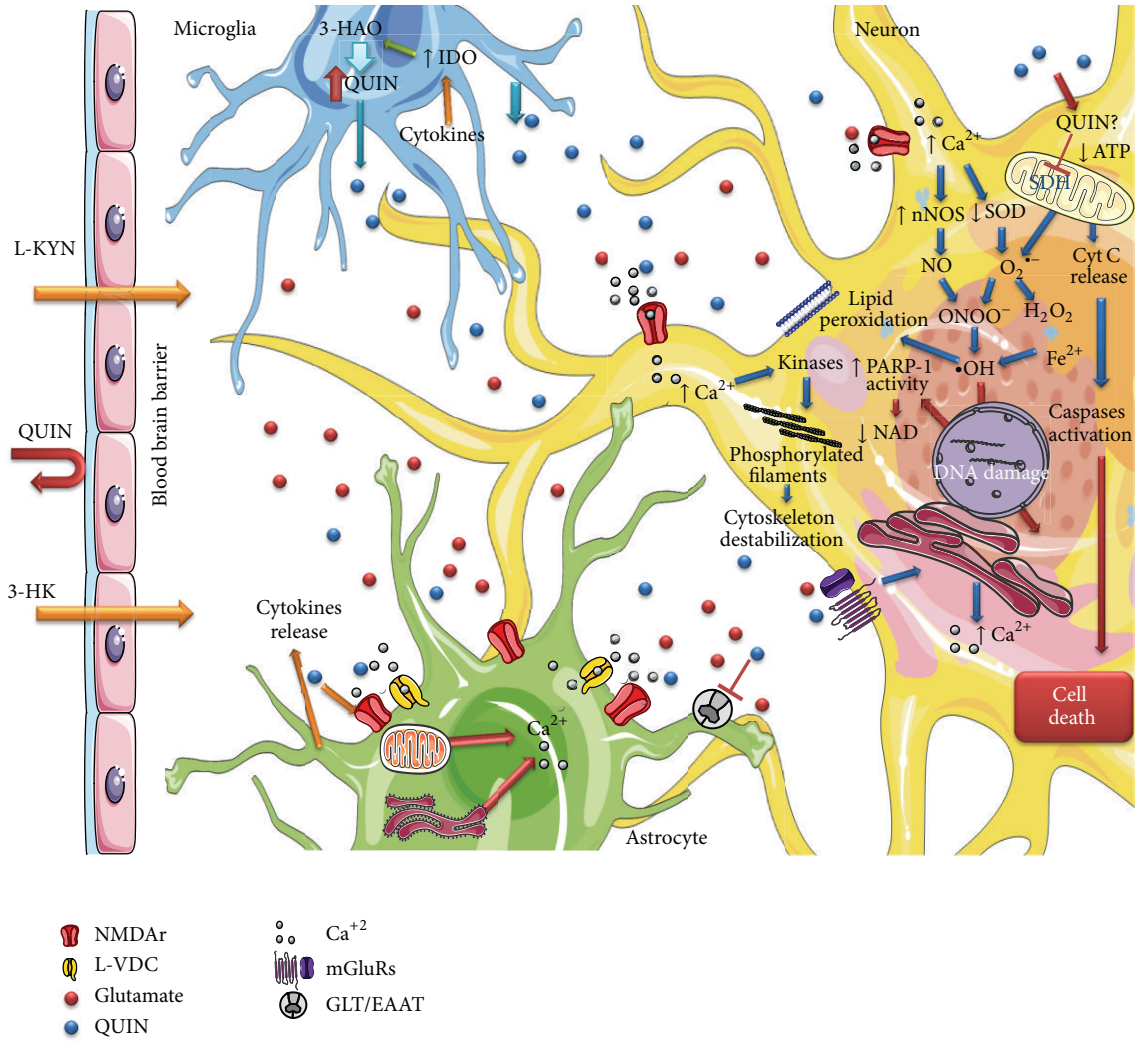


Figure 2: Multiple mechanisms leading to QUIN cytotoxicity. One of the principal toxicity mechanism of QUIN is through the over stimulation of the NMDA receptor which is powered by the lack of uptake of QUIN from the extracellular space. Additionally, QUIN enhances the release of synaptosomal glutamate as a consequence of the inhibition of glutamate uptake into the astrocytes that will lead to overstimulation of receptors. Furthermore, QUIN can decrease the activity of antioxidant enzymes promoting ROS production and generating lipid peroxidation. Also, QUIN may inhibit the activity of mitochondrial complexes leading to energetic deficit, activating caspases and releasing cytochrome c. All these factors induce cytoskeleton destabilization, DNA damage, and cell death.

to destabilization of the cytoskeleton by phosphorylating structural proteins [25, 44]. The cytoskeleton plays a key role in maintaining the neuronal cell shape and is essential for its normal functions, such as neurite outgrowth, synapse formation, and internal transport of various molecules.

QUIN resulted in neurons that displayed a nonapoptotic pattern of chromatin condensation and early disruption of cytoplasmic organelles. QUIN-injured neurons underwent changes in mitochondria and endoplasmic reticulum [133]. It was also demonstrated that QPRT-depleted cells had an increased intracellular active-caspase-3 activity and were highly sensitive to spontaneous cell death [134].

QUIN has been demonstrated to induce neuronal and astrocytic apoptosis involving the activation of caspase 3 [135–137]. Another study demonstrated that intrastriatal injection of QUIN in rat brain leads to the hyperphosphorylation of cytoskeletal intermediate filament proteins in astrocytes and neurons [44]. It has been shown that intrastriatal injection of different doses of QUIN causes apoptotic cell death [138–140], and in striatal cells, this kind of death is mediated by an increase in Bax and a decrease in Bcl-2 protein levels, leading to reduced levels of Bax:Bcl-2 heterodimers [141]. In fact, Bcl-2 and Bcl-xL protein levels were downregulated at later times after QUIN injection suggesting that apoptotic cell death may, in part, be related to reduced levels of antiapoptotic proteins [141]. Besides striatal NMDA receptor stimulation by QUIN promotes the selective degradation of IκB-α, this degradation appears to be mediated by caspase-3-like protease and promotes an apoptotic response that involves the NF-κB activation [142]. Recently,

it was shown that during the process of neuronal cell death induced by QUIN, upregulation of p53 and proapoptotic p53 target genes PUMA (p53-upregulated modulator of apoptosis) and Bax and downregulation of antiapoptotic protein Bcl-2 were observed. Moreover, QUIN induced the expression of damage-regulated autophagy modulator (DRAM), beclin 1, and LC3-II, proteins that are involved in autophagy [143]. All this evidence suggests that NF-κB-dependent p53 induction contributes to QUIN-induced death of striatal neurons through both apoptotic and autophagic mechanisms.

8. QUIN and Neurodegenerative Disease

It is known that kynurenine pathway is found in glial cells of the CNS and in inflammatory cells of the circulation, and it is regulated by redox components as well as by inflammatory components. The fact that different neuropathologies present excitotoxicity, oxidative stress, and inflammation as common factors, suggests that KP metabolites may be altered. In this context, different groups have been shown that in some brain pathologies as well as in experimental models of neurodegeneration, an inappropriate activation of KP may lead to increased QUIN levels. Alteration in QUIN levels has been implicated in different pathologies such as Alzheimer's, Huntington's, and Parkinson's diseases as well as in experimental models of those diseases in which QUIN plays a special role acting on the neurodegenerative cascade (Table 2).

9. Conclusion

According to the information that has been reviewed, the mechanisms by which QUIN produces neurotoxicity include overactivation of the NMDA receptor, energy deficit, oxidative stress, and cell death. A sequence of these events is described in Figure 2. Far from being excluding, all these factors are somehow closely related and also act synergistically to induce neurodegeneration. Taking into account that QUIN has been implicated in neurodegenerative diseases and some of their toxic mechanisms are still unknown, the challenges for the future research should be directed to clarify all the possible routes that can promote or contribute to the damage induced by this metabolite. This may help to explain the physiopathological events occurring in several neurodegenerative diseases in which the levels of QUIN are increased.

Acknowledgments

This work was supported by CONACYT Grant 183867.

References

[1] L. Musajo and C. A. Benassi, "Aspects of disorders of the Kynurenine pathway of Tryptophan metabolism in man," *Advances in Clinical Chemistry*, vol. 7, pp. 63–135, 1964.

[2] H. Wolf, "The effect of hormones and vitamin B6 on urinary excretion of metabolites of the kynurenine pathway," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 136, pp. 1–186, 1974.

[3] R. M. Anderson, K. J. Bitterman, J. G. Wood et al., "Manipulation of a nuclear NAD+ salvage pathway delays aging without altering steady-state NAD+ levels," *The Journal of Biological Chemistry*, vol. 277, no. 21, pp. 18881–18890, 2002.

[4] H. Massudi, R. Grant, G. J. Guillemin, and N. Braidy, "NAD+ metabolism and oxidative stress: the golden nucleotide on a crown of thorns," *Redox Report*, vol. 17, no. 1, pp. 28–46, 2012.

[5] D. A. Bender and G. M. McCreanor, "The preferred route of kynurenine metabolism in the rat," *Biochimica et Biophysica Acta*, vol. 717, no. 1, pp. 56–60, 1982.

[6] A. C. Foster, R. J. White, and R. Schwarcz, "Synthesis of quinolinic acid by 3-hydroxyanthranilic acid oxygenase in rat brain tissue in vitro," *Journal of Neurochemistry*, vol. 47, no. 1, pp. 23–30, 1986.

[7] N. Braidy, R. Grant, B. J. Brew, S. Adams, T. Jayasena, and G. J. Guillemin, "Effects of kynurenine pathway metabolites on intracellular NAD+ synthesis and cell death in human primary astrocytes and neurons," *International Journal of Tryptophan Research*, vol. 2, no. 1, pp. 61–69, 2009.

[8] T. W. Stone, "Neuropharmacology of quinolinic and kynurenic acids," *Pharmacological Reviews*, vol. 45, no. 3, pp. 309–379, 1993.

[9] R. C. Roberts, K. E. McCarthy, F. Du, P. Ottersen, E. Okuno, and R. Schwarcz, "3-Hydroxyanthranilic acid oxygenase-containing astrocytic processes surround glutamate-containing axon terminals in the rat striatum," *The Journal of Neuroscience*, vol. 15, no. 2, pp. 1150–1161, 1995.

[10] A. C. Foster, W. O. Whetsell Jr., E. D. Bird, and R. Schwarcz, "Quinolinic acid phosphoribosyltransferase in human and rat brain: activity in Huntington's disease and in quinolinate-lesioned rat striatum," *Brain Research*, vol. 336, no. 2, pp. 207–214, 1985.

[11] A. C. Foster, W. C. Zinkand, and R. Schwarcz, "Quinolinic acid phosphoribosyltransferase in rat brain," *Journal of Neurochemistry*, vol. 44, no. 2, pp. 446–454, 1985.

[12] H. G. McDaniel, W. J. Reddy, and B. R. Boshell, "The mechanism of inhibition of phosphoenolpyruvate carboxylase by quinolinic acid," *Biochimica et Biophysica Acta*, vol. 276, no. 2, pp. 543–550, 1972.

[13] M. P. Heyes, C. L. Achim, C. A. Wiley, E. O. Major, K. Saito, and S. P. Markey, "Human microglia convert L-tryptophan into the neurotoxin quinolinic acid," *The Biochemical Journal*, vol. 320, no. 2, pp. 595–597, 1996.

[14] G. J. Guillemin, D. G. Smith, G. A. Smythe, P. J. Armati, and B. J. Brew, "Expression of the kynurenine pathway enzymes in human microglia and macrophages," *Advances in Experimental Medicine and Biology*, vol. 527, pp. 105–112, 2003.

[15] G. J. Guillemin, G. Smythe, O. Takikawa, and B. J. Brew, "Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons," *GLIA*, vol. 49, no. 1, pp. 15–23, 2005.

[16] F. Moroni, G. Lombardi, V. Carla, and G. Moneti, "The excitotoxin quinolinic acid is present and unevenly distributed in the rat brain," *Brain Research*, vol. 295, no. 2, pp. 352–355, 1984.

[17] F. Moroni, G. Lombardi, G. Moneti, and C. Aldinio, "The excitotoxin quinolinic acid is present in the brain of several mammals and its cortical content increases during the aging process," *Neuroscience Letters*, vol. 47, no. 1, pp. 51–55, 1984.

[18] Y. Chen and G. J. Guillemin, "Kynurenine pathway metabolites in humans: disease and healthy states," *International Journal of Tryptophan Research*, vol. 2, no. 1, pp. 1–19, 2009.

[19] A. C. Foster, L. P. Miller, W. H. Oldendorf, and R. Schwarcz, "Studies on the disposition of quinolinic acid after intracerebral or systemic administration in the rat," *Experimental Neurology*, vol. 84, no. 2, pp. 428–440, 1984.

[20] C. Kohler, L. G. Eriksson, P. R. Flood, J. A. Hardie, E. Okuno, and R. Schwarcz, "Quinolinic acid metabolism in the rat brain. Immunohistochemical identification of 3-hydroxyanthranilic acid oxygenase and quinolinic acid phosphoribosyltransferase in the hippocampal region," *The Journal of Neuroscience*, vol. 8, no. 3, pp. 975–987, 1988.

[21] A. C. Foster, E. Okuno, D. S. Brougher, and R. Schwarcz, "A radioenzymatic assay for quinolinic acid," *Analytical Biochemistry*, vol. 158, no. 1, pp. 98–103, 1986.

[22] A. C. Müller, A. Dairam, J. L. Limson, and S. Daya, "Mechanisms by which acyclovir reduces the oxidative neurotoxicity and biosynthesis of quinolinic acid," *Life Sciences*, vol. 80, no. 10, pp. 918–925, 2007.

[23] E. K. Stachowski and R. Schwarcz, "Regulation of quinolinic acid neosynthesis in mouse, rat and human brain by iron and iron chelators in vitro," *Journal of Neural Transmission*, vol. 119, no. 2, pp. 123–131, 2012.

[24] G. J. Guillemin, "Quinolinic acid: neurotoxicity," *The FEBS Journal*, vol. 279, no. 8, p. 1355, 2012.

[25] A. Rahman, K. Ting, K. M. Cullen, N. Braidy, B. J. Brew, and G. J. Guillemin, "The excitotoxin quinolinic acid induces tau phosphorylation in human neurons," *PLoS ONE*, vol. 4, no. 7, Article ID e6344, 2009.

[26] G. J. Guillemin, B. J. Brew, C. E. Noonan, O. Takikawa, and K. M. Cullen, "Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus," *Neuropathology and Applied Neurobiology*, vol. 31, no. 4, pp. 395–404, 2005.

[27] T. W. Stone and J. H. Connick, "Quinolinic acid and other kynurenines in the central nervous system," *Neuroscience*, vol. 15, no. 3, pp. 597–617, 1985.

[28] I. P. Lapin, "Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice," *Journal of Neural Transmission*, vol. 42, no. 1, pp. 37–43, 1978.

[29] T. W. Stone and M. N. Perkins, "Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS," *European Journal of Pharmacology*, vol. 72, no. 4, pp. 411–412, 1981.

[30] R. Schwarcz, W. O. Whetsell Jr., and R. M. Mangano, "Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain," *Science*, vol. 219, no. 4582, pp. 316–318, 1983.

[31] L. Prado de Carvalho, P. Bochet, and J. Rossier, "The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits," *Neurochemistry International*, vol. 28, no. 4, pp. 445–452, 1996.

[32] R. Schwarcz and C. Kohler, "Differential vulnerability of central neurons of the rat to quinolinic acid," *Neuroscience Letters*, vol. 38, no. 1, pp. 85–90, 1983.

[33] S. Nakanishi, "Molecular diversity of glutamate receptors and implications for brain function," *Science*, vol. 258, no. 5082, pp. 597–603, 1992.

[34] A. C. Foster, J. F. Collins, and R. Schwarcz, "On the excitotoxic properties of quinolinic acid, 2,3-piperidine dicarboxylic acids and structurally related compounds," *Neuropharmacology*, vol. 22, no. 12, pp. 1331–1342, 1983.

[35] M. F. Beal, N. W. Kowall, D. W. Ellison, M. F. Mazurek, K. J. Swartz, and J. B. Martin, "Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid," *Nature*, vol. 321, no. 6066, pp. 168–171, 1986.

[36] R. G. Tavares, C. I. Tasca, C. E. S. Santos, M. Wajner, D. O. Souza, and C. S. Dutra-Filho, "Quinolinic acid inhibits glutamate uptake into synaptic vesicles from rat brain," *NeuroReport*, vol. 11, no. 2, pp. 249–253, 2000.

[37] R. G. Tavares, C. I. Tasca, C. E. S. Santos et al., "Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes," *Neurochemistry International*, vol. 40, no. 7, pp. 621–627, 2002.

[38] G. Baverel, G. Martin, and C. Michoudet, "Glutamine synthesis from aspartate in guinea-pig renal cortex," *The Biochemical Journal*, vol. 268, no. 2, pp. 437–442, 1990.

[39] K. K. Ting, B. J. Brew, and G. J. Guillemin, "Effect of quinolinic acid on human astrocytes morphology and functions: implications in Alzheimer's disease," *Journal of Neuroinflammation*, vol. 6, article 36, 2009.

[40] G. J. Guillemin, K. M. Cullen, C. K. Lim et al., "Characterization of the kynurenine pathway in human neurons," *The Journal of Neuroscience*, vol. 27, no. 47, pp. 12884–12892, 2007.

[41] Y. Sei, L. Fossom, G. Goping, P. Skolnick, and A. S. Basile, "Quinolinic acid protects rat cerebellar granule cells from glutamate-induced apoptosis," *Neuroscience Letters*, vol. 241, no. 2-3, pp. 180–184, 1998.

[42] W. O. Whetsell Jr. and R. Schwarcz, "Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system," *Neuroscience Letters*, vol. 97, no. 3, pp. 271–275, 1989.

[43] S. J. Kerr, P. J. Armati, G. J. Guillemin, and B. J. Brew, "Chronic exposure of human neurons to quinolinic acid results in neuronal changes consistent with AIDS dementia complex," *AIDS*, vol. 12, no. 4, pp. 355–363, 1998.

[44] P. Pierozan, A. Zamoner, Â. Krombauer Soska et al., "Acute intrastriatal administration of quinolinic acid provokes hyperphosphorylation of cytoskeletal intermediate filament proteins in astrocytes and neurons of rats," *Experimental Neurology*, vol. 224, no. 1, pp. 188–196, 2010.

[45] P. Pierozan, A. Zamoner, Â. K. Soska et al., "Signaling mechanisms downstream of quinolinic acid targeting the cytoskeleton of rat striatal neurons and astrocytes," *Experimental Neurology*, vol. 233, no. 1, pp. 391–399, 2012.

[46] A. M. A. P. Fernandes, A. M. Landeira-Fernandez, P. Souza-Santos, P. C. Carvalho-Alves, and R. F. Castilho, "Quinolinate-induced rat striatal excitotoxicity impairs endoplasmic reticulum $Ca2^{+}$-ATPase function," *Neurochemical Research*, vol. 33, no. 9, pp. 1749–1758, 2008.

[47] M. Naoi, R. Ishiki, Y. Nomura, S. Hasegawa, and T. Nagatsu, "Quinolinic acid: an endogenous inhibitor specific for type B monoamine oxidase in human brain synaptosomes," *Neuroscience Letters*, vol. 74, no. 2, pp. 232–236, 1987.

[48] Y. M. Bordelon, M.-F. Chesselet, D. Nelson, F. Welsh, and M. Erecińska, "Energetic dysfunction in quinolinic acid-lesioned rat striatum," *Journal of Neurochemistry*, vol. 69, no. 4, pp. 1629–1639, 1997.

[49] Y. M. Bordelon, M.-F. Chesselet, M. Erecińska, and I. A. Silver, "Effects of intrastriatal injection of quinolinic acid on electrical activity and extracellular ion concentrations in rat striatum in vivo," *Neuroscience*, vol. 83, no. 2, pp. 459–469, 1998.

[50] F. Pérez-Severiano, B. Escalante, and C. Ríos, "Nitric oxide synthase inhibition prevents acute quinolinate-induced striatal

neurotoxicity," *Neurochemical Research*, vol. 23, no. 10, pp. 1297–1302, 1998.

[51] J. Cabrera, R. J. Reiter, D.-X. Tan et al., "Melatonin reduces oxidative neurotoxicity due to quinolinic acid: in vitro and in vivo findings," *Neuropharmacology*, vol. 39, no. 3, pp. 507–514, 2000.

[52] M. Ganzella, F. M. Jardim, C. R. Boeck, and D. Vendite, "Time course of oxidative events in the hippocampus following intracerebroventricular infusion of quinolinic acid in mice," *Neuroscience Research*, vol. 55, no. 4, pp. 397–402, 2006.

[53] C. A. Ribeiro, V. Grando, C. S. Dutra Filho, C. M. Wannmacher, and M. Wajner, "Evidence that quinolinic acid severely impairs energy metabolism through activation of NMDA receptors in striatum from developing rats," *Journal of Neurochemistry*, vol. 99, no. 6, pp. 1531–1542, 2006.

[54] P. F. Schuck, A. Tonin, G. da Costa Ferreira et al., "In vitro effect of quinolinic acid on energy metabolism in brain of young rats," *Neuroscience Research*, vol. 57, no. 2, pp. 277–288, 2007.

[55] T. Blanco-Ayala, I. Serratos-Alvarez, M. Orozco-Ibarra et al., "Effect of some kynurenines under succinate deshidrogenase activity and ATP production: in vitro experimental and computational tools," in *Neuroscience Meeting*, vol. 60.09, Society for Neuroscience, New Orleans, LA, USA, 2012.

[56] I. P. Lapin, S. M. Mirzaev, I. V. Ryzov, and G. F. Oxenkrug, "Anticonvulsant activity of melatonin against seizures induced by quinolinate, kainate, glutamate, NMDA, and pentylenetetrazole in mice," *Journal of Pineal Research*, vol. 24, no. 4, pp. 215–218, 1998.

[57] G. S. Southgate, S. Daya, and B. Potgieter, "Melatonin plays a protective role in quinolinic acid-induced neurotoxicity in the rat hippocampus," *Journal of Chemical Neuroanatomy*, vol. 14, no. 3-4, pp. 151–156, 1998.

[58] E. Antunes Wilhelm, C. Ricardo Jesse, C. Folharini Bortolatto, and C. Wayne Nogueira, "Correlations between behavioural and oxidative parameters in a rat quinolinic acid model of Huntington's disease: protective effect of melatonin," *European Journal of Pharmacology*, vol. 701, no. 1–3, pp. 65–72, 2013.

[59] W. M. H. Behan, M. McDonald, L. G. Darlington, and T. W. Stone, "Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl," *British Journal of Pharmacology*, vol. 128, no. 8, pp. 1754–1760, 1999.

[60] N. Nakao and P. Brundin, "Effects of α-phenyl-tert-butyl nitrone on neuronal survival and motor function following intrastriatal injections of quinolinate or 3-nitropropionic acid," *Neuroscience*, vol. 76, no. 3, pp. 749–761, 1996.

[61] N. A. V. Bellé, G. D. Dalmolin, G. Fonini, M. A. Rubin, and J. B. T. Rocha, "Polyamines reduces lipid peroxidation induced by different pro-oxidant agents," *Brain Research*, vol. 1008, no. 2, pp. 245–251, 2004.

[62] S. Stipek, F. Stastny, J. Platenik, J. Crkovska, and T. Zima, "The effect of quinolinate on rat brain lipid peroxidation is dependent on iron," *Neurochemistry International*, vol. 30, no. 2, pp. 233–237, 1997.

[63] A. Santamaría, S. Galván-Arzate, V. Lisý et al., "Quinolinic acid induces oxidative stress in rat brain synaptosomes," *NeuroReport*, vol. 12, no. 4, pp. 871–874, 2001.

[64] A. Santamaría, B. Vázquez-Román, V. Pérez-De La Cruz et al., "Selenium reduces the proapoptotic signaling associated to NF-κB pathway and stimulates glutathione peroxidase activity during excitotoxic damage produced by quinolinate in rat corpus striatum," *Synapse*, vol. 58, no. 4, pp. 258–266, 2005.

[65] A. Santamaría, R. Salvatierra-Sánchez, B. Vázquez-Román et al., "Protective effects of the antioxidant selenium on quinolinic acid-induced neurotoxicity in rats: in vitro and in vivo studies," *Journal of Neurochemistry*, vol. 86, no. 2, pp. 479–488, 2003.

[66] P. D. Maldonado, V. Perez-De La Cruz, M. Torres-Ramos et al., "Selenium-induced antioxidant protection recruits modulation of thioredoxin reductase during excitotoxic/pro-oxidant events in the rat striatum," *Neurochemistry International*, vol. 61, no. 2, pp. 195–206, 2012.

[67] J. I. Rossato, G. Zeni, C. F. Mello, M. A. Rubin, and J. B. T. Rocha, "Ebselen blocks the quinolinic acid-induced production of thiobarbituric acid reactive species but does not prevent the behavioral alterations produced by intra-striatal quinolinic acid administration in the rat," *Neuroscience Letters*, vol. 318, no. 3, pp. 137–140, 2002.

[68] S. S. Swathy and M. Indira, "The Ayurvedic drug, Ksheerabala, ameliorates quinolinic acid-induced oxidative stress in rat brain," *International Journal of Ayurveda Research*, vol. 1, no. 1, pp. 4–9, 2010.

[69] H. Kalonia, P. Kumar, A. Kumar, and B. Nehru, "Protective effect of montelukast against quinolinic acid/malonic acid induced neurotoxicity: possible behavioral, biochemical, mitochondrial and tumor necrosis factor-α level alterations in rats," *Neuroscience*, vol. 171, no. 1, pp. 284–299, 2010.

[70] H. Kalonia, P. Kumar, and A. Kumar, "Pioglitazone ameliorates behavioral, biochemical and cellular alterations in quinolinic acid induced neurotoxicity: possible role of peroxisome proliferator activated receptor-υ (PPARυ) in Huntington's disease," *Pharmacology Biochemistry and Behavior*, vol. 96, no. 2, pp. 115–124, 2010.

[71] H. Kalonia, P. Kumar, and A. Kumar, "Licofelone attenuates quinolinic acid induced Huntington like symptoms: possible behavioral, biochemical and cellular alterations," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 35, no. 2, pp. 607–615, 2011.

[72] M. Misztal, T. Frankiewicz, C. G. Parsons, and W. Danysz, "Learning deficits induced by chronic intraventricular infusion of quinolinic acid—protection by MK-801 and memantine," *European Journal of Pharmacology*, vol. 296, no. 1, pp. 1–8, 1996.

[73] H. Kalonia, P. Kumar, B. Nehru, and A. Kumar, "Neuroprotective effect of MK-801 against intra-striatal quinolinic acid induced behavioral, oxidative stress and cellular alterations in rats," *Indian Journal of Experimental Biology*, vol. 47, no. 11, pp. 880–892, 2009.

[74] Y. Chen, B. J. Brew, and G. J. Guillemin, "Characterization of the kynurenine pathway in NSC-34 cell line: implications for amyotrophic lateral sclerosis," *Journal of Neurochemistry*, vol. 118, no. 5, pp. 816–825, 2011.

[75] H. Kalonia, P. Kumar, A. Kumar, and B. Nehru, "Effects of caffeic acid, rofecoxib, and their combination against quinolinic acid-induced behavioral alterations and disruption in glutathione redox status," *Neuroscience Bulletin*, vol. 25, no. 6, pp. 343–352, 2009.

[76] H. Kalonia, P. Kumar, A. Kumar, and B. Nehru, "Protective effect of rofecoxib and nimesulide against intra-striatal quinolinic acid-induced behavioral, oxidative stress and mitochondrial dysfunctions in rats," *NeuroToxicology*, vol. 31, no. 2, pp. 195–203, 2010.

[77] N. Braidy, R. Grant, S. Adams, B. J. Brew, and G. J. Guillemin, "Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons," *Neurotoxicity Research*, vol. 16, no. 1, pp. 77–86, 2009.

[78] J. H. Connick and T. W. Stone, "Quinolinic acid effects on amino acid release from the rat cerebral cortex in vitro and in vivo," *British Journal of Pharmacology*, vol. 93, no. 4, pp. 868–876, 1988.

[79] V. Pérez-De La Cruz, M. Konigsberg, J. Pedraza-Chaverri et al., "Cytoplasmic calcium mediates oxidative damage in an excitotoxic/energetic deficit synergic model in rats," *The European Journal of Neuroscience*, vol. 27, no. 5, pp. 1075–1085, 2008.

[80] D. Silva-Adaya, V. Pérez-De La Cruz, M. N. Herrera-Mundo et al., "Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine," *Journal of Neurochemistry*, vol. 105, no. 3, pp. 677–689, 2008.

[81] D. Elinos-Calderón, Y. Robledo-Arratia, V. Pérez-De La Cruz, J. Pedraza-Chaverrí, S. F. Ali, and A. Santamaría, "Early nerve ending rescue from oxidative damage and energy failure by l-carnitine as post-treatment in two neurotoxic models in rat: recovery of antioxidant and reductive capacities," *Experimental Brain Research*, vol. 197, no. 3, pp. 287–296, 2009.

[82] A. Dairam, P. Chetty, and S. Daya, "Non-steroidal anti-inflammatory agents, tolmetin and sulindac, attenuate oxidative stress in rat brain homogenate and reduce quinolinic acid-induced neurodegeneration in rat hippocampal neurons," *Metabolic Brain Disease*, vol. 21, no. 2-3, pp. 221–233, 2006.

[83] A. Dairam, A. C. Müller, and S. Daya, "Non-steroidal anti-inflammatory agents, tolmetin and sulindac attenuate quinolinic acid (QA)-induced oxidative stress in primary hippocampal neurons and reduce QA-induced spatial reference memory deficits in male Wistar rats," *Life Sciences*, vol. 80, no. 15, pp. 1431–1438, 2007.

[84] A. C. Müller, H. Maharaj, D. S. Maharaj, and S. Daya, "Aciclovir protects against quinolinic-acid-induced oxidative neurotoxicity," *Journal of Pharmacy and Pharmacology*, vol. 57, no. 7, pp. 883–888, 2005.

[85] A. Santamaría, D. Santamaría, M. Díaz-Muñoz, V. Espinoza-González, and C. Ríos, "Effects of Nω-nitro-L-arginine and L-arginine on quinolinic acid-induced lipid peroxidation," *Toxicology Letters*, vol. 93, no. 2-3, pp. 117–124, 1997.

[86] V. Pérez-De La Cruz, C. González-Cortés, S. Galván-Arzate et al., "Excitotoxic brain damage involves early peroxynitrite formation in a model of Huntington's disease in rats: protective role of iron porphyrinate 5,10,15,20-tetrakis (4-sulfonatophenyl)porphyrinate iron (III)," *Neuroscience*, vol. 135, no. 2, pp. 463–474, 2005.

[87] D. Santiago-López, B. Vázquez-Román, V. Pérez-de la Cruz et al., "Peroxynitrite decomposition catalyst, iron metalloporphyrin, reduces quinolinate-induced neurotoxicity in rats," *Synapse*, vol. 54, no. 4, pp. 233–238, 2004.

[88] C. González-Cortés, C. Salinas-Lara, M. A. Gómez-López et al., "Iron porphyrinate Fe(TPPS) reduces brain cell damage in rats intrastriatally lesioned by quinolinate," *Neurotoxicology and Teratology*, vol. 30, no. 6, pp. 510–519, 2008.

[89] H. R. Sadeghnia, M. Kamkar, E. Assadpour, M. T. Boroushaki, and A. Ghorbani, "Protective effect of Safranal, a constituent of crocus sativus, on quinolinic acid-induced oxidative damage in rat hippocampus," *Iranian Journal of Basic Medical Sciences*, vol. 16, no. 1, pp. 73–82, 2013.

[90] N. Braidy, R. Grant, S. Adams, and G. J. Guillemin, "Neuroprotective effects of naturally occurring polyphenols on quinolinic acid-induced excitotoxicity in human neurons," *The FEBS Journal*, vol. 277, no. 2, pp. 368–382, 2010.

[91] E. Gulaj, K. Pawlak, B. Bien, and D. Pawlak, "Kynurenine and its metabolites in Alzheimer's disease patients," *Advances in Medical Sciences*, vol. 55, no. 2, pp. 204–211, 2010.

[92] W. Wu, J. A. Nicolazzo, L. Wen et al., "Expression of tryptophan 2, 3-dioxygenase and production of kynurenine pathway metabolites in triple transgenic mice and human Alzheimer's disease brain," *PloS One*, vol. 8, no. 4, Article ID e59749, 2013.

[93] G. J. Guillemin, G. A. Smythe, L. A. Veas, O. Takikawa, and B. J. Brew, "A beta 1-42 induces production of quinolinic acid by human macrophages and microglia," *Neuroreport*, vol. 14, no. 18, pp. 2311–2315, 2003.

[94] P. Guidetti, R. E. Luthi-Carter, S. J. Augood, and R. Schwarcz, "Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease," *Neurobiology of Disease*, vol. 17, no. 3, pp. 455–461, 2004.

[95] P. Guidetti, G. P. Bates, R. K. Graham et al., "Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington disease mice," *Neurobiology of Disease*, vol. 23, no. 1, pp. 190–197, 2006.

[96] M. P. Heyes, K. Saito, A. Lackner, C. A. Wiley, C. L. Achim, and S. P. Markey, "Sources of the neurotoxin quinolinic acid in the brain of HIV-1-infected patients and retrovirus-infected macaques," *FASEB Journal*, vol. 12, no. 10, pp. 881–896, 1998.

[97] M. P. Heyes, B. J. Brew, K. Saito et al., "Inter-relationships between quinolinic acid, neuroactive kynurenines, neopterin and β2-microglobulin in cerebrospinal fluid and serum of HIV-1-infected patients," *Journal of Neuroimmunology*, vol. 40, no. 1, pp. 71–80, 1992.

[98] G. J. Guillemin, S. J. Kerr, and B. J. Brew, "Involvement of quinolinic acid in aids dementia complex," *Neurotoxicity Research*, vol. 7, no. 1-2, pp. 103–123, 2005.

[99] S. Erhardt, C. K. Lim, K. R. Linderholm et al., "Connecting inflammation with glutamate agonism in suicidality," *Neuropsychopharmacology*, vol. 38, no. 5, pp. 743–752, 2013.

[100] J. Steiner, M. Walter, T. Gos et al., "Severe depression is associated with increased microglial quinolinic acid in subregions of the anterior cingulate gyrus: evidence for an immune-modulated glutamatergic neurotransmission?" *Journal of Neuroinflammation*, vol. 8, article 94, 2011.

[101] A. W. Zimmerman, H. Jyonouchi, A. M. Comi et al., "Cerebrospinal fluid and serum markers of inflammation in autism," *Pediatric Neurology*, vol. 33, no. 3, pp. 195–201, 2005.

[102] Y. Chen, R. Stankovic, K. M. Cullen et al., "The kynurenine pathway and inflammation in amyotrophic lateral sclerosis," *Neurotoxicity Research*, vol. 18, no. 2, pp. 132–142, 2010.

[103] E. M. Flanagan, J. B. Erickson, O. H. Viveros, S. Y. Chang, and J. F. Reinhard Jr., "Neurotoxin quinolinic acid is selectively elevated in spinal cords of rats with experimental allergic encephalomyelitis," *Journal of Neurochemistry*, vol. 64, no. 3, pp. 1192–1196, 1995.

[104] J. Füvesi, C. Rajda, K. Bencsik, J. Toldi, and L. Vécsei, "The role of kynurenines in the pathomechanism of amyotrophic lateral sclerosis and multiple sclerosis: therapeutic implications," *Journal of Neural Transmission*, vol. 119, no. 2, pp. 225–234, 2012.

[105] H. Iwahashi, H. Kawamori, and K. Fukushima, "Quinolinic acid, α-picolinic acid, fusaric acid, and 2,6-pyridinedicarboxylic acid enhance the Fenton reaction in phosphate buffer," *Chemico-Biological Interactions*, vol. 118, no. 3, pp. 201–215, 1999.

[106] K. Goda, R. Kishimoto, S. Shimizu, Y. Hamane, and M. Ueda, "Quinolinic acid and active oxygens. Possible contribution of active Oxygens during cell death in the brain," *Advances in Experimental Medicine and Biology*, vol. 398, pp. 247–254, 1996.

[107] V. Perez-De La Cruz, D. Elinos-Calderón, P. Carrillo-Mora et al., "Time-course correlation of early toxic events in three models of striatal damage: modulation by proteases inhibition," *Neurochemistry International*, vol. 56, no. 6-7, pp. 834–842, 2010.

[108] E. Rodríguez-Martínez, A. Camacho, P. D. Maldonado et al., "Effect of quinolinic acid on endogenous antioxidants in rat corpus striatum," *Brain Research*, vol. 858, no. 2, pp. 436–439, 2000.

[109] A. Santamaría, M. E. Jiménez-Capdeville, A. Camacho, E. Rodríguez-Martínez, A. Flores, and S. Galván-Arzate, "In vivo hydroxyl radical formation after quinolinic acid infusion into rat corpus striatum," *NeuroReport*, vol. 12, no. 12, pp. 2693–2696, 2001.

[110] G. Leipnitz, C. Schumacher, K. Scussiato et al., "Quinolinic acid reduces the antioxidant defenses in cerebral cortex of young rats," *International Journal of Developmental Neuroscience*, vol. 23, no. 8, pp. 695–701, 2005.

[111] H. Noack, J. Lindenau, F. Rothe, K. Asayama, and G. Wolf, "Differential expression of superoxide dismutase isoforms in neuronal and glial compartments in the course of excitotoxically mediated neurodegeneration: relation to oxidative and nitrergic stress," *Glia*, vol. 23, no. 4, pp. 285–297, 1998.

[112] C. Tronel, G. Y. Rochefort, N. Arlicot, S. Bodard, S. Chalon, and D. Antier, "Oxidative stress is related to the deleterious effects of heme oxygenase-1 in an in vivo neuroinflammatory rat model," *Oxidative Medicine and Cellular Longevity*, vol. 2013, Article ID 264935, 10 pages, 2013.

[113] M. P. Heyes, C. Y. Chen, E. O. Major, and K. Saito, "Different kynurenine pathway enzymes limit quinolinic acid formation by various human cell types," *The Biochemical Journal*, vol. 326, no. 2, pp. 351–356, 1997.

[114] M. G. Espey, J. R. Moffett, and M. A. A. Namboodiri, "Temporal and spatial changes of quinolinic acid immunoreactivity in the immune system of lipopolysaccharide stimulated mice," *Journal of Leukocyte Biology*, vol. 57, no. 2, pp. 199–206, 1995.

[115] R. Hanbury, Z. D. Ling, J. Wuu, and J. H. Kordower, "GFAP knockout mice have increased levels of GDNF that protect striatal neurons from metabolic and excitotoxic insults," *Journal of Comparative Neurology*, vol. 461, no. 3, pp. 307–316, 2003.

[116] G. J. Guillemin, J. Croitoru-Lamoury, D. Dormont, P. J. Armati, and B. J. Brew, "Quinolinic acid upregulates chemokine production and chemokine receptor expression in astrocytes," *GLIA*, vol. 41, no. 4, pp. 371–381, 2003.

[117] K. E. Beagles, P. F. Morrison, and M. P. Heyes, "Quinolinic acid in vivo synthesis rates, extracellular concentrations, and intercompartmental distributions in normal and immune-activated brain as determined by multiple-isotope microdialysis," *Journal of Neurochemistry*, vol. 70, no. 1, pp. 281–291, 1998.

[118] F. Block and M. Schwarz, "Expression of GFAP in the striatum and its projection areas in response to striatal quinolinic acid lesion in rats," *NeuroReport*, vol. 5, no. 17, pp. 2237–2240, 1994.

[119] J. Schiefer, R. Töpper, W. Schmidt et al., "Expression of interleukin 6 in the rat striatum following stereotaxic injection of quinolinic acid," *Journal of Neuroimmunology*, vol. 89, no. 1-2, pp. 168–176, 1998.

[120] H. Kalonia and A. Kumar, "Suppressing inflammatory cascade by cyclo-oxygenase inhibitors attenuates quinolinic acid induced Huntington's disease-like alterations in rats," *Life Sciences*, vol. 88, no. 17-18, pp. 784–791, 2011.

[121] S. G. Wilt, E. Milward, J. M. Zhou et al., "In vitro evidence for a dual role of tumor necrosis factor-α in human immunodeficiency virus type 1 encephalopathy," *Annals of Neurology*, vol. 37, no. 3, pp. 381–394, 1995.

[122] W. Cammer, "Oligodendrocyte killing by quinolinic acid in vitro," *Brain Research*, vol. 896, no. 1-2, pp. 157–160, 2001.

[123] J. S. Pober and R. S. Cotran, "Cytokines and endothelial cell biology," *Physiological Reviews*, vol. 70, no. 2, pp. 427–451, 1990.

[124] K. Schulze-Osthoff, A. C. Bakker, B. Vanhaesebroeck, R. Beyaert, W. A. Jacob, and W. Fiers, "Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation," *The Journal of Biological Chemistry*, vol. 267, no. 8, pp. 5317–5323, 1992.

[125] M. Hayashi, Y. Luo, J. Laning, R. M. Strieter, and M. E. Dorf, "Production and function of monocyte chemoattractant protein-1 and other β-chemokines in murine glial cells," *Journal of Neuroimmunology*, vol. 60, no. 1-2, pp. 143–150, 1995.

[126] M. P. Heyes, "Quinolinic acid and inflammation," *Annals of the New York Academy of Sciences*, vol. 679, pp. 211–216, 1993.

[127] P. R. Sanberg, S. F. Calderon, M. Giordano, J. M. Tew, and A. B. Norman, "The quinolinic acid model of Huntington's disease: locomotor abnormalities," *Experimental Neurology*, vol. 105, no. 1, pp. 45–53, 1989.

[128] V. Pérez-De La Cruz, D. Elinos-Calderón, Y. Robledo-Arratia et al., "Targeting oxidative/nitrergic stress ameliorates motor impairment, and attenuates synaptic mitochondrial dysfunction and lipid peroxidation in two models of Huntington's disease," *Behavioural Brain Research*, vol. 199, no. 2, pp. 210–217, 2009.

[129] D. A. Shear, J. Dong, K. L. Haik-Creguer, T. J. Bazzett, R. L. Albin, and G. L. Dunbar, "Chronic administration of quinolinic acid in the rat striatum causes spatial learning deficits in a radial arm water maze task," *Experimental Neurology*, vol. 150, no. 2, pp. 305–311, 1998.

[130] E. M. Vazey, K. Chen, S. M. Hughes, and B. Connor, "Transplanted adult neural progenitor cells survive, differentiate and reduce motor function impairment in a rodent model of Huntington's disease," *Experimental Neurology*, vol. 199, no. 2, pp. 384–396, 2006.

[131] E. G. McGeer and E. Singh, "Neurotoxic effects of endogenous materials: quinolinic acid, L-pyroglutamic acid, and thyroid releasing hormone (TRH)," *Experimental Neurology*, vol. 86, no. 2, pp. 410–413, 1984.

[132] A. P. Kells, R. A. Henry, and B. Connor, "AAV-BDNF mediated attenuation of quinolinic acid-induced neuropathology and motor function impairment," *Gene Therapy*, vol. 15, no. 13, pp. 966–977, 2008.

[133] C. Portera-Cailliau, D. L. Price, and L. J. Martin, "Non-NMDA and NMDA receptor-mediated excitotoxic neuronal deaths in adult brain are morphologically distinct: further evidence for an apoptosis-necrosis continuum," *The Journal of Comparative Neurology*, vol. 378, no. 1, pp. 88–104, 1997.

[134] K. Ishidoh, N. Kamemura, T. Imagawa, M. Oda, J. Sakurai, and N. Katunuma, "Quinolinate phosphoribosyl transferase, a key enzyme in de novo NAD+ synthesis, suppresses spontaneous cell death by inhibiting overproduction of active-caspase-3," *Biochimica et Biophysica Acta*, vol. 1803, no. 5, pp. 527–533, 2010.

[135] A. Macaya, F. Munell, R. M. Gubits, and R. E. Burke, "Apoptosis in substantia nigra following developmental striatal excitotoxic injury," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 17, pp. 8117–8121, 1994.

[136] B. S. Jeon, N. G. Kholodilov, T. F. Oo et al., "Activation of caspase-3 in developmental models of programmed cell death in neurons of the substantia nigra," *Journal of Neurochemistry*, vol. 73, no. 1, pp. 322–333, 1999.

[137] G. J. Guillemin, L. Wang, and B. J. Brew, "Quinolinic acid selectively induces apoptosis of human astocytes: potential role in AIDS dementia complex," *Journal of Neuroinflammation*, vol. 2, article 16, 2005.

[138] P. E. Hughes, T. Alexi, T. Yoshida, S. S. Schreiber, and B. Knusel, "Excitotoxic lesion of rat brain with quinolinic acid induces expression of p53 messenger RNA and protein and p53-inducible genes Bax and Gadd-45 in brain areas showing DNA fragmentation," *Neuroscience*, vol. 74, no. 4, pp. 1143–1160, 1996.

[139] Y. M. Bordelon, L. Mackenzie, and M. F. Chesselet, "Morphology and compartmental location of cells exhibiting DNA damage after quinolinic acid injections into rat striatum," *The Journal of Comparative Neurology*, vol. 412, no. 1, pp. 38–50, 1999.

[140] H. Wei, Z.-H. Qin, V. V. Senatorov et al., "Lithium suppresses excitotoxicity-induced striatal lesions in a rat model of Huntington's disease," *Neuroscience*, vol. 106, no. 3, pp. 603–612, 2001.

[141] E. Pérez-Navarro, N. Gavaldà, E. Gratacòs, and J. Alberch, "Brain-derived neurotrophic factor prevents changes in Bcl-2 family members and caspase-3 activation induced by excitotoxicity in the striatum," *Journal of Neurochemistry*, vol. 92, no. 3, pp. 678–691, 2005.

[142] Z.-H. Qin, Y. Wang, and T. N. Chasea, "A caspase-3-like protease is involved in NF-κB activation induced by stimulation of N-methyl-d-aspartate receptors in rat striatum," *Molecular Brain Research*, vol. 80, no. 2, pp. 111–122, 2000.

[143] Y. Wang, X.-X. Dong, Y. Cao et al., "P53 induction contributes to excitotoxic neuronal death in rat striatum through apoptotic and autophagic mechanisms," *The European The Journal of Neuroscience*, vol. 30, no. 12, pp. 2258–2270, 2009.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 898034, 12 pages
http://dx.doi.org/10.1155/2013/898034

Review Article

Cadmium and Its Neurotoxic Effects

Bo Wang[1] and Yanli Du[2]

[1] *Department of Pathology, The Second Clinical Medical School of Inner Mongolia University for the Nationalities (Inner Mongolia Forestry General Hospital), Yakeshi 022150, Inner Mongolia, China*
[2] *Department of Neurosurgery, The Second Clinical Medical School of Inner Mongolia University for the Nationalities (Inner Mongolia Forestry General Hospital), Yakeshi 022150, Inner Mongolia, China*

Correspondence should be addressed to Yanli Du; 13804705515@163.com

Received 24 April 2013; Revised 28 June 2013; Accepted 30 June 2013

Academic Editor: Renata Santos

Cadmium (Cd) is a heavy metal that has received considerable concern environmentally and occupationally. Cd has a long biological half-life mainly due to its low rate of excretion from the body. Thus, prolonged exposure to Cd will cause toxic effect due to its accumulation over time in a variety of tissues, including kidneys, liver, central nervous system (CNS), and peripheral neuronal systems. Cd can be uptaken from the nasal mucosa or olfactory pathways into the peripheral and central neurons; for the latter, Cd can increase the blood brain barrier (BBB) permeability. However, mechanisms underlying Cd neurotoxicity remain not completely understood. Effect of Cd neurotransmitter, oxidative damage, interaction with other metals such as cobalt and zinc, estrogen-like, effect and epigenetic modification may all be the underlying mechanisms. Here, we review the *in vitro* and *in vivo* evidence of neurotoxic effects of Cd. The available finding indicates the neurotoxic effects of Cd that was associated with both biochemical changes of the cell and functional changes of central nervous system, suggesting that neurotoxic effects may play a role in the systemic toxic effects of the exposure to Cd, particularly the long-term exposure.

1. Introduction

Cadmium (Cd) is a toxic, nonessential transition metal and classified as a human carcinogen by the National Toxicology Program [1]. There are several sources of human exposure to Cd, including employment in primary metal industries, production of certain batteries, some electroplating processes (about 29% of year production), and consumption of tobacco products [2]. It is also of interest since the natural biogeochemical cycle of Cd has been overwhelmed. First, the concept of provisional tolerable weekly intake (PTWI) was established. The Joint FAO/WHO Expert Committee on Food Additives defines the PTWI for a chemical with no intended function as an estimate of the amount of the chemical that can be ingested weekly over a lifetime without appreciable health risk [3]. The first Cd PTWI was 400–500 μg per person per week. This level was based on a critical concentration of 200 mg Cd/g kidney cortex, attainable after a dietary Cd intake of 140–260 μg/d for over 50 years or 2000 mg over a lifetime. For several decades, the PTWI has been expressed more rationally in terms of the intake per kg body weight, and the value was constantly changed (Table 1). In 2010, the Consumer Product Safety Commission (CPSC) released a staff report recommending new guidance on Cd, that is, an acceptable daily intake level of 0.1 $\mu g\,kg^{-1}$ body weight per day for chronic exposure [4]. In most studies, the half-life of Cd in humans is estimated at a range of 15 to 20 years [5]. Epidemiological and experimental studies have linked the occupational Cd exposure with lung cancer and other cancers such as the prostate, renal, liver, hematopoietic system, urinary bladder, pancreatic, testis, and stomach cancers [6–8]. Exposure to Cd also severely affects the function of the nervous system [9, 10], with symptoms including headache and vertigo, olfactory dysfunction, parkinsonian-like symptoms, slowing of vasomotor functioning, peripheral neuropathy, decreased equilibrium, decreased ability to concentrate, and learning disabilities [11–13]. The neurotoxicity of Cd in children was investigated in several studies in the 1970s and 1980s but has received little attention since then. In case-control studies in which the hair concentration of Cd in a clinically defined group was compared with that of a reference group, higher concentrations of hair Cd were reported in

Table 1: Summary of the "safe" level for human contacted of Cd.

Organization/Authors	Year	Approach	Dose	Reference
FAO/WHO	1988	Intake	400–500 μg/week	[3]
WHO	1992	Intake	7 $\mu g \cdot kg^{-1}$ b.w./week or 1 $\mu g \cdot kg^{-1}$ b.w./day	[38]
FAO/WHO	1993	Intake	7 $\mu g \cdot kg^{-1}$ b.w./week	[39]
Satarug et al.	2000	Intake	30 μg/day	[40]
Nasreddine and Parent-Massin	2002	Intake	10–30 μg/day	[41]
WHO	2004	Drinking-water	3 μg/L	[42]
FAO/WHO	2006	Food	0.4 mg/kg of the BMDL of R-Cd standard	[43]
ACGIH.	2007	Blood	5 μg/L	[44]
EFSA	2009	Intake	2.5 $\mu g \cdot kg^{-1}$ b.w./week	[45]
FAO/WHO	2010	Intake	5.8 $\mu g \cdot kg^{-1}$ b.w./week	[46]
CPSC	2010	Intake	0.1 $\mu g \cdot kg^{-1}$ b.w./day	[4]

ACGIH: American conference of governmental industrial hygienists; CPSC: Consumer Product Safety Commission; FAO: Food and Agriculture Organization; WHO: World Health Organization; b.w.: body weight.

children with mental retardation [14] and learning difficulties or dyslexia [11, 15]. In cohort studies, Thatcher et al. reported that the concentration of Cd in hair was inversely related to adjust Intelligence Quotient (IQ) [16, 17]. Other investigators [18] also reported associations between hair Cd concentrations and children's performance on visual-motor tasks. These studies clearly indicate the association of increased total Cd concentration with mental retardation and reduced visual motor abilities. Effect of Cd neurotransmitter, oxidative damage, interaction with other metals such as cobalt and zinc, estrogen-like effect, and epigenetic modification may be the underlying mechanisms (Figure 1). However, the exact mechanism(s) through which Cd elicits its neurotoxic effects is still unresolved. In this review, therefore, we focus on recent evidence from experimental and epidemiological studies, showing that Cd exposure can induce its neurotoxin effects.

2. The Absorption of Cd in the Nervous System and Distribution

The Cd exposure rate (based on meconium analysis) in infants was 8.5%, and the median concentration of the pollutants in the positive samples was 13.37 mg/mL. In most of these studies, the concentration of Cd in hair was measured. The CNS is especially vulnerable to damage during early neonatal development; Cd is able to readily pass to the fetus via the placenta and was detected in milk during lactation [19]. Cd can be uptaken from the nasal mucosa or olfactory pathways into the CNS; thus, the CNS is subjected to Cd toxicity [20–22]. Under normal conditions, Cd barely reaches the brain in adults due to the presence of the blood brain barrier (BBB); however, this structure is not fully developed in young animals [23]. The anatomical and physiological bases on which the choroids plexus becomes the target of xenobiotics have been examined. Cd tends to accumulate in the choroids plexus at concentrations much greater than those found in the cerebrospinal fluid (CSF) and elsewhere in brain tissues. A postmortem human study revealed that the Cd concentration in the choroids plexus was about 2-3 times higher than that found in the brain cortex [24]. As a general choroids plexus toxicant, Cd can directly damage the choroids plexus ultrastructure. Due to differences in the BBB integrity [25], Cd is thus more toxic to newborn and young rats than to adult rats. Cd can increase permeability of the BBB in rats [26] to penetrate and accumulate in the brain of developing and adult rats [27, 28], leading to brain intracellular accumulation, cellular dysfunction, and cerebral edema.

As a barrier, the choroidal epithelia are often the first and the most frequent ones to encounter metal insults from blood. Two mechanisms may aid resistance of choroids plexus to blood-borne toxicities. First, the choroids plexus contains abundant metal binding ligands that effectively sequester metal ions. Moreover, the concentration of cystine in the choroids plexus is 4-fold higher than that in the brain cortex [29]. Second, the choroids plexus owns an active defense system. The activities of superoxide dismutase and catalase are significantly higher in the choroids plexus than in cerebrum and cerebellum. Taken together, the choroids plexus likely forms the first line of defense against neurotoxicants. It must be kept in mind, however, that none of the cellular defense mechanisms would operate in an unlimited capacity. The pathophysiological changes can occur either as the threshold above which the protective capacity of the choroids plexus is exceeded or saturated, or as a direct result of barrier dysfunction. Thus, the need arises for a more comprehensive understanding of the detoxification mechanisms, such as antioxidant systems, induction of protective macromolecules (heat shock proteins, etc.), formation of specific metal inclusion bodies or binding proteins, and biotransformation reactions (methylation, conjugation, etc.) that operate in the choroids plexus.

Cd is transported along the primary olfactory neurons to their terminations in the olfactory bulbs, thereby bypassing the intact BBB. The olfactory route could therefore be a likely way to reach the brain and should be taken into account for occupational risk assessments for this metal [30–32]. Occupational inhalation of Cd can be toxic to the olfactory sense [30]. Primary olfactory neurons are regularly

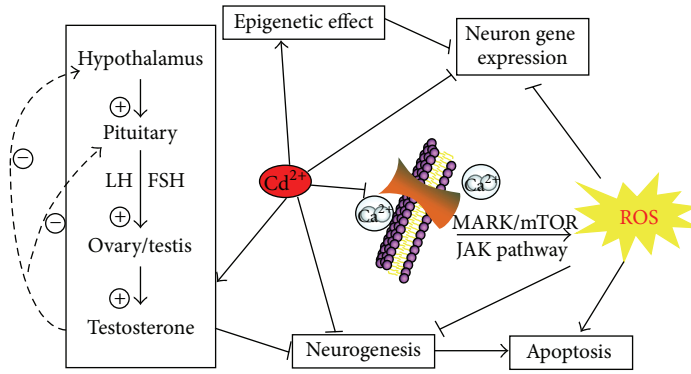


Figure 1: Mechanistic illustration of the neuronal toxicity of Cd. (1) Cd-induced neuron cell apoptosis and ROS (reactive oxygen species) are mediated through Ca^{2+}-mitochondria signaling and Ca^{2+}-membrane channels. (2) Cd impaired neurogenesis. (3) Cd accumulation in the brain leads to the altered gene expression and epigenetic effect. (4) Cd has estrogen-like effect, which can induce endocrine disruption by affecting the hypothalamic-pituitary-gonadal (HPG) axis in different aspects. Meanwhile, these potential mechanisms have possible interactions. The solid black arrows represent the stimulation, the solid black line segments indicate the inhibition, and the dotted lines represent the negative feedback control of the HPG axis.

replaced throughout the life span. Functional recovery and regeneration of olfactory neurons has been demonstrated in laboratory animals but not confirmed in humans [33]. Intranasal exposure to Cd has been related to olfactory dysfunction in humans and to nasal epithelial damage and altered odorant-guided behavior in rodent models. The pathophysiology underlying these deficits has been partly elucidated. Optical imaging revealed significant reductions in odorant-evoked release from the olfactory nerve at a Cd chloride dose two orders of magnitude less than that required to induce morphological changes in the nerve in the same animals, demonstrating that it is a more sensitive technique for assessing the consequences of intranasal neurotoxicant exposure. This approach is potentially useful in exploring the effects of any putative neurotoxicant that can be delivered intranasal [32].

3. The Influence of Cd to Central Nervous System (CNS)

3.1. The Morphology Change of CNS in Response to Cd. Cd-treated embryos developed a smaller head with unclear boundaries between the brain subdivisions, particularly in the mid-hindbrain region. Embryos display normal anterior to posterior regionalization; however, the commitment of neural progenitor cells was affected by Cd [34]. Cd has been shown to produce free radicals in the brain, which may potentially damage both neurons and oligodendrocytes (OLG). OLGs are the glial cells which myelinate axons in the CNS. An early study reported that Cd toxicity affected CNS white matter [35], and one laboratory demonstrated that OLGs are direct targets of this structure [36]. Experimental studies have shown that Cd can be a potent neurotoxicant for the peripheral nervous system. Moreover, Cd has a half-life of more than 15 years in humans. Elderly workers may be more susceptible to an increased Cd body burden and may develop a peripheral polyneuropathy (PNP) over time [37]. The primary olfactory neuron is the only sensory cell directly in contact with the environment and therefore potentially exposed to airborne toxicants; so olfactory damage may represent the early action of a toxicant on the nervous system; the primary olfactory neuron may represent the early target for airborne Cd toxic action.

In cortical neurons cell culture from fetal rats at 19 days of gestation, Cd modified the neuronal morphology after a 6 h treatment in a serum-free medium with 10 μM of Cd, whereas at 24 h it showed a great loss of neuronal integrity mainly evidenced by the almost complete disappearance of the axons. Lower concentrations of Cd than 1 μM could induce cell apoptosis and higher concentrations of Cd than 1 μM could induce necrotic cell death [9]. Cd affected the cell morphology; the morphological changes were mainly located in the neural extensions (axons and dendrites), which almost disappeared after 24 h of treatment with 1 μM of Cd in a serum-free medium [9, 47]. Such morphological changes induced by Cd have also been described in other cells [48]. Cd inhibited neurite outgrowth at concentrations that decreased viability in Neuroscreen-1 cells (NS-1) models, a subclone of PC12 cells, using high content screening. The previous studies have shown that cultured oligodendrocytes are directly damaged by Cd exposure, and continuous exposure (18–48 h) of OLPs to low micromolar concentrations (0.001–25 μM) of Cd significantly decreased mitochondrial metabolic activity, increased LDH leakage starting at 5 mM, and maximally activated caspase-3. These results suggest that Cd induces OLP cell death mainly by apoptosis, and at higher concentrations or with prolonged exposure to the heavy metal there is an increase in cytoplasmic membrane damage, an index of necrosis. More importantly, transient exposure to Cd is sufficient to damage OLPs and could in principle impair myelination in the neonate. Although the effects of Cd on neuronal cells in culture have been described in several papers, its effects on human central nervous system neurons *in vivo* have not yet been demonstrated. However, the recently described Cd-induced apoptosis of the motor neurons of the ventral horns in cultured explants from human fetal spinal

cords (10-11 weeks gestational age) [49] suggests that the effects observed *in vitro* could actually occur also *in vivo*.

Increasing evidence has demonstrated that Cd is a possible etiological factor of neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) [50, 51]. Cerebral cortical neurons have been identified as targets of Cd-mediated toxicity and Cd-induced cell apoptosis [52, 53]. Apoptotic morphological changes induced by Cd in cerebral cortical neurons were assessed in some studies [53, 54].

In vivo study, the age and species of experimental animals could be effected the CNS results of damage. Perinatal exposure to Cd (50 ppm in drinking water) reduced the brain weights of pups and inhibited the activities of enzymes in nervous system, for example acetylcholine esterase, K^+-ATPase, CNP (cyclic nucleotide phosphodiesterase), and 50-nucleotidase [55]. The Cd concentration in the choroids plexus was about 2-3 times higher than that found in the brain cortex. Cd-produced deterioration of the plexus structure can be characterized by the loss of microvilli, a rupture of the apical surface, and an increased number of blebs. Cellular debris present in the ventricular lumen may result from the breaking of the apical membrane. The epithelial cells display an abnormally high number of cytoplasmic vacuoles and lysosomes with condensed or irregular nuclei. As a general choroids plexus toxicant, Cd directly destroys the plexus ultrastructure. In both chronic (22 weeks) and acute (1–24 days) exposure models, the levels of Cd in the choroids plexus were high, while Cd in the CSF fell below the detection limit [29].

3.2. The Biochemical Changes of CNS in Response to Cd. The cholinergic system, with acetylcholine (ACh) as the neurotransmitter, is involved in cognitive processes, through the activation of metabotropic muscarinic and ionotropic nicotinic cholinergic receptors. The reaction responsible for the maintenance of levels of ACh is catalyzed by two cholinesterases (ChE): acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE is an important biomarker for several environmental contaminants in zebrafish [56, 57]. In addition, it is also known that this enzyme plays an important role in diseases with an increasing incidence in the elderly population, such as Alzheimer disease. Zebrafish (Danio rerio) is an emergent vertebrate model for studying several biological events, such as neurochemical alterations promoted by heavy metal toxicity. This teleost possesses only the gene for AChE, which is responsible for the whole ACh degradation, being the BuChE absent. The AChE gene has already been identified, cloned, and functionally detected in the zebrafish brain. The recently reported on the effects of long-term dietary-induced exposure to Cd on the AChE activity of adult rodents' brain regions. The authors studied the changes in the activities of AChE and Na^+, K^+-ATPase in the cerebral cortex, hippocampus, hypothalamus, cerebellum, and striatum of adult Wistar rats, following a 5-month (long-term) exposure to an experimental diet supplemented with low levels of Cd salt or with Cd-contaminated potato tubers. The authors also assessed the behavioral (cognitive-, motor-, and anxiety-related) outcomes following the above-mentioned treatment [58]. But the other research term queried that their study can be "regarded as a significant contribution to the field, as there is paucity of information on the AChE activity in brain regions following exposure to Cd" and "the experimental protocol used in the aforementioned study [58] is exceptionally well designed in order to simulate long-term dietary-induced exposure to Cd" [59].

Cd can affect the degree and balance of excitation inhibition in synaptic neurotransmission as well as the antioxidant levels in animal brain [28]. Cd increases the serotonin sensibility in the CNS [60]. Cd inhibits the release of acetylcholine, probably by interfering with calcium metabolism [61]. A remarkable reduction in the concentration of brain galactosylceramide (30%–43%) and 3′-sulphogalactosylceramide (24%–37%) at 21, 30, and 45 days of age was observed following pre- and postnatal exposure of Cd in comparison to the respective controls. The ontogenic profile of different brain phospholipids in the Cd-exposed group showed an increase in the levels of phosphatidylethanolamine at 21 (31%), 30 (25%) and 45 (19%) days of age; the phosphatidylcholine contents increased at day 21 (14%), followed by a significant decrease at 30 (14%) and 45 (19%) days compared to age-matched controls. Brain phosphatidylinositol, phosphatidylserine, and sphingomyelin did not show any alteration at early periods of exposure but decreased significantly following continued exposure by 34%, 45%, and 21%, respectively, at 45 days of age [62].

Numerous studies have shown that cadmium caused a decrease in depolarization-evoked exocytotic release of glutamate (as well as other neurotransmitters) from nerve terminals [73]. Cd^{2+} can stimulate [^{3}H]-glutamate binding in human platelets. Cd^{2+} can increase lipid peroxidation levels and reactive oxygen species (ROS) measurement in platelets. Glutamatergic system may be used as a potential biomarker for neurotoxic action of Cd in humans [74].

3.3. The Central Activity Changes in Response to Cd. Cd may alter the stimulus properties of morphine in adult male rat model [75]. As shown in Table 2, perinatal Cd exposure has been shown to alter behaviors and reduce learning ability. In addition, high levels of Cd and lead in children's hair were associated with learning disabilities. Motor and perceptual abilities of children exposed to Cd in uteri were significantly affected [76]. Behavioral defects, neurochemical changes, and brain lesions were reported in experimental animals, while in humans acute Cd poisoning produced Parkinsonism symptoms [50]. Blood Cd in motor neuron disease (MND, with limited disability) was higher than controls [77, 78]. Plasma Cd levels in the sporadic motor neuron disease (SMND) group were significantly increased compared to controls [78]. A recent study using a fully automated, observer-independent procedure to study morphological alterations of the central nervous system demonstrated that myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) patients had an average decrease of the volume of the gray matter of about 8% compared to matched healthy controls [79]. The gray matter volume reduction was significantly associated with objective reduction of physical activity in ME/CFS patients. Deficits in learning and altered

Table 2: Literature review of Cd neurotoxicity in humans and rats.

Year	Study design	Age group	E/C (*n*)	Exposure to Cd	Expose pathways	Effects	Reference
1961	Cross-sectional	Male worker	106E/84C	—	Occupational exposure	Anosmia	[63]
1977	Cross- sectional	Children	31E/22C	CdH	Daily life	Neurological disorders, such as learning disabilities and hyperactivity	[11]
1981	Cross-sectional	Children	73E/44C	CdH	Daily life	Dyslexic, learning disorder	[15]
1981	Cross-sectional	Workers	49E	CdU	Occupational exposure	Polyneuropathy	[64]
1982	Cross-sectional	Children	149	CdH	Daily life	Effect on verbal I.Q.	[16]
1985	Case-control	Young men	40	CdH	Daily life	Behavioural difficulty	[65]
1985	Cross-sectional	Children	69	CdH	Daily life	Nonadaptive classroom behavior, affected behavioral development visuomotor skills ↓	[18]
1989	Cross-sectional	Male workers	31E	CdU	Occupational exposure	↓ Attention, memory, and psychomotor speed	[66]
1992	Cross-sectional	Worker	38E	CdU	Occupational exposure	90% Headache; 42% dizzy spells 21% weakness; 16% brain atrophy	[67]
1992	Cross-sectional	Worker	55E/16C	CdU	Occupational exposure	Hyposmia	[68]
1997	Case report	Old man	1	Multiple organ failure	Occupational exposure, acute	Parkinsonism	[50]
1999	Cross-sectional	Worker	13E/19C	CdU	Occupational exposure	Polyneuropathy	[37]
2000	Cross-sectional	Adult worker	42E/47C	CdU	Occupational exposure	↓ Motor speed, attention, memory ↑ equilibrium, PNP, and concentration complaints	[69]
2006	Case report	Adult workers	1	CdU	Inhale the fumes	Peripheral neuropathy	[70]
2009	Cross-sectional	Children	549	CdH	Daily life	Withdrawal, social problems and attention problems associated	[71]
2012	Wistar rats	Male	20E/20C	Intratracheal instillation	Experiment exposure	Dose- and time-dependent shift from slower to faster waves	[72]

E: exposed subjects, C: control subjects; CdU: urinary cadmium concentration; CdH: concentration of cadmium in hair; I.Q.: Intelligence Quotient.

behaviors and activities were also observed in offspring exposed to Cd during gestational and/or lactational period. Cd induced neuronal death in cortical neurons through a combined mechanism of apoptosis and necrosis involving reactive oxygen species generation and lipid peroxidation [80]. With regard to the assessment of behavioral disorders, the cross-sectional study by Bao et al., from China, revealed a higher frequency of withdrawal, social problems, and attention problems associated with higher levels of cadmium in hair of children aged 7–16 years [71]. But another study did not find any significant association between cadmium exposure and ADHD [81].

In animal study, the role of the nanosized Cd in the causation of nervous system damages and shows the possibility of modeling human neurotoxic damage in rats [72].

4. The Mechanism by Which Cd Affects CNS

4.1. Cd and Oxidative Damage. Cd-induced injury in the cerebral microvessels is thought to be associated with oxidative stress. Following *in vivo* Cd exposure, there was an early increase followed by a later decrease in microvessel enzymes involved in cellular redox reactions, such as superoxide dismutase, glutathione peroxidase, and catalase. Thus, a depletion of microvessel antioxidant defense systems and a resultant increase in lipid peroxidation (LPO) may provoke microvessel damage [82]. Upon exposure, Cd has been shown to induce heavy metal-binding proteins such as metallothionein (MT) in various organs. MT is a low-molecular-weight protein, 6,500 Da with high cysteine content and high metal affinity, which plays a major role in the kinetics and metabolism of Cd. Four isoforms have been identified, namely, MT-I to MT-IV. Metallothionein-3 (MT-III) is specifically expressed in the brain; however, it is down-regulated, and thus deficient in Alzheimer's disease [83]. The choroids plexus also expresses MT proteins. Nishimura et al. observed a strong MT immunostaining in ependymal cells and choroids plexus epithelium in younger rats (1–3 weeks old) poisoned with Cd. Thus, the sequestration of Cd by MT may partly contribute to the high accumulation of Cd in the

choroids plexus [84]. Cd significantly increases the levels of lipid peroxidation in parietal cortex, striatum, and cerebellum as compared to a control group, and dexamethasone (Dx) treatment prevented the increase in LPO levels associated to Cd exposure, probably through the increase in MT content [85]. So MT as a protective mechanism against Cd-induced neurotoxicity is suggested.

In addition, mitochondria play a role in stress responses and can produce ROS when damaged. Mitochondria are indeed a major source of ROS. The enzyme COX can serve as an indicator of mitochondrial function. This is because COX dysfunction increases ROS, reduces energy stores, and impairs energy metabolism [86]. Accumulating evidences indicate that Cd-induced neuronal toxicity is due to induction of ROS, which leads to oxidative stress [54, 87]. Recently, Cd induced ROS generation in a time- and concentration-dependent manner in PC12 and SH-SY5Y cells [54], which causes apoptosis of neuronal cells via activation of MAPKs (mitogen-activated protein kinases) and mTOR (mammalian target of rapamycin) signaling pathways [54, 87, 88].

4.2. Interaction and/or Impact of Other Metals Including Calcium, Zinc, and Cobalt on Cd Neurotoxicity. Cerebral cortical neurons have been identified as targets of Cd-mediated toxicity [52]. And Cd-induced cerebral cortical neurons apoptosis occurs through Ca^{2+}-mitochondria signaling [52, 53]. Cd disrupts intracellular free calcium ([$Ca^{(2+)}$] i) homeostasis, leading to apoptosis in a variety of cells including primary murine neurons. Calcium is a ubiquitous intracellular ion which acts as a signaling mediator in numerous cellular processes including cell proliferation, differentiation, and survival/death. Few studies have focused on the interaction between Cd and Ca-binding molecules, such as calmodulin.

Cd may block the influx of Ca^{2+} through membrane channels into the nerve terminal following the action potential; these decreases in calcium influx caused by Cd would be associated with an altered transmitter release [89]. Of the receptor agonists tested partially inhibited by Cd^{2+} [90]. Cd ions are often used to block high-threshold Ca^{2+} currents [91] but may also block low-threshold Ca^{2+} currents [92]. When 100 μM Cd was added to the external solution, a minor fraction, 8.6% ± 8.9%, of the low-threshold current at −40 mV (holding potential −90 mV) was blocked [93]. Further studies are needed to define which molecular effects are indeed elicited by Cd-calmodulin and/or whether Cd^{2+} binds to other regulators of the Ca^{2+}-induced signaling pathways.

In a new study, Cd-induced apoptosis is associated with calcium-induced massive production of ROS, dissipation of mitochondrial membrane potential ($\Delta\Psi m$), cleavage of caspase-9, caspase-3, and PARP. And the results demonstrate that Cd-induced apoptosis is mediated by calcium signaling pathway, and calcium-mediated apoptosis occurs through the mitochondria-caspase signaling pathway [53].

Although Cd is not accumulated in significant quantities into the brain following exposure, it disturbs the metabolism of Cu and Zn. Because zinc (Zn) and Cd are cations of similar size and charge, and Cd has been shown to inhibit Zn uptake in a variety of systems, Cd is using transport systems that normally function to regulate Zn levels in brain. Metal analysis in the brain showed a reduction in zinc and copper levels at 15 and 21 days of age in Cd-exposed animals. It may be concluded that an early exposure of Cd may produce alteration in the development of different lipids, which may produce CNS dysfunctions with a possibility of being manifested even in later life [62]. The most well studied metallothioneins are isoforms of metallothioneins I and II that are expressed in almost all mammalian tissues. Metallothionein III is expressed in brain and is rich in zinc. Since the blood-brain barrier keeps Cd outside the CNS, reported neurotoxic effects of Cd during development are likely to be secondary to an interference of Cd with Zn-metabolism and not a direct effect of Cd on brain cells. It is therefore of importance to investigate whether neurotoxicity induced by Cd is related to mechanisms involving MT III in brain [5].

Very few articles study neurotoxin effects about the effect of cobalt (Co^{2+}). This study addition of inorganic Ca^{2+} channel blockers (Cd^{2+} or Co^{2+}) was used to demonstrate the Ca^{2+} dependence of Ca^{2+}-activated k^{+} currents [94]. Cd^{2+} and Co^{2+} were the nonspecific calcium channel antagonists in the study [95, 96]. Co^{2+} did not modify significantly the ATP-evoked current [97]. Application of high potassium media to growth cones inhibited neurite outgrowth, an effect that was blocked by 2 mM cobalt or 100 μM Cd, suggesting that Ca^{2+} influx via voltage-gated channels contributes to glutamate-induced regulation of neuritis outgrowth [98].

4.3. Cd and Neurogenesis. There is evidence that relates arsenic and manganese exposure with neurodevelopmental problems in children, but there is little information on cadmium exposure. Cd-induced neurotoxicity might be caused by impaired neurogenesis, resulting in markedly reduced neuronal differentiation and axonogenesis, leading to neuronal cell death [99]. The present study compares the sensitivity of human (ReN CX) and mouse (mCNS) neuroprogenitor cell lines to chemicals using a multiplex assay for proliferation and apoptosis, endpoints that are critical for neural development. Cells were exposed to 0.001–100 μM concentrations of Cd to affect proliferation and/or apoptosis. Cd decreased proliferation by at least 50% of control in either the ReN CX or mCNS cells. Compared to control, Cd decreased cell viability (ATP levels) by at least 50% in the ReN CX cells, while Cd decreased viability by at least 50% in the mCNS cells. Based on these results, BrdU is an appropriate marker for assessing chemical effects on proliferation, and human cells are more sensitive than mouse cells for this endpoint [100]. In the mammalian brain, the complex molecular pathways underlying neurogenesis provide a variety of possible targets that might be impacted by Cd exposure and identifying which pathways are disrupted is difficult.

Gender-related differences in susceptibility to chemical exposure to neurotoxicants have not received sufficient attention. There is abundant available information on the gender-specific health effects of mercury and lead, and exposure to lead seems to affect boys more than girls [101]. The internal

cadmium dose is generally higher in women than in men, due to a higher gastrointestinal absorption at low iron stores. This was probably one major reason why Itai-itai disease was mainly a woman's disease. Yet, data are sparse regarding the risk for women relative to men to develop cadmium-induced kidney damage in populations exposed to low levels of cadmium [102]. Information regarding gender differences in susceptibility of cadmium is still too scarce to draw any definite conclusion. More research is highly warranted about this matter. Environmental epidemiological studies should be designed to quantify differential gender-based exposures and outcomes, and this may provide new insights into prevention strategies [101].

4.4. Cd and Gene Expression. Cd accumulation prior to and at birth, however, might cause irreversible or lasting changes in the brain, which in turn leads to the altered gene expression [103, 104]. The expression of several proneuronal genes including ngn1 in cell clusters, zash1a in the developing optic tectum, and zash1b in the telencephalon and tectum. Cd-treated embryos also have fewer differentiated neurons and glia in the facial sensory ganglia as indicated by decreased zn-12 expression. Also, a lower transcription level of neurogenic genes, ngn1 and neuroD, is observed in neurons [34]. Cd-induced neurotoxicity can be caused by impaired neurogenesis, resulting in markedly reduced neuronal differentiation and axonogenesis.

By contrast, caspase 3 and p53 were altered by environmental chemicals in mouse, but not in human cells. Therefore, these markers are not appropriate to assess the ability of environmental chemicals to induce apoptosis in the ReN CX cells [100]. Among the brain-specific genes, neurogranin (RC3) and myelin basic protein (MBP) are regulated by serum thyroid hormones (THs) [105]. Since Cd exposure affects THs, Cd exposure will alter expression of these genes in the brain, and furthermore, those effects might be enhanced by a hypothyroid state. In conclusion, Cd combined with MMI decreased the RC mRNA expression in the brain of female offspring. The reduced expression of RC3 mRNA may explain the effect of Cd on brain function. Perinatal Cd exposure disrupted the reproductive function of offspring indicated by the reduced expression of ERs and PgR mRNA, which was not considered to be estrogenic action.

Cd-induced apoptosis in the neuronal cells has a time- and concentration-dependent manner. Cd induces apoptosis of neuronal cells by activation of JNK, Erk1/2, and mTOR signaling network. These findings support the notion that inhibitors of these pathways may be exploited for prevention of Cd-induced Parkinson's disease, Alzheimer's disease, and other neurodegenerative disorders [88].

4.5. Estrogen-Like Effect (Hormones Regulating Shaft). Cd can modify hormone levels by affecting the hypothalamic-pituitary-testicular axis in different aspects, not only via its effects on Leydig cells. Cd affected the circadian pattern release of noradrenaline, a regulator of hypothalamus hormone secretion, which resulted in changes in the daily pattern of plasma testosterone and LH levels [106]. In addition, plasma levels of pituitary hormones (e.g., LH, FSH, prolactin, ACTH) were also modified after Cd exposure [107]. Nevertheless, it remains to be investigated if Cd acts as an endocrine modulator by interacting with ERs or ARs in the testis and/or Leydig cells. The study of the hypothalamic-pituitary-gonadal (HPG) axis in animals exposed to the metal is of great interest since the levels of Cd in air, water, soil, and foods have increased by several folds in many parts of the world as a result of emissions from industrial activities. Cd accumulation increased in the hypothalamus and testes in all the Cd-treated animals, whereas the accumulation of Cd in the pituitary was found only in postpubertal rats. These data suggest that Cd exerts age-dependent effects on the hypothalamic-pituitary-testicular (HPT) axis function, and a disruption of the regulatory mechanisms of the HPG axis emerges [108]. Previous studies have shown that the heavy metal Cd mimics the effects of estradiol in estrogen-responsive breast cancer cell lines. Cd activates ER-alpha through an interaction with the hormone-binding domain of the receptor [109]. Cd globally effects HPT axis function by acting at the three levels analyzed and that an interaction between Cd exposure and age emerge [110]. The effects of Cd on sGnRH and rtERa gene expression in the brain in rainbow trout model, and these genes are strongly involved in reproduction [111]. But one study show that the effect of Cd on the ability of medaka gonads to produce steroids without the presence of physiological signals from brain, pituitary or circulating blood was examined *in vitro* [112]. Plasma levels of luteinizing hormone (LH) were not modified by Cd in both age groups, but follicle stimulating hormone (FSH) levels decreased in postpubertal rats, and was not altered in pubertal rats. Plasma levels of testosterone increased in pubertal rats but decreased in postpubertal rats [108].

In summary, it is important to note that the endocrine disruption induced by Cd is likely to be multi-factorial, mediated via its effects on Leydig cells and/or the hypothalamic-pituitary-gonadal axis.

4.6. Epigenetic Effect. Cd binds DNA in a weak fashion, indicating this is not a primary mode of action. Because DNA sequence is static, genetic susceptibility from DNA sequence variation cannot explain the mechanisms by which prenatal or early childhood metal exposures impact cognition and behavior later in life. Cd may well act as an epigenetic or indirectly genotoxic carcinogen since it is, in general, poorly mutagenic [6, 113]. There is growing evidence that exposure to toxicants in early life may cause later health effects. Children of women exposed to Cd during pregnancy display lower motor and perceptual abilities. High Cd body burden in children is also related to impaired intelligence and lowered school achievement. One possible mechanistic pathway for this phenomenon, which has yet to be fully explored in humans, is epigenetics. Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence. Such changes can have influences as profound as those exerted by mutations but, unlike mutations, are reversible and responsive to environmental influences. DNA methylation is the best studied of the epigenetic processes that regulate gene silencing. DNA methylation results in the addition of a methyl group to

the 5′position of the cytosine ring in the context of CpG dinucleotides (or island) to form 5-methylcytosine (5-MeC) [114]. Several studies have demonstrated that DNA methylation was facilitated by long-term exposure to Cd [115, 116]. Oxidative stress may be a unifying process to explain these findings across different metals. Metals are known to increase reactive oxygen species production in a catalytic fashion via redox cycling. Oxidative DNA damage can interfere with the ability of methyltransferases to interact with DNA [117], thus resulting in a generalized hypomethylation of cytosine residues at CpG sites [118]. Cd-induced alterations in methylation metabolism could initiate a cascade of events including gene-specific DNA hypo- or hyper-methylation [119], resulting in aberrant gene expression and also in diminished glutathione activity leaving cells more vulnerable to oxidative stress. Although the results of these epigenetic changes on neurodevelopment have remained unexplored, given the clear importance of DNA methylation to processes of neurodevelopment, the metal-induced disruption of DNA methylation clearly deserves further study. Neurotoxicity is a common health endpoint for excess Cd exposure. Finally, there is intriguing evidence that epigenetic phenomena may underlie observed effects of fetal or early life exposure and late onset of disease. In addition, Cd inhibited DNA methyltransferases in a manner that was noncompetitive with respect to the DNA substrate. This finding is suggestive of interference in enzyme DNA interaction, possibly through an interaction of Cd with the methyltransferase DNA binding domain [115]. Failure of DNA methylation systems in the brain leads to clinical syndromes such as mental retardation and autistic-like behaviors [120]. Animal studies increasingly demonstrate that environmental factors can alter DNA methylation patterns and that these changes correlate with animal behavior [121]. Further research in Cd should include the role of epigenetics in determining long-term and late-onset health effects from metal exposure.

5. Summary

Cd plays a critical role in neurobiology; a growing number of clinical investigations have pointed to Cd intoxication as a possible etiological factor of neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and Huntington's disease [50, 122, 123]. Many individuals in Europe and Asian already exceed these exposure levels, and the margin is very narrow for large groups. The question remains open about the impact of long term low level exposure to Cd on vulnerable subgroups of the general population (foetuses, pregnant women, young children, and elderly people) living in historically polluted areas where nonferrous industries are or were in operation. The neurotoxic effects of Cd were complex associated with both biochemical changes of the cell and functional changes of central nervous system, suggesting that neurotoxic effects may play a role in the systemic toxic effects of the exposure to Cd, particularly the long-term exposure. So the mechanism of Cd neurotoxicity should be enhancing, and measures should be taken to reduce cadmium exposure in the general population in order to minimize the risk of adverse health effects. The recent report on toxicity testing in the 21st century by the National Research Council of the National Academy of Sciences recommends the use of cell lines of human origin. The future of environmental research on Cd should include the role of neurotoxic in determining the long-term and late-onset health effects following Cd exposure.

Abbreviations

5-MeC: 5-Methylcytosine
ACGIH: American conference of governmental industrial hygienists
Ach: Acetylcholine
AChE: Acetylcholinesterase
AD: Alzheimer's disease
ADHD: Attention deficit hyperactivity disorder
BBB: Blood brain barrier
BuChE: Butyrylcholinesterase
B.W.: Body weight
Cd: Cadmium
ChE: Cholinesterases
CFS: Chronic fatigue syndrome
CNP: Cyclic nucleotide phosphodiesterase
CPSC: Consumer product safety commission
CNS: Central nervous system
CSF: Cerebrospinal fluid
Dx: Dexamethasone
FAO: Food and Agriculture Organization
FSH: Follicle stimulating hormone
HPG: Hypothalamus-pituitary-gonadal axis
HPT: Hypothalamic-pituitary-testicular
Im: Intramuscular
IQ: Intelligence quotient
LDH: Lactate dehydrogenase
LH: Luteinizing hormone
LPO: Lipid peroxidation
MAPKs: Mitogen-activated protein kinases
mTOR: Mammalian target of rapamycin
MBP: Myelin basic protein
Mcns: Mouse cortical neural stem cells
ME: Myalgic encephalomyelitis
MND: Motor neuron disease
MT: Metallothionein
NS-1: Neuroscreen-1 cells
OLP: Oligodendrocyte progenitors
PNP: Polyneuropathy
PTWI: Provisional tolerable weekly intake
ReN CX: Human ReNcell CX
Sc: Subcutaneous
SMND: Sporadic motor neuron disease
THs: Thyroid hormones
WHO: World Health Organization
Zn: Zinc.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgment

This paper was supported by National Natural Science Foundation of China (NSFC, no. 81260310).

References

[1] ATSDR, "Guidance Manual for the Joint Toxic Action of Chemical Mixtures," 2004, http://www.atsdr.cdc.gov/interactionprofiles/ipga.html.

[2] "Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. Working Group views and expert opinions," *IARC Monographs on the Evaluation of Carcinogenic Risks To Humans*, vol. 58, pp. 1–415, 1993.

[3] FAO/WHO, "Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives)," WHO Technical Report Series No. 776, WHO, Geneva, Switzerland, 1988.

[4] M. N. Mead, "Cadmium confusion do consumers need protection?" *Environmental Health Perspectives*, vol. 118, no. 12, pp. A528–A534, 2010.

[5] T. Jin, J. Lu, and M. Nordberg, "Toxicokinetics and biochemistry of cadmium with special emphasis on the role of metallothionein," *NeuroToxicology*, vol. 19, no. 4-5, pp. 529–536, 1998.

[6] M. P. Waalkes, "Cadmium carcinogenesis in review," *Journal of Inorganic Biochemistry*, vol. 79, no. 1–4, pp. 241–244, 2000.

[7] B. Pesch, J. Haerting, U. Ranft, A. Klimpel, B. Oelschlägel, and W. Schill, "Occupational risk factors for renal cell carcinoma: agent-specific results from a case-control study in Germany. MURC Study Group. Multicenter urothelial and renal cancer study," *International Journal of Epidemiology*, vol. 29, no. 6, pp. 1014–1024, 2000.

[8] P. Joseph, T. K. Muchnok, M. L. Klishis et al., "Cadmium-induced cell transformation and tumorigenesis are associated with transcriptional activation of c-fos, c-jun, and c-myc protooncogenes: role of cellular calcium and reactive oxygen species," *Toxicological Sciences*, vol. 61, no. 2, pp. 295–303, 2001.

[9] E. López, S. Figueroa, M. J. Oset-Gasque, and M. P. González, "Apoptosis and necrosis: two distinct events induced by cadmium in cortical neurons in culture," *British Journal of Pharmacology*, vol. 138, no. 5, pp. 901–911, 2003.

[10] Y. Cao, A. Chen, J. Radcliffe et al., "Postnatal cadmium exposure, neurodevelopment, and blood pressure in children at 2, 5, and 7 years of age," *Environmental Health Perspectives*, vol. 117, no. 10, pp. 1580–1586, 2009.

[11] R. O. Pihl and M. Parkes, "Hair element content in learning disabled children," *Science*, vol. 198, no. 4313, pp. 204–206, 1977.

[12] S. D. Kim, C. K. Moon, S.-Y. Eun, P. D. Ryu, and S. A. Jo, "Identification of ASK1, MKK4, JNK, c-Jun, and caspase-3 as a signaling cascade involved in cadmium-induced neuronal cell apoptosis," *Biochemical and Biophysical Research Communications*, vol. 328, no. 1, pp. 326–334, 2005.

[13] R. K. Monroe and S. W. Halvorsen, "Cadmium blocks receptor-mediated Jak/STAT signaling in neurons by oxidative stress," *Free Radical Biology and Medicine*, vol. 41, no. 3, pp. 493–502, 2006.

[14] M. Marlowe, J. Errera, and J. Jacobs, "Increased lead and cadmium burdens among mentally retarded children and children with borderline intelligence," *American Journal of Mental Deficiency*, vol. 87, no. 5, pp. 477–483, 1983.

[15] I. D. Capel, M. H. Pinnock, and H. M. Dorrell, "Comparison of concentrations of some trace, bulk, and toxic metals in the hair of normal and dyslexic children," *Clinical Chemistry*, vol. 27, no. 6, pp. 879–881, 1981.

[16] R. W. Thatcher, M. L. Lester, R. McAlaster, and R. Horst, "Effects of low levels of cadmium and lead on cognitive functioning in children," *Archives of Environmental Health*, vol. 37, no. 3, pp. 159–166, 1982.

[17] R. W. Thatcher, R. McAlaster, and M. L. Lester, "Evoked potentials related to hair cadmium and lead in children," *Annals of the New York Academy of Sciences*, vol. 425, pp. 384–390, 1984.

[18] M. Marlowe, A. Cossairt, and C. Moon, "Main and interaction effects of metallic toxins on classroom behavior," *Journal of Abnormal Child Psychology*, vol. 13, no. 2, pp. 185–198, 1985.

[19] H. Korpela, R. Loueniva, E. Yrjanheikki, and A. Kauppila, "Lead and cadmium concentrations in maternal and umbilical cord blood, amniotic fluid, placenta, and amniotic membranes," *American Journal of Obstetrics and Gynecology*, vol. 155, no. 5, pp. 1086–1089, 1986.

[20] A. Lafuente and A. I. Esquifino, "Cadmium effects on hypothalamic activity and pituitary hormone secretion in the male," *Toxicology Letters*, vol. 110, no. 3, pp. 209–218, 1999.

[21] A. I. Esquifino, N. Márquez, E. Alvarez-Demanuel, and A. Lafuente, "Effects of chronic alternating cadmium exposure on the episodic secretion of prolactin in male rats," *Journal of Trace Elements in Medicine and Biology*, vol. 12, no. 4, pp. 205–210, 1999.

[22] A. Lafuente, N. Márquez, S. Piquero, and A. I. Esquifino, "Cadmium affects the episodic luteinizing hormone secretion in male rats: possible age-dependent effects," *Toxicology Letters*, vol. 104, no. 1-2, pp. 27–33, 1999.

[23] R. Pal, R. Nath, and K. D. Gill, "Influence of ethanol on cadmium accumulation and its impact on lipid peroxidation and membrane bound functional enzymes (Na^+, K^+-ATPase and acetylcholinesterase) in various regions of adult rat brain," *Neurochemistry International*, vol. 23, no. 5, pp. 451–458, 1993.

[24] W. I. Manton and J. D. Cook, "High accuracy (stable isotope dilution) measurements of lead in serum and cerebrospinal fluid," *British Journal of Industrial Medicine*, vol. 41, no. 3, pp. 313–319, 1984.

[25] M. T. Antonio, L. Corredor, and M. L. Leret, "Study of the activity of several brain enzymes like markers of the neurotoxicity induced by perinatal exposure to lead and/or cadmium," *Toxicology Letters*, vol. 143, no. 3, pp. 331–340, 2003.

[26] G. S. Shukla and S. V. Chandra, "Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats," *Archives of Environmental Contamination and Toxicology*, vol. 16, no. 3, pp. 303–310, 1987.

[27] J. F. Gonçalves, A. M. Fiorenza, R. M. Spanevello et al., "N-acetylcysteine prevents memory deficits, the decrease in acetylcholinesterase activity and oxidative stress in rats exposed to cadmium," *Chemico-Biological Interactions*, vol. 186, no. 1, pp. 53–60, 2010.

[28] M. Mendez-Armenta and C. Rios, "Cadmium neurotoxicity," *Environmental Toxicology and Pharmacology*, vol. 23, no. 3, pp. 350–358, 2007.

[29] W. Zheng, D. F. Perry, D. L. Nelson, and H. V. Aposhian, "Choroid plexus protects cerebrospinal fluid against toxic metals," *FASEB Journal*, vol. 5, no. 8, pp. 2188–2193, 1991.

[30] H. Tjalve and J. Henriksson, "Uptake of metals in the brain via olfactory pathways," *NeuroToxicology*, vol. 20, no. 2-3, pp. 181–195, 1999.

[31] J.-R. Bondier, G. Michel, A. Propper, and P.-M. Badot, "Harmful effects of cadmium on olfactory system in mice," *Inhalation Toxicology*, vol. 20, no. 13, pp. 1169–1177, 2008.

[32] L. A. Czarnecki, A. H. Moberly, T. Rubinstein, D. J. Turkel, J. Pottackal, and J. P. McGann, "In vivo visualization of olfactory pathophysiology induced by intranasal cadmium instillation in mice," *NeuroToxicology*, vol. 32, no. 4, pp. 441–449, 2011.

[33] P. Mascagni, D. Consonni, G. Bregante, G. Chiappino, and F. Toffoletto, "Olfactory function in workers exposed to moderate airborne cadmium levels," *NeuroToxicology*, vol. 24, no. 4-5, pp. 717–724, 2003.

[34] E. S. H. Chow, M. N. Y. Hui, C. C. Lin, and S. H. Cheng, "Cadmium inhibits neurogenesis in zebrafish embryonic brain development," *Aquatic Toxicology*, vol. 87, no. 3, pp. 157–169, 2008.

[35] R. Fern, J. A. Black, B. R. Ransom, and S. G. Waxman, "Cd^{2+}-induced injury in CNS white matter," *Journal of Neurophysiology*, vol. 76, no. 5, pp. 3264–3273, 1996.

[36] G. Almazan, H.-N. Liu, A. Khorchid, S. Sundararajan, A. K. Martinez-Bermudez, and S. Chemtob, "Exposure of developing oligodendrocytes to cadmium causes HSP72 induction, free radical generation, reduction in glutathione levels, and cell death," *Free Radical Biology and Medicine*, vol. 29, no. 9, pp. 858–869, 2000.

[37] M. K. Viaene, H. A. Roels, J. Leenders et al., "Cadmium: a possible etiological factor in peripheral polyneuropathy," *NeuroToxicology*, vol. 20, no. 1, pp. 7–16, 1999.

[38] WHO, *Cadmium-Environmental Aspects (Environmental Health Criteria 135)*, . WHO, Geneva, Switzerland, 1992.

[39] FAO/WHO, "Toxicology evaluation of certain food additives and contaminants," WHO Technical Report Series No. 837, WHO, Geneva, Switzerland, 1993.

[40] S. Satarug, M. R. Haswell-Elkins, and M. R. Moore, "Safe levels of cadmium intake to prevent renal toxicity in human subjects," *British Journal of Nutrition*, vol. 84, no. 6, pp. 791–802, 2000.

[41] L. Nasreddine and D. Parent-Massin, "Food contamination by metals and pesticides in the European Union. Should we worry?" *Toxicology Letters*, vol. 127, no. 1–3, pp. 29–41, 2002.

[42] WHO, *Guidelines For Drinking-Water Quality*, Geneva, Switzerland, 3rd edition, 2004, http://www.who.int/water_sanitation_health/dwq/gdwq3/en/.

[43] FAO/WHO, "Codex Alimentarius Commission," in *Proceedings of the 29th Session*, International Conference Centre, Geneva, Switzerland, July 2006.

[44] ACGIH, "Cadmium. Threshold limit values for chemical substances and physical agents and biological exposure indices," in *Proceedings of the American Conference of Governmental Industrial Hygienists*, p. 17, Cincinnati, OH, USA, 2007.

[45] EFSA, *Scientific Opinion of the Panel on Contaminants in the Food Chain on a Request From the European Commission on cadmium in Food*, 2009.

[46] FAO/WHO, "The Summary report of the 73rd JECFA meeting," Tech. Rep., WHO, Geneva, Switzerland, 2010.

[47] S. Yoshida, "Re-evaluation of acute neurotoxic effects of Cd^{2+} on mesencephalic trigeminal neurons of the adult rat," *Brain Research*, vol. 892, no. 1, pp. 102–110, 2001.

[48] L. Bucio, V. Souza, A. Albores et al., "Cadmium and mercury toxicity in a human fetal hepatic cell line (WRL-68 cells)," *Toxicology*, vol. 102, no. 3, pp. 285–299, 1995.

[49] E. Sarchielli, S. Pacini, G. Morucci et al., "Cadmium induces alterations in the human spinal cord morphogenesis," *BioMetals*, vol. 25, no. 1, pp. 63–74, 201.

[50] B. Okuda, Y. Iwamoto, H. Tachibana, M. Sugita et al., "Parkinsonism after acute cadmium poisoning," *Clinical Neurology and Neurosurgery*, vol. 99, no. 4, pp. 263–265, 1997.

[51] L.-F. Jiang, T.-M. Yao, Z.-L. Zhu, C. Wang, and L.-N. Ji, "Impacts of Cd(II) on the conformation and self-aggregation of Alzheimer's tau fragment corresponding to the third repeat of microtubule-binding domain," *Biochimica et Biophysica Acta*, vol. 1774, no. 11, pp. 1414–1421, 2007.

[52] B. Xu, S. Chen, Y. Luo et al., "Calcium signaling is involved in cadmium-induced neuronal apoptosis via induction of reactive oxygen species and activation of MAPK/mTOR network," *PLoS One*, vol. 6, no. 4, Article ID e19052, 2011.

[53] Y. Yuan, C.-Y. Jiang, X. H. Xu et al., "Cadmium-induced apoptosis in primary rat cerebral cortical neurons culture is mediated by a calcium signaling pathway," *PLoS One*, vol. 8, no. 5, Article ID e64330, 2013.

[54] L. Chen, L. Liu, and S. Huang, "Cadmium activates the mitogen-activated protein kinase (MAPK) pathway via induction of reactive oxygen species and inhibition of protein phosphatases 2A and 5," *Free Radical Biology and Medicine*, vol. 45, no. 7, pp. 1035–1044, 2008.

[55] A. Gupta and S. V. Chandra, "Gestational cadmium exposure and brain development: a biochemical study," *Industrial Health*, vol. 29, no. 2, pp. 65–71, 1991.

[56] E. P. Rico, D. B. Rosemberg, M. R. Senger et al., "Methanol alters ecto-nucleotidases and acetylcholinesterase in zebrafish brain," *Neurotoxicology and Teratology*, vol. 28, no. 4, pp. 489–496, 2006.

[57] M. R. Senger, D. B. Rosemberg, E. P. Rico et al., "In vitro effect of zinc and cadmium on acetylcholinesterase and ectonucleotidase activities in zebrafish (Danio rerio) brain," *Toxicology in Vitro*, vol. 20, no. 6, pp. 954–958, 2006.

[58] J. F. Goncalves, F. T. Nicoloso, P. da Costa et al., "Behavior and brain enzymatic changes after long-term intoxication with cadmium salt or contaminated potatoes," *Food and Chemical Toxicology*, vol. 50, no. 10, pp. 3709–3718, 2012.

[59] A. Zarros, K. Kalopita, S. Tsakiris, and G. S. Baillie, "Can acetylcholinesterase activity be considered as a reliable biomarker for the assessment of cadmium-induced neurotoxicity?" *Food and Chemical Toxicology*, vol. 56, pp. 406–410, 2013.

[60] E. Herba, D. Pojda-Wilczek, S. M. Pojda, A. Plech, and R. Brus, "The effect of serotonin on flash visual evoked potential in the rat prenatally exposed to cadmium," *Klinika Oczna*, vol. 103, no. 2-3, pp. 81–84, 2001.

[61] I. Desi, L. Nagymajtenyi, and H. Schulz, "Behavioural and neurotoxicological changes caused by cadmium treatment of rats during development," *Journal of Applied Toxicology*, vol. 18, no. 1, pp. 63–70, 1998.

[62] A. Gupta and G. S. Shukla, "Ontogenic profile of brain lipids following perinatal exposure to cadmium," *Journal of Applied Toxicology*, vol. 16, no. 3, pp. 227–233, 1996.

[63] R. G. Adams and N. Crabtree, "Anosmia in alkaline battery workers," *British Journal of Industrial Medicine*, vol. 18, pp. 216–221, 1961.

[64] A. MNusioł, K. Szyrocka-Szwed, J. Wojczuk, and E. Kudybka, "Evaluation of the neurological status and EEG findings in workers chronically exposed to cadmium," *Wiadomosci Lekarskie*, vol. 34, no. 19, pp. 1615–1620, 1981.

[65] R. E. Struempler, G. E. Larson, and B. Rimland, "Hair mineral analysis and disruptive behavior in clinically normal young men," *Journal of Learning Disabilities*, vol. 18, no. 10, pp. 609–612, 1985.

[66] R. P. Hart, C. S. Rose, and R. M. Hamer, "Neuropsychological effects of occupational exposure to cadmium," *Journal of Clinical and Experimental Neuropsychology*, vol. 11, no. 6, pp. 933–943, 1989.

[67] S. Bar-Sela, M. Levy, J. B. Westin, R. Laster, and E. D. Richter, "Medical findings in nickel-cadmium battery workers," *Israel Journal of Medical Sciences*, vol. 28, no. 8-9, pp. 578–583, 1992.

[68] C. S. Rose, P. G. Heywood, and R. M. Costanzo, "Olfactory impairment after chronic occupational cadmium exposure," *Journal of Occupational Medicine*, vol. 34, no. 6, pp. 600–605, 1992.

[69] M. K. Viaene, R. Masschelein, J. Leenders, L. J. V. C. Swerts, M. de Groof, and H. A. Roels, "Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study," *Occupational and Environmental Medicine*, vol. 57, no. 1, pp. 19–27, 2000.

[70] P. K. Sethi and D. Khandelwal, "Cadmium exposure: health hazards of silver cottage industry in developing countries," *Journal of Medical Toxicology*, vol. 2, no. 1, pp. 14–15, 2006.

[71] Q.-S. Bao, C.-Y. Lu, H. Song et al., "Behavioural development of school-aged children who live around a multi-metal sulphide mine in Guangdong province, China: a cross-sectional study," *BMC Public Health*, vol. 9, article 217, 2009.

[72] A. Papp, G. Oszlánczi, E. Horváth et al., "Consequences of subacute intratracheal exposure of rats to cadmium oxide nanoparticles: electrophysiological and toxicological effects," *Toxicology & Industrial Health*, vol. 28, no. 10, pp. 933–941, 2012.

[73] T. Borisova, N. Krisanova, R. Sivko et al., "Presynaptic malfunction: the neurotoxic effects of cadmium and lead on the proton gradient of synaptic vesicles and glutamate transport," *Neurochemistry International*, vol. 59, no. 2, pp. 272–279, 2011.

[74] V. C. Borges, F. W. Santos, J. B. T. Rocha, and C. W. Nogueira, "Heavy metals modulate glutamatergic system in human platelets," *Neurochemical Research*, vol. 32, no. 6, pp. 953–958, 2007.

[75] J. R. Nation, D. K. Miller, and G. R. Bratton, "Dietary cadmium exposure alters characteristics of training, substitution, and tolerance when morphine is used as a discriminative stimulus," *NeuroToxicology*, vol. 21, no. 4, pp. 553–567, 2000.

[76] C. Bonithon-Kopp, G. Huel, and C. Grasmick, "Effects of pregnancy on the inter-individual variations in blood levels of lead, cadmium and mercury," *Biological Research in Pregnancy and Perinatology*, vol. 7, no. 1, pp. 37–42, 1986.

[77] M. Vinceti, D. Guidetti, M. Bergomi et al., "Lead, cadmium, and selenium in the blood of patients with sporadic amyotrophic lateral sclerosis," *Italian Journal of Neurological Sciences*, vol. 18, no. 2, pp. 87–92, 1997.

[78] R. Pamphlett, R. McQuilty, and K. Zarkos, "Blood levels of toxic and essential metals in motor neuron disease," *NeuroToxicology*, vol. 22, no. 3, pp. 401–410, 2001.

[79] F. P. de Lange, J. S. Kalkman, G. Bleijenberg, P. Hagoort, J. W. M. Van Der Meer, and I. Toni, "Gray matter volume reduction in the chronic fatigue syndrome," *NeuroImage*, vol. 26, no. 3, pp. 777–781, 2005.

[80] E. López, C. Arce, M. J. Oset-Gasque, S. Cañadas, and M. P. González, "Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture," *Free Radical Biology and Medicine*, vol. 40, no. 6, pp. 940–951, 2006.

[81] S. Yousef, A. Adem, T. Zoubeidi, M. Kosanovic, A. A. Mabrouk, and V. Eapen, "Attention deficit hyperactivity disorder and environmental toxic metal exposure in the United Arab Emirates," *Journal of Tropical Pediatrics*, vol. 57, no. 6, Article ID fmq121, pp. 457–460, 2011.

[82] A. Shukla, G. S. Shukla, and R. C. Srimal, "Cadmium-induced alterations in blood—brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat," *Human and Experimental Toxicology*, vol. 15, no. 5, pp. 400–405, 1996.

[83] Y. Uchida, K. Takio, K. Titani, Y. Ihara, and M. Tomonaga, "The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein," *Neuron*, vol. 7, no. 2, pp. 337–347, 1991.

[84] N. Nishimura, H. Nishimura, A. Ghaffar, and C. Tohyama, "Localization of metallothionein in the brain of rat and mouse," *Journal of Histochemistry and Cytochemistry*, vol. 40, no. 2, pp. 309–315, 1992.

[85] M. Méndez-Armenta, J. Villeda-Hernández, R. Barroso-Moguel, C. Nava-Ruíz, M. E. Jiménez-Capdeville, and C. Ríos, "Brain regional lipid peroxidation and metallothionein levels of developing rats exposed to cadmium and dexamethasone," *Toxicology Letters*, vol. 144, no. 2, pp. 151–157, 2003.

[86] I. G. Onyango, J. P. Bennett Jr., and J. B. Tuttle, "Endogenous oxidative stress in sporadic Alzheimer's disease neuronal cybrids reduces viability by increasing apoptosis through pro-death signaling pathways and is mimicked by oxidant exposure of control cybrids," *Neurobiology of Disease*, vol. 19, no. 1-2, pp. 312–322, 2005.

[87] L. Chen, B. Xu, L. Liu et al., "Cadmium induction of reactive oxygen species activates the mTOR pathway, leading to neuronal cell death," *Free Radical Biology and Medicine*, vol. 50, no. 5, pp. 624–632, 2011.

[88] L. Chen, L. Liu, Y. Luo, and S. Huang, "MAPK and mTOR pathways are involved in cadmium-induced neuronal apoptosis," *Journal of Neurochemistry*, vol. 105, no. 1, pp. 251–261, 2008.

[89] M. T. Antonio, I. Corpas, and M. L. Leret, "Neurochemical changes in newborn rat's brain after gestational cadmium and lead exposure," *Toxicology Letters*, vol. 104, no. 1-2, pp. 1–9, 1999.

[90] S. Hayat, C. B. Wigley, and J. Robbins, "Intracellular calcium handling in rat olfactory ensheathing cells and its role in axonal regeneration," *Molecular and Cellular Neuroscience*, vol. 22, no. 2, pp. 259–270, 2003.

[91] A. P. Fox, M. C. Nowycky, and R. W. Tsien, "Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones," *Journal of Physiology*, vol. 394, pp. 149–172, 1987.

[92] J. R. Huguenard, "Low-threshold calcium currents in central nervous system neurons," *Annual Review of Physiology*, vol. 58, pp. 329–348, 1996.

[93] A. K. Sundgren-Andersson and S. Johansson, "Calcium spikes and calcium currents in neurons from the medial preoptic nucleus of rat," *Brain Research*, vol. 783, no. 2, pp. 194–209, 1998.

[94] T. Aoki and S. C. Baraban, "Properties of a calcium-activated K^+ current on interneurons in the developing rat hippocampus," *Journal of Neurophysiology*, vol. 83, no. 6, pp. 3453–3461, 2000.

[95] X. Liu, J.-L. Zhou, K. Chung, and J. M. Chung, "Ion channels associated with the ectopic discharges generated after segmental spinal nerve injury in the rat," *Brain Research*, vol. 900, no. 1, pp. 119–127, 2001.

[96] Y.-C. Hsieh, E.-L. Hsu, Y.-S. Chow, and R. Kou, "Effects of calcium channel antagonists on the corpora allata of adult

male loreyi leafworm Mythimna loreyi: Juvenile hormone acids release and intracellular calcium level," *Archives of Insect Biochemistry and Physiology*, vol. 48, no. 2, pp. 89–99, 2001.

[97] C. Acuña-Castillo, B. Morales, and J. P. Huidobro-Toro, "Zinc and copper modulate differentially the P2X4 receptor," *Journal of Neurochemistry*, vol. 74, no. 4, pp. 1529–1537, 2000.

[98] S. K. Ryan, L. R. Shotts, S.-K. Hong et al., "Glutamate regulates neurite outgrowth of cultured descending brain neurons from larval lamprey," *Developmental Neurobiology*, vol. 67, no. 2, pp. 173–188, 2007.

[99] J. Son, S.-E. Lee, B.-S. Park et al., "Biomarker discovery and proteomic evaluation of cadmium toxicity on a collembolan species, Paronychiurus kimi (Lee)," *Proteomics*, vol. 11, no. 11, pp. 2294–2307, 2011.

[100] M. E. Culbreth, J. A. Harrill, T. M. Freudenrich, W. R. Mundy, and T. J. Shafer, "Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells," *Neurotoxicology*, vol. 33, no. 6, pp. 1499–1510, 2012.

[101] S. Llop, M. J. Lopez-Espinosa, M. Rebagliato, and F. Ballester, "Gender differences in the neurotoxicity of metals in children," *Toxicology*, 2013.

[102] M. Vahter, A. Åkesson, C. Lidén, S. Ceccatelli, and M. Berglund, "Gender differences in the disposition and toxicity of metals," *Environmental Research*, vol. 104, no. 1, pp. 85–95, 2007.

[103] H. Ishitobi, K. Mori, K. Yoshida, and C. Watanabe, "Effects of perinatal exposure to low-dose cadmium on thyroid hormone-related and sex hormone receptor gene expressions in brain of offspring," *NeuroToxicology*, vol. 28, no. 4, pp. 790–797, 2007.

[104] H. Ishitobi and C. Watanabe, "Effects of low-dose perinatal cadmium exposure on tissue zinc and copper concentrations in neonatal mice and on the reproductive development of female offspring," *Toxicology Letters*, vol. 159, no. 1, pp. 38–46, 2005.

[105] A. Farsetti, T. Mitsuhashi, B. Desvergne, J. Robbins, and V. M. Nikodem, "Molecular basis of thyroid hormone regulation of myelin basic protein gene expression in rodent brain," *Journal of Biological Chemistry*, vol. 266, no. 34, pp. 23226–23232, 1991.

[106] A. Lafuente, A. González-Carracedo, A. Romero, P. Cano, and A. I. Esquifino, "Cadmium exposure differentially modifies the circadian patterns of norepinephrine at the median eminence and plasma LH, FSH and testosterone levels," *Toxicology Letters*, vol. 146, no. 2, pp. 175–182, 2004.

[107] A. Lafuente, P. Cano, and A. I. Esquifino, "Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent?" *BioMetals*, vol. 16, no. 2, pp. 243–250, 2003.

[108] A. Lafuente, N. Márquez, M. Pérez-Lorenzo, D. Pazo, and A. I. Esquifino, "Pubertal and postpubertal cadmium exposure differentially affects the hypothalamic-pituitary-testicular axis function in the rat," *Food and Chemical Toxicology*, vol. 38, no. 10, pp. 913–923, 2000.

[109] A. Stoica, B. S. Katzenellenbogen, and M. B. Martin, "Activation of estrogen receptor-α by the heavy metal cadmium," *Molecular Endocrinology*, vol. 14, no. 4, pp. 545–553, 2000.

[110] A. Lafuente, N. Márquez, M. Pérez-Lorenzo, D. Pazo, and A. I. Esquifino, "Cadmium effects on hypothalamic-pituitary-testicular axis in male rats," *Experimental Biology and Medicine*, vol. 226, no. 6, pp. 605–611, 2001.

[111] A. Vetillard and T. Bailhache, "Cadmium: an endocrine disrupter that affects gene expression in the liver and brain of juvenile rainbow trout," *Biology of Reproduction*, vol. 72, no. 1, pp. 119–126, 2005.

[112] S. C. Tilton, C. M. Foran, and W. H. Benson, "Effects of cadmium on the reproductive axis of Japanese medaka (*Oryzias latipes*)," *Comparative Biochemistry and Physiology C*, vol. 136, no. 3, pp. 265–276, 2003.

[113] H. Koyama, H. Kitoh, M. Satoh, and C. Tohyama, "Low dose exposure to cadmium and its health effects (1). Genotoxicity and carcinogenicity," *Japanese Journal of Hygiene*, vol. 57, no. 3, pp. 547–555, 2002.

[114] O. J. Miller, W. Schnedl, J. Allen, and B. F. Erlanger, "5 Methylcytosine localised in mammalian constitutive heterochromatin," *Nature*, vol. 251, no. 5476, pp. 636–637, 1974.

[115] M. Takiguchi, W. E. Achanzar, W. Qu, G. Li, and M. P. Waalkes, "Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation," *Experimental Cell Research*, vol. 286, no. 2, pp. 355–365, 2003.

[116] L. Benbrahim-Tallaa, R. A. Waterland, A. L. Dill, M. M. Webber, and M. P. Waalkes, "Tumor suppressor gene inactivation during cadmium-induced malignant transformation of human prostate cells correlates with overexpression of de Novo DNA methyltransferase," *Environmental Health Perspectives*, vol. 115, no. 10, pp. 1454–1459, 2007.

[117] V. Valinluck, H.-H. Tsai, D. K. Rogstad, A. Burdzy, A. Bird, and L. C. Sowers, "Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2)," *Nucleic Acids Research*, vol. 32, no. 14, pp. 4100–4108, 2004.

[118] P. W. Turk, A. Laayoun, S. S. Smith, and S. A. Weitzman, "DNA adduct 8-hydroxyl-2′-deoxyguanosine (8-hydroxyguanine) affects function of human DNA methyltransferase," *Carcinogenesis*, vol. 16, no. 5, pp. 1253–1255, 1995.

[119] B. Wang, Y. Li, Y. Tan et al., "Low-dose Cd induces hepatic gene hypermethylation, along with the persistent reduction of cell death and increase of cell proliferation in rats and mice," *PLoS One*, vol. 7, no. 3, Article ID e33853, 2012.

[120] M. D. Shahbazian and H. Y. Zoghbi, "Rett syndrome and MeCP2: linking epigenetics and neuronal function," *American Journal of Human Genetics*, vol. 71, no. 6, pp. 1259–1272, 2002.

[121] B. S. Schwartz, B.-K. Lee, K. Bandeen-Roche et al., "Occupational lead exposure and longitudinal decline in neurobehavioral test scores," *Epidemiology*, vol. 16, no. 1, pp. 106–113, 2005.

[122] S. Johnson, "Gradual micronutrient accumulation and depletion in Alzheimer's disease," *Medical Hypotheses*, vol. 56, no. 6, pp. 595–597, 2001.

[123] A. E. Panayi, N. M. Spyrou, B. S. Iversen, M. A. White, and P. Part, "Determination of cadmium and zinc in Alzheimer's brain tissue using Inductively Coupled Plasma Mass Spectrometry," *Journal of the Neurological Sciences*, vol. 195, no. 1, pp. 1–10, 2002.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 836760, 12 pages
http://dx.doi.org/10.1155/2013/836760

Review Article

SOD1 and DJ-1 Converge at Nrf2 Pathway: A Clue for Antioxidant Therapeutic Potential in Neurodegeneration

Pamela Milani,[1,2] Giulia Ambrosi,[3,4] Omar Gammoh,[1,2,5] Fabio Blandini,[3] and Cristina Cereda[1]

[1] *Laboratory of Experimental Neurobiology, National Neurological Institute C. Mondino, IRCCS, Via Mondino 2, 27100 Pavia, Italy*
[2] *Department of Public Health, Neuroscience, Experimental and Forensic Medicine, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy*
[3] *Laboratory of Functional Neurochemistry, Center for Research in Neurodegenerative Diseases, National Neurological Institute C. Mondino, IRCCS, Via Mondino 2, 27100 Pavia, Italy*
[4] *Department of Brain and Behavioral Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy*
[5] *Faculty of Health Sciences, American University of Madaba, Madaba, Jordan*

Correspondence should be addressed to Pamela Milani; pame.milani@gmail.com

Received 26 April 2013; Revised 3 July 2013; Accepted 3 July 2013

Academic Editor: Renata Santos

Neurodegenerative diseases share diverse pathological features and among these oxidative stress (OS) plays a leading role. Impaired activity and reduced expression of antioxidant proteins have been reported as common events in several aging-associated disorders. In this review paper, we first provide an overview of the involvement of reactive oxygen species- (ROS-) induced oxidative damage in Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). Subsequently, we focus on DJ-1 and SOD1 proteins, which are involved in PD and ALS and also exert a prominent role in the interaction between redox homeostasis and neurodegeneration. Interestingly, recent studies demonstrated that DJ-1 and SOD1 are both tightly connected with Nrf2 protein, a transcriptional factor and master regulator of the expression of many antioxidant/detoxification genes. Nrf2 is emerging as a key neuroprotective protein in neurodegenerative diseases, since it helps neuronal cells to cope with toxic insults and OS. We herein summarize the recent literature providing a detailed picture of the promising therapeutic efficacy of Nrf2 natural and synthetic inducers as disease-modifying molecules for the treatment of neurodegenerative diseases.

1. Introduction

Oxidative stress (OS) is a crucial player in several diseases, including age-dependent neurodegenerative disorders such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). OS accumulation in postmitotic neurons during aging represents a phenomenon of significant relevance since it can trigger a vicious cycle of intracellular damages, ultimately resulting in neuronal cell death.

The involvement of OS in several neurodegenerative conditions has been demonstrated by the identification of pathological mutations in genes prominently featuring in defensive pathways as well as OS markers in patients' samples (as reviewed in [1–4]). Nevertheless, in many cases it is not clear whether this kind of stress is a primary cause or rather an ongoing downstream event associated with the progression of the neurodegenerative process.

OS is typically defined as the imbalance between the production of reactive oxygen species (ROS) and the efficient removal of these species by cellular defensive mechanisms, which include both enzymatic scavengers (e.g., superoxide dismutases, catalase, glutathione peroxidase, glutathione reductases, and peroxiredoxins) and low-molecular-weight reductants (e.g., vitamin E, glutathione, and ascorbate). Mitochondria use approximately the 85–90% of total oxygen, thus representing the major site of oxygen consumption as well as a primary and continuous source of cellular ROS. ROS such as superoxide (O^{-2}) and hydrogen peroxide (H_2O_2) principally

originate as by-products of aerobic metabolism, due to electron "leakage" from the mitochondrial respiratory chain during oxidative phosphorylation with the consequent incomplete reduction of molecular oxygen. A more limited percentage of intracellular ROS arise from the activity of oxidative enzymes, including the cytochrome P450 system associated with the endoplasmic reticulum, the cytoplasmatic xanthine oxidase, the membrane enzyme NADPH oxidase [5], and p66Shc, an important regulator of intracellular redox balance, mitochondrial permeability, and apoptosis [6]. Superoxide itself is not highly dangerous; nevertheless it can rapidly react with the mild oxidant Nitric Oxide (NO), produced by the nitric oxide synthase (NOS), to generate the more harmful peroxynitrite ($ONOO^-$) [7, 8]. Likewise, H_2O_2 is a weak oxidant but it gradually decomposes to generate the hydroxyl radical ($^\bullet OH$), one of the most toxic-free radicals in biological systems. Both $ONOO^-$ and $^\bullet OH$ impair the function of biomolecules by affecting several targets inside the cell. Specifically, ROS attack the backbone and the side chains of proteins causing the formation of carbonyl groups and methionine sulfoxide and often determining protein misfolding and aggregation. In addition, they attack nucleic acids, leading to DNA single- and double-strand breaks, DNA-protein crosslinks, and/or modification of purine and pyrimidine bases, and to oxidative modification in both protein-coding RNAs and noncoding RNAs. Furthermore, ROS cause lipid peroxidation, a complex phenomenon involving the interaction between unstable free radicals and polyunsaturated fatty acids, yielding highly reactive products, such as malondialdehyde, 4-hydroxy-2-trans-nonenal (HNE), acrolein, and thiobarbituric acid reactive substances (TBARS) [9]. In synthesis, OS causes a cascade of damaging processes eventually leading to cell death.

Although all the aerobic cells are subjected to oxidative damage, neurons are particularly vulnerable to the injuring effects of by-products derived from the oxidative metabolism. This susceptibility can be ascribed to their high metabolic requirements and oxygen demand combined with a relatively low expression of antioxidant proteins, in particular catalase (as reviewed in [1, 10]), and their limited regenerative capacity.

While an exaggerate production of ROS is typically associated with broad deleterious effects for neuronal cell functions and viability, increasing body of evidence is demonstrating that changes in redox environment, including generation of oxidants, also exert crucial roles in regulating specific signalling events. In particular, ROS have been shown to be involved in kinase cascade activation [11], calcium mobilization and signalling [12, 13], fine-tuned control of redox-sensitive gene expression [14, 15], and, more recently, in neural stem cell differentiation [16] and neurogenesis [17].

Consequently, a better understanding of ROS involvement in determining the fate of neuronal cells may yield clues to the pathogenesis of neurodegenerative diseases and may offer the possibility to pharmacologically manipulate intracellular molecular pathways, redox-sensitive transcriptional events, and antioxidant systems as promising neuroprotective therapies.

2. 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 Alzheimer's disease (AD) [18]. The majority of cases (90%) are sporadic, while about 10% show monogenic inheritance [19].

PD is caused by the degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNc) and although there is still no clear explanation for the intrinsic vulnerability of these neurons, it is known that they are more prone and susceptible to OS. In fact, several prooxidant factors constitutively challenge SNc dopaminergic neurons: in particular pacemaking activity, sustained calcium buffering, dopamine self-oxidation, and iron oxidation [20]. PD pathogenesis is indeed complex and multifactorial and selective vulnerability of SNc dopaminergic neurons can be further stressed by converging pathogenic mechanisms that include a predisposing genetic background, exposure to environmental neurotoxins, defective proteolytic systems, and impaired mitochondrial integrity and function [21]. In particular, mitochondrial defects lead to impaired energy and ROS production and therefore to altered bioenergetic and redox balance.

Consistent evidence from both genetic and epidemiological studies shows that disrupted mitochondrial integrity and OS play a pivotal role in PD pathogenesis and disease progression. Genes such as *PARK2*, *PARK6*, and *PARK7* encoding, respectively, for Parkin, PINK1, and DJ-1 are associated with early-onset familial forms of PD and mutations in all of those genes affect mitochondrial health and function thereby causing neuronal death [22]. Epidemiological data and animal models demonstrate that environmental toxins and pesticides that inhibit mitochondrial complex I, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone, are responsible for bioenergetic crisis and, most importantly, increased ROS production and OS, eventually causing loss of dopaminergic neurons in the SNc [23].

Furthermore, markers of OS are typically found in brain biopsies, peripheral cells, and biological fluids derived from patients with PD, indicating that indeed OS is a key factor in PD pathogenesis. In postmortem brains derived from PD patients increased accumulation of both carbonylated proteins and markers of lipid peroxidation such as TBARS were detected [24]. Similarly, markers of lipid peroxidation were increased in plasma and cerebrospinal fluid (CSF) derived from PD patients as compared to controls [25]. Nonetheless, 8-hydroxy-2′-deoxyguanosine (8-OHdG), an indicator of nucleic acid oxidation, in particular of nuclear and mitochondrial DNA damage, was increased in CSF of patients with PD, together with augmented levels of oxidized Coenzyme Q10 [26]. 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 in blood cells from PD patients as compared to controls and concentrations of other antioxidant molecules and catalase activity are decreased [27]. Furthermore, several studies have shown that activation of antioxidant genes expression, in particular those under the control

of the Nrf2/ARE system (i.e., NQO1 and GST; see Section 5.1), has neuroprotective effects in different models of PD [28, 29].

Finally, OS can also drive PD progression through the activation of excitotoxic phenomena and neuroinflammatory processes. Excitotoxicity is due to the hyperactivation of glutamatergic receptors, in particular *N*-methyl-D-aspartate (NMDA). Activation of these receptors leads to intracellular calcium overload which triggers ROS formation and the release of proapoptotic factors [30]. The activation of neuroinflammatory processes is mediated by glial cells, astrocytes, and more severely microglia. After a primary neuronal insult, microglia remains persistently activated and contributes to the release of free radicals which exacerbate the neurodegenerative process and accelerate its progression [31].

3. Amyotrophic Lateral Sclerosis (ALS)

ALS is a rare adult-onset neurodegenerative disease characterized by the selective degeneration of motor neurons in the motor cortex, brainstem, and spinal cord. Most of the cases (90%) are sporadic (SALS), while the remainder presents a family history (FALS).

Although the exact cause of ALS is still unknown, a major step forward in the understanding of the pathogenetic events involved in ALS was provided in 1993 by the observation that mutations in the gene coding for the antioxidant enzyme Cu/Zn superoxide dismutase (SOD1) are carried by the 15–20% of FALS patients [32].

Growing evidence suggests that ALS is a complex and multifactorial disease characterized by the involvement of several interconnected pathogenic events, such as OS, mitochondrial dysfunction, inflammation, glutamate excitotoxicity, protein misfolding and aggregation, aberrant RNA metabolism, and altered gene expression [33–37]. In particular, OS is one of the most detrimental contributors of disease onset and progression. In fact, several distinctive oxidation markers have been observed in both nervous and peripheral tissues in SALS and FALS patients [38–40]. Elevated protein carbonyl and 3-nitrotyrosine levels have been detected in spinal cord and motor cortex from SALS and FALS patients, particularly in large ventral motor neurons [38, 41–43]. Lipid oxidation has also been identified in motor neurons, astrocytes, and microglia of SALS patients compared to control individuals [44, 45]. Elevated levels of HNE have been detected also in CSF [46] and in sera [47] from ALS patients. Additionally, mitochondrial defects have been reported as a major hallmark in motor neuron degeneration in ALS [48, 49]. 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 [50] and in lymphocytes [51] from SALS patients, in SOD1 transgenic mice and cellular models [52]. Dynamic and morphological abnormalities, such as swelling and vacuolization, along with metabolic deficits in the activities of the respiratory chain complexes have been also described both in SALS and FALS patients [53]. These defects lead to both bioenergetic failure and increased ROS generation.

Notably, impairment in defensive mechanisms has also been revealed in ALS, including downregulation of members of glutathione S-transferase family [54, 55], peroxiredoxins [56], and, in particular, the transcriptional factor Nrf2 [57–60].

4. DJ-1 and SOD1 Involvement in Oxidative Stress and Neurodegeneration

4.1. DJ-1. DJ-1 is a ubiquitously expressed protein encoded by *PARK7* gene, initially identified as an oncogene functionally associated with cancer and male infertility [61, 62]. Mutations in *PARK7* gene, leading to loss of function of the protein, were later associated with early-onset recessive forms of PD [63].

DJ-1 is a small homodimeric protein composed of two subunits of 189 amino acid residues with a molecular weight of approximately 20 kDa. The crystal structure of this protein was independently solved by several groups [64–67], providing a helpful framework for a better understanding of its possible molecular activities. Specifically, it has a flavodoxin-like core fold, composed of α-helical layers sandwiching a six-strand parallel β-sheet. PD-associated mutations lead to diverse levels of protein folding defects or structural perturbations with consequent functional alterations of the protein [68]. DJ-1 is mainly localized in the cytoplasm, but under OS the protein can be recruited either to the mitochondria or to the nucleus [69–71]. It has been reported that after oxidative insult, DJ-1 translocates to the mitochondria within 3 hours and to the nucleus after 12 [72], suggesting that timing after oxidative challenge is essential to determine the subcellular compartment where the protein is active. Moreover, DJ-1 stimulates the cytoprotective pathway mediated by extracellular-signal-regulated kinase (ERK1/2) and its substrate ETS domain-containing protein (Elk1) (Figure 1). It was demonstrated by Gu and colleagues in two different cell lines that overexpression of wild-type DJ-1 increases ERK1/2 phosphorylation leading to Elk1 activation, thereby decreasing cell susceptibility to H_2O_2 and increasing cell viability [73].

Although the exact function of DJ-1 remains unclear, increasing studies have revealed that the protein is involved in various biological processes, in particular control of ROS levels and OS-induced apoptosis. DJ-1 responds to increased ROS levels by oxidizing itself, in particular at Cystine 106 (C106). Site-directed mutagenesis of C106, performed in both cellular and animal models, established a clear role for C106 oxidation in DJ-1 function, since mutations interfered with the antioxidant, antiapoptotic, and eventually neuroprotective effect of the protein [74–76].

Through self-oxidation, DJ-1 acts as a sensor of cellular redox state [77] and also as a redox-activated peroxidase with peroxiredoxin-like activity [74]. DJ-1 is therefore a signalling molecule responsive to cellular redox state which exerts antioxidant functions because of its capability of buffering oxidized molecules not only by physically interacting with them at C106, but also by inducing the expression of antioxidant defences [78, 79]. Finally, according to its oxidative state,

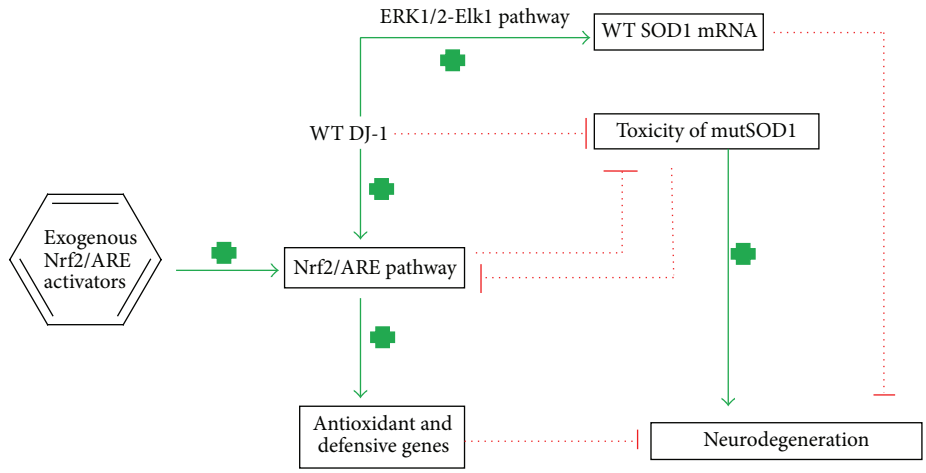


Figure 1: WT DJ-1 directly protects from PD and activates ERK1/2-ELK1 pathway thus upregulating WT-SOD1 expression. Also WT DJ-1 activates Nrf2/ARE pathway hence inducing antioxidant genes. Furthermore, both WT DJ-1 and Nrf2/ARE inhibit the toxicity of mutSOD1 that causes ALS, and vice versa, Nrf2/ARE pathway is inhibited by mutSOD1. Finally, exogenous Nrf2/ARE activators represent a powerful tool in the induction of antioxidant and defensive genes.

DJ-1 counteracts the induction of apoptotic mechanisms [80] and protects neuron viability by acting as a chaperone [81] and autophagy modulator [82].

Therefore, the essential role of DJ-1 is to protect cells from apoptotic death triggered by harmful levels of ROS by immediately protecting mitochondrial integrity and, after sustained oxidative challenge, by modulating transcription of antioxidant genes.

4.2. SOD1. One of the primary cellular defensive systems for oxidative insults is the antioxidant enzyme SOD1. It is one of the three human superoxide dismutases identified and characterized in mammals: copper-zinc superoxide dismutase (Cu/ZnSOD or SOD1), manganese superoxide dismutase (MnSOD or SOD2), and extracellular superoxide dismutase (ECSOD or SOD3). It is a 32 kDa homodimer of a 153-residue polypeptide with one copper- and one zinc-binding site per subunit. Specifically, each monomer possesses a β-barrel motif and two large functionally important loops, called the electrostatic and zinc loops, which encase the metal-binding region. It catalyzes the reaction of superoxide anion (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) at a bound copper ion [83]. The intracellular concentration of SOD1 is high (ranging from 10 to 100 μM) [84] counting for 1% of the total protein content in central nervous system (CNS). The protein is localized not only in the cytoplasm but also in nucleus, lysosomes, peroxisomes, and mitochondrial intermembrane spaces in eukaryotic cells [84, 85].

As mentioned before, the first evidence of the involvement of SOD1 in familial ALS was provided by Rosen and coworkers [32]; currently, more than 150 different mutations distributed throughout the 153-amino acid SOD1 polypeptide have been linked to ALS. These are predominantly single amino acid substitutions although deletions, insertions, and C-terminal truncations also occur. Initial hypothesis regarding its involvement in mediating motor neuron degeneration suggested that mutSOD1 displays reduced activity, promoting accumulation of toxic superoxide radicals [32]. However, several molecular and functional studies performed in animal and cellular models showed that SOD1 pathogenic variants cause FALS by gain rather than loss of function. In this regard, Pesaresi and coworkers [86] demonstrated that mutSOD1 activates p66Shc, which is known to affect mitochondrial function and mitochondria-dependent oxidative balance. Furthermore p66Shc activation inhibits the activity of Rac1, an intracellular transducer which mediates several pathways associated with gene expression regulation, cell proliferation, and cytoskeleton organization [87], thereby causing further OS. Interestingly, another group showed that SOD1 creates a self-regulated redox cycle by directly interacting with Rac1 and orchestrating NADPH oxidase-dependent superoxide production. The study therefore suggests that some mutations in SOD1 might contribute to a gain of function of the protein through the disruption of such redox control [88]. Other hypotheses concerning the role of SOD1 in FALS postulate that mutSOD1 acquires toxic properties that are independent of its normal physiological function. The investigation on the toxic function acquired by mutSOD1 led to the proposal of two main hypotheses [33]. In the aberrant redox chemistry model, mutSOD1 is unstable and through aberrant chemistry interacts with nonconventional substrates causing ROS overproduction. In the protein toxicity model, unstable, misfolded SOD1 aggregates into cytoplasmic inclusion bodies, sequestering proteins crucial for cellular processes. These two hypotheses, however, are not mutually exclusive. Indeed, it has been shown that oxidation of selected histidine residues that bind metals in the active site mediates SOD1 aggregation [89].

4.3. DJ-1 Modulates MutSOD1 Motor Neuron Degeneration Processes in ALS. Recent observations described a connection between DJ-1 and mutSOD1 in ALS (Figure 1). The first evidence about such connection was described by Lev and coworkers [90], who detected increased DJ-1 mRNA and

protein levels in the brains and spinal cords of SOD1-G93A transgenic mice, a widely employed model of ALS. The upregulation was detectable starting from the very early stages of disease progression. In addition, enhanced levels of DJ-1 acidic isoforms were found, indicating that more oxidized DJ-1 amounts were present in the CNS of transgenic mice. In 2010, Yamashita and collaborators [91] proved, both *in vitro* and *in vivo*, the existence of direct association between DJ-1 and SOD1: the two proteins interacted in GST pull-down assays and formed complexes that colocalized in mice primary motor neuron culture. A better colocalization was obtained between DJ-1 and mutSOD1 rather than WT-SOD1. Notably, the overexpression of exogenous DJ-1 in stably mutSOD1-expressing cells reduced cell toxicity and OS markers as compared to cells expressing control vector protein. These data strongly suggest that the described interaction plays a protective role, although further investigation is required to shed light on the functional events activated by DJ-1. Indeed, the exact molecular mechanisms by which DJ-1 accomplishes its defensive function(s) in ALS are still largely unknown.

5. Nrf2/ARE Pathway: Relationship with DJ-1 and SOD1 Proteins

5.1. Nrf2 Pathway. NF-E2-related factor 2 (Nrf2) belongs to the Cap'n'collar (Cnc) transcription factor family and is considered the "master regulator" of the antioxidant response since it modulates the expression and the coordinated induction of an array of defensive genes encoding phase II detoxifying enzymes and antioxidant proteins, such as NAD(P)H: quinine oxidoreductases (NQOs), heme oxygenase-1 (HO-1), the glutathione S-transferase (GST) family, multidrug resistance-associated proteins (Mrps), the UDP-glucuronosyltransferase (UGT) family, ferritin proteins, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) [60, 92]. 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 redox homeostasis disturbance. Under basal condition, Keap1 retains Nrf2 in the cytoplasm, linking this transcriptional factor to the actin cytoskeleton and driving its degradation. Specifically, Keap1 acts as a linker protein between Nrf2 and the Cul3-based E3-ubiquitin ligase complex, promoting Nrf2 ubiquitination and consequent degradation by the 26S proteasome [93, 94].

This quenching interaction between the two proteins is a dynamic process controlled by specific intracellular cascades that allow for a fine-tuned regulation of inducible expression of Nrf2 target genes under OS or after exposure to toxic electrophiles. In fact, activation of Nrf2 requires its cytosolic stabilization via oxidative modification of distinct Keap1 cysteine residues and/or Keap1 ubiquitination and proteasomal degradation. It has been largely demonstrated that also Nrf2 phosphorylation facilitates its dissociation from Keap1. Therefore, several signaling pathways, such as the activation of mitogen-activated protein kinase (MAPK) cascade, phosphatidylinositol 3-kinase (PI3K), and protein kinase C (PKC), favour Nrf2 detachment from its repressors and the consequent translocation to the nucleus. In the nuclear compartment Nrf2 forms a heterodimer with its partner small Maf and binds specific *cis*-acting antioxidant response element (ARE) sequences, ultimately transactivating a battery of highly inducible cytoprotective genes thus allowing cell to efficiently cope with endogenous stress and exogenous toxicants [95]. Nrf2 has also been shown to modulate the transcription of genes promoting mitochondrial biogenesis, such as mitochondrial transcription factors (TFAM) [96], and consequently to be directly involved in mitochondrial maintenance.

Considering the pivotal defensive role exerted by the Nrf2/ARE pathway, it is evident that the dysregulation of Nrf2-regulated genes offers a logical explanation for the direct and indirect association between OS and several neurodegenerative conditions.

5.2. DJ-1 and Nrf2 Pathway. DJ-1 is a redox-sensitive protein which triggers activation of antioxidant defences in particular through Nrf2/ARE system (Figure 1). The first evidence of a connection between DJ-1 and Nrf2 was reported by Clements and collaborators who showed in different cellular models that DJ-1 affects the stability and the transcriptional functions of Nrf2. In particular they found that DJ-1 stabilizes Nrf2 by interfering with its ubiquitination and facilitates Nrf2 translocation to the nucleus by preventing the binding with Keap1 [97]. Similar results from another group confirmed the role of DJ-1 in controlling Nrf2 turnover in both cellular and animal models [98]. A very recent study showed that mutations in *PARK7* gene and protein loss of function significantly reduce thioredoxin1 (trx1) expression. Thioredoxin1 is a disulfide oxidoreductase, whose transcription is under the control of a promoter containing typical ARE sequence and is therefore expressed after Nrf2 activation. According to the authors, these findings further stress the association between DJ-1 and Nrf2 and support the hypothesis that DJ-1 action against OS in the nucleus is carried out by Nrf2 [79]. Nonetheless, Nrf2/ARE pathway activation has recently obtained growing attention and interest as a major target to develop new disease-modifying, neuroprotective strategies in PD [99].

5.3. SOD1 and Nrf2. The first evidence of a relationship between SOD1 and Nrf2 was reported by Kirby and coworkers [57], who showed by microarray analysis that the presence of mutSOD1 (G93A) in mouse motor neuron-like hybrid cell line NSC34 caused a reduction in Nrf2 mRNA expression as well as a global downregulation of a battery of Nrf2 target genes (Figure 1). Notably, also Nrf2 mRNA expression is reduced as compared to cells transfected with WT-SOD1. Several further studies attempted to shed light on Nrf2 signalling cascade in models for SOD1-associated FALS. Diminished Nrf2 levels have been observed in embryonic motor neuron cultures from SOD1-G93A transgenic mice. These cells were more susceptible to apoptosis caused by exposure to nerve growth factor (NGF) [100]. Opposed to these studies carried out in motor neurons, Kraft and collaborators reported a significant activation of Nrf2 in distal muscles

of mutSOD1 mice during the early stages of the pathology. Nrf2-ARE activation appeared to later propagate in a retrograde manner also along the motor pathway during disease progression and was interpreted as a reactive attempt to counteract broad pathogenic signalling cascades induced by mutSOD1 toxicity [101].

Although the literature data present some contrasts and gaps on the molecular details underlying mutSOD1-Nrf2 interaction, these findings suggest that the toxic gain of function of mutSOD1 may lead to a perturbation of Nrf2 pathway activation.

On the other hand, though, pharmacological treatment aiming at activating Nrf2 pathway might counteract the negative effect exerted by mutSOD1 and might represent a promising therapeutic perspective for ALS. In support of this hypothesis, a neuroprotective role played by Nrf2 pathway activation in mutSOD1-associated ALS has been demonstrated by Vargas and coworkers who generated SOD1-G93A mice overexpressing Nrf2 specifically in astrocytes [122] and in neurons or type II skeletal muscle fibers [123]. In fact, Nrf2 overexpressing astrocytes isolated from SOD1-G93A transgenic mice could protect cocultured nontransgenic motor neurons from mutSOD1 toxicity by increasing the production/secretion of glutathione. Additionally, the overexpression of Nrf2 in astrocytes of SOD1-G93A transgenic mice increased the median survival in these animals [122]. Differently, Nrf2 overexpression in either neurons or type II skeletal muscle fibers in the same ALS mouse model could delay disease onset but could not extend life span [123]. A similar result has been reported by Guo and colleagues [124], who demonstrated that Nrf2 knockout in SOD1-G93A transgenic mice only modestly impacted the course of ALS. Taken together, these findings suggest that the antioxidant and prosurvival effects exerted by Nrf2 activation are important in modulating ALS phenotype but cell type specificity represents a critical factor to take into consideration when designing effective Nrf2-based pharmacological strategies for ALS treatment.

6. Modulators of Nrf2/ARE Pathway

The Nrf2/ARE pathway can be pharmacologically activated by molecules of both natural derivation (nutraceuticals) and chemical synthesis. Sulforaphane (SFN), polyphenols, epigallocatechin 3-gallate (EGCG), and 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, alias curcumin, are among Nrf2/ARE activators of natural origin, whereas chemical Nrf2/ARE activators include triterpenoids and N-(4-(2-pyridyl)(1,3-thiazol-2-yl))-2-(2,4,6-trimethylphenoxy) acetamide, alias CPN-9.

A variety of nutraceuticals have demonstrated antioxidant and neuroprotective activity through Nrf2/ARE pathway induction. SFN, a naturally occurring isothiocyanate derived from cruciferous vegetables such as broccoli, activates Nrf2 via modification of reactive cysteine residues of Keap1 [102, 103], thereby providing protection in various models of neurodegeneration. In particular, it was shown that SFN is able to cross the blood brain barrier, activate Nrf2-dependent gene expression in the basal ganglia, and protect nigral dopaminergic neurons from cell death induced by MPTP [28]. Important Nrf2/ARE pathway activators are also EGCG and resveratrol, belonging to the family of polyphenols. Taking into account their abundance and multiple antioxidant mechanisms, polyphenols are considered to be important nutraceuticals. EGCG, a flavonoid polyphenol, is the main antioxidant molecule present in green tea. It displayed notable antioxidant and neuroprotective functions in cultured motoneuron-neuroblastoma hybrid cell line transfected with mutSOD1 [110] and in PC12 cells exposed to paraquat [111]. Furthermore, EGCG was shown to be neuroprotective in mice model of ALS: oral administration to mice expressing mutSOD1 delayed symptoms onset [112, 113]. EGCG was shown to activate Nrf2/ARE through protein kinase cascades [125]. Notably, it was demonstrated that EGCG activated HO-1 expression via Nrf2/ARE pathway, protecting rat neurons against oxidative insult [114]. Resveratrol, a polyphenolic compound present in red wine, demonstrated protective effects against hypoxic injury in rat spinal cord dorsal column by activating Nrf2 pathway [115]. Similarly, Nrf2 stabilization mediated by resveratrol protected dorsal root ganglion (DRG) neurons from glucose-induced injury [116]. Curcumin, a member of the curcuminoid family isolated from turmeric, the yellow rhizome of the plant *Curcuma longa*, showed Nrf2-dependent antioxidant properties in primary spinal cord astrocytes exposed to H_2O_2 [104] and in ischemic brain injury models [105]. Other natural activators of Nrf2/ARE pathway, such as naphthazarin, genistein, and carnosic acid, showed positive effects in several models of neurodegenerative and cardiovascular diseases implicating OS as a pathogenic factor [106–109, 126–128]. A summary of nutraceutical activators of Nrf2/ARE pathway described in this paragraph is reported in Table 1.

Together with molecules of natural origin, several synthetic Nrf2/ARE activators were recently developed. Notably, important obstacles in the identification of Nrf2/ARE chemical activators were encountered, mainly because of the highly time- and money-demanding conventional screening methods and the lack of structural similarities among developed molecules, thereby preventing coherent and convenient structure activity relationships (SAR) applications.

Recently, triterpenoids emerged as a potent class of Nrf2/ARE inducers. The triterpenoid family consists in three chemically related members: 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid ethylamide (CDDO-EA), CDDO trifluoroethylamide (CDDO-TFEA), and CDDO methylamide (CDDO-MA). Triterpenoids have shown a paramount potency in Nrf2 induction, the ability to attenuate dopaminergic neurodegeneration in MPTP mouse model of PD [117], and increase the life span in ALS mouse models [118]. Another interesting small 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 also significantly suppressed ROS levels through the induction of several defensive genes [119]. Finally it is worth mentioning that some well-established therapeutics such as bromocriptine [120] and azathioprine [121] were

Table 1: Natural activators of Nrf2/ARE signalling pathway.

Molecule	Model	Reference
Sulforaphanes (SFN)	Keap-1 purified protein	Dinkova-Kostova et al., 2002 [102]
	Keap-1 protein (*in vitro* study)	Hong et al., 2005 [103]
	Dopaminergic neurons (basal ganglia) after exposure to MPTP	Jazwa et al. 2011 [28]
Curcumin	Spinal cord primary astrocytes after exposure to H_2O_2	Jiang et al., 2011 [104]
	Primary cortical neurons after oxygen-glucose deprivation/reoxygenation (model of brain ischemia)	Wu et al., 2013 [105]
Naphthazarin	ARE-bla Hep G2 cell line and primary neuron and astrocyte cultures	Son et al., 2013 [106]
Genistein	PC12 cells after incubation with beta-amyloid peptides 25–35	Ma et al., 2010 [127]
	bEND.3 cells after incubation with beta-amyloid peptides 25–35	Xi et al., 2012 [128]
	Rat hippocampal CA1 neurons after cerebral ischemia	Wang et al., 2013 [107]
Carnosic acid	Primary cortical neurons	Satoh et al., 2008 [108]
	SH-SY5Y cells after exposure to 6-hydroxydopamine	Chen et al., 2012 [109]
Polyphenols		
Epigallocatechin 3-gallate (EGCG)	mutSOD1-transfected motoneurons	Koh et al., 2004 [110]
	PC12 cells after exposure to paraquat	Hou et al., 2008 [111]
	SOD1-G93A transgenic mice (model of ALS)	Koh et al., 2006 [112]
	SOD1-G93A transgenic mice (model of ALS)	Xu et al., 2006 [113]
	Rat immortalized neurons (H19-7)	Romeo et al., 2009 [114]
Resveratrol	Spinal cord from adult rats after hypoxic injury	Kesherwani et al., 2013 [115]
	Dorsal root ganglionic cells after glucose-induced injury	Vincent et al., 2009 [116]

Table 2: Synthetic activators of Nrf2/ARE signalling pathway.

Molecule	Model	Reference
Triterpenoids		
CDDO-EA	MPTP-treated mice (model of PD)	Kaidery et al., 2013 [117]
CDDO-TFEA	Cellular models and SOD1-G93A transgenic mice (model of ALS)	Neymotin et al., 2011 [118]
CPN-9	Cellular models and $SOD1^{H46R}$ transgenic mice (model of ALS)	Kanno et al., 2012 [119]
Bromocriptine	PC12 cells	Lim et al., 2008 [120]
Azathioprine	Cellular and transgenic mice (models of skin and liver carcinoma)	Kalra et al., 2011 [121]

reported to induce the Nrf2/ARE pathway, therefore providing insight into a possible development of new synthetic cutting edge Nrf2 activators. A summary of synthetic activators of Nrf2/ARE pathway described in this paragraph is reported in Table 2.

7. Conclusion

OS is a hallmark of neurodegeneration and both PD and ALS report deregulated antioxidant defences and increased levels of OS markers in neural cells, biological fluids, and peripheral tissues.

Proteins such as DJ-1 and SOD1 are critical in PD and ALS pathogenesis and are also major players in the association between the neurodegenerative process and redox homeostasis. In fact, DJ-1 plays a protective role against OS and pathogenic mutations in *PARK7* gene lead to PD because of loss of this function. Differently, mutSOD1, through the gain of prooxidant and toxic function, aggregates and accumulates in the cell eventually causing ALS. According to recent studies, DJ-1 antioxidant capacity is not restricted to the protection of the neurons affected in PD but is rather a general function. Therefore, the activation of DJ-1 antioxidant downstream targets could be potentiated in order to obtain beneficial effects also in other neurodegenerative conditions.

Several studies showed that both DJ-1 and SOD1 have a remarkable connection with the antioxidant Nrf2/ARE pathway, with DJ-1 being an upstream activator and SOD1 a target. As we explained in this review, the importance of Nrf2/ARE pathway lies in the ability to activate the expression of crucial antioxidant and detoxifying genes. Modulation of Nrf2/ARE pathway in neurodegenerative diseases, either with

nutraceuticals or chemically synthesized molecules, might therefore augment cellular defences against OS, thus leading to neuroprotection [60].

In line with other studies, we conclude that pharmacological activation of Nrf2/ARE pathway represents an attractive neuroprotective therapy which may hold superior power as compared to conventional antioxidant routes and might represent a disease-modifying treatment to counteract neuronal loss in different degenerative pathologies.

Authors' Contribution

Pamela Milani and Giulia Ambrosi have equally contributed to the work.

References

[1] B. Halliwell, "Oxidative stress and neurodegeneration: where are we now?" *Journal of Neurochemistry*, vol. 97, no. 6, pp. 1634–1658, 2006.

[2] A. Melo, L. Monteiro, R. M. F. Lima, D. M. de Oliveira, M. D. de Cerqueira, and R. S. El-Bachá, "Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives," *Oxidative Medicine and Cellular Longevity*, Article ID 467180, 2011.

[3] S. Gandhi and A. Y. Abramov, "Mechanism of oxidative stress in neurodegeneration," *Oxidative Medicine and Cellular Longevity*, vol. 2012, Article ID 428010, 11 pages, 2012.

[4] M. Jimenez-Del-Rio and C. Velez-Pardo, "The bad, the good, and the ugly about oxidative stress," *Oxidative Medicine and Cellular Longevity*, vol. 2012, Article ID 163913, 13 pages, 2012.

[5] M. S. Hernandes and L. R. Britto, "NADPH oxidase and neurodegeneration," *Current Neuropharmacology*, vol. 10, no. 4, pp. 321–327, 2012.

[6] F. Orsini, M. Moroni, C. Contursi et al., "Regulatory effects of the mitochondrial energetic status on mitochondrial p66Shc," *Biological Chemistry*, vol. 387, no. 10-11, pp. 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," *American Journal of Physiology*, vol. 268, no. 5, pp. L699–L722, 1995.

[8] M. C. Martínez and R. Andriantsitohaina, "Reactive nitrogen species: molecular mechanisms and potential significance in health and disease," *Antioxidants and Redox Signaling*, vol. 11, no. 3, pp. 669–702, 2009.

[9] D. Trachootham, W. Lu, M. A. Ogasawara, N. R.-D. Valle, and P. Huang, "Redox regulation of cell survival," *Antioxidants and Redox Signaling*, vol. 10, no. 8, pp. 1343–1374, 2008.

[10] B. Halliwell, "Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment," *Drugs and Aging*, vol. 18, no. 9, pp. 685–716, 2001.

[11] Y. Son, Y. K. Cheong, N. H. Kim, H. T. Chung, D. G. Kang, and H. O. Pae, "Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways?" *Journal of Signal Transduction*, vol. 2011, Article ID 792639, 6 pages, 2011.

[12] Y. Yan, C.-L. Wei, W.-R. Zhang, H.-P. Cheng, and J. Liu, "Cross-talk between calcium and reactive oxygen species signaling," *Acta Pharmacologica Sinica*, vol. 27, no. 7, pp. 821–826, 2006.

[13] R. F. Feissner, J. Skalska, W. E. Gaum, and S.-S. Sheu, "Crosstalk signaling between mitochondrial Ca2+ and ROS," *Frontiers in Bioscience*, vol. 14, no. 4, pp. 1197–1218, 2009.

[14] Q. Ma, "Transcriptional responses to oxidative stress: pathological and toxicological implications," *Pharmacology and Therapeutics*, vol. 125, no. 3, pp. 376–393, 2010.

[15] R. G. Allen and M. Tresini, "Oxidative stress and gene regulation," *Free Radical Biology and Medicine*, vol. 28, no. 3, pp. 463–499, 2000.

[16] H. L. A. Vieira, P. M. Alves, and A. Vercelli, "Modulation of neuronal stem cell differentiation by hypoxia and reactive oxygen species," *Progress in Neurobiology*, vol. 93, no. 3, pp. 444–455, 2011.

[17] K. A. M. Kennedy, S. D. E. Sandiford, I. S. Skerjanc, and S. S.-C. Li, "Reactive oxygen species and the neuronal fate," *Cellular and Molecular Life Sciences*, vol. 69, no. 2, pp. 215–221, 2012.

[18] L. M. de Lau and M. M. Breteler, "Epidemiology of Parkinson's disease," *The Lancet Neurology*, vol. 5, no. 6, pp. 525–535, 2006.

[19] G. Xiromerisiou, E. Dardiotis, V. Tsimourtou et al., "Genetic basis of Parkinson disease," *Neurosurgical Focus*, vol. 28, no. 1, pp. E7.1–E7.7, 2010.

[20] 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," *International Journal of Molecular Sciences*, vol. 13, no. 8, pp. 10478–10504, 2012.

[21] J. T. Greenamyre and T. G. Hastings, "Parkinsons-divergent causes convergent mechanisms," *Science*, vol. 304, no. 5674, pp. 1120–1122, 2004.

[22] R. K. Chaturvedi and M. F. Beal, "Mitochondrial diseases of the brain," *Free Radical Biology and Medicine*, vol. 63, pp. 1–29, 2013.

[23] A. H. V. Schapira, A. Hartley, M. W. J. Cleeter, and J. M. Cooper, "Free radicals and mitochondrial dysfunction in Parkinson's disease," *Biochemical Society Transactions*, vol. 21, no. 2, pp. 367–370, 1993.

[24] 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 Radical Biology and Medicine*, vol. 46, no. 12, pp. 1574–1580, 2009.

[25] 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," *Neurobiology of Disease*, vol. 15, no. 1, pp. 160–170, 2004.

[26] C. Isobe, T. Abe, and Y. Terayama, "Levels of reduced and oxidized coenzymeQ-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," *Neuroscience Letters*, vol. 469, no. 1, pp. 159–163, 2010.

[27] S. Nikam, P. Nikam, S. K. Ahaley, and A. V. Sontakke, "Oxidative stress in Parkinson's disease," *Indian Journal of Clinical Biochemistry*, vol. 24, no. 1, pp. 98–101, 2009.

[28] A. Jazwa, A. I. Rojo, N. G. Innamorato, M. Hesse, J. Fernández-Ruiz, and A. Cuadrado, "Pharmacological targeting of the transcription factor NRf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism," *Antioxidants and Redox Signaling*, vol. 14, no. 12, pp. 2347–2360, 2011.

[29] T. P. Williamson, D. A. Johnson, and 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*, vol. 33, no. 3, pp. 272–279, 2012.

[30] A. Mehta, M. Prabhakar, P. Kumar, R. Deshmukh, and P. L. Sharma, "Excitotoxicity: bridge to various triggers in neurodegenerative disorders," *European Journal of Pharmacology*, vol. 698, no. 1–3, pp. 6–18, 2013.

[31] L. J. Peterson and P. M. Flood, "Oxidative stress and microglial cells in Parkinson's disease," *Mediators of Inflammation*, vol. 2012, Article ID 401264, 12 pages, 2012.

[32] D. R. Rosen, T. Siddique, D. Patterson et al., "Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis," *Nature*, vol. 362, no. 6415, pp. 59–62, 1993.

[33] P. Pasinelli and R. H. Brown, "Molecular biology of amyotrophic lateral sclerosis: insights from genetics," *Nature Reviews Neuroscience*, vol. 7, no. 9, pp. 710–723, 2006.

[34] M. Cozzolino, M. G. Pesaresi, V. Gerbino, J. Grosskreutz, and M. T. Carri, "Amyotrophic lateral sclerosis: new insights into underlying molecular mechanisms and opportunities for therapeutic intervention," *Antioxidants & Redox Signaling*, vol. 17, no. 9, pp. 1277–1330, 2012.

[35] S. Gagliardi, P. Milani, V. Sardone, O. Pansarasa, and C. Cereda, "From transcriptome to noncoding RNAs: implications in ALS mechanism," *Neurology Research International*, vol. 2012, Article ID 278725, 7 pages, 2012.

[36] L. Rossi, C. Valle, and M. T. Carri, "Altered gene expression, mitochondrial damage and oxidative stress: converging routes in motor neuron degeneration," *International Journal of Cell Biology*, vol. 2012, Article ID 908724, 9 pages, 2012.

[37] M. J. Strong, "The evidence for altered RNA metabolism in amyotrophic lateral sclerosis (ALS)," *Journal of the Neurological Sciences*, vol. 288, no. 1-2, pp. 1–12, 2010.

[38] R. J. Ferrante, S. E. Browne, L. A. Shinobu et al., "Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis," *Journal of Neurochemistry*, vol. 69, no. 5, pp. 2064–2074, 1997.

[39] S. C. Barber, R. J. Mead, and P. J. Shaw, "Oxidative stress in ALS: a mechanism of neurodegeneration and a therapeutic target," *Biochimica et Biophysica Acta*, vol. 1762, no. 11-12, pp. 1051–1067, 2006.

[40] S. C. Barber and P. J. Shaw, "Oxidative stress in ALS: key role in motor neuron injury and therapeutic target," *Free Radical Biology and Medicine*, vol. 48, no. 5, pp. 629–641, 2010.

[41] K. Abe, L.-H. Pan, M. Watanabe, T. Kato, and Y. Itoyama, "Induction of nitrotyrsone-like immunoreactivity in the lower motor neuron of amyotrophic lateral sclerosis," *Neuroscience Letters*, vol. 199, no. 2, pp. 152–154, 1995.

[42] M. F. Beal, R. J. Ferrante, S. E. Browne, R. T. Matthews, N. W. Kowall, and R. H. Brown Jr., "Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis," *Annals of Neurology*, vol. 42, no. 4, pp. 644–654, 1997.

[43] P. J. Shaw, P. G. Ince, G. Falkous, and D. Mantle, "Oxidative damage to protein in sporadic motor neuron disease spinal cord," *Annals of Neurology*, vol. 38, no. 4, pp. 691–695, 1995.

[44] R. G. Cutler, W. A. Pedersen, S. Camandola, J. D. Rothstein, and M. P. Mattson, "Evidence that accumulation of ceramides and cholesterol esters mediates oxidative stress—induced death of motor neurons in amyotrophic lateral sclerosis," *Annals of Neurology*, vol. 52, no. 4, pp. 448–457, 2002.

[45] 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," *Annals of Neurology*, vol. 44, no. 5, pp. 819–824, 1998.

[46] R. G. Smith, Y. K. Henry, M. P. Mattson, and S. H. Appel, "Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis," *Annals of Neurology*, vol. 44, no. 4, pp. 696–699, 1998.

[47] E. P. Simpson, Y. K. Henry, J. S. Henkel, R. G. Smith, and S. H. Appel, "Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden," *Neurology*, vol. 62, no. 10, pp. 1758–1765, 2004.

[48] M. T. Carrì and M. Cozzolino, "SOD1 and mitochondria in ALS: a dangerous liaison," *Journal of Bioenergetics and Biomembranes*, vol. 43, no. 6, pp. 593–599, 2011.

[49] L. J. Martin, "Mitochondrial pathobiology in ALS," *Journal of Bioenergetics and Biomembranes*, vol. 43, no. 6, pp. 569–579, 2011.

[50] G. Manfredi and Z. Xu, "Mitochondrial dysfunction and its role in motor neuron degeneration in ALS," *Mitochondrion*, vol. 5, no. 2, pp. 77–87, 2005.

[51] D. Curti, A. Malaspina, G. Facchetti et al., "Amyotrophic lateral sclerosis: oxidative energy metabolism and calcium homeostasis in peripheral blood lymphocytes," *Neurology*, vol. 47, no. 4, pp. 1060–1064, 1996.

[52] J. Kong and Z. Xu, "Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1," *Journal of Neuroscience*, vol. 18, no. 9, pp. 3241–3250, 1998.

[53] F. R. Wiedemann, G. Manfredi, C. Mawrin, M. F. Beal, and E. A. Schon, "Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients," *Journal of Neurochemistry*, vol. 80, no. 4, pp. 616–625, 2002.

[54] E. Usarek, B. Gajewska, B. Kaźmierczak, M. Kuźma, D. Dziewulska, and A. Barańczyk-Kuźma, "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," *Neurochemical Research*, vol. 30, no. 8, pp. 1003–1007, 2005.

[55] M. Kuzma, Z. Jamrozik, and A. Baranczyk-Kuzma, "Activity and expression of glutathione S-transferase pi in patients with amyotrophic lateral sclerosis," *Clinica Chimica Acta*, vol. 364, no. 1-2, pp. 217–221, 2006.

[56] 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 Neuropathologica*, vol. 110, no. 2, pp. 101–112, 2005.

[57] J. Kirby, E. Halligan, M. J. Baptista et al., "Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS," *Brain*, vol. 128, no. 7, pp. 1686–1706, 2005.

[58] A. Sarlette, K. Krampfl, C. Grothe, N. V. Neuhoff, R. Dengler, and S. Petri, "Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis," *Journal of Neuropathology and Experimental Neurology*, vol. 67, no. 11, pp. 1055–1062, 2008.

[59] 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 Research*, vol. 1446, pp. 109–118, 2012.

[60] S. Petri, S. Korner, and M. Kiaei, "Nrf2/ARE signaling pathway: key mediator in oxidative stress and potential therapeutic target in ALS," *Neurology Research International*, vol. 2012, Article ID 878030, 7 pages, 2012.

[61] D. Nagakubo, T. Taira, H. Kitaura et al., "DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras," *Biochemical and Biophysical Research Communications*, vol. 231, no. 2, pp. 509–513, 1997.

[62] A. Wagenfeld, J. Gromoll, and T. G. Cooper, "Molecular cloning and expression of rat contraception associated protein 1 (CAP1), a protein putatively involved in fertilization," *Biochemical and Biophysical Research Communications*, vol. 251, no. 2, pp. 545–549, 1998.

[63] V. Bonifati, P. Rizzu, M. J. van Baren et al., "Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism," *Science*, vol. 299, no. 5604, pp. 256–259, 2003.

[64] 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," *Journal of Biological Chemistry*, vol. 278, no. 33, pp. 31380–31384, 2003.

[65] Q. Huai, Y. Sun, H. Wang et al., "Crystal structure of DJ-1/RS and implication on familial Parkinson's disease," *FEBS Letters*, vol. 549, no. 1–3, pp. 171–175, 2003.

[66] X. Tao and L. Tong, "Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease," *Journal of Biological Chemistry*, vol. 278, no. 33, pp. 31372–31379, 2003.

[67] M. A. Wilson, J. L. Collins, Y. Hod, D. Ringe, and G. A. Petsko, "The 1.1-Å resolution crystal structure of DJ-1, the protein mutated in autosomal recessive early onset Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 16, pp. 9256–9261, 2003.

[68] M. Lakshminarasimhan, M. T. Maldonado, W. Zhou, A. L. Fink, and M. A. Wilson, "Structural impact of three Parkinsonism-associated missense mutations on human DJ-1," *Biochemistry*, vol. 47, no. 5, pp. 1381–1392, 2008.

[69] I. Irrcher, H. Aleyasin, E. L. Seifert et al., "Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics," *Human Molecular Genetics*, vol. 19, no. 19, Article ID ddq288, pp. 3734–3746, 2010.

[70] N. J. Larsen, G. Ambrosi, S. J. Mullett, S. B. Berman, and D. A. Hinkle, "DJ-1 knock-down impairs astrocyte mitochondrial function," *Neuroscience*, vol. 196, pp. 251–264, 2011.

[71] S. J. Kim, Y. J. Park, I. Y. Hwang, M. B. Youdim, K. S. Park, and Y. J. Oh, "Nuclear translocation of DJ-1 during oxidative stress-induced neuronal cell death," *Free Radical Biology and Medicine*, vol. 53, no. 4, pp. 936–950, 2012.

[72] E. Junn, W. H. Jang, X. Zhao, B. S. Jeong, and M. M. Mouradian, "Mitochondrial localization of DJ-1 leads to enhanced neuroprotection," *Journal of Neuroscience Research*, vol. 87, no. 1, pp. 123–129, 2009.

[73] L. Gu, T. Cui, C. Fan et al., "Involvement of ERK1/2 signaling pathway in DJ-1-induced neuroprotection against oxidative stress," *Biochemical and Biophysical Research Communications*, vol. 383, no. 4, pp. 469–474, 2009.

[74] E. Andres-Mateos, C. Perier, L. Zhang et al., "DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 37, pp. 14807–14812, 2007.

[75] 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," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 24, pp. 9103–9108, 2004.

[76] J. Waak, S. S. Weber, K. Görner et al., "Oxidizable residues mediating protein stability and cytoprotective interaction of DJ-1 with apoptosis signal-regulating kinase 1," *Journal of Biological Chemistry*, vol. 284, no. 21, pp. 14245–14257, 2009.

[77] M. A. Wilson, "The role of cysteine oxidation in DJ-1 function and dysfunction," *Antioxidants and Redox Signaling*, vol. 15, no. 1, pp. 111–122, 2011.

[78] N. Zhong and J. Xu, "Synergistic activation of the human MnSOD promoter by DJ-1 and PGC-1α: regulation by SUMOylation and oxidation," *Human Molecular Genetics*, vol. 17, no. 21, pp. 3357–3367, 2008.

[79] J. Y. Im, K. W. Lee, J. M. Woo, E. Junn, and M. M. Mouradian, "DJ-1 induces thioredoxin 1 expression through the Nrf2 pathway," *Human Molecular Genetics*, vol. 21, no. 13, pp. 3013–3024, 2012.

[80] H. Ren, K. Fu, D. Wang, C. Mu, and G. Wang, "Oxidized DJ-1 interacts with the mitochondrial protein BCL-XL," *Journal of Biological Chemistry*, vol. 286, no. 40, pp. 35308–35317, 2011.

[81] W. Zhou, M. Zhu, M. A. Wilson, G. A. Petsko, and A. L. Fink, "The oxidation state of DJ-1 regulates its chaperone activity toward α-synuclein," *Journal of Molecular Biology*, vol. 356, no. 4, pp. 1036–1048, 2006.

[82] M. K. McCoy and M. R. Cookson, "DJ-1 regulation of mitochondrial function and autophagy through oxidative stress," *Autophagy*, vol. 7, no. 5, pp. 531–532, 2011.

[83] J. M. McCord and I. Fridovich, "Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein)," *Journal of Biological Chemistry*, vol. 244, no. 22, pp. 6049–6055, 1969.

[84] J. Lindenau, H. Noack, H. Possel, K. Asayama, and G. Wolf, "Cellular distribution of superoxide dismutases in the rat CNS," *Glia*, vol. 29, no. 1, pp. 25–34, 2000.

[85] N. Kurobe, F. Suzuki, K. Okajima, and K. Kato, "Sensitive enzyme immunoassay for human Cu/Zn superoxide dismutase," *Clinica Chimica Acta*, vol. 187, no. 1, pp. 11–20, 1990.

[86] M. G. Pesaresi, I. Amori, C. Giorgi et al., "Mitochondrial redox signalling by p66Shc mediates ALS-like disease through Rac1 inactivation," *Human Molecular Genetics*, vol. 20, no. 21, Article ID ddr347, pp. 4196–4208, 2011.

[87] S. Etienne-Manneville and A. Hall, "Rho GTPases in cell biology," *Nature*, vol. 420, no. 6916, pp. 629–635, 2002.

[88] M. M. Harraz, J. J. Marden, W. Zhou et al., "SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model," *Journal of Clinical Investigation*, vol. 118, no. 2, pp. 659–670, 2008.

[89] R. Rakhit, P. Cunningham, A. Furtos-Matei et al., "Oxidation-induced misfolding and aggregation of superoxide dismutase and its implications for amyotrophic lateral sclerosis," *Journal of Biological Chemistry*, vol. 277, no. 49, pp. 47551–47556, 2002.

[90] N. Lev, D. Ickowicz, Y. Barhum, E. Melamed, and D. Offen, "DJ-1 changes in G93A-SOD1 transgenic mice: implications for oxidative stress in ALS," *Journal of Molecular Neuroscience*, vol. 38, no. 2, pp. 94–102, 2009.

[91] S. Yamashita, A. Mori, E. Kimura et al., "DJ-1 forms complexes with mutant SOD1 and ameliorates its toxicity," *Journal of Neurochemistry*, vol. 113, no. 4, pp. 860–870, 2010.

[92] K.-A. Jung and M.-K. Kwak, "The Nrf2 system as a potential target for the development of indirect antioxidants," *Molecules*, vol. 15, no. 10, pp. 7266–7291, 2010.

[93] M. Kobayashi and M. Yamamoto, "Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation," *Antioxidants and Redox Signaling*, vol. 7, no. 3-4, pp. 385–394, 2005.

[94] N. F. Villeneuve, A. Lau, and D. D. Zhang, "Regulation of the Nrf2-keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases," *Antioxidants and Redox Signaling*, vol. 13, no. 11, pp. 1699–1712, 2010.

[95] P. Milani, S. Gagliardi, E. Cova, and C. Cereda, "SOD1 transcriptional and posttranscriptional regulation and its potential implications in ALS," *Neurology Research International*, vol. 2011, Article ID 458427, 9 pages, 2011.

[96] P. Yenki, F. Khodagholi, and F. Shaerzadeh, "Inhibition of phosphorylation of JNK suppresses Abeta-induced ER stress and upregulates prosurvival mitochondrial proteins in rat hippocampus," *Journal of Molecular Neuroscience*, vol. 49, no. 2, pp. 262–269, 2013.

[97] C. M. Clements, R. S. McNally, B. J. Conti, T. W. Mak, and J. P.-Y. Ting, "DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 41, pp. 15091–15096, 2006.

[98] L. Gan, D. A. Johnson, and J. A. Johnson, "Keap1-Nrf2 activation in the presence and absence of DJ-1," *European Journal of Neuroscience*, vol. 31, no. 6, pp. 967–977, 2010.

[99] O. Hwang, "Role of oxidative stress in Parkinson's disease," *Experimental Neurobiology*, vol. 22, pp. 11–17, 2013.

[100] M. Pehar, M. R. Vargas, K. M. Robinson et al., "Mitochondrial superoxide production and nuclear factor erythroid 2-related factor 2 activation in p75 neurotrophin receptor-induced motor neuron apoptosis," *Journal of Neuroscience*, vol. 27, no. 29, pp. 7777–7785, 2007.

[101] A. D. Kraft, J. M. Resch, D. A. Johnson, and J. A. Johnson, "Activation of the Nrf2-ARE pathway in muscle and spinal cord during ALS-like pathology in mice expressing mutant SOD1," *Experimental Neurology*, vol. 207, no. 1, pp. 107–117, 2007.

[102] 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," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 18, pp. 11908–11913, 2002.

[103] F. Hong, M. L. Freeman, and D. C. Liebler, "Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane," *Chemical Research in Toxicology*, vol. 18, no. 12, pp. 1917–1926, 2005.

[104] H. Jiang, X. Tian, Y. Guo, W. Duan, H. Bu, and C. Li, "Activation of nuclear factor erythroid 2-related factor 2 cytoprotective signaling by curcumin protect primary spinal cord astrocytes against oxidative toxicity," *Biological and Pharmaceutical Bulletin*, vol. 34, no. 8, pp. 1194–1197, 2011.

[105] J. Wu, Q. Li, X. Wang et al., "Neuroprotection by curcumin in ischemic brain injury involves the akt/nrf2 pathway," *PLoS One*, vol. 8, Article ID e59843, 2013.

[106] T. G. Son, E. M. Kawamoto, Q. S. Yu, N. H. Greig, M. P. Mattson, and S. Camandola, "Naphthazarin protects against glutamate-induced neuronal death via activation of the Nrf2/ARE pathway," *Biochemical and Biophysical Research Communications*, vol. 433, no. 4, pp. 602–606, 2013.

[107] R. Wang, J. Tu, Q. Zhang et al., "Genistein attenuates ischemic oxidative damage and behavioral deficits via eNOS/Nrf2/HO-1 signaling," *Hippocampus*, vol. 23, no. 7, pp. 634–647, 2013.

[108] 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," *Journal of Neurochemistry*, vol. 104, no. 4, pp. 1116–1131, 2008.

[109] J. H. Chen, H. P. Ou, C. Y. Lin et al., "Carnosic acid prevents 6-hydroxydopamine-induced cell death in SH-SY5Y cells via mediation of glutathione synthesis," *Chemical Research in Toxicology*, vol. 25, no. 9, pp. 1893–1901, 2012.

[110] S.-H. Koh, H. Kwon, K. S. Kim et al., "Epigallocatechin gallate prevents oxidative-stress-induced death of mutant Cu/Zn-superoxide dismutase (G93A) motoneuron cells by alteration of cell survival and death signals," *Toxicology*, vol. 202, no. 3, pp. 213–225, 2004.

[111] R.-R. Hou, J.-Z. Chen, H. Chen, X.-G. Kang, M.-G. Li, and B.-R. Wang, "Neuroprotective effects of (-)-epigallocatechin-3-gallate (EGCG) on paraquat-induced apoptosis in PC12 cells," *Cell Biology International*, vol. 32, no. 1, pp. 22–30, 2008.

[112] S.-H. Koh, S. M. Lee, H. Y. Kim et al., "The effect of epigallocatechin gallate on suppressing disease progression of ALS model mice," *Neuroscience Letters*, vol. 395, no. 2, pp. 103–107, 2006.

[113] Z. Xu, S. Chen, X. Li, G. Luo, L. Li, and W. Le, "Neuroprotective effects of (-)-epigallocatechin-3-gallate in a transgenic mouse model of amyotrophic lateral sclerosis," *Neurochemical Research*, vol. 31, no. 10, pp. 1263–1269, 2006.

[114] 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," *Journal of the American College of Nutrition*, vol. 28, supplement, pp. 492S–499S, 2009.

[115] V. Kesherwani, F. Atif, S. Yousuf, and S. K. Agrawal, "Resveratrol protects spinal cord dorsal column from hypoxic injury by activating Nrf-2," *Neuroscience*, vol. 241, pp. 80–88, 2013.

[116] A. M. Vincent, K. Kato, L. L. McLean, M. E. Soules, and E. L. Feldman, "Sensory neurons and schwann cells respond to oxidative stress by increasing antioxidant defense mechanisms," *Antioxidants and Redox Signaling*, vol. 11, no. 3, pp. 425–438, 2009.

[117] 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," *Antioxidants & Redox Signaling*, vol. 18, no. 2, pp. 139–157, 2013.

[118] 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 Radical Biology and Medicine*, vol. 51, no. 1, pp. 88–96, 2011.

[119] 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 Radical Biology and Medicine*, vol. 53, no. 11, pp. 2028–2042, 2012.

[120] J. H. Lim, K.-M. Kim, S. W. Kim, O. Hwang, and H. J. Choi, "Bromocriptine activates NQO1 via Nrf2-PI3K/Akt signaling: novel cytoprotective mechanism against oxidative damage," *Pharmacological Research*, vol. 57, no. 5, pp. 325–331, 2008.

[121] S. Kalra, Y. Zhang, E. V. Knatko, S. Finlayson, M. Yamamoto, and A. T. Dinkova-Kostova, "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 Prevention Research*, vol. 4, no. 10, pp. 1665–1674, 2011.

[122] M. R. Vargas, D. A. Johnson, D. W. Sirkis, A. Messing, and J. A. Johnson, "Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis," *Journal of Neuroscience*, vol. 28, no. 50, pp. 13574–13581, 2008.

[123] 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*, vol. 8, article e56625, 2013.

[124] Y. Guo, Y. Zhang, D. Wen et al., "The modest impact of transcription factor Nrf2 on the course of disease in an ALS animal model," *Laboratory Investigation*, vol. 93, no. 7, pp. 825–833, 2013.

[125] H.-K. Na and Y.-J. Surh, "Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG," *Food and Chemical Toxicology*, vol. 46, no. 4, pp. 1271–1278, 2008.

[126] N. A. Kelsey, H. M. Wilkins, and D. A. Linseman, "Nutraceutical antioxidants as novel neuroprotective agents," *Molecules*, vol. 15, no. 11, pp. 7792–7814, 2010.

[127] 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," *International Journal of Developmental Neuroscience*, vol. 28, no. 4, pp. 289–295, 2010.

[128] 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," *Current Neurovascular Research*, vol. 9, no. 1, pp. 32–41, 2012.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 415078, 10 pages
http://dx.doi.org/10.1155/2013/415078

Review Article

Sulforaphane as a Potential Protective Phytochemical against Neurodegenerative Diseases

Andrea Tarozzi,[1] Cristina Angeloni,[1] Marco Malaguti,[1] Fabiana Morroni,[2] Silvana Hrelia,[1] and Patrizia Hrelia[2]

[1] *Department for Life Quality Studies, Alma Mater Studiorum, University of Bologna, Corso d'Augusto 237, 47900 Rimini, Italy*
[2] *Department of Pharmacy and Biotechnology, Alma Mater Studiorum, University of Bologna, Via Irnerio 48, 40126 Bologna, Italy*

Correspondence should be addressed to Cristina Angeloni; cristina.angeloni@unibo.it

Received 30 April 2013; Revised 28 June 2013; Accepted 3 July 2013

Academic Editor: Cláudio M. Gomes

A wide variety of acute and chronic neurodegenerative diseases, including ischemic/traumatic brain injury, Alzheimer's disease, and Parkinson's disease, share common characteristics such as oxidative stress, misfolded proteins, excitotoxicity, inflammation, and neuronal loss. As no drugs are available to prevent the progression of these neurological disorders, intervention strategies using phytochemicals have been proposed as an alternative form of treatment. Among phytochemicals, isothiocyanate sulforaphane, derived from the hydrolysis of the glucosinolate glucoraphanin mainly present in *Brassica* vegetables, has demonstrated neuroprotective effects in several in vitro and in vivo studies. In particular, evidence suggests that sulforaphane beneficial effects could be mainly ascribed to its peculiar ability to activate the Nrf2/ARE pathway. Therefore, sulforaphane appears to be a promising compound with neuroprotective properties that may play an important role in preventing neurodegeneration.

1. Introduction

Acute and chronic neurodegenerative diseases, including stroke, traumatic brain injury (TBI), Alzheimer's disease (AD), and Parkinson's disease (PD), are illnesses associated with high morbidity and mortality, and few or no effective options are available for their treatment [1, 2]. These diseases result in acute, as well as gradual and progressive neurodegeneration, leading to brain dysfunction and neuronal death. Although molecular mechanisms involved in the pathogenesis of acute and chronic neurodegenerative diseases remain elusive, oxidative stress, misfolding, aggregation, accumulation of proteins, perturbed Ca^{2+} homeostasis, excitotoxicity, inflammation, and apoptosis have been implicated as possible causes of neurodegeneration in the previously mentioned neurological disorders [3, 4]. In addition, recent studies demonstrated that acute brain injuries are also environmental risk factors associated with chronic neurodegenerative diseases [5–7].

In the last few years, there has been a growing interest in a number of pharmacological approaches aimed at preventing and counteracting the neuronal dysfunction and death associated with neurodegenerative diseases. However, while enormous efforts have been made to identify agents that could be used to alleviate debilitating neurodegenerative disorders, a source of potentially beneficial agents, namely, phytochemicals, would appear to have significant benefits in counteracting neurodegenerative diseases. Phytochemicals have long been recognized as exerting different biological effects, including antioxidant, antiallergic, antiinflammatory, antiviral, antiproliferative, and anticarcinogenic effects [8–10]. Considering that these age-related neurological disorders are multifactorial and that no drugs are available to stop their progression, intervention strategies using phytochemicals have been proposed as an alternative form of treatment for their prevention. Among phytochemicals, sulforaphane (isothiocyanato-4-(methylsulfinyl)-butane) (SF) has been demonstrated to have neuroprotective effects in several experimental paradigms. Reports in the literature have shown a pleiotropic role of this natural compound, thanks to its ability to address different targets and to modulate different pathways in neuronal/glial cells.

In this review, we will discuss the most recent experimental evidence on the role of SF in counteracting brain oxidative stress in both acute and chronic neurodegenerative diseases. SF bioavailability is also considered, since it is a fundamental aspect in the evaluation of the "in vivo" bioactivity of a nutritional compound.

2. Sulforaphane Bioavailability

Various Brassica vegetables and especially broccoli contain glucoraphanin. Following cutting or chewing, it is hydrolyzed into the corresponding isothiocyanate SF either by the plant thioglucosidase myrosinase or by bacterial thioglucosidases in the colon [11].

Because of its lipophilicity [12] and molecular size, SF is likely to passively diffuse into the enterocytes [13]. After absorption, SF is conjugated with glutathione (SF-GSH) by glutathione-S-transferase (GST) leading to maintenance of a concentration gradient and facilitating a fast passive absorption into the cell [14]. It is metabolized via the mercapturic acid pathway, producing predominantly cysteinylglycine (SF-CG), cysteine (SF-Cys), and N-acetyl-cysteine (SF-NAC) conjugates that are excreted in the urine [15].

Pharmacokinetic studies in both humans and animals showed that the plasma concentration of SF and its metabolites increased rapidly, reaching a maximum between 1 and 3 h after administration of either SF, glucosinolate, or broccoli [16–21]. In particular, Veeranki and colleagues [21] reported the ability of SF and its metabolites to reach different tissues in the gastrointestinal and genitourinary tracts and other organs such as liver, pancreas, lung, and heart, in vastly different concentrations and that bioactivity, in terms of induction of cytoprotective phase II enzymes, may differ significantly among organs. Both plasma and tissue levels of these SF metabolites are rapidly eliminated through urinary excretion within 12–24 h reflecting the rapid elimination of SF. The in vivo bioactivity of each SF metabolite is still unclear, although many in vitro studies have shown the ability of SF-Cys, and SF-NAC metabolites to exert some bioactivity [22–24]. These data suggest the hypothesis that repeated consumption of SF or cruciferous vegetables is required to maintain the SF metabolite concentration in tissues.

Interestingly, more recent SF bioavailability studies in human subjects consuming broccoli showed its bioconversion into isothiocyanate erucin (isothiocyanato-4-(methylthio)-butane) (ER), a sulfide analog [25, 26]. Whether this conversion from SF to ER is important for the health promoting effects of glucosinolate still remains to be determined although some reports provide a glimpse into the possibility of differing activities between these two isothiocyanates [27–29].

In order to exert protective effects towards neurodegenerative disorders or improve brain function, SF must traverse the blood-brain barrier (BBB) and accumulate in the central nervous system (CNS). As reported in the following sections of this review, various studies in animal models of neurodegeneration suggest the ability of SF to reach CNS and to display protective effects at this level. In this context, Jazwa et al. [30] demonstrated in mice that after SF gavage, SF is able to cross the BBB and to accumulate in cerebral tissues such as the ventral midbrain and striatum, with a maximum increase and disappearance after 15 min and 2 h, respectively. Interestingly, Clarke et al. [19] also detected SF-GSH, SF-Cys and SF-NAC metabolites, but not SF alone, in the CNS in a similar experimental in vivo model after 2 h and 6 h. However, the authors suggest that low levels of the various SF metabolites recorded in the CNS indicate their poor ability to cross the BBB. These results show the ability of SF to quickly reach the CNS and the potential contribution of SF metabolites to prolong the presence of SF at this level because they are unstable under physiological conditions and readily dissociate back to SF [21, 30].

3. Protective Effects of Sulforaphane against Oxidative Stress

Oxidative stress results from an imbalance of pro-oxidant/antioxidant homeostasis that leads to an abnormal production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The main ROS/RNS involved in neurodegeneration are superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), the highly reactive hydroxyl radical ($^{\bullet}OH$), and nitric oxide (NO) that can react with superoxide anion to produce peroxynitrite [31]. At high levels, ROS can react with different cell molecules, causing damage to DNA, lipids, and proteins and modulate intracellular signaling pathways, leading to cellular degeneration and apoptosis. ROS can also initiate proinflammatory pathways, further exacerbating the deleterious oxidized environment. The brain is particularly vulnerable to oxidative stress because of its high oxygen consumption, high content of oxidizable polyunsatured fatty acids, and low antioxidant defense capacities especially in aging brains [32–34]. Oxidative stress is involved in many neurodegenerative diseases and is a proposed mechanism for age-related degenerative processes as a whole [35, 36]. Numerous studies have provided compelling evidence that oxidative stress is an important causative factor in PD [2, 37–40], AD [41–43], amyotrophic lateral sclerosis (ALS) [44, 45], and multiple sclerosis (MS) [46, 47].

Cells possess a complex network of nonenzymatic and enzymatic components to counteract oxidative stress. GSH is the major nonenzymatic regulator of intracellular redox homeostasis. On the other hand, enzymatic antioxidants include glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), NAD(P)H-quinone oxidoreductase 1 (NQO1), thioredoxin reductase (TR), heme oxygenase 1 (HO1), peroxiredoxins, and many others. These enzymes are now recognized as primary defense mechanisms against many degenerative and chronic disease conditions [48]. These antioxidants and cytoprotective enzymes are regulated by a common mechanism that involves two proteins: nuclear factor erythroid 2-related factor 2 (Nrf2) and Kelch-like-ECH-associated protein 1 (Keap1) [49, 50]. Under basal conditions, Nrf2 is sequestered in the cytoplasm by its repressor protein Keap1 [51]. Keap1 contains several reactive cysteine residues that serve as sensors of the intracellular redox state. Nrf2 is released from Keap1

upon oxidative or covalent modification of thiols in some of these cysteine residues. Nrf2 translocates to the nucleus where it heterodimerizes with small Maf proteins before binding to the antioxidant responsive element (ARE) [35, 52] within the promoter regions of many cytoprotective genes [36]. In addition, Nrf2 has a key role against inflammation thanks to its ability to antagonize the transcription factor nuclear factor-κB (NF-κB) which regulates the expression of inflammatory genes [37].

ARE induction by chemical activators has been shown to protect neuronal cell lines against various oxidative damages induced by dopamine, hydrogen peroxide (H_2O_2), and glutamate [38–40]. SF has been demonstrated to increase many ARE-dependent antioxidant enzymes in different cell systems [41–43], such as GR, GPx, glutaredoxin (GLRX), thioredoxin (TX), TR, HO1, and NQO1. It has been shown that SF directly interacts with Keap1 by covalent binding to its thiol groups [44].

Negi et al. [45] demonstrated that SF increased the expression of Nrf2 and of downstream targets HO-1 and NQO-1 in Neuro2a cells and the sciatic nerve of diabetic animals. SF was also effective in counteracting oxidative stress induced by antipsychotic drugs in human neuroblastoma SK-N-SH cells, increasing GSH levels and inducing NQO1 activity [46].

Sulforaphane prevented oxidative stress-induced cytotoxicity in rat striatal cultures by raising the intracellular GSH content via an increase in γ-GCS expression induced by the activation of the Nrf2-antioxidant responsive element pathway [47].

It has also been observed that oxidative stress can inactivate peroxiredoxins, an important family of cysteine-based antioxidant enzymes that exert neuroprotective effects in several models of neurodegeneration [48, 53–55]. Interestingly, in both neurons and glia, SF treatment upregulates sulfiredoxin, an enzyme responsible for reducing hyperoxidized peroxiredoxins [56]. SF pretreatment also leads to attenuation of the tetrahydrobiopterin (BH4) induced ROS production thanks to the increase in mRNA levels and enzymatic activity of NQO1 in DAergic cell lines CATH.a and SK-N-BE(2)C [57].

Kraft et al. [58] demonstrated the importance of ARE activation in astrocytes of a mixed primary culture system. They observed that SF induced an ARE-mediated genetic response that is highly selective for astrocytes over neurons and conveys neuroprotection from oxidative insults initiated by H_2O_2 or nonexcitotoxic glutamate toxicity. Innamorato et al. [59] observed a direct association between the protective effect of SF against oxidative stress induced by lipopolysaccharide with HO-1 induction in BV2 microglial cells.

Oxidative stress induces Ca^{2+}-dependent opening of the mitochondrial inner membrane permeability transition pore (PTP), causing bioenergetic failure and subsequent death in different cell models, including those related to acute brain injury [60–62]. Intraperitoneal injection of rats with a nontoxic level of SF resulted in resistance of isolated nonsynaptic brain mitochondria to peroxide-induced PTP opening [63], and this could contribute to the neuroprotection observed with SF.

BBB damage following oxidative stress has been extensively investigated [64]. Postinjury induction of Nrf2-driven genes by SF treatment attenuated the loss of endothelial cells and tight junction proteins and reduced BBB permeability and cerebral edema [65]. Another study demonstrated that SF administration reduced BBB permeability in a rat subarachnoid hemorrhage model likely through the antioxidative effects of the activated Nrf2-ARE pathway [66].

Less attention has been focused on oxidative damage at the blood-cerebrospinal fluid (CSF) barrier (BCSFB) located at the choroid plexus (CP) epithelium. Even modest changes in the CPs may have a marked impact on the brain. For example, changes in CP function have been implicated in Alzheimer's disease [67]. A study by Xiang et al. [68] demonstrated that SF can protect the BCSFB in vitro from damage caused by H_2O_2 and reduced H_2O_2-induced cell death in primary CP epithelial cells and a CP cell line Z310.

Summarizing, the observed protective effects of SF against brain oxidative stress are mainly associated with Nrf2 activation and the resulting upregulation of antioxidant cytoprotective proteins and elevation of GSH (Figure 1).

4. Protective Effects of Sulforaphane against Acute Neurodegeneration

4.1. Ischemic Brain Injury. The pathophysiology of ischemic brain injury involves various biochemical mechanisms, such as glutamate-mediated excitotoxicity, the generation of ROS, apoptosis, and inflammation [69]. In adults, brain ischemic insults typically result from stroke or cardiac arrest, while in infants, cerebral ischemia is mediated by complications during labor and delivery, resulting in neonatal hypoxic-ischemic encephalopathy. In both groups, restoring blood flow to the ischemic brain is essential to salvage neurons. However, reperfusion itself causes additional and substantial brain damage referred to as "reperfusion injury."

In a neonatal hypoxia/ischemia brain injury model, Ping et al. [70] observed that SF significantly increased Nrf2 and HO-1 expression which was accompanied by reduced infarct volume. In particular, SF treatment reduced the number of apoptotic neurons, activated macroglia, and some oxidative parameters such as the amount of 8-hydroxy-2-deoxyguanosine and MDA level. In a similar model of ischemia/reperfusion induced by either oxygen and glucose deprivation or hemin in immature mouse hippocampal neurons, SF treatment activated the ARE/Nrf2 pathway of antioxidant defenses and protected immature neurons from delayed cell death [71]. Zhao et al. [69] demonstrated that delayed administration of a single dose of SF significantly decreased cerebral infarct volume in rats following focal ischemia. Moreover, in rat cortical astrocytes, SF treatment before or after oxygen and glucose deprivation significantly reduced cell death, stimulating the Nrf2 pathway of antioxidant gene expression [72]. In contrast to these data, Porritt et al. [73] showed that SF treatment initiated after photothrombosis-induced permanent cerebral ischemia in mice did not interfere with key cellular mechanisms involved in tissue damage. The authors suggest that the small volume of

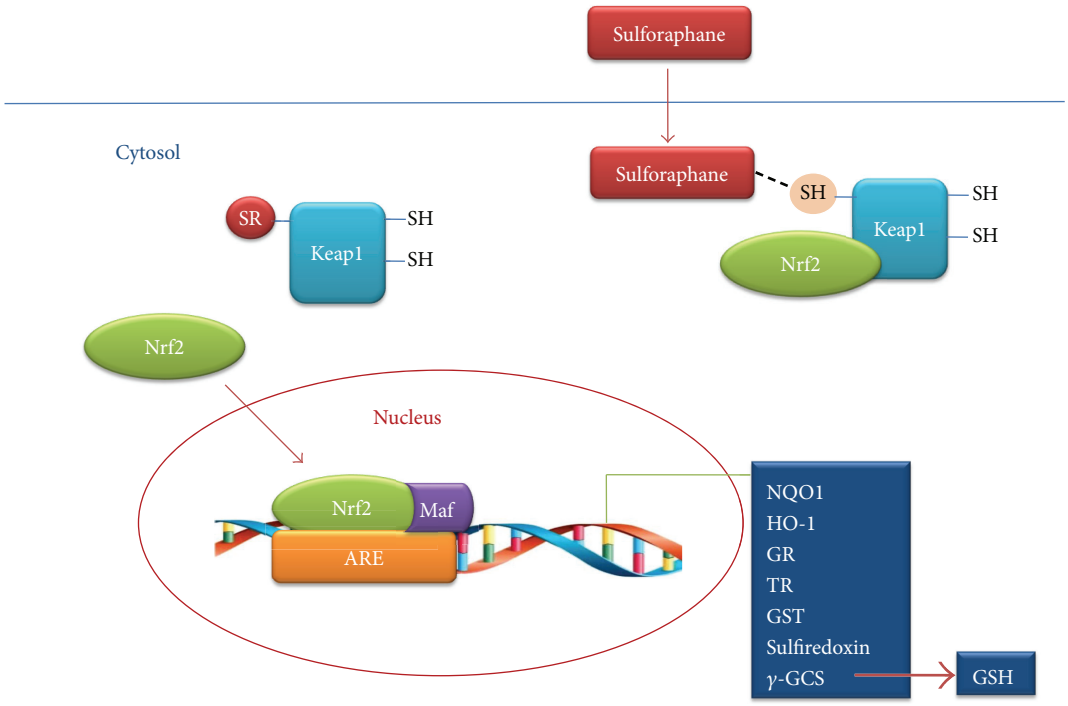


Figure 1: Proposed mechanism of neuroprotective effects provided by SF through Keap1/Nrf2 transcriptional activation of the antioxidant system. Adapted from [124].

infarcted cortical tissue resulting from the photothrombosis injury might result in the generation of relatively smaller amounts of ROS and may explain why they did not observe any neuroprotection after SF administration. In addition, Srivastava et al. [74] recorded that the pretreatment of rats with SF decreased the nuclear accumulation of Nrf2 following cerebral ischemia/reperfusion injury. On this topic, the authors speculate that rapid accumulation of SF in the brain and subsequent upregulation of Nrf2 and antioxidant enzymes may reduce the need for the later adaptive increase in Nrf2 expression following stroke.

These lines of evidence indicate that SF may counteract ischemia/reperfusion due to its ability to modulate Nrf2 and intracellular redox signaling.

4.2. Traumatic Brain Injury. Traumatic brain injury (TBI) is defined as damage to the brain caused by external mechanical force [75]. Survivors of TBI are left with long-term disabilities, and even a mild TBI can leave people with cognitive impairments, difficulty in concentrating, headaches, and fatigue [76]. TBI is a complex disease process [77] that results in early phase of mechanical damage of brain tissue and a secondary phase of cellular and molecular events that cause oxidative damage and brain cell death [78, 79]. Despite advances in prevention measures, surgical, and diagnostic techniques, no pharmacological treatment has so far been found to confer neuroprotection by targeting secondary injury mechanisms [76].

Recent studies in a rat model of TBI showed that postinjury administration of SF reduces the BBB impairment and cerebral edema after TBI [65, 80]. In particular, Zhao et al. [80] showed that SF attenuated aquaporin-4 (AQP4) channel loss in the injury core and further increased AQP4 protein levels in the penumbra region at 24 h and 3 days following TBI. In contrast to the early increase of AQP4 levels, the decrease in cerebral edema was observed only at 3 days, confirming the important role of AQP4 channels to clear the water in excess and to maintain the brain water homeostasis [81]. However, the authors suggest that the observed SF neuroprotective effect may be due to a combination of mechanisms that include decreased BBB permeability, enhanced cell survival, and/or increased AQP4 channel levels. In particular, the restoration of AQP4 channel activity prevented the impaired clearance of extracellular potassium with neuronal depolarization and glutamate release. It should be noted that the glutamate release is involved in an important sequel of CNS injury [80]. In the same rat model of TBI, Zhao et al. [65] demonstrated that postinjury administration of SF preserved BBB function through the reduction of endothelial cell markers and tight junction protein loss. These protective effects were mediated by the activity of Nrf2. In particular, SF increased the expression of Nrf2-driven cytoprotective genes such as GSTα3, GPx, and HO-1 in the parietal cortex and brain microvessels. More recent papers confirmed these findings in both rat and mice models of TBI [82]. Interestingly, Dash et al. [83] showed that in addition to vascular protection of SF, postinjury SF treatment preserved neurological function in injured animals. This improvement was demonstrated by enhanced learning and memory and by improved performance in a working memory task. The authors propose that the ability of SF to improve the hippocampal- and

prefrontal cortex-dependent cognitive function could be ascribed to its ability to protect the neurons and other cell types of the neurovascular unit from the oxidative damage elicited by TBI. Taken together, these findings suggest that SF may protect against the various pathophysiological consequences of TBI and other neurological traumatic injuries. On this topic, a recent study demonstrated that SF provides neuroprotective effects in the spinal cord after contusive injury [84].

5. Protective Effects of Sulforaphane against Chronic Neurodegeneration

5.1. Alzheimer's Disease. Alzheimer's disease (AD) is the most common neurodegenerative disease that accounts for most cases of dementia experienced by older people and is characterized by a progressive decline in memory and impairment of at least one other cognitive function [85].

This neurodegenerative disease is characterized by the accumulation of amyloid beta (Aβ) peptides that result in oxidative damage, inflammation and increased intracellular calcium levels [86, 87]. Two major hallmarks of AD are the extracellular aggregation of Aβ peptides and the intracellular precipitation/aggregation of hyperphosphorylated Tau (forming neurofibrillary tangles) protein [87]. In particular, Aβ 1–40 and Aβ 1–42 peptides, produced by the cleavage of the precursor protein, can exist in multiple aggregation forms, including soluble oligomers or protofibrils, and insoluble fibrils, which are responsible for various pathological effects [88, 89].

Several studies showed that increased oxidative stress, the impaired protein-folding function of the endoplasmatic reticulum, and deficient proteasome- and autophagic-mediated clearance of damaged proteins accelerated the accumulation of Aβ peptides and Tau protein in AD [90, 91].

In this context, Kwak et al. [92] demonstrated that the neuroprotective effects of SF against oxidative stress, in terms of protein carbonyl formation and cytotoxicity elicited by hydrogen peroxide, could be ascribed to its ability to induce proteasome expression in murine neuroblastoma Neuro2A cells. In similar cellular models, Park et al. [93] confirmed the ability of SF to enhance the proteasome activities and to protect the neuronal cells from Aβ1–42-mediated cytoxicity. More recent studies reported that SF induced the expression of heat shock protein 27, demonstrating that SF-stimulated proteasome activity may contribute to cytoprotection [94]. These data suggest that induction of proteasome by SF may facilitate the clearance of the Aβ1–42 peptides and lead to the improvement of protein misfolding in AD. Kim et al. [95] investigated the potential neuroprotective effects of SF in an Aβ1–40 peptide-induced AD acute mouse model. In particular, they recorded the ability of SF to ameliorate the cognitive function impairment although it did not directly interact with Aβ. These findings reinforce the indirect neuroprotective effects of SF against Aβ toxicity.

5.2. Parkinson's Disease. Parkinson's disease (PD) is an age-related neurodegenerative disease with progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta and with accumulation of neuronal inclusions known as Lewy bodies [96]. The exact etiology of PD remains to be fully elucidated, but the most reliable theories propose either an environmental [97, 98] or a genetic [99] origin, or a combination of both. Genetic studies have demonstrated that α-synuclein protein, a principal component of Lewy body inclusions [100], is a key participant in the pathogenesis of this disorder [101–103]. The exact biological function of α-synuclein and the mechanism by which mutations in this gene lead to neuron loss are still not clear, although it has been observed that an excess of α-synuclein protein can cause DA neuron loss [104].

Overwhelming evidence indicates that oxidative damage induced by ROS participates in the progression of DA neurons. In particular, the metabolism of dopamine (DA) might be responsible for the high basal levels of oxidative stress in the SN. Autooxidation of dopamine leads to the formation of neurotoxic species such as electrophilic DA quinone and ROS including superoxide anion ($O_2^{\bullet}$) and H_2O_2 [105]. DA quinone is also thought to cause mitochondrial dysfunction [106] and to mediate α-synuclein-associated neurotoxicity in PD by covalently modifying α-synuclein monomer [107] and by stabilizing the toxic protofibrillar α-synuclein [108].

Using a *Drosophila* model of α-synucleinopathy, Trinh et al. [109] observed that the neuronal death accompanying α-synuclein expression is enhanced by loss-of-function mutations in genes involved in the phase II detoxification pathway, specifically, glutathione metabolism. This neuronal loss can be overcome by pharmacological inducers, including SF, that increase glutathione synthesis or glutathione conjugation activity. They also observed similar neuroprotective effects of SF in *Drosophila* parkin mutants, another loss-of-function model of PD.

Several in vitro studies showed that SF was able to significantly reduce DA quinone levels in dopaminergic cell lines, such as CATH.a and SK-N-BE(2)C, as well as in mesencephalic dopaminergic neurons, evoked by 6-hydroxydopamine (6-OHDA) and BH4 [110]. In particular, Han et al. [57] demonstrated that SF can protect dopaminergic cells from the cytotoxicity of 6-OHDA and BH4 by removal of intracellular DA quinone, because NQO1 enzyme activity and mRNA level are increased by SF treatment and quinone-modified proteins are decreased.

In addition, DA quinone may yield neurotoxic species following its reaction with cellular thiols to form the 5-S-cysteinyl-dopamine (CysDA) [111–113]. CysDA adducts have been reported in human brain tissue and are elevated in the brains of patients suffering from PD [114]. We have demonstrated that SF is able to protect primary cortical neurons against CysDA-induced injury. In particular, we found that the protection exerted by SF against this neurotoxin is linked to the activation of ERK1/2, to the associated release of Nrf2 from Keap1, and to a subsequent increase in the expression and activity of specific detoxifying phase II enzymes [115]. Moreover, we demonstrated that SF prevented the dopaminergic-like neuroblastoma SH-SY5Y cell death, in terms of apoptosis and necrosis, induced by oxidant compounds, such as H_2O_2 and 6-OHDA, by its abilities

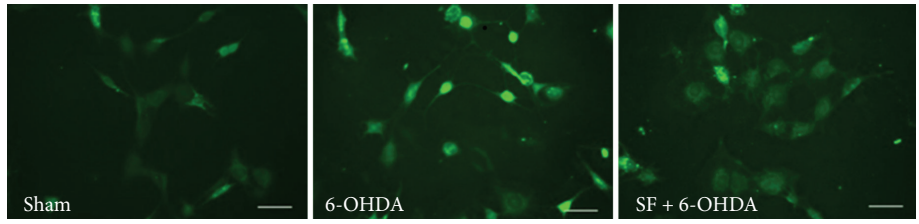


Figure 2: SF prevents 6-OHDA-induced ROS formation in SH-SY5Y cells. Representative images of SH-SY5Y cells incubated with SF for 24 h and then treated with 6-OHDA for 3 h. At the end of incubation, ROS formation was determined by fluorescence probe, 2′,7′-dichlorofluorescein-diacetate (DCFH-DA). Scale bar: 100 μm.

to increase endogenous GSH, enzymes involved in GSH metabolism including GST and GR, and to normalize the intracellular redox status (Figure 2) [116]. Interestingly, we recorded similar in vitro neuroprotective effects also with the erucin generated by bioconversion of the SF suggesting a neuroprotective role of SF metabolites in PD [117].

Deng et al. [118] observed that SF inhibited 6-OHDA-induced cytotoxicity in SH-SY5Y cells through increasing Nrf2 nuclear translocation and HO-1 expression in a PI3 K/Akt-dependent manner. Further, other authors confirmed that Nrf2 activation by SF may play an important role in DA neuron protection against 6-OHDA-induced toxicity in rat organotypical nigrostriatal cocultures [119]. As regards in vivo neurodegeneration models, Jazwa et al. [30] demonstrated that SF induced an Nrf2-dependent phase II response in the basal ganglia and protected against nigral dopaminergic cell death, astrogliosis, and microgliosis in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of PD. Further, we reported the ability of SF to exert neuroprotective effects on DA neurons in 6-OHDA-lesioned mice. In particular, these effects may be attributed to SF ability to enhance GSH levels and its dependent enzymes, including GST and GR, and to modulate neuronal survival pathways, such as ERK1/2 [120].

6. Conclusions

Several in vitro and in vivo studies have demonstrated the ability of SF to prevent various neurodegenerative processes that underlie stroke, traumatic brain injury, AD, and PD. The ability of SF to exert neuroprotective effects in different acute and chronic neurodegenerative diseases could be ascribed to its peculiar ability to activate the Nrf2/ARE pathway. Nrf2 is a recent therapeutic target in neurodegenerative diseases because it regulates several genes that have been implicated in protection against neurodegenerative conditions [121, 122]. In this context, SF presents many advantages, such as good pharmacokinetics and safety after oral administration as well as the potential ability to penetrate the BBB and deliver its neuroprotective effects in the central nervous system [123]. Based on these considerations, SF appears to be a promising compound with neuroprotective properties that may play an important role in preventing neurodegenerative diseases.

Authors' Contribution

Silvana Hrelia and Patrizia Hrelia contributed equally.

Acknowledgments

This work was supported by MIUR-FIRB (Project RBAP11HSZS), MIUR-PRIN (Project 2010PWNJXK_002), and "Fondazione del Monte di Bologna e Ravenna (Italy)."

References

[1] K. Ritchie and S. Lovestone, "The dementias," *The Lancet*, vol. 360, no. 9347, pp. 1759–1766, 2002.

[2] A. Akhlaq and A. Farooqui, *Neurochemical Aspects of Neurotraumatic and Neurodegenerative Diseases*, Springer, 2010.

[3] S. Mandel, E. Grünblatt, P. Riederer, M. Gerlach, Y. Levites, and M. B. H. Youdim, "Neuroprotective strategies in Parkinson's disease: an update on progress," *CNS Drugs*, vol. 17, no. 10, pp. 729–762, 2003.

[4] W. Dauer and S. Przedborski, "Parkinson's disease: mechanisms and models," *Neuron*, vol. 39, no. 6, pp. 889–909, 2003.

[5] P. C. Lee, Y. Bordelon, J. Bronstein, and B. Ritz, "Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease," *Neurology*, vol. 79, no. 20, pp. 2061–2066, 2012.

[6] J. Campdelacreu, "Parkinson disease and Alzheimer disease: environmental risk factors," *Neurologia*. In press.

[7] R. Pluta, "From brain ischemia-reperfusion injury to possible sporadic Alzheimer's disease," *Current Neurovascular Research*, vol. 1, no. 5, pp. 441–453, 2004.

[8] E. Middleton Jr., "Effect of plant flavonoids on immune and inflammatory cell function," *Advances in Experimental Medicine and Biology*, vol. 439, pp. 175–182, 1998.

[9] P. C. H. Hollman and M. B. Katan, "Health effects and bioavailability of dietary flavonols," *Free Radical Research*, vol. 31, supplement, pp. S75–S80, 1999.

[10] M. A. Eastwood, "Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease?" *Quarterly Journal of Medicine*, vol. 92, no. 9, pp. 527–530, 1999.

[11] N. V. Matusheski, J. A. Juvik, and E. H. Jeffery, "Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli," *Phytochemistry*, vol. 65, no. 9, pp. 1273–1281, 2004.

[12] D. A. Cooper, D. R. Webb, and J. C. Peters, "Evaluation of the potential for olestra to affect the availability of dietary phytochemicals," *Journal of Nutrition*, vol. 124, no. 8, pp. 1699S–1709S, 1997.

[13] S. Winiwarter, N. M. Bonham, F. Ax, A. Hallberg, H. Lennernäs, and A. Karlén, "Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach," *Journal of Medicinal Chemistry*, vol. 41, no. 25, pp. 4939–4949, 1998.

[14] Y. Zhang and E. C. Callaway, "High cellular accumulation of sulphoraphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate," *Biochemical Journal*, vol. 364, part 1, pp. 301–307, 2002.

[15] K. Kassahun, M. Davis, P. Hu, B. Martin, and T. Baillie, "Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat: identification of phase I metabolites and glutathione conjugates," *Chemical Research in Toxicology*, vol. 10, no. 11, pp. 1228–1233, 1997.

[16] A. V. Gasper, A. Al-Janobi, J. A. Smith et al., "Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli," *American Journal of Clinical Nutrition*, vol. 82, no. 6, pp. 1283–1291, 2005.

[17] T. A. Shapiro, J. W. Fahey, K. L. Wade, K. K. Stephenson, and P. Talalay, "Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans," *Cancer Epidemiology Biomarkers and Prevention*, vol. 10, no. 5, pp. 501–508, 2001.

[18] P. A. Egner, J. G. Chen, J. B. Wang et al., "Bioavailability of sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China," *Cancer Prevention Research*, vol. 4, no. 3, pp. 384–395, 2011.

[19] J. D. Clarke, A. Hsu, D. E. Williams et al., "Metabolism and tissue distribution of sulforaphane in Nrf2 knockout and wild-type mice," *Pharmaceutical Research*, vol. 28, no. 12, pp. 3171–3179, 2011.

[20] A. T. Dinkova-Kostova and R. V. Kostov, "Glucosinolates and isothiocyanates in health and disease," *Trends in Molecular Medicine*, vol. 18, no. 6, pp. 337–347, 2012.

[21] O. L. Veeranki, A. Bhattacharya, J. R. Marshall, and Y. Zhang, "Organ-specific exposure and response to sulforaphane, a key chemopreventive ingredient in broccoli: implications for cancer prevention," *British Journal of Nutrition*, vol. 109, no. 1, pp. 25–32, 2013.

[22] M. C. Myzak, P. A. Karplus, F.-L. Chung, and R. H. Dashwood, "A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase," *Cancer Research*, vol. 64, no. 16, pp. 5767–5774, 2004.

[23] E. S. Hwang and E. H. Jeffery, "Induction of quinone reductase by sulforaphane and sulforaphane N-acetylcysteine conjugate in murine hepatoma cells," *Journal of Medicinal Food*, vol. 8, no. 2, pp. 198–203, 2005.

[24] L. Tang, G. Li, L. Song, and Y. Zhang, "The principal urinary metabolites of dietary isothiocyanates, N-acetylcysteine conjugates, elicit the same anti-proliferative response as their parent compounds in human bladder cancer cells," *Anti-Cancer Drugs*, vol. 17, no. 3, pp. 297–305, 2006.

[25] J. D. Clarke, A. Hsu, K. Riedl et al., "Bioavailability and interconversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design," *Pharmacological Research*, vol. 64, no. 5, pp. 456–463, 2011.

[26] S. Saha, W. Hollands, B. Teucher et al., "Isothiocyanate concentrations and interconversion of sulforaphane to erucin in human subjects after consumption of commercial frozen broccoli compared to fresh broccoli," *Molecular Nutrition & Food Research*, vol. 56, no. 12, pp. 1906–1916, 2012.

[27] J. Jakubíková, J. Sedlák, R. Mithen, and Y. Bao, "Role of PI3K/Akt and MEK/ERK signaling pathways in sulforaphane- and erucin-induced phase II enzymes and MRP2 transcription, G2/M arrest and cell death in Caco-2 cells," *Biochemical Pharmacology*, vol. 69, no. 11, pp. 1543–1552, 2005.

[28] N. Hanlon, N. Coldham, M. J. Sauer, and C. Ioannides, "Modulation of rat pulmonary carcinogen-metabolising enzyme systems by the isothiocyanates erucin and sulforaphane," *Chemico-Biological Interactions*, vol. 177, no. 2, pp. 115–120, 2009.

[29] A. Melchini, C. Costa, M. Traka et al., "Erucin, a new promising cancer chemopreventive agent from rocket salads, shows anti-proliferative activity on human lung carcinoma A549 cells," *Food and Chemical Toxicology*, vol. 47, no. 7, pp. 1430–1436, 2009.

[30] A. Jazwa, A. I. Rojo, N. G. Innamorato, M. Hesse, J. Fernández-Ruiz, and A. Cuadrado, "Pharmacological targeting of the transcription factor NRf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism," *Antioxidants and Redox Signaling*, vol. 14, no. 12, pp. 2347–2360, 2011.

[31] A. Melo, L. Monteiro, R. M. F. Lima, D. M. de Oliveira, M. D. de Cerqueira, and R. S. El-Bachá, "Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives," *Oxidative Medicine and Cellular Longevity*, vol. 2011, Article ID 467180, 14 pages, 2011.

[32] B. Halliwell, "Reactive oxygen species and the central nervous system," *Journal of Neurochemistry*, vol. 59, no. 5, pp. 1609–1623, 1992.

[33] M. L. Hamilton, H. van Remmen, J. A. Drake et al., "Does oxidative damage to DNA increase with age?" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 18, pp. 10469–10474, 2001.

[34] O. I. Aruoma, "Neuroprotection by dietary antioxidants: new age of research," *Nahrung*, vol. 46, no. 6, pp. 381–382, 2002.

[35] T. Nguyen, H. C. Huang, and C. B. Pickett, "Transcriptional regulation of the antioxidant response element. Activation by Nrf2 and repression by MafK," *The Journal of Biological Chemistry*, vol. 275, no. 20, pp. 15466–15473, 2000.

[36] K. Itoh, T. Chiba, S. Takahashi et al., "An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements," *Biochemical and Biophysical Research Communications*, vol. 236, no. 2, pp. 313–322, 1997.

[37] W. Jin, H. Wang, W. Yan et al., "Disruption of Nrf2 enhances upregulation of nuclear factor-κB activity, proinflammatory cytokines, and intercellular adhesion molecule-1 in the brain after traumatic brain injury," *Mediators of Inflammation*, vol. 2008, Article ID 725174, 7 pages, 2008.

[38] T. H. Murphy, M. J. de Long, and J. T. Coyle, "Enhanced NAD(P)H: quinone reductase activity prevents glutamate toxicity produced by oxidative stress," *Journal of Neurochemistry*, vol. 56, no. 3, pp. 990–995, 1991.

[39] S. Duffy, A. So, and T. H. Murphy, "Activation of endogenous antioxidant defenses in neuronal cells prevents free radical-mediated damage," *Journal of Neurochemistry*, vol. 71, no. 1, pp. 69–77, 1998.

[40] J. Li, J. M. Lee, and J. A. Johnson, "Microarray analysis reveals an antioxidant responsive element-driven gene set involved in conferring protection from an oxidative stress-induced apoptosis in IMR-32 cells," *The Journal of Biological Chemistry*, vol. 277, no. 1, pp. 388–394, 2002.

[41] N. Juge, R. F. Mithen, and M. Traka, "Molecular basis for chemoprevention by sulforaphane: a comprehensive review," *Cellular and Molecular Life Sciences*, vol. 64, no. 9, pp. 1105–1127, 2007.

[42] A. T. Dinkova-Kostova and P. Talalay, "Direct and indirect antioxidant properties of inducers of cytoprotective proteins," *Molecular Nutrition and Food Research*, vol. 52, no. supplement 1, pp. S128–S138, 2008.

[43] C. Angeloni, E. Leoncini, M. Malaguti, S. Angelini, P. Hrelia, and S. Hrelia, "Modulation of phase II enzymes by sulforaphane: implications for its cardioprotective potential," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 12, pp. 5615–5622, 2009.

[44] 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," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 18, pp. 11908–11913, 2002.

[45] G. Negi, A. Kumar, and S. S. Sharma, "Nrf2 and NF-κB modulation by sulforaphane counteracts multiple manifestations of diabetic neuropathy in rats and high glucose-induced changes," *Current Neurovascular Research*, vol. 8, no. 4, pp. 294–304, 2011.

[46] S. Mas, P. Gassó, G. Trias, M. Bernardo, and A. Lafuente, "Sulforaphane protects SK-N-SH cells against antipsychotic-induced oxidative stress," *Fundamental & Clinical Pharmacology*, vol. 26, no. 6, pp. 712–721, 2012.

[47] K. Mizuno, T. Kume, C. Muto et al., "Glutathione biosynthesis via activation of the nuclear factor E2-related factor 2 (Nrf2)—antioxidant-response element (ARE) pathway is essential for neuroprotective effects of sulforaphane and 6-(methylsulfinyl) hexyl isothiocyanate," *Journal of Pharmacological Sciences*, vol. 115, no. 3, pp. 320–328, 2011.

[48] M. F. Sánchez-Font, J. Sebastià, C. Sanfeliu, R. Cristòfol, G. Marfany, and R. Gonzàlez-Duarte, "Peroxiredoxin 2 (PRDX2), an antioxidant enzyme, is under-expressed in Down syndrome fetal brains," *Cellular and Molecular Life Sciences*, vol. 60, no. 7, pp. 1513–1523, 2003.

[49] P. Talalay, M. J. de Long, and H. J. Prochaska, "Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 21, pp. 8261–8265, 1988.

[50] K. Itoh, N. Wakabayashi, Y. Katoh et al., "Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain," *Genes and Development*, vol. 13, no. 1, pp. 76–86, 1999.

[51] L. Baird and A. T. Dinkova-Kostova, "The cytoprotective role of the Keap1-Nrf2 pathway," *Archives of Toxicology*, vol. 85, no. 4, pp. 241–272, 2011.

[52] M. G. Marini, K. Chan, L. Casula, Y. W. Kan, A. Cao, and P. Moi, "hMAF, a small human transcription factor that heterodimerizes specifically with Nrf1 and Nrf2," *The Journal of Biological Chemistry*, vol. 272, no. 26, pp. 16490–16497, 1997.

[53] J. Yao, M. Taylor, F. Davey et al., "Interaction of amyloid binding alcohol dehydrogenase/Aβ mediates up-regulation of peroxiredoxin II in the brains of Alzheimer's disease patients and a transgenic Alzheimer's disease mouse model," *Molecular and Cellular Neuroscience*, vol. 35, no. 2, pp. 377–382, 2007.

[54] F. Hattori, N. Murayama, T. Noshita, and S. Oikawa, "Mitochondrial peroxiredoxin-3 protects hippocampal neurons from excitotoxic injury in vivo," *Journal of Neurochemistry*, vol. 86, no. 4, pp. 860–868, 2003.

[55] S. Boulos, B. P. Meloni, P. G. Arthur, C. Bojarski, and N. W. Knuckey, "Peroxiredoxin 2 overexpression protects cortical neuronal cultures from ischemic and oxidative injury but not glutamate excitotoxicity, whereas Cu/Zn superoxide dismutase 1 overexpression protects only against oxidative injury," *Journal of Neuroscience Research*, vol. 85, no. 14, pp. 3089–3097, 2007.

[56] F. X. Soriano, F. Léveillé, S. Papadia et al., "Induction of sulfiredoxin expression and reduction of peroxiredoxin hyperoxidation by the neuroprotective Nrf2 activator 3H-1,2-dithiole-3-thione," *Journal of Neurochemistry*, vol. 107, no. 2, pp. 533–543, 2008.

[57] J. M. Han, Y. J. Lee, S. Y. Lee et al., "Protective effect of sulforaphane against dopaminergic cell death," *Journal of Pharmacology and Experimental Therapeutics*, vol. 321, no. 1, pp. 249–256, 2007.

[58] A. D. Kraft, D. A. Johnson, and J. A. Johnson, "Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult," *Journal of Neuroscience*, vol. 24, no. 5, pp. 1101–1112, 2004.

[59] N. G. Innamorato, A. I. Rojo, Á. J. García-Yagüe, M. Yamamoto, M. L. de Ceballos, and A. Cuadrado, "The transcription factor Nrf2 is a therapeutic target against brain inflammation," *The Journal of Immunology*, vol. 181, no. 1, pp. 680–689, 2008.

[60] A. Navarro and A. Boveris, "Brain mitochondrial dysfunction and oxidative damage in Parkinson's disease," *Journal of Bioenergetics and Biomembranes*, vol. 41, no. 6, pp. 517–521, 2009.

[61] A. A. Starkov, C. Chinopoulos, and G. Fiskum, "Mitochondrial calcium and oxidative stress as mediators of ischemic brain injury," *Cell Calcium*, vol. 36, no. 3-4, pp. 257–264, 2004.

[62] K. Niizuma, M. Fujimura, T. Takahashi, A. Takahashi, M. Watanabe, and T. Tominaga, "Exclusively extradural arteriovenous malformation with neurogenic claudication: case illustration," *Journal of Neurosurgery*, vol. 100, no. 4, p. 397, 2004.

[63] T. Greco and G. Fiskum, "Brain mitochondria from rats treated with sulforaphane are resistant to redox-regulated permeability transition," *Journal of Bioenergetics and Biomembranes*, vol. 42, no. 6, pp. 491–497, 2010.

[64] P. B. L. Pun, J. Lu, and S. Moochhala, "Involvement of ROS in BBB dysfunction," *Free Radical Research*, vol. 43, no. 4, pp. 348–364, 2009.

[65] J. Zhao, A. N. Moore, J. B. Redell, and P. K. Dash, "Enhancing expression of Nrf2-driven genes protects the blood-brain barrier after brain injury," *Journal of Neuroscience*, vol. 27, no. 38, pp. 10240–10248, 2007.

[66] G. Chen, Q. Fang, J. Zhang, D. Zhou, and Z. Wang, "Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage," *Journal of Neuroscience Research*, vol. 89, no. 4, pp. 515–523, 2011.

[67] B. R. Ott, R. A. Cohen, A. Gongvatana et al., "Brain ventricular volume and cerebrospinal fluid biomarkers of Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 20, no. 2, pp. 647–657, 2010.

[68] J. Xiang, G. N. Alesi, N. Zhou, and R. F. Keep, "Protective effects of isothiocyanates on blood-CSF barrier disruption induced by oxidative stress," *American Journal of Physiology*, vol. 303, no. 1, pp. R1–R7, 2012.

[69] J. Zhao, N. Kobori, J. Aronowski, and P. K. Dash, "Sulforaphane reduces infarct volume following focal cerebral ischemia in rodents," *Neuroscience Letters*, vol. 393, no. 2-3, pp. 108–112, 2006.

[70] Z. Ping, W. Liu, Z. Kang et al., "Sulforaphane protects brains against hypoxic-ischemic injury through induction of Nrf2-dependent phase 2 enzyme," *Brain Research*, vol. 1343, pp. 178–185, 2010.

[71] L. Soane, W. Li Dai, G. Fiskum, and L. L. Bambrick, "Sulforaphane protects immature hippocampal neurons against

death caused by exposure to hemin or to oxygen and glucose deprivation," *Journal of Neuroscience Research*, vol. 88, no. 6, pp. 1355–1363, 2010.

[72] C. A. Danilov, K. Chandrasekaran, J. Racz, L. Soane, C. Zielke, and G. Fiskum, "Sulforaphane protects astrocytes against oxidative stress and delayed death caused by oxygen and glucose deprivation," *Glia*, vol. 57, no. 6, pp. 645–656, 2009.

[73] M. J. Porritt, H. C. Andersson, L. Hou et al., "Photothrombosis-induced infarction of the mouse cerebral cortex is not affected by the Nrf2-activator sulforaphane," *PLoS ONE*, vol. 7, no. 7, Article ID e41090, 2012.

[74] S. Srivastava, A. Alfieri, R. C. Siow, G. E. Mann, and P. A. Fraser, "Temporal and spatial distribution of Nrf2 in rat brain following stroke: quantitation of nuclear to cytoplasmic Nrf2 content using a novel immunohistochemical technique," *The Journal of Physiology*, 2013.

[75] A. I. Maas, N. Stocchetti, and R. Bullock, "Moderate and severe traumatic brain injury in adults," *The Lancet Neurology*, vol. 7, no. 8, pp. 728–741, 2008.

[76] T. Woodcock and M. C. Morganti-Kossmann, "The role of markers of inflammation in traumatic brain injury," *Frontiers in Neurology*, vol. 4, p. 18, 2013.

[77] B. E. Masel and D. S. DeWitt, "Traumatic brain injury: a disease process, not an event," *Journal of Neurotrauma*, vol. 27, no. 8, pp. 1529–1540, 2010.

[78] M. Gaetz, "The neurophysiology of brain injury," *Clinical Neurophysiology*, vol. 115, no. 1, pp. 4–18, 2004.

[79] H. M. Bramlett and W. D. Dietrich, "Progressive damage after brain and spinal cord injury: pathomechanisms and treatment strategies," *Progress in Brain Research*, vol. 161, pp. 125–141, 2007.

[80] J. Zhao, A. N. Moore, G. L. Clifton, and P. K. Dash, "Sulforaphane enhances aquaporin-4 expression and decreases cerebral edema following traumatic brain injury," *Journal of Neuroscience Research*, vol. 82, no. 4, pp. 499–506, 2005.

[81] G. T. Manley, D. K. Binder, M. C. Papadopoulos, and A. S. Verkman, "New insights into water transport and edema in the central nervous system from phenotype analysis of aquaporin-4 null mice," *Neuroscience*, vol. 129, no. 4, pp. 983–991, 2004.

[82] Y. Hong, W. Yan, S. Chen et al., "The role of Nrf2 signaling in the regulation of antioxidants and detoxifying enzymes after traumatic brain injury in rats and mice," *Acta Pharmacologica Sinica*, vol. 31, no. 11, pp. 1421–1430, 2010.

[83] P. K. Dash, J. Zhao, S. A. Orsi, M. Zhang, and A. N. Moore, "Sulforaphane improves cognitive function administered following traumatic brain injury," *Neuroscience Letters*, vol. 460, no. 2, pp. 103–107, 2009.

[84] A. L. Benedict, A. Mountney, A. Hurtado et al., "Neuroprotective effects of sulforaphane after contusive spinal cord injury," *Journal of Neurotrauma*, vol. 29, no. 16, pp. 2576–2586, 2012.

[85] R. K. Tannenberg, H. L. Scott, A. E. G. Tannenberg, and P. R. Dodd, "Selective loss of synaptic proteins in Alzheimer's disease: evidence for an increased severity with *APOE* $\varepsilon 4$," *Neurochemistry International*, vol. 49, no. 7, pp. 631–639, 2006.

[86] H. W. Querfurth and F. M. LaFerla, "Alzheimer's disease," *The New England Journal of Medicine*, vol. 362, no. 4, pp. 329–344, 2010.

[87] C. A. Loewen and M. B. Feany, "The unfolded protein response protects from tau neurotoxicity in vivo," *PLoS ONE*, vol. 5, no. 9, Article ID e13084, 2010.

[88] A. Tarozzi, F. Morroni, A. Merlicco et al., "Neuroprotective effects of cyanidin 3-O-glucopyranoside on amyloid beta (25–35) oligomer-induced toxicity," *Neuroscience Letters*, vol. 473, no. 2, pp. 72–76, 2010.

[89] Y. Liu, R. Dargusch, C. Banh, C. A. Miller, and D. Schubert, "Detecting bioactive amyloid β peptide species in Alzheimer's disease," *Journal of Neurochemistry*, vol. 91, no. 3, pp. 648–656, 2004.

[90] J. J. M. Hoozemans, R. Veerhuis, E. S. van Haastert et al., "The unfolded protein response is activated in Alzheimer's disease," *Acta Neuropathologica*, vol. 110, no. 2, pp. 165–172, 2005.

[91] M. Lopez Salon, L. Morelli, E. M. Castano, E. F. Soto, and J. M. Pasquini, "Defective ubiquitination of cerebral proteins in Alzheimer's disease," *Journal of Neuroscience Research*, vol. 62, no. 2, pp. 302–310, 2000.

[92] M. K. Kwak, J. M. Cho, B. Huang, S. Shin, and T. W. Kensler, "Role of increased expression of the proteasome in the protective effects of sulforaphane against hydrogen peroxide-mediated cytotoxicity in murine neuroblastoma cells," *Free Radical Biology and Medicine*, vol. 43, no. 5, pp. 809–817, 2007.

[93] H. M. Park, J. A. Kim, and M. K. Kwak, "Protection against amyloid beta cytotoxicity by sulforaphane: role of the proteasome," *Archives of Pharmacal Research*, vol. 32, no. 1, pp. 109–115, 2009.

[94] N. Gan, Y. C. Wu, M. Brunet et al., "Sulforaphane activates heat shock response and enhances proteasome activity through up-regulation of Hsp27," *The Journal of Biological Chemistry*, vol. 285, no. 46, pp. 35528–35536, 2010.

[95] H. V. Kim, H. Y. Kim, H. Y. Ehrlich, S. Y. Choi, D. J. Kim, and Y. Kim, "Amelioration of Alzheimer's disease by neuroprotective effect of sulforaphane in animal model," *Amyloid*, vol. 20, no. 1, pp. 7–12, 2013.

[96] T. M. Dawson and V. L. Dawson, "Molecular pathways of neurodegeneration in Parkinson's disease," *Science*, vol. 302, no. 5646, pp. 819–822, 2003.

[97] S. Calne, B. Schoenberg, W. Martin, R. J. Uitti, P. Spencer, and D. B. Calne, "Familial Parkinson's disease: possible role of environmental factors," *Canadian Journal of Neurological Sciences*, vol. 14, no. 3, pp. 303–305, 1987.

[98] B. S. Schoenberg, "Environmental risk factors for Parkinson's disease: the epidemiologic evidence," *Canadian Journal of Neurological Sciences*, vol. 14, no. 3, pp. 407–413, 1987.

[99] T. Kitada, S. Asakawa, N. Hattori et al., "Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism," *Nature*, vol. 392, no. 6676, pp. 605–608, 1998.

[100] M. G. Spillantini, M. L. Schmidt, V. M. Lee, J. Q. Trojanowski, R. Jakes, and M. Goedert, "α-synuclein in Lewy bodies," *Nature*, vol. 388, no. 6645, pp. 839–840, 1997.

[101] M. H. Polymeropoulos, C. Lavedan, E. Leroy et al., "Mutation in the α-synuclein gene identified in families with Parkinson's disease," *Science*, vol. 276, no. 5321, pp. 2045–2047, 1997.

[102] R. Krüger, W. Kuhn, T. Müller et al., "Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease," *Nature Genetics*, vol. 18, no. 2, pp. 106–108, 1998.

[103] J. J. Zarranz, J. Alegre, J. C. Gómez-Esteban et al., "The new mutation, E46K, of α-synuclein causes Parkinson and Lewy body dementia," *Annals of Neurology*, vol. 55, no. 2, pp. 164–173, 2004.

[104] A. M. Cuervo, L. Stafanis, R. Fredenburg, P. T. Lansbury, and D. Sulzer, "Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy," *Science*, vol. 305, no. 5688, pp. 1292–1295, 2004.

[105] P. Jenner, "Oxidative stress in Parkinson's disease," *Annals of Neurology*, vol. 53, supplement 3, pp. S26–S38, 2003.

[106] S. Y. Lee, Y. Moon, D. Hee Choi, H. Jin Choi, and O. Hwang, "Particular vulnerability of rat mesencephalic dopaminergic neurons to tetrahydrobiopterin: relevance to Parkinson's disease," *Neurobiology of Disease*, vol. 25, no. 1, pp. 112–120, 2007.

[107] S. B. Dunnett and A. Björklund, "Prospects for new restorative and neuroprotective treatments in Parkinson's disease," *Nature*, vol. 399, no. 6738, supplement, pp. A32–A39, 1999.

[108] K. A. Conway, J.-C. Rochet, R. M. Bieganski, and P. T. Lansbury Jr., "Kinetic stabilization of the α-synuclein protofibril by a dopamine-α-synuclein adduct," *Science*, vol. 294, no. 5545, pp. 1346–1349, 2001.

[109] K. Trinh, K. Moore, P. D. Wes et al., "Induction of the phase II detoxification pathway suppresses neuron loss in Drosophila models of Parkinson's disease," *Journal of Neuroscience*, vol. 28, no. 2, pp. 465–472, 2008.

[110] N. S. Yoon, Y. Cho, S. Y. Lee, H. J. Choi, and O. Hwang, "Inactivation of aconitase by tetrahydrobiopterin in DArgic cells: relevance to PD," *Experimental Neurobiology*, vol. 19, no. 1, pp. 23–29, 2010.

[111] J. P. E. Spencer, M. Whiteman, P. Jenner, and B. Halliwell, "5-s-cysteinyl-conjugates of catecholamines induce cell damage, extensive DNA base modification and increases in caspase-3 activity in neurons," *Journal of Neurochemistry*, vol. 81, no. 1, pp. 122–129, 2002.

[112] D. Vauzour, G. Ravaioli, K. Vafeiadou, A. Rodriguez-Mateos, C. Angeloni, and J. P. E. Spencer, "Peroxynitrite induced formation of the neurotoxins 5-S-cysteinyl-dopamine and DHBT-1: implications for Parkinson's disease and protection by polyphenols," *Archives of Biochemistry and Biophysics*, vol. 476, no. 2, pp. 145–151, 2008.

[113] D. Vauzour, K. Vafeiadou, and J. P. E. Spencer, "Inhibition of the formation of the neurotoxin 5-S-cysteinyl-dopamine by polyphenols," *Biochemical and Biophysical Research Communications*, vol. 362, no. 2, pp. 340–346, 2007.

[114] J. P. E. Spencer, P. Jenner, S. E. Daniel, A. J. Lees, D. C. Marsden, and B. Halliwell, "Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species," *Journal of Neurochemistry*, vol. 71, no. 5, pp. 2112–2122, 1998.

[115] D. Vauzour, M. Buonfiglio, G. Corona et al., "Sulforaphane protects cortical neurons against 5-S-cysteinyl-dopamine-induced toxicity through the activation of ERK1/2, NrF-2 and the upregulation of detoxification enzymes," *Molecular Nutrition and Food Research*, vol. 54, no. 4, pp. 532–542, 2010.

[116] A. Tarozzi, F. Morroni, A. Merlicco et al., "Sulforaphane as an inducer of glutathione prevents oxidative stress-induced cell death in a dopaminergic-like neuroblastoma cell line," *Journal of Neurochemistry*, vol. 111, no. 5, pp. 1161–1171, 2009.

[117] A. Tarozzi, F. Morroni, C. Bolondi et al., "Neuroprotective effects of erucin against 6-hydroxydopamine-induced oxidative damage in a dopaminergic-like neuroblastoma cell line," *International Journal of Molecular Sciences*, vol. 13, no. 9, pp. 10899–10910, 2012.

[118] C. Deng, R. Tao, S. Z. Yu, and H. Jin, "Sulforaphane protects against 6-hydroxydopamine-induced cytotoxicity by increasing expression of heme oxygenase-1 in a PI3K/Akt-dependent manner," *Molecular Medicine Reports*, vol. 5, no. 3, pp. 847–851, 2012.

[119] A. Siebert, V. Desai, K. Chandrasekaran, G. Fiskum, and M. S. Jafri, "Nrf2 activators provide neuroprotection against 6-hydroxydopamine toxicity in rat organotypic nigrostriatal cocultures," *Journal of Neuroscience Research*, vol. 87, no. 7, pp. 1659–1669, 2009.

[120] F. Morroni, A. Tarozzi, G. Sita et al., "Neuroprotective effect of sulforaphane in 6-hydroxydopamine-lesioned mouse model of Parkinson's disease," *Neurotoxicology*, vol. 36, pp. 63–71, 2013.

[121] Q. Ma and X. He, "Molecular basis of electrophilic and oxidative defense: promises and perils of Nrf2," *Pharmacological Reviews*, vol. 64, no. 4, pp. 1055–1081, 2012.

[122] M. J. Calkins, D. A. Johnson, J. A. Townsend et al., "The Nrf2/ARE pathway as a potential therapeutic target in neurodegenerative disease," *Antioxidants and Redox Signaling*, vol. 11, no. 3, pp. 497–508, 2009.

[123] T. A. Shapiro, J. W. Fahey, A. T. Dinkova-Kostova et al., "Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study," *Nutrition and Cancer*, vol. 55, no. 1, pp. 53–62, 2006.

[124] Y. J. Surh, "Cancer chemoprevention with dietary phytochemicals," *Nature Reviews Cancer*, vol. 3, no. 10, pp. 768–780, 2003.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 316523, 10 pages
http://dx.doi.org/10.1155/2013/316523

Review Article

Oxidative Stress and the Pathogenesis of Alzheimer's Disease

Yan Zhao[1] and Baolu Zhao[2]

[1] *Department of Bioengineering, Harbin Institute of Technology at Weihai, Weihai, Shandong 264209, China*
[2] *State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*

Correspondence should be addressed to Yan Zhao; ywz12@yahoo.com and Baolu Zhao; zhaobl@sun5.ibp.ac.cn

Received 25 May 2013; Accepted 3 July 2013

Academic Editor: Cláudio M. Gomes

Alzheimer's disease (AD) is the most common neurodegenerative disease that causes dementia in the elderly. Patients with AD suffer a gradual deterioration of memory and other cognitive functions, which eventually leads to a complete incapacity and death. A complicated array of molecular events has been implicated in the pathogenesis of AD. The major pathological characteristics of AD brains are the presence of senile plaques, neurofibrillary tangles, and neuronal loss. Growing evidence has demonstrated that oxidative stress is an important factor contributing to the initiation and progression of AD. However, the mechanisms that lead to the disruption of redox balance and the sources of free radicals remain elusive. The excessive reactive oxygen species may be generated from mechanisms such as mitochondria dysfunction and/or aberrant accumulation of transition metals, while the abnormal accumulation of Abeta and tau proteins appears to promote the redox imbalance. The resulted oxidative stress has been implicated in Abeta- or tau-induced neurotoxicity. In addition, evidence has suggested that oxidative stress may augment the production and aggregation of Abeta and facilitate the phosphorylation and polymerization of tau, thus forming a vicious cycle that promotes the initiation and progression of AD.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease that causes dementia in the elderly. It is characterized by the gradual deterioration of memory and other cognitive functions, which eventually leads to a complete incapacity and death of the patients within 3 to 9 years after diagnosis [1]. Increasing age is a major risk factor for sporadic forms of AD. As the elderly population of the world continues to increase, the prevalence of AD has increased remarkably worldwide, and AD has become one of the leading causes of disability and death among the elderly [2–5]. Despite the tremendous progress that has been made in AD research in the past few decades, the exact cause and pathogenesis of AD are not completely understood, and currently, there is no effective treatment for the disease.

The major pathological characteristics of AD brains are the presence of senile plaques, neurofibrillary tangles (NFTs), and neuronal loss [1, 6]. Senile plaques are mainly composed of beta-amyloid peptide (Abeta) that is produced from proteolytic cleavage of the transmembrane amyloid precursor protein (APP). NFTs are formed by arrays of paired helical filaments (PHFs) structures, which contain mainly self-aggregated hyperphosphorylated tau, a multifunctional protein involved in microtubule assembly and stabilization [6]. Accumulating evidence has shown that the presence of extensive oxidative stress is a characteristic of AD brains in addition to the established pathology of senile plaques and NFT [7]. It has been demonstrated that the levels of protein carbonyls and 3-nitrotyrosine, which are resulted from protein oxidation, and markers of oxidative damage to DNA and RNA, such as 8-hydroxydeoxyguanosine (8-OHdG) and 8-hydroxyguanosine, are elevated in AD brains [8–12]. Products of lipid peroxidation, such as malondialdehyde (MDA), 4-hydroxynonenal, and F_2-isoprostanes, are also increased in multiple brain regions and cerebrospinal fluid (CSF) of patients with AD or mild cognitive impairment (MCI) [13–17]. In addition to the accumulation of free radical damage, alterations in the activities or expression of antioxidant enzymes such as superoxide dismutase (SOD) and catalase have been observed in both central nervous system and peripheral tissues of AD patients [17–20]. Moreover, in AD and MCI brains, the increased oxidative damage to lipids and proteins and the decline of glutathione and antioxidant

enzyme activities are more localized to the synapses and correlate with the severity of the disease, suggesting an involvement of oxidative stress in AD-related synaptic loss [21]. Importantly, many of the previously mentioned studies show elevations of oxidative stress in MCI, which is proposed as an intermediate state between normal aging and dementia, indicating that the oxidative stress damage in AD may occur preceding the onset of the disease. These results suggest that oxidative stress may be one of the earliest alterations that occur during the initiation and development of AD.

While oxidative stress has emerged as one of the important factors in AD pathogenesis, the mechanisms by which the redox balance is altered and the sources of free radicals remain elusive. The present paper reviews the involvement of abnormal accumulation of Abeta and tau proteins in the induction of redox imbalance and the generation of free radicals through mechanisms such as mitochondria dysfunction and/or transition metal homeostasis imbalance and discusses the mechanisms by which oxidative stress promotes Abeta- and tau-mediated neurotoxicity.

2. Oxidative Stress and Abeta-Induced Toxicity

Abeta is produced via sequential proteolytic cleavages of APP by two membrane-bound proteases, beta-secretase, also known as beta-site APP cleaving enzyme 1 (BACE1), and gamma-secretase, a multiprotein complex consisting of presenilin (PS), nicastrin (NCT), anterior pharynx-defective 1 (APH-1), and presenilin enhancer protein 2 (PEN-2) [1, 6, 22]. BACE1 cleaves APP at the N-terminal end, producing a 99 amino acid APP C-terminal fragment, which is further cleaved within the transmembrane domain by gamma-secretase, resulting in the release of Abeta peptides [1, 6, 23]. Several peptides of varying lengths can be generated from the cleavages by beta- and gamma-secretases; among them, the 42-amino acid form of Abeta (Abeta42) is more toxic than the more abundantly produced 40-amino acid form of Abeta (Abeta40), possibly because of its faster self-aggregation into oligomers [1, 23, 24]. In fact, multiple lines of evidence suggest that soluble Abeta oligomers are the most neurotoxic, whose levels correlate with the severity of the cognitive decline in AD [23, 24]. Cleavage of APP by a third enzyme, alpha-secretase, precludes the formation of toxic Abeta peptides [25]. Increased production and/or decreased clearance of Abeta peptides leads to the accumulation of Abeta, which stimulates diverse cell signaling pathways, eventually resulting in synaptic degeneration, neuronal loss and decline in cognitive function [6, 23, 24, 26–28].

A great deal of research has implicated oxidative stress in Abeta-induced neurotoxicity [29]. *In vitro* experiments using cell models showed that Abeta treatment could increase the levels of hydrogen peroxide and lipid peroxides [30]. Consistently, in various AD transgenic mouse models carrying mutants of APP and PS-1, increased hydrogen peroxide and nitric oxide production as well as elevated oxidative modifications of proteins and lipids were correlated with the age-associated Abeta accumulation, confirming that Abeta promotes oxidative stress [31–35]. In hippocampal neuronal cell cultures, the induction of reactive oxygen species (ROS) by soluble Abeta oligomers required the activation of N-methyl-D-aspartate (NMDA) receptor and was associated with a rapid increase in neuronal calcium levels, suggesting a possible role of soluble Abeta oligomers as proximal neurotoxins and the involvement of oxidative stress in the synaptic impairment and neuronal loss induced by soluble Abeta oligomers [36]. Consistently, it has been demonstrated in AD cell and animal models that natural antioxidants, such as EGb 761, curcumin, and green tea catechins, can exert neuroprotective functions by attenuating Abeta-induced ROS generation and neuronal apoptosis [37–40].

In addition to mediating Abeta-induced cytotoxity, numerous studies have suggested that oxidative stress promotes the production of Abeta. It was demonstrated that defects in antioxidant defense system caused elevated oxidative stress and significantly increased Abeta deposition in transgenic mice overexpressing APP mutant [41, 42], while dietary antioxidants such as curcumin lowered the elevation of oxidized proteins and decreased brain Abeta levels and Abeta plaque burden [43]. Moreover, the increased Abeta deposition and its associated earlier onset and more severe cognitive dysfunction induced by the defect in antioxidant defense system could be ameliorated by antioxidant supplementation [42]. In line with these findings, overexpression of manganese superoxide dismutase (MnSOD) in Tg19959 transgenic mice overexpressing APP mutant decreased protein oxidation and increased antioxidant defense capability in brains while reducing Abeta plaque burden and restoring the memory deficit [44]. Furthermore, deletion of cytoplasmic copper/zinc superoxide dismutase (Cu-Zn-SOD, SOD1) in Tg2576 APP-overexpressing AD mouse model was found to increase Abeta oligomerization while accelerating the loss of spatial learning and memory as compared with control AD mice, suggesting a possible role of oxidative damage in Abeta oligomerization [45]. These results suggest that the enhancement of Abeta production/plaque formation as well as Abeta oligomerization by oxidative stress is important for the initiation and development of AD.

Studies on how oxidative stress enhances Abeta production have revealed that oxidative stress decreases the activity of alpha-secretase while promoting the expression and activation of beta- and gamma-secretase, enzymes critical for the generation of Abeta from APP [46–50]. The induction of BACE1 and PS1 expression and the activation of gamma-secretase by oxidative stress were found to be dependent on the activation of c-Jun N-terminal kinase (JNK) pathway, a major cell signaling cascade that is stimulated by oxidative stress [51, 52]. In fact, the promoter and 5′ untranslated region of BACE gene contain binding sites for multiple transcription factors including the redox-sensitive activator protein (AP1) and nuclear factor (NF)-kappa B, activation of which by oxidative stress may in turn enhance BACE expression [53]. In AD brains, both the activation of JNK signaling cascade [54–56] and the elevation of BACE1 and PS1 expression/activity have been detected [57–59]; thus, it is possible that the increased oxidative stress in AD brains may initiate the activation of a cascade of redox-sensitive cell signal pathways including JNK, which promotes the expression of BACE1 and PS1, eventually enhancing the production of

Abeta and the deterioration of cognitive function. As JNK has also been implicated in Abeta-induced neuronal apoptosis [60], oxidative stress may enhance Abeta production as well as mediate Abeta-induced neurotoxicity through the activation of redox-sensitive signaling pathways such as JNK.

Alternatively, the augmentation of Abeta production by oxidative stress may be a compensatory reaction to oxidative stress. It was found that neuronal oxidative damage was more pronounced in AD subjects with lesser amounts of Abeta deposition or in AD subjects with a shorter disease duration [61, 62], and there was an inverse relationship between the levels of neuronal oxidative damage to nucleic acids and the amounts of intraneuronal Abeta42 in the hippocampus and the subiculum of AD brains [63]. These unexpected observations have led to the hypothesis that Abeta may potentially play a protective role against neuronal oxidative stress [64]. Indeed, evidence has suggested that picomolar or low nanomolar levels of Abeta can be neurotrophic or neuroprotective [65, 66]. Physiological concentrations of Abeta were shown to efficiently inhibit autooxidation of lipoproteins in CSF and plasma [67] and markedly increase hippocampal long-term potentiation [68], whereas the high nanomolar concentrations of Abeta caused the well-established toxic effects. In addition, it appeared that the dualistic effects of Abeta depended on the aggregation state of Abeta and were Abeta size-form specific [66, 69]. Taken together, low levels of Abeta may have a role in the normal function of neuronal cells and could beneficially influence the cellular redox status, while the abnormal accumulation and aggregation of specific forms of Abeta, which can be enhanced by oxidative stress, may impair neuronal function and further exacerbate neuronal oxidative damage, contributing to the pathological development of AD. A better understanding of the pathological as well as the physiological role of Abeta may lead to more effective strategies for AD interventions.

3. Mitochondria Dysfunction and Oxidative Stress in AD

Mitochondria are unique organelles that are pivotal for a variety of cellular functions including ATP synthesis, calcium homeostasis, and cell survival and death. Meanwhile, mitochondrial respiratory chain is a major site of ROS production in the cell, and mitochondria are particularly vulnerable to oxidative stress [70, 71]. Extensive studies have demonstrated that mitochondria dysfunction is an important factor involved in the pathogenesis of AD. A number of mitochondrial and metabolic abnormalities have been identified in the hippocampal neurons of AD compared to age-matched controls [72–74]. Morphometric analysis of biopsies from AD brains showed a significant reduction of mitochondria, while the mitochondrial DNA and protein were increased in the cytoplasm and in the vacuoles associated with lipofuscin, a lysosome suggested as the site of mitochondrial degradation by autophagy [72, 73]. These mitochondrial abnormalities were found accompanied by oxidative damage marked by 8-hydroxyguanosine and nitrotyrosine, indicating that the mitochondria were damaged during the progression of AD [72]. In line with this, a significant decrease of mitochondrial cytochrome oxidase (complex IV) activity in the cortical regions of AD brains was reported [74]. Deficiency in this key electron transport enzyme could lead to the increase in ROS production and reduction in energy stores, eventually contributing to the neurodegenerative process [74].

Evidence suggests that Abeta may directly disrupt mitochondria function and contribute to the deficiency of energy metabolism and neuronal death seen in AD. It was found that Abeta was localized to mitochondria in brains of AD patients and transgenic mice as well as in neuroblastoma cells stably expressing human mutant APP [34, 75]. The presence of Abeta in mitochondria was associated with impaired mitochondrial metabolism and increased mitochondrial ROS production [34, 75]. In fact, in isolated mitochondria, Abeta treatment could cause oxidative injury to mitochondrial membrane, disrupt lipid polarity and protein mobility and inhibit key enzymes of the mitochondria respiratory chain, leading to increased mitochondrial membrane permeability and cytochrome c release [76, 77]. MnSOD, a primary antioxidant enzyme protecting mitochondria against superoxide, was found to be a target of nitration and inactivation in a double homozygous knock-in mouse model expressing APP and PS-1 mutants [78]. The decreased activity of antioxidant defense enzymes such as MnSOD may further increase ROS levels and compromise mitochondria function, contributing to the loss of mitochondrial membrane potential and eventually caspase activation and apoptosis [78]. Abeta has also been shown to alter other cellular protective mechanisms against oxidative damage to mitochondria. Uncoupling proteins (UCPs) are a family of mitochondrial anion carrier proteins that are located on the inner mitochondrial membrane with diverse physiological functions [79]. It has been demonstrated that UCP2 and UCP3 can be activated by ROS or products of lipid peroxidation to diminish proton motive force and reduce mitochondrial membrane potential and ATP production, causing mitochondria uncoupling and decrease of ROS generation from mitochondria [80]. Therefore, the expression and activation of UCPs are considered to be a protective mechanism in response to oxidative stress. This protective mechanism appears dysfunctional in AD brains where the expression of UCP2, 4, and 5 is significantly decreased [81]. In SH-SY5Y neuroblastoma cells overexpressing APP or APP mutant, it was found that the upregulation of UCP2 and UCP4 protein levels in response to the exposure of superoxide was abrogated; although the mechanisms are unclear, it suggests that Abeta accumulation may lead to irreversible cellular alterations that render the cell more susceptible to oxidative stress [82]. Moreover, the UCP2- and UCP4-dependent upregulation of mitochondrial free calcium in response to superoxide treatment was found to be diminished in cells overexpressing APP or APP mutant, indicating that the Abeta accumulation may be associated with a dysfunction of mitochondria as a reserve pool of intracellular calcium that leads to an increased cell sensitivity to the loss of calcium homeostasis [82].

It is noted that the mitochondria-associated Abeta along with the increase in hydrogen peroxide and decrease in cytochrome oxidase activity was detected prior to the appearance of Abeta plaques, suggesting the defect in mitochondria

occurs earlier in the pathogenesis of AD. Therefore, early mitochondrially targeted therapeutic interventions may be effective in delaying the onset and progression of AD [34, 75].

4. Metal Homeostasis and Oxidative Stress in AD

Transition metals such as copper (Cu), zinc (Zn), and iron (Fe) play important catalytic roles in many enzymes and are essential for a broad range of biological processes in human body including brain functions. Both Cu and Zn have been shown to participate in regulating synaptic function. Following NMDA receptor activation, Cu is released from the neuron and regulates neuronal activation by functionally blocking NMDA receptors and limiting calcium entry into the cell [83]. Zn also has a neuromodulatory role; it is released from presynaptic nerve terminals into the synaptic cleft upon neuronal activation and has been shown to inhibit excitatory NMDA receptors [84]. Fe is crucial for neuronal processes such as myelination, synaptogenesis, and synaptic plasticity (SP). It is well documented that deficiency of Fe can induce a series of neurochemical alterations that may eventually lead to cognitive deficits [85]. While these transition metals play essential roles in neural functions, their levels and transport are strictly regulated, as aberrant metal homeostasis can result in neurotoxic free-radical production. For example, excess Fe or Cu can directly interact with oxygen to produce superoxide ion, hydrogen peroxide, and hydroxyl radical, which may lead to oxidative stress and a cascade of biochemical alterations that eventually cause neuronal cell death [86]. In fact, growing evidence has shown that there is a close relationship between the disruption of metal homeostasis and AD [86]. Abnormal levels of Cu, Zn, and Fe have been observed in AD hippocampus and amygdala, areas showing severe histopathologic alterations [87]. Moreover, these transition metals have been detected within the amyloid deposits in AD patients as well as transgenic mouse models [88–90]. These data suggest that the aberrant accumulation of transition metals may be inextricably linked with Abeta pathology in AD; however, the precise cause and the nature of the involvement of brain metal dyshomeostasis in AD are still largely unknown.

The presence of transition metals within the amyloid deposits in AD patients indicates that transition metals may directly interact with Abeta [88–90]. Indeed, both Cu^{2+} and Zn^{2+} can bind to Abeta monomers via three histidine residues (His^{6}, His^{13}, and His^{14}) and a tyrosine residue (Tyr^{10}), causing conformational changes of the peptide that promote its aggregation [91, 92]. Consistently, *in vitro* data showed that Cu and Zn rapidly induced the aggregation of soluble Abeta peptides [93, 94]. Therefore, the disturbance of metal homeostasis and the abnormal interactions of Abeta with metal ions may be directly involved in the process of Abeta deposition in AD brains [93–96]. In addition, the aberrant interaction between transition metals and Abeta may be a source of ROS generation. Abeta binds Cu^{2+} with high affinity, forming a cuproenzyme-like complex [91]. Electrons can be transferred from Abeta to Cu, reducing Cu^{2+} to Cu^{+} and forming positively charged Abeta radical ($Abeta^{+\bullet}$) [97]. Cu^{+} then donates two electrons to oxygen, generating H_2O_2 [97, 98], setting up conditions to further produce hydroxyl radicals (Fenton-type reaction) [99, 100]. After electron donation to O_2, the radicalized $\cdot Cu^{2+}$ complex may be restored to $Abeta{\cdot}Cu^{2+}$ by electron transfer from biological reducing agents such as cholesterol, catecholamines, and vitamin C [97]. The efficiency for generation of H_2O_2 is greater for Abeta42 than Abeta40, correlating with their cytotoxic activity [98, 99]. Similar to copper-Abeta interaction, binding of Fe to Abeta results in reduction of Fe^{3+} to Fe^{2+} and the generation of H_2O_2 [101]. These data suggest that ROS generated from the interaction of transition metals with Abeta are key contributors to the oxidative stress in Abeta-mediated neurotoxicity and AD pathogenesis.

Additionally, there is a close association between Fe/Cu homeostasis and the production and processing of APP. In SH-SY5Y cells overexpressing the Swedish mutant form of human APP (APPsw), Fe treatment induced the release of Abeta42 [102, 103]. Moreover, an iron-responsive element (IRE-Type II) was identified within the $5'$-untranslated region ($5'$-UTR) of APP transcript, which was selectively downregulated in response to intracellular Fe chelation; thus, in addition to promotion of Abeta generation from APP, increases in Fe levels may lead to upregulation of APP protein translation through binding of Fe regulatory proteins to the APP IRE [104]. On the contrary, Cu treatment was shown to promote the nonamyloidogenic pathway of APP and decrease the release of Abeta [105]. This may be related to the finding that Cu is abnormally distributed in AD brain, with accumulation of Cu in amyloid plaques but a deficiency of Cu in neighboring cells [87, 88].

Studies have shown that metal transporters such as Zn transporters and divalent metal transporter 1 (DMT1) are increased in the cortex and hippocampus of APP/PS1 transgenic mice; and similar to transition metals, these metal transporters are colocalized with Abeta in senile plaques in the cortex of AD brains [103, 106, 107]. This leads to the speculation that metal transporters may play important roles in the aberrant metal homeostasis in AD. DMT1, also known as natural resistance-associated macrophage protein 2 (Nramp2) or divalent cation transporter 1 (DCT1), is a newly discovered proton-coupled metal-ion transport protein responsible for the uptake of a broad range of divalent metal ions, including Fe, Cu, and Zn [108]. In APPsw cells, where a significant increase in DMT1 levels was found when compared to the control cells [103], it was observed that the intracellular Fe was significantly elevated along with the increased oxidative stress and cell toxicity [102, 103]. Silencing of endogenous DMT1 by RNA interference (RNAi) decreased the protein levels of DMT1 and also reduced bivalent ion influx into the cells, suggesting that the elevation of DMT1 might be involved in the disruption of Fe homeostasis seen in APPsw cells [102, 103].

APP, which has a Cu binding site at N-terminal cysteine-rich region [109], generates free radicals when interacting with Cu. In addition, APP has been shown to modulate Cu homeostasis. Overexpression of APP in Tg2576 transgenic

mice caused a significant reduction in Cu levels [110]. In APP knockout mice, Cu levels were significantly elevated in cerebral cortex, a region of the brain particularly involved in AD [111, 112]. This leads to the speculation that the secreted APP and/or Abeta may promote the efflux of Cu or prevent its uptake, thus reducing its levels [110]. In SH-SY5Y cells, it was found that endogenous APP had a partial colocalization with the Golgi marker GM130, while the elevation of cellular Cu levels promoted the exit of APP from the Golgi to a wider distribution throughout the cytoplasm and to the plasma membrane [113]. It was suggested that the increase in cell surface APP was resulted from a concomitant increase in exocytosis and reduction in endocytosis [113]. The copper-responsive trafficking of APP is therefore consistent with a role for APP in Cu efflux pathways [111, 113]. These observations suggest that there is an interdependent relationship between APP metabolism and Cu homeostasis, perturbations of either may cause alteration of the other, eventually promoting the accumulation of Abeta and the generation of free radicals [112].

As aberrant metal homeostasis plays an important role in several important aspects of AD pathogenesis including the production and aggregation of Abeta and the oxidative stress mediated by Abeta, AD therapy-targeted metal-Abeta interaction is rapidly emerging as a promising therapeutic option. Various metal chelating compounds have been tested for their efficacy [114]. One of these compounds, clioquinol (CQ), an orally bioavailable Cu/Zn chelator, was shown to block Abeta-induced production of H_2O_2 and Zn^{2+}/Cu^{2+}-induced precipitation of synthetic Abeta *in vitro* and significantly reduce the level of Abeta deposition in brains of Tg2576 transgenic mice [96, 115]. In a randomized, double-blind, placebo-controlled clinical intervention using clioquinol in patients with moderately severe AD, CQ treatment lowered plasma Abeta42 levels and slowed cognitive impairment in the more severely affected patients (baseline cognitive subscale score of the AD Assessment Scale, ≥25) during a 36-week period [116].

Since the generation of ROS is an important factor in AD pathogenesis promoted by the aberrant accumulation of transition metals, natural antioxidants may have protective effects against AD pathogenesis induced by disruption of metal homeostasis [117]. As expected, treatment with antioxidant nicotine attenuated the copper-facilitated neurotoxicity induced by Abeta in APPsw cells while decreasing the intracellular Cu concentration [90]. Consistently, nicotine treatment significantly lowered the Cu and Zn concentrations in senile plaques and a subfield of the hippocampus CA1 region in the brains of APPV717I (London mutant form of APP) transgenic mice, and these effects were found to be independent of the activation of nicotinic acetylcholine receptors [90]. These results suggest that antioxidants such as nicotine may also have a role in regulating metal homeostasis.

5. Oxidative Stress and Tau Pathology

Hyperphosphorylated tau protein is the major component of NFT, another hallmark of AD pathology that correlates with neurodegeneration and cognitive decline [6]. Abnormal hyperphosphorylation of tau impairs its binding with tubulin and its capacity to promote microtubule assembly, resulting in its self-aggregation into filaments [118]. A number of protein kinases and protein phosphatases have been implicated in the abnormal phosphorylation of tau including glycogen synthase kinase-3 beta (GSK-3 beta), cyclin-dependent kinase 5, mitogen-activated protein kinase (MAPK), calcium-calmodulin kinase, and protein kinase C [119]. It has been suggested that the accumulation of Abeta may appear before the tau pathology and that Abeta aggregates may be one of a cascade of molecular events leading to tau hyperphosphorayltion [120–122]. On the other hand, it was reported that overexpression of tau inhibited kinesin-dependent transport of peroxisomes, neurofilaments, and Golgi-derived vesicles into neurites, causing transport defects in primary neuronal cells including the trafficking of APP [123]. In particular, the transport of APP into axons and dendrites was blocked, causing its accumulation in the cell body [123].

Although less well studied, evidence has shown that oxidative stress is interlinked with tau pathology. It was also shown that the cells overexpressing tau protein had increased susceptibility against oxidative stress, perhaps due to the depletion of peroxisomes [123]. In a drosophila model of human tauopathies expressing a disease-related mutant form of human tau (tau R406W), reduction of gene dosage of thioredoxin reductase (TrxR) or mitochondrial SOD2 enhanced tau-induced neurodegenerative histological abnormalities and neuronal apoptosis [124]. In contrast, overexpression of these antioxidant enzymes or treatment with vitamin E attenuated tau-induced neuronal cell death [124]. Moreover, in cortical neurons derived from a transgenic rat model expressing a human truncated variant form of tau protein, it was observed that the levels of ROS were increased when compared to control nontransgenic neurons, while antioxidants such as vitamin C significantly eliminated the elevation of ROS [125, 126]. These observations suggest that tau-induced neurotoxicity is at least partially mediated by oxidative damage [124]. The linkage between oxidative stress and tau pathology was further demonstrated in P301S and P301L transgenic mouse models carrying the human tau gene with P301S or P301L mutations, which exhibit an accumulation of hyperphosphorylated tau and develop neurofibrillary tangles and neurodegeneration [127]. Mitochondrial dysfunction together with reduced NADH-ubiquinone oxidoreductase activity was found in P301L tau transgenic mice, which was associated with increased ROS production, impaired mitochondrial respiration and ATP synthesis in aged animals [128]. Similarly, the brains of P301S transgenic mice exhibited signs of elevated oxidative stress including increased protein carbonyl levels in cortex mitochondria, alterations in the activity and content of mitochondrial enzymes involved in ROS formation and energy metabolism, suggesting that oxidative stress and mitochondrial dysfunction might play an important role in tau pathology [129]. Consistently, administration of P301S mice with coenzyme Q10, an antioxidant and a key component of the electron transport chain, significantly increased complex I activity and reduced lipid peroxidation while improving survival and behavioral deficits of the mice

[130]. Furthermore, the convergence of Abeta and tau pathologies on mitochondria dysfunction was demonstrated in a triple transgenic mouse model [pR5/APP/PS2] (^{triple}AD), which exhibits both Abeta and tau pathologic features of the disease in the brain of the animal [131]. Proteomics analyses of the ^{triple}AD brain samples demonstrated a massive deregulation of 24 proteins, of which one third were mitochondrial proteins mainly related to complexes I and IV of the oxidative phosphorylation system [132]. Notably, deregulation of mitochondrial complex IV was shown to be Abeta dependent, while deregulation of complex I was tau dependent [132]. The effects of Abeta and tau on mitochondrial function were found to be synergistic and age associated, resulting in the decrease of the mitochondrial respiratory capacity and the reduction of ATP synthesis, which finally led to the synaptic loss, and neuronal death [132].

Growing evidence has also shown that oxidative stress may have a role in the hyperphosphoryaltion and polymerization of tau. The oxidation of fatty acids, which is found to be elevated in AD brains, was reported to facilitate the polymerization of tau, and thus might serve as a possible link between oxidative stress and the formation of the fibrillar pathology in AD [133]. In Tg2576 AD transgenic mice, deficiency in mitochondrial SOD2 [134] or reduction of cytoplasmic SOD1 induced tau phosphorylation, suggesting that ROS may play a critical role in the hyperphosphoryaltion of tau [45]. p38 MAPK, which can be activated by oxidative stress, is capable of phosphorylating tau protein *in vitro* [135]. In hippocampal and cortical brain regions of AD patients, activated p38 is found exclusively localized to NFT and coimmunoprecipitated with PHF-tau, suggesting that it might be involved in the phosphorylation of tau *in vivo* [136]. Thus, p38 may be a candidate that links the phosphorylation of tau with increased oxidative stress in AD.

6. Conclusions

In summary, evidence has demonstrated that oxidative stress is inextricably linked with several major pathological processes in AD including Abeta-induced neurotoxicity, tau pathology, mitochondria dysfunction, and metal dyshomeostasis. Excessive ROS may be generated from mitochondria dysfunction and/or aberrant accumulation of transition metals, perhaps caused by a combination of abnormal Abeta accumulation and tau pathology, eventually resulting in oxidative stress. Oxidative stress, which mediates the neurotoxicity induced by abnormal accumulation of Abeta and tau proteins, may augment Abeta production and aggregation as well as facilitate tau phosphorylation and polymerization, further enhancing a variety of neurotoxic events including ROS production, thus forming a vicious cycle that promotes the initiation and progression of AD. Regardless a primary or secondary event, oxidative stress is an important factor contributing to the development of AD. Removal of ROS or prevention of their formation may delay the onset or slow down the progression of AD through multiple mechanisms including, but not limited to, reduction of oxidative stress-mediated neuronal toxicity, inhibition of Abeta production and aggregation, decrease of tau phosphorylation and polymerization, and restoration of mitochondria function and metal homeostasis. Therefore, AD prevention or treatment with natural antioxidant may be an approach that is capable of targeting a number of different molecular events implicated in the pathogenesis of AD.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (30930036, 30870587, and 31201338) and 973 (2006CB500706). Yan Zhao is also supported by the Fundamental Research Funds for the Central Universities (HIT.NSRIF.2009147), research funds from Harbin Institute of Technology at Weihai [HIT(WH)Y200902], and Weihai Science and Technology Development Program (2009-3-93 and 2011DXGJ14).

References

[1] H. W. Querfurth and F. M. LaFerla, "Alzheimer's disease," *The New England Journal of Medicine*, vol. 362, no. 4, pp. 329–344, 2010.

[2] "2010 Alzheimer's disease facts and figures," *Alzheimer's & Dementia*, vol. 6, pp. 158–194, 2010.

[3] M.-J. Dong, B. Peng, X.-T. Lin, J. Zhao, Y.-R. Zhou, and R.-H. Wang, "The prevalence of dementia in the People's Republic of China: a systematic analysis of 1980–2004 studies," *Age and Ageing*, vol. 36, no. 6, pp. 619–624, 2007.

[4] C. P. Ferri, M. Prince, C. Brayne et al., "Global prevalence of dementia: a Delphi consensus study," *The Lancet*, vol. 366, no. 9503, pp. 2112–2117, 2005.

[5] R. N. Kalaria, G. E. Maestre, R. Arizaga et al., "Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors," *The Lancet Neurology*, vol. 7, no. 9, pp. 812–826, 2008.

[6] M. Goedert and M. G. Spillantini, "A century of Alzheimer's disease," *Science*, vol. 314, no. 5800, pp. 777–781, 2006.

[7] D. Praticò, "Oxidative stress hypothesis in Alzheimer's disease: a reappraisal," *Trends in Pharmacological Sciences*, vol. 29, no. 12, pp. 609–615, 2008.

[8] A. Nunomura, G. Perry, M. A. Pappolla et al., "RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease," *Journal of Neuroscience*, vol. 19, no. 6, pp. 1959–1964, 1999.

[9] S. P. Gabbita, M. A. Lovell, and W. R. Markesbery, "Increased nuclear DNA oxidation in the brain in Alzheimer' s disease," *Journal of Neurochemistry*, vol. 71, no. 5, pp. 2034–2040, 1998.

[10] P. Mecocci, U. MacGarvey, and M. F. Beal, "Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease," *Annals of Neurology*, vol. 36, no. 5, pp. 747–751, 1994.

[11] D. A. Butterfield and J. Kanski, "Brain protein oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins," *Mechanisms of Ageing and Development*, vol. 122, no. 9, pp. 945–962, 2001.

[12] M. F. Beal, "Oxidatively modified proteins in aging and disease," *Free Radical Biology and Medicine*, vol. 32, no. 9, pp. 797–803, 2002.

[13] D. Praticò and S. Sung, "Lipid peroxidation and oxidative imbalance: early functional events in Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 6, no. 2, pp. 171–175, 2004.

[14] M. A. Lovell, W. D. Ehmann, S. M. Butler, and W. R. Markesbery, "Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease," *Neurology*, vol. 45, no. 8, pp. 1594–1601, 1995.

[15] W. R. Markesbery and M. A. Lovell, "Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease," *Neurobiology of Aging*, vol. 19, no. 1, pp. 33–36, 1998.

[16] T. I. Williams, B. C. Lynn, W. R. Markesbery, and M. A. Lovell, "Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease," *Neurobiology of Aging*, vol. 27, no. 8, pp. 1094–1099, 2006.

[17] D. L. Marcus, C. Thomas, C. Rodriguez et al., "Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease," *Experimental Neurology*, vol. 150, no. 1, pp. 40–44, 1998.

[18] R. A. Omar, Y.-J. Chyan, A. C. Andorn, B. Poeggeler, N. K. Robakis, and M. A. Pappolla, "Increased expression but reduced activity of antioxidant enzymes in Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 1, no. 3, pp. 139–145, 1999.

[19] A. Furuta, D. L. Price, C. A. Pardo et al., "Localization of superoxide dismutases in Alzheimer's disease and Down's syndrome neocortex and hippocampus," *American Journal of Pathology*, vol. 146, no. 2, pp. 357–367, 1995.

[20] M. Padurariu, A. Ciobica, L. Hritcu, B. Stoica, W. Bild, and C. Stefanescu, "Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease," *Neuroscience Letters*, vol. 469, no. 1, pp. 6–10, 2010.

[21] M. A. Ansari and S. W. Scheff, "Oxidative stress in the progression of alzheimer disease in the frontal cortex," *Journal of Neuropathology and Experimental Neurology*, vol. 69, no. 2, pp. 155–167, 2010.

[22] H. Steiner, "Uncovering gamma-secretase," *Current Alzheimer Research*, vol. 1, no. 3, pp. 175–181, 2004.

[23] D. M. Walsh and D. J. Selkoe, "Aβ oligomers—a decade of discovery," *Journal of Neurochemistry*, vol. 101, no. 5, pp. 1172–1184, 2007.

[24] B. A. Yankner and T. Lu, "Amyloid β-protein toxicity and the pathogenesis of Alzheimer disease," *Journal of Biological Chemistry*, vol. 284, no. 8, pp. 4755–4759, 2009.

[25] W. P. Esler and M. S. Wolfe, "A portrait of Alzheimer secretases—new features and familiar faces," *Science*, vol. 293, no. 5534, pp. 1449–1454, 2001.

[26] E. D. Roberson and L. Mucke, "100 Years and counting: prospects for defeating Alzheimer's disease," *Science*, vol. 314, no. 5800, pp. 781–784, 2006.

[27] R. B. Maccioni, J. P. Muñoz, and L. Barbeito, "The molecular bases of Alzheimer's disease and other neurodegenerative disorders," *Archives of Medical Research*, vol. 32, no. 5, pp. 367–381, 2001.

[28] K. Hsiao, P. Chapman, S. Nilsen et al., "Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice," *Science*, vol. 274, no. 5284, pp. 99–102, 1996.

[29] M. P. Mattson, "Cellular actions of β-amyloid precursor protein and its soluble and fibrillogenic derivatives," *Physiological Reviews*, vol. 77, no. 4, pp. 1081–1132, 1997.

[30] C. Behl, J. B. Davis, R. Lesley, and D. Schubert, "Hydrogen peroxide mediates amyloid β protein toxicity," *Cell*, vol. 77, no. 6, pp. 817–827, 1994.

[31] Y. Matsuoka, M. Picciano, J. La Francois, and K. Duff, "Fibrillar β-amyloid evokes oxidative damage in a transgenic mouse model of Alzheimer's disease," *Neuroscience*, vol. 104, no. 3, pp. 609–613, 2001.

[32] M. A. Smith, K. Hirai, K. Hsiao et al., "Amyloid-β deposition in Alzheimer transgenic mice is associated with oxidative stress," *Journal of Neurochemistry*, vol. 70, no. 5, pp. 2212–2215, 1998.

[33] H. Mohmmad Abdul, R. Sultana, J. N. Keller, D. K. St. Clair, W. R. Markesbery, and D. A. Butterfield, "Mutations in amyloid precursor protein and presenilin-1 genes increase the basal oxidative stress in murine neuronal cells and lead to increased sensitivity to oxidative stress mediated by amyloid β-peptide (1-42), H_2O_2 and kainic acid: implications for Alzheimer's disease," *Journal of Neurochemistry*, vol. 96, no. 5, pp. 1322–1335, 2006.

[34] M. Manczak, T. S. Anekonda, E. Henson, B. S. Park, J. Quinn, and P. H. Reddy, "Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression," *Human Molecular Genetics*, vol. 15, no. 9, pp. 1437–1449, 2006.

[35] J. Apelt, M. Bigl, P. Wunderlich, and R. Schliebs, "Aging-related increase in oxidative stress correlates with developmental pattern of beta-secretase activity and beta-amyloid plaque formation in transgenic Tg2576 mice with Alzheimer-like pathology," *International Journal of Developmental Neuroscience*, vol. 22, no. 7, pp. 475–484, 2004.

[36] F. G. De Felice, P. T. Velasco, M. P. Lambert et al., "Aβ oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine," *Journal of Biological Chemistry*, vol. 282, no. 15, pp. 11590–11601, 2007.

[37] G. M. Cole, B. Teter, and S. A. Frautschy, "Neuroprotective effects of curcumin," *Advances in Experimental Medicine and Biology*, vol. 595, pp. 197–212, 2007.

[38] S. A. Mandel, T. Amit, L. Kalfon, L. Reznichenko, and M. B. H. Youdim, "Targeting multiple neurodegenerative diseases etiologies with multimodal-acting green tea catechins," *Journal of Nutrition*, vol. 138, no. 8, 2008.

[39] Y. Zhao and B. Zhao, "Natural antioxidants in prevention and management of Alzheimer's disease," *Frontiers in Bioscience*, vol. 4, pp. 794–808, 2012.

[40] J. V. Smith and Y. Luo, "Studies on molecular mechanisms of Ginkgo biloba extract," *Applied Microbiology and Biotechnology*, vol. 64, no. 4, pp. 465–472, 2004.

[41] F. Li, N. Y. Calingasan, F. Yu et al., "Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice," *Journal of Neurochemistry*, vol. 89, no. 5, pp. 1308–1312, 2004.

[42] Y. Nishida, T. Yokota, T. Takahashi, T. Uchihara, K.-I. Jishage, and H. Mizusawa, "Deletion of vitamin E enhances phenotype of Alzheimer disease model mouse," *Biochemical and Biophysical Research Communications*, vol. 350, no. 3, pp. 530–536, 2006.

[43] G. P. Lim, T. Chu, F. Yang, W. Beech, S. A. Frautschy, and G. M. Cole, "The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse," *Journal of Neuroscience*, vol. 21, no. 21, pp. 8370–8377, 2001.

[44] M. Dumont, E. Wille, C. Stack, N. Y. Calingasan, M. F. Beal, and M. T. Lin, "Reduction of oxidative stress, amyloid deposition, and memory deficit by manganese superoxide dismutase overexpression in a transgenic mouse model of Alzheimer's disease," *The FASEB Journal*, vol. 23, no. 8, pp. 2459–2466, 2009.

[45] K. Murakami, N. Murata, Y. Noda et al., "SOD1 (copper/zinc superoxide dismutase) deficiency drives amyloid β protein

oligomerization and memory loss in mouse model of Alzheimer disease," *Journal of Biological Chemistry*, vol. 286, no. 52, pp. 44557–44568, 2011.

[46] E. Tamagno, P. Bardini, A. Obbili et al., "Oxidative stress increases expression and activity of BACE in NT2 neurons," *Neurobiology of Disease*, vol. 10, no. 3, pp. 279–288, 2002.

[47] A. Oda, A. Tamaoka, and W. Araki, "Oxidative stress up-regulates presenilin 1 in lipid rafts in neuronal cells," *Journal of Neuroscience Research*, vol. 88, no. 5, pp. 1137–1145, 2010.

[48] M.-H. Yoo, X. Gu, X.-M. Xu et al., "Delineating the role of glutathione peroxidase 4 in protecting cells against lipid hydroperoxide damage and in alzheimer's disease," *Antioxidants and Redox Signaling*, vol. 12, no. 7, pp. 819–827, 2010.

[49] L. Chen, R. Na, M. Gu, A. Richardson, and Q. Ran, "Lipid peroxidation up-regulates BACE1 expression in vivo: a possible early event of amyloidogenesis in Alzheimer's disease," *Journal of Neurochemistry*, vol. 107, no. 1, pp. 197–207, 2008.

[50] R. Quiroz-Baez, E. Rojas, and C. Arias, "Oxidative stress promotes JNK-dependent amyloidogenic processing of normally expressed human APP by differential modification of α-, β- and γ-secretase expression," *Neurochemistry International*, vol. 55, no. 7, pp. 662–670, 2009.

[51] E. Tamagno, M. Guglielmotto, M. Aragno et al., "Oxidative stress activates a positive feedback between the γ- and β-secretase cleavages of the β-amyloid precursor protein," *Journal of Neurochemistry*, vol. 104, no. 3, pp. 683–695, 2008.

[52] C. Shen, Y. Chen, H. Liu et al., "Hydrogen peroxide promotes Aβ production through JNK-dependent activation of γ-secretase," *Journal of Biological Chemistry*, vol. 283, no. 25, pp. 17721–17730, 2008.

[53] K. Sambamurti, R. Kinsey, B. Maloney, Y.-W. Ge, and D. K. Lahiri, "Gene structure and organization of the human β-secretase (BACE) promoter," *The FASEB Journal*, vol. 18, no. 9, pp. 1034–1036, 2004.

[54] X. Zhu, A. K. Raina, C. A. Rottkamp et al., "Activation and redistribution of c-Jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease," *Journal of Neurochemistry*, vol. 76, no. 2, pp. 435–441, 2001.

[55] X. Zhu, O. Ogawa, Y. Wang, G. Perry, and M. A. Smith, "JKK1, an upstream activator of JNK/SAPK, is activated in Alzheimer's disease," *Journal of Neurochemistry*, vol. 85, no. 1, pp. 87–93, 2003.

[56] S. Lagalwar, A. L. Guillozet-Bongaarts, R. W. Berry, and L. I. Binder, "Formation of phospho-SAPK/JNK granules in the hippocampus is an early event in Alzheimer disease," *Journal of Neuropathology and Experimental Neurology*, vol. 65, no. 5, pp. 455–464, 2006.

[57] H. Fukumoto, B. S. Cheung, B. T. Hyman, and M. C. Irizarry, "β-secretase protein and activity are increased in the neocortex in Alzheimer disease," *Archives of Neurology*, vol. 59, no. 9, pp. 1381–1389, 2002.

[58] L.-B. Yang, K. Lindholm, R. Yan et al., "Elevated β-secretase expression and enzymatic activity detected in sporadic Alzheimer disease," *Nature Medicine*, vol. 9, no. 1, pp. 3–4, 2003.

[59] T. Matsui, M. Ingelsson, H. Fukumoto et al., "Expression of APP pathway mRNAs and proteins in Alzheimer's disease," *Brain Research*, vol. 1161, no. 1, pp. 116–123, 2007.

[60] M. Yao, T.-V. V. Nguyen, and C. J. Pike, "β-Amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase-dependent downregulation of Bcl-w," *Journal of Neuroscience*, vol. 25, no. 5, pp. 1149–1158, 2005.

[61] A. Nunomura, G. Perry, G. Aliev et al., "Oxidative damage is the earliest event in Alzheimer disease," *Journal of Neuropathology and Experimental Neurology*, vol. 60, no. 8, pp. 759–767, 2001.

[62] A. Nunomura, S. Chiba, C. F. Lippa et al., "Neuronal RNA oxidation is a prominent feature of familial Alzheimer's disease," *Neurobiology of Disease*, vol. 17, no. 1, pp. 108–113, 2004.

[63] A. Nunomura, T. Tamaoki, K. Tanaka et al., "Intraneuronal amyloid β accumulation and oxidative damage to nucleic acids in Alzheimer disease," *Neurobiology of Disease*, vol. 37, no. 3, pp. 731–737, 2010.

[64] A. Nunomura, R. J. Castellani, X. Zhu, P. I. Moreira, G. Perry, and M. A. Smith, "Involvement of oxidative stress in Alzheimer disease," *Journal of Neuropathology and Experimental Neurology*, vol. 65, no. 7, pp. 631–641, 2006.

[65] Y. Luo, T. Sunderland, G. S. Roth, and B. Wolozin, "Physiological levels of β-amyloid peptide promote PC12 cell proliferation," *Neuroscience Letters*, vol. 217, no. 2-3, pp. 125–128, 1996.

[66] L. D. Plant, J. P. Boyle, I. F. Smith, C. Peers, and H. A. Pearson, "The production of amyloid β peptide is a critical requirement for the viability of central neurons," *Journal of Neuroscience*, vol. 23, no. 13, pp. 5531–5535, 2003.

[67] A. Kontush, C. Berndt, W. Weber et al., "Amyloid-β is an antioxidant for lipoproteins in cerebrospinal fluid and plasma," *Free Radical Biology and Medicine*, vol. 30, no. 1, pp. 119–128, 2001.

[68] D. Puzzo, L. Privitera, E. Leznik et al., "Picomolar amyloid-β positively modulates synaptic plasticity and memory in hippocampus," *Journal of Neuroscience*, vol. 28, no. 53, pp. 14537–14545, 2008.

[69] K. Zou, J.-S. Gong, K. Yanagisawa, and M. Michikawa, "A novel function of monomeric amyloid β-protein serving as an antioxidant molecule against metal-induced oxidative damage," *Journal of Neuroscience*, vol. 22, no. 12, pp. 4833–4841, 2002.

[70] S. Tan, Y. Sagara, Y. Liu, P. Maher, and D. Schubert, "The regulation of reactive oxygen species production during programmed cell death," *Journal of Cell Biology*, vol. 141, no. 6, pp. 1423–1432, 1998.

[71] V. G. Grivennikova and A. D. Vinogradov, "Generation of superoxide by the mitochondrial Complex I," *Biochimica et Biophysica Acta*, vol. 1757, no. 5-6, pp. 553–561, 2006.

[72] K. Hirai, G. Aliev, A. Nunomura et al., "Mitochondrial abnormalities in Alzheimer's disease," *Journal of Neuroscience*, vol. 21, no. 9, pp. 3017–3023, 2001.

[73] X. Zhu, G. Perry, P. I. Moreira et al., "Mitochondrial abnormalities and oxidative imbalance in Alzheimer disease," *Journal of Alzheimer's Disease*, vol. 9, no. 2, pp. 147–153, 2006.

[74] E. M. Mutisya, A. C. Bowling, and M. F. Beal, "Cortical cytochrome oxidase activity is reduced in Alzheimer's disease," *Journal of Neurochemistry*, vol. 63, no. 6, pp. 2179–2184, 1994.

[75] C. Caspersen, N. Wang, J. Yao et al., "Mitochondrial Aβ: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease," *The FASEB Journal*, vol. 19, no. 14, pp. 2040–2041, 2005.

[76] C. M. P. Rodrigues, S. Solá, M. A. Brito, C. D. Brondino, D. Brites, and J. J. G. Moura, "Amyloid β-peptide disrupts mitochondrial membrane lipid and protein structure: protective role of tauroursodeoxycholate," *Biochemical and Biophysical Research Communications*, vol. 281, no. 2, pp. 468–474, 2001.

[77] C. S. Casley, L. Canevari, J. M. Land, J. B. Clark, and M. A. Sharpe, "β-Amyloid inhibits integrated mitochondrial respiration and key enzyme activities," *Journal of Neurochemistry*, vol. 80, no. 1, pp. 91–100, 2002.

[78] M. Anantharaman, J. Tangpong, J. N. Keller et al., "β-amyloid mediated nitration of manganese superoxide dismutase: implication for oxidative stress in a APPNLh/NLh X PS-1 P264L/P264L double knock-in mouse model of Alzheimer's disease," *American Journal of Pathology*, vol. 168, no. 5, pp. 1608–1618, 2006.

[79] S. Rousset, M.-C. Alves-Guerra, J. Mozo et al., "The biology of mitochondrial uncoupling proteins," *Diabetes*, vol. 53, no. 1, pp. S130–S135, 2004.

[80] K. S. Echtay, "Mitochondrial uncoupling proteins—what is their physiological role?" *Free Radical Biology and Medicine*, vol. 43, no. 10, pp. 1351–1371, 2007.

[81] S. M. de la Monte and J. R. Wands, "Molecular indices of oxidative stress and mitochondrial dysfunction occur early and often progress with severity of Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 9, no. 2, pp. 167–181, 2006.

[82] Z. Wu, J. Zhang, and B. Zhao, "Superoxide anion regulates the mitochondrial free ca2+ through uncoupling proteins," *Antioxidants and Redox Signaling*, vol. 11, no. 8, pp. 1805–1818, 2009.

[83] M. L. Schlief and J. D. Gitlin, "Copper homeostasis in the CNS: a novel link between the NMDA receptor and copper homeostasis in the hippocampus," *Molecular Neurobiology*, vol. 33, no. 2, pp. 81–90, 2006.

[84] T. G. Smart, A. M. Hosie, and P. S. Miller, "Zn2+ ions: modulators of excitatory and inhibitory synaptic activity," *Neuroscientist*, vol. 10, no. 5, pp. 432–442, 2004.

[85] P. Munoz and A. Humeres, "Iron deficiency on neuronal function," *Biometals*, vol. 25, no. 4, pp. 825–835, 2012.

[86] V. B. Kenche and K. J. Barnham, "Alzheimer's disease & metals: therapeutic opportunities," *British Journal of Pharmacology*, vol. 163, no. 2, pp. 211–219, 2011.

[87] M. A. Deibel, W. D. Ehmann, and W. R. Markesbery, "Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress," *Journal of the Neurological Sciences*, vol. 143, no. 1-2, pp. 137–142, 1996.

[88] M. A. Lovell, J. D. Robertson, W. J. Teesdale, J. L. Campbell, and W. R. Markesbery, "Copper, iron and zinc in Alzheimer's disease senile plaques," *Journal of the Neurological Sciences*, vol. 158, no. 1, pp. 47–52, 1998.

[89] J. Y. Lee, I. Mook-Jung, and J. Y. Koh, "Histochemically reactive zinc in plaques of the Swedish mutant beta-amyloid precursor protein transgenic mice," *The Journal of Neuroscience*, vol. 19, no. 11, p. RC10, 1999.

[90] J. Zhang, Q. Liu, Q. Chen et al., "Nicotine attenuates beta-amyloid-induced neurotoxicity by regulating metal homeostasis," *The FASEB Journal*, vol. 20, no. 8, pp. 1212–1214, 2006.

[91] C. C. Curtain, F. Ali, I. Volitakis et al., "Alzheimer's disease amyloid-β binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits," *Journal of Biological Chemistry*, vol. 276, no. 23, pp. 20466–20473, 2001.

[92] L. Hesse, D. Beher, C. L. Masters, and G. Multhaup, "The βA4 amyloid precursor protein binding to copper," *FEBS Letters*, vol. 349, no. 1, pp. 109–116, 1994.

[93] C. S. Atwood, R. D. Moir, X. Huang et al., "Dramatic aggregation of alzheimer by Cu(II) is induced by conditions representing physiological acidosis," *Journal of Biological Chemistry*, vol. 273, no. 21, pp. 12817–12826, 1998.

[94] A. I. Bush, W. H. Pettingell, G. Multhaup et al., "Rapid induction of Alzheimer Aβ amyloid formation by zinc," *Science*, vol. 265, no. 5177, pp. 1464–1467, 1994.

[95] D. Strausak, J. F. B. Mercer, H. H. Dieter, W. Stremmel, and G. Multhaup, "Copper in disorders with neurological symptoms: Alzheimer's, Menkes, and Wilson diseases," *Brain Research Bulletin*, vol. 55, no. 2, pp. 175–185, 2001.

[96] A. I. Bush, "Metal complexing agents as therapies for Alzheimer's disease," *Neurobiology of Aging*, vol. 23, no. 6, pp. 1031–1038, 2002.

[97] C. Opazo, X. Huang, R. A. Cherny et al., "Metalloenzyme-like activity of Alzheimer's disease β-amyloid: Cu-dependent catalytic conversion of dopamine, cholesterol, and biological reducing agents to neurotoxic H_2O_2," *Journal of Biological Chemistry*, vol. 277, no. 43, pp. 40302–40308, 2002.

[98] X. Huang, M. P. Cuajungco, C. S. Atwood et al., "Cu(II) potentiation of Alzheimer aβ neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction," *Journal of Biological Chemistry*, vol. 274, no. 52, pp. 37111–37116, 1999.

[99] X. Huang, C. S. Atwood, M. A. Hartshorn et al., "The Aβ peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction," *Biochemistry*, vol. 38, no. 24, pp. 7609–7616, 1999.

[100] T. Lynch, R. A. Cherny, and A. I. Bush, "Oxidative processes in Alzheimer's disease: the role of Aβ-metal interactions," *Experimental Gerontology*, vol. 35, no. 4, pp. 445–451, 2000.

[101] C. A. Rottkamp, A. K. Raina, X. Zhu et al., "Redox-active iron mediates amyloid-β toxicity," *Free Radical Biology and Medicine*, vol. 30, no. 4, pp. 447–450, 2001.

[102] L. Wan, G. Nie, J. Zhang et al., "β-Amyloid peptide increases levels of iron content and oxidative stress in human cell and Caenorhabditis elegans models of Alzheimer disease," *Free Radical Biology and Medicine*, vol. 50, no. 1, pp. 122–129, 2011.

[103] W. Zheng, N. Xin, Z.-H. Chi et al., "Divalent metal transporter 1 is involved in amyloid precursor protein processing and Aβ generation," *The FASEB Journal*, vol. 23, no. 12, pp. 4207–4217, 2009.

[104] J. T. Rogers, J. D. Randall, C. M. Cahill et al., "An iron-responsive element type II in the 5′-untranslated region of the Alzheimer's amyloid precursor protein transcript," *Journal of Biological Chemistry*, vol. 277, no. 47, pp. 45518–45528, 2002.

[105] T. Borchardt, J. Camakaris, R. Cappai, C. L. Masters, K. Beyreuther, and G. Multhaup, "Copper inhibits β-amyloid production and stimulates the non-amyloidogenic pathway of amyloid-precursor-protein secretion," *Biochemical Journal*, vol. 344, no. 2, pp. 461–467, 1999.

[106] L.-H. Zhang, X. Wang, Z.-H. Zheng et al., "Altered expression and distribution of zinc transporters in APP/PS1 transgenic mouse brain," *Neurobiology of Aging*, vol. 31, no. 1, pp. 74–87, 2010.

[107] L.-H. Zhang, X. Wang, M. Stoltenberg, G. Danscher, L. Huang, and Z.-Y. Wang, "Abundant expression of zinc transporters in the amyloid plaques of Alzheimer's disease brain," *Brain Research Bulletin*, vol. 77, no. 1, pp. 55–60, 2008.

[108] H. Li, F. Li, H. Sun, and Z. M. Qian, "Membrane-inserted conformation of transmembrane domain 4 of divalent-metal transporter," *Biochemical Journal*, vol. 372, no. 3, pp. 757–766, 2003.

[109] K. J. Barnham, W. J. McKinstry, G. Multhaup et al., "Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. A regulator of neuronal copper homeostasis," *Journal of Biological Chemistry*, vol. 278, no. 19, pp. 17401–17407, 2003.

[110] C. J. Maynard, R. Cappai, I. Volitakis et al., "Overexpression of Alzheimer's disease amyloid-β opposes the age-dependent

elevations of brain copper and iron," *Journal of Biological Chemistry*, vol. 277, no. 47, pp. 44670–44676, 2002.

[111] S. A. Bellingham, G. D. Ciccotosto, B. E. Needham et al., "Gene knockout of amyloid precursor protein and amyloid precursor-like protein-2 increases cellular copper levels in primary mouse cortical neurons and embryonic fibroblasts," *Journal of Neurochemistry*, vol. 91, no. 2, pp. 423–428, 2004.

[112] A. R. White, R. Reyes, J. F. B. Mercer et al., "Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice," *Brain Research*, vol. 842, no. 2, pp. 439–444, 1999.

[113] K. M. Acevedo, Y. H. Hung, A. H. Dalziel et al., "Copper promotes the trafficking of the amyloid precursor protein," *Journal of Biological Chemistry*, vol. 286, no. 10, pp. 8252–8262, 2011.

[114] S. Bolognin, L. Messori, and P. Zatta, "Metal ion physiopathology in neurodegenerative disorders," *NeuroMolecular Medicine*, vol. 11, no. 4, pp. 223–238, 2009.

[115] R. A. Cherny, C. S. Atwood, M. E. Xilinas et al., "Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice," *Neuron*, vol. 30, no. 3, pp. 665–676, 2001.

[116] C. W. Ritchie, A. I. Bush, A. Mackinnon et al., "Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Aβ amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial," *Archives of Neurology*, vol. 60, no. 12, pp. 1685–1691, 2003.

[117] B. Zhao and L. Wan, "Metal metabolic homeostasis disruption and early initiation of mechanism for Alzheimer's disease," *Progress in Biochemistry and Biophysics*, vol. 39, pp. 756–763, 2012.

[118] K. Iqbal, F. Liu, C.-X. Gong, A. C. del Alonso, and I. Grundke-Iqbal, "Mechanisms of tau-induced neurodegeneration," *Acta Neuropathologica*, vol. 118, no. 1, pp. 53–69, 2009.

[119] M. L. Billingsley and R. L. Kincaid, "Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration," *Biochemical Journal*, vol. 323, no. 3, pp. 577–591, 1997.

[120] W.-H. Zheng, S. Bastianetto, F. Mennicken, W. Ma, and S. Kar, "Amyloid β peptide induces tau phosphorylation and loss of cholinergic neurons in rat primary septal cultures," *Neuroscience*, vol. 115, no. 1, pp. 201–211, 2002.

[121] J. Lewis, D. W. Dickson, W.-L. Lin et al., "Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP," *Science*, vol. 293, no. 5534, pp. 1487–1491, 2001.

[122] J. Götz, F. Chen, J. Van Dorpe, and R. M. Nitsch, "Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Aβ42 fibrils," *Science*, vol. 293, no. 5534, pp. 1491–1495, 2001.

[123] K. Stamer, R. Vogel, E. Thies, E. Mandelkow, and E.-M. Mandelkow, "Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress," *Journal of Cell Biology*, vol. 156, no. 6, pp. 1051–1063, 2002.

[124] D. Dias-Santagata, T. A. Fulga, A. Duttaroy, and M. B. Feany, "Oxidative stress mediates tau-induced neurodegeneration in Drosophila," *Journal of Clinical Investigation*, vol. 117, no. 1, pp. 236–245, 2007.

[125] M. Cente, P. Filipcik, M. Pevalova, and M. Novak, "Expression of a truncated tau protein induces oxidative stress in a rodent model of tauopathy," *European Journal of Neuroscience*, vol. 24, no. 4, pp. 1085–1090, 2006.

[126] M. Cente, P. Filipcik, S. Mandakova, N. Zilka, G. Krajciova, and M. Novak, "Expression of a truncated human tau protein induces aqueous-phase free radicals in a rat model of tauopathy: implications for targeted antioxidative therapy," *Journal of Alzheimer's Disease*, vol. 17, no. 4, pp. 913–920, 2009.

[127] Y. Yoshiyama, M. Higuchi, B. Zhang et al., "Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model," *Neuron*, vol. 53, no. 3, pp. 337–351, 2007.

[128] D. C. David, S. Hauptmann, I. Scherping et al., "Proteomic and functional analyses reveal a mitochondrial dysfunction in P301L tau transgenic mice," *Journal of Biological Chemistry*, vol. 280, no. 25, pp. 23802–23814, 2005.

[129] M. Dumont, C. Stack, C. Elipenahli et al., "Behavioral deficit, oxidative stress, and mitochondrial dysfunction precede tau pathology in P301S transgenic mice," *The FASEB Journal*, vol. 25, no. 11, pp. 4063–4072, 2011.

[130] C. Elipenahli, C. Stack, S. Jainuddin et al., "Behavioral improvement after chronic administration of coenzyme Q10 in P301S transgenic mice," *Journal of Alzheimer's Disease*, vol. 28, no. 1, pp. 173–182, 2012.

[131] A. Eckert, K. L. Schulz, V. Rhein, and J. Götz, "Convergence of amyloid-β and tau pathologies on mitochondria in vivo," *Molecular Neurobiology*, vol. 41, no. 2-3, pp. 107–114, 2010.

[132] V. Rhein, X. Song, A. Wiesner et al., "Amyloid-β and tau synergistically impair the oxidative phosphorylation system in triple transgenic Alzheimer's disease mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 47, pp. 20057–20062, 2009.

[133] T. C. Gamblin, M. E. King, J. Kuret, R. W. Berry, and L. I. Binder, "Oxidative regulation of fatty acid-induced tau polymerization," *Biochemistry*, vol. 39, no. 46, pp. 14203–14210, 2000.

[134] S. Melov, P. A. Adlard, K. Morten et al., "Mitochondrial oxidative stress causes hyperphosphorylation of tau," *PLoS ONE*, vol. 2, no. 6, article e536, 2007.

[135] M. Goedert, M. Hasegawa, R. Jakes, S. Lawler, A. Cuenda, and P. Cohen, "Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases," *FEBS Letters*, vol. 409, no. 1, pp. 57–62, 1997.

[136] X. Zhu, C. A. Rottkamp, H. Boux, A. Takeda, G. Perry, and M. A. Smith, "Activation of p38 kinase links tau phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease," *Journal of Neuropathology and Experimental Neurology*, vol. 59, no. 10, pp. 880–888, 2000.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 487534, 10 pages
http://dx.doi.org/10.1155/2013/487534

Review Article

Neurodegeneration in Friedreich's Ataxia: From Defective Frataxin to Oxidative Stress

Cláudio M. Gomes[1] and Renata Santos[2]

[1] *Instituto Tecnologia Química e Biológica, Universidade Nova de Lisboa, Avenida da República, 2784-505 Oeiras, Portugal*
[2] *Development of the Nervous System, IBENS, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France*

Correspondence should be addressed to Renata Santos; rsantos@biologie.ens.fr

Received 26 May 2013; Accepted 14 June 2013

Academic Editor: Anne-Laure Bulteau

Friedreich's ataxia is the most common inherited autosomal recessive ataxia and is characterized by progressive degeneration of the peripheral and central nervous systems and cardiomyopathy. This disease is caused by the silencing of the *FXN* gene and reduced levels of the encoded protein, frataxin. Frataxin is a mitochondrial protein that functions primarily in iron-sulfur cluster synthesis. This small protein with an α/β sandwich fold undergoes complex processing and imports into the mitochondria, generating isoforms with distinct N-terminal lengths which may underlie different functionalities, also in respect to oligomerization. Missense mutations in the *FXN* coding region, which compromise protein folding, stability, and function, are found in 4% of FRDA heterozygous patients and are useful to understand how loss of functional frataxin impacts on FRDA physiopathology. In cells, frataxin deficiency leads to pleiotropic phenotypes, including deregulation of iron homeostasis and increased oxidative stress. Increasing amount of data suggest that oxidative stress contributes to neurodegeneration in Friedreich's ataxia.

1. Friedreich's Ataxia: Origin, Clinical Features, and Neurodegeneration

The main feature of Friedreich's ataxia (FRDA) disease is progressive and unremitting ataxia [1–3]. The first symptoms appear at puberty but onset of the disease can occur from infancy to after 60 years [4]. Major neurologic signs include gait and limb ataxia, tendon areflexia, dysarthria, sensory loss, and pyramidal signs [5]. Cardiomyopathy is a frequent symptom and is associated with a severe prognosis, particularly in young patients [6]. Some patients can also develop skeletal deformation, ocular abnormalities, hearing loss, and diabetes [5]. The neuropathology of FRDA involves degeneration of the dorsal root ganglia, peripheral nerves, the spinal cord, and the dentate nucleus in the cerebellum [7]. Patient nerves show axonal neuropathy with loss of large myelinated fibers and an increase in the number of small unmyelinated fibers [8, 9].

FRDA is caused by a GAA trinucleotide repeat expansion in the first intron of the *FXN* gene [10]. The majority of patients are homozygous for the trinucleotide expansion but in 4% of patients, one allele presents point mutations in the coding region. Expanded alleles lead to the inhibition of *FXN* expression resulting in decreased levels of the encoded protein, frataxin [10, 11]. The transcriptional repression of the *FXN* gene induced by the GAA expansion is due to arrest of RNA polymerase II progression and to heterochromatin-mediated gene silencing [12–16]. Frataxin is a mitochondrial protein involved in cellular iron use and maintenance of the redox status [4]. Although the function of frataxin has been a matter of debate since its discovery, it is now generally accepted that its primary function is in iron-sulfur cluster biosynthesis [17–20].

Frataxin is expressed in all cells of eukaryotic organisms. However, the levels of *FXN* mRNA and frataxin show tissue specificity that partially correlates with the sites of disease. In humans, the highest levels of expression are found in the heart and spinal cord and lower levels are observed in the cerebellum, liver, skeletal muscle, and pancreas [10]. The differential sensitivity of the tissues to frataxin deficiency is not clear but may depend on the cellular metabolism and/or on the somatic instability of expanded GAA triplet repeats

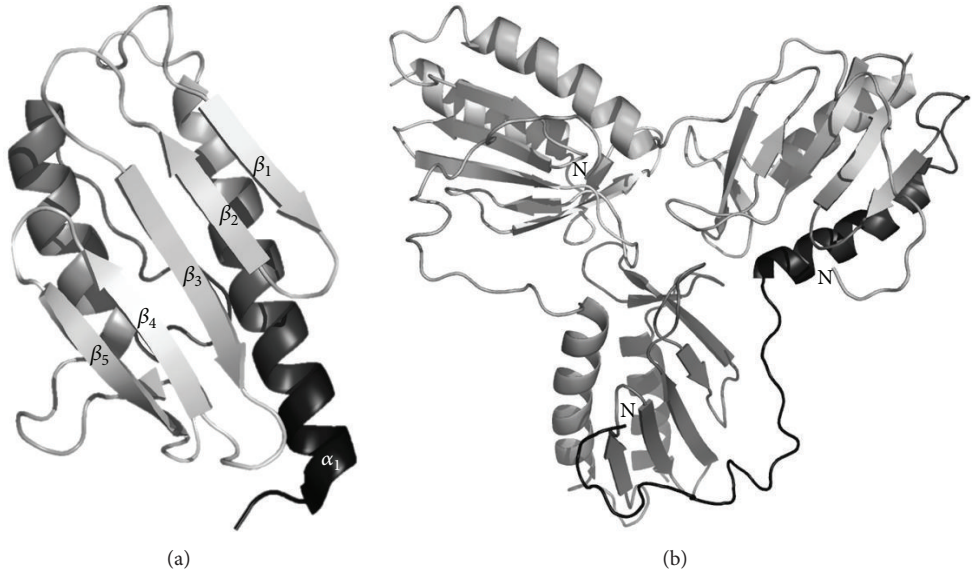


Figure 1: Structures of the frataxin monomer and trimer, denoting the typical α/β fold in which the α-helices pack against the β-sheet strands. (a) Structure of the human frataxin monomer (PDB: 3S4M). (b) Structure of the yeast frataxin trimer (PDB: 3OEQ). Note that in the trimer structure, unwinding of the N-terminal α-helix affords an interaction with a β-sheet from the nearby subunit.

[4, 21]. For dorsal root ganglia, one of the first tissues affected in FRDA patients, it was shown that somatic instability starts during embryonic development and continues throughout life, resulting in progressive, age-dependent accumulation of larger GAA triplet repeat expansions [21, 22]. Nevertheless, understanding why only certain tissues are sensitive to frataxin depletion will contribute to a better understanding of the pathophysiology of the disease. Oxidative stress has been suggested to be one of the major inducers of neurodegeneration, but the underlying mechanisms are not fully understood [4, 23, 24]. Antioxidant therapy has been tested since the discovery of the gene with different molecules with limited success in stopping the progression of the disease; however, it is a potential target to treat the disease, and new molecules are being tested.

2. The Frataxin Protein: Structure and Function

Human frataxin (FXN) is a small protein, which is involved in the mitochondrial biogenesis of iron-sulfur clusters (ISCs). These inorganic structures are essential redox cofactors found in several respiratory and metabolic enzymes within mitochondria. Although frataxin function remains to be fully elucidated at the molecular level, a wealth of biochemical data and the determination of three-dimensional structures from several homologues of FXN, particularly yeast frataxin (Yfh1), have contributed to substantial progress on understanding frataxin function [25–29].

The frataxin fold is characterized by a planar α-β sandwich motif, comprising two terminal α-helices, alongside with five antiparallel β-strands that make up two planes which are intersected by additional β-strands, thus composing the structure (Figure 1(a)). Several frataxins have been shown to undergo oligomerization reactions, assembling into trimers, hexamers, and larger order 24- and 48-mer assemblies, although there is no consensus in the community regarding the possibility of these reactions taking place *in vivo* or on their functional relevance (Figure 1(b)). Frataxin function involves participating in larger molecular assemblies with the several components from the mitochondrial ISC assembly machinery, and the molecular details of these interactions are only now starting to be elucidated with the disclosure of possible interactions taking place in such quaternary complexes [30–32]. Nevertheless, other still controversial roles have been proposed for frataxin, including delivery of iron to ferrochelatase for heme synthesis, recovery of the oxidatively damaged [3Fe4S] cluster in aconitase, and iron storage (reviewed in [17]).

One important region in the frataxin fold is the so-called acidic region, which comprises a set of acidic residues located within the first α-helix and the edge of the first β-strand (α1 and β1 in Figure 1(a)), that are involved in low affinity iron-binding [33]. Nevertheless, iron-binding is essential for the interaction between Yfh1 frataxin and the Isu scaffold protein from the ISC machinery [34]. Studies on Yfh1 focusing on a set of functional mutants in the acidic region have shown that charge-to-neutral alterations in the ridge do not abolish iron-binding, but rather decrease binding affinity [35]. Indeed, frataxin iron-binding capacity is quite robust, as even upon changing five of the most conserved residues from the putative iron-binding region, at least two iron atoms per monomer can be bound. This study has also elicited an interesting trade-off between frataxin function and stability of the fold. Although these negative charges have a functional role, at the same time, they significantly impair Yfh1 stability as their replacement results in a dramatic increase of protein stability while reducing conformational plasticity. The acidic

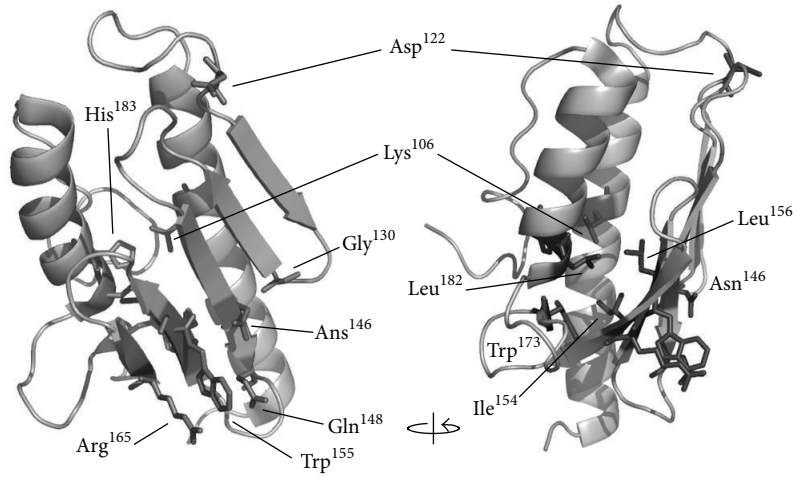


Figure 2: Mapping of amino acids mutated in FRDA compound heterozygous patients. FRDA mutations indicated on the human frataxin structure (PDB: 3S4M): Lys106Ser, Asp122Tyr, Gly130Val, Asn146Lys, Gln148Arg, Ile154Phe, Trp155Arg, Leu156Pro, Arg165Cys, Trp173Gly, Leu182Phe, Leu182His, and His183Arg. The arrow denotes a 90° rotation.

ridge has thus evolved to favor function over stability, therefore highlighting its importance on frataxin structure. On the other hand, mutations of conserved residues of the portion of the acid ridge found in the β-sheet, affected interaction with the scaffold protein Isu, but did not affect overall protein stability [35].

Frataxin processing and cleavage upon import into the mitochondria results, in some cases, in the production of diverse isoforms with distinct structural properties, especially in what concerns oligomerization propensity [36–38]. Human frataxin is a 210-amino acid protein which upon expression in the cytosol is imported into the mitochondria matrix, where it undergoes a complex processing resulting in isoforms with different lengths at the N-terminus [36–38]. Identified isoforms include shorter frataxin versions (processed at positions 42- and 56-) and longer ones (processed at positions 78- and 81-). In humans, the most abundant products seem to be the monomeric form FXN(81-201) which predominates in fibroblasts, alongside with the oligomerization prone longer FXN(42-120) isoform, which is abundant in the heart, cerebellum, and dividing fibroblasts [39]. In fact, distinct N-terminal processing of human frataxin has been suggested to result in proteins with distinct functionalities in what concerns interactions with the ISC machinery proteins and iron-binding properties [17, 39]. In yeast, one major form of processed frataxin is detected in the mitochondria (corresponding to Yfh1 52-174), which seems to be present mostly in the form of trimers, unless protein overexpression takes place which then generates larger oligomers [40]. Stress and increased iron uptake seem also to induce the formation of higher order Yfh1 oligomers [41].

One aspect which remains to be fully investigated is whether frataxin may be itself a target for oxidative modifications in the context of FRDA in which very low frataxin levels are present with iron accumulation in an environment prone to oxidative reactions. To establish a proof of principle, the susceptibility of frataxin to amino acid carbonylation and nitration was investigated [42]. The results on FXN(91-210) showed that residues in the β-sheet surface (Tyr143, Tyr174, Tyr205, and Trp155) were preferential targets, and that modifications did not alter substantially iron binding or protein stability. Interestingly, the strictly conserved Trp155, which is mutated in early onset patients, is a hot spot for modifications leading to the speculation that this could be an effective mechanism to modulate frataxin interactions and thus its function [42].

3. FRDA-Related Frataxin Mutations Result in a Loss of Function Misfolding Disease

A small fraction of FRDA patients (around 4%) are compound heterozygotes for the intronic GAA trinucleotide repeat expansion, carrying in the other allele a missense mutation in the coding region of the *FXN* gene (Figure 2). These mutations are useful to understand how loss of functional frataxin impacts on FRDA physiopathology. Some of these mutations can be grouped according to FRDA symptoms severity: for example, whereas the FXN-p.Ile154Phe and FXN-p.Trp155Arg mutations lead to severe FRDA, the mutations FXN-p.Gly130Val and the FXN-p.Asp122Tyr account for milder clinical symptoms, although the latter has a very low prevalence [43]. Overall, the link between a point mutation in frataxin and the disease physiopathology remains unclear. Whereas first approaches shed light onto possible molecular mechanisms of disease by analyzing effects of mutations on protein folding and stability [44, 45], more recently mutations have also been studied in the context of cell models [46] and distinct frataxin isoforms with different N-terminal processing [47], and for some mutants, crystal structure information has been obtained [48].

The initial studies on FRDA frataxin mutants focused on the aggressive phenotype FXN-p.Ile154Phe and FXN-p.Trp155Arg variants, studied in the background of the

shorter processed FXN(91-210) protein [44]. The former is among the most common clinical mutation, and affects an isoleucine residue at the protein core, and thus is expected to directly affect the structure and stability of the protein fold. The latter affects a conserved tryptophan residue at the protein surface in the exposed region of a β-sheet. The study has shown that these FRDA frataxin variants retained the native fold under physiological conditions but were thermodynamically destabilized in respect to normal frataxin had a higher tendency towards proteolytic degradation, and iron-binding was only partly impaired, nevertheless with concomitant protein aggregation [44]. The implication of these findings was that the severe mutations did not abolish the expression of the FRDA frataxin variants, suggesting that these proteins are formed under physiological conditions. Interestingly, a FRDA murine fibroblast cell model based on the FXN-p.Ile154Phe has corroborated this observation *in vivo*, as transgenic expression of this pathological mutation partly rescued endogenous frataxin deficiency [46]. Further, these studies also evidenced decreased activity of iron-sulfur proteins and accumulation of iron [46], in agreement with the poorer iron-binding ability which had been observed *in vitro* [44].

The evidence that the pathogenic mechanism in FRDA mutant frataxin results from frataxin misfolding and instability suggests that, although quite different from other neurodegenerative diseases involving toxic aggregation and metal ions [49], FRDA in compound heterozygous patients can be classified as a protein misfolding disease [50]. In order to further explore this possibility, a broader study encompassing also frataxin mutations yielding the milder FRDA forms FXN-p.Asp122Tyr and FXN-p.Gly130Val was undertaken [45]. The conclusion from those studies showed that FRDA frataxins exhibit distinct degrees of conformational defects that impair folding and function. For example, protein degradation propensity does not correlate necessarily with disease severity, as the two milder mutations were found for example to undergo proteolysis at higher rates than the severe ones, which rather seem to expose more particular part of the protein. An exploration of folding defects caused by mutations resulting in a change in the soluble : insoluble ratio upon recombinant expression showed that the severe FXN-p.Trp155Arg and FXN-p.Ile154Phe variants are mostly expressed as insoluble peptides, indicating that these mutations affect early folding [45]. Interestingly these results were corroborated by an investigation of the effects of these mutations in the context of the isoforms FXN(42-210) and FXN(81-210) in cell-free and different cellular models [47]. In addition, this study has shown that the FXN-p.Trp155Arg mutation destabilizes FXN(42-210) to a greater extent, as the mutation in this form makes the protein more susceptible to *in vivo* degradation than in the FXN(81-210) form. Likewise, the FXN-p.Ile154Phe variant in FXN(42-210) has a strongly compromised solubility; however, in FXN(81-210) yields a folded polypeptide when expressed in different cells types [47], in agreement with the results obtained *in vitro* for the purified recombinant mutant variant [45].

4. Oxidative Stress in Frataxin-Deficient Cells and Neurodegeneration

Oxidative stress is a central feature of FRDA disease and still a privileged target for therapy [4, 23, 24]. An increasing amount of data from different organisms support the hypothesis that frataxin-deficiency causes a deregulation in the antioxidant defenses, which result in oxidative stress and pathology [4, 24, 51, 52]. Increased levels of prooxidant molecules such as H_2O_2 and superoxide have been detected in yeast, *Drosophila* and FRDA patient cells [40, 53–58]. In addition, frataxin deficiency increases the cellular sensitivity to a wide variety of prooxidants in yeast [58, 59], *Caenorhabditis elegans* [60], *Drosophila* [56, 61, 62], mouse [63], and patient FRDA cells [64–68].

The eukaryotic cellular response to oxidative stress involves the induction of detoxifying enzymes such as superoxide dismutases (SODs), an increase in glutathione and NADPH synthesis, a decrease in the reduced to oxidized glutathione ratio and glutathionylation of target proteins [69]. SODs convert superoxide into H_2O_2 and are upregulated upon oxidant insult. However, in fibroblasts from FRDA patients, unlike those from healthy controls, SODs are not upregulated in response to low doses of H_2O_2, oligomycin, and iron [57, 65, 66]. In yeast, the anaerobiosis to aerobiosis transition is an inducer of oxidative stress in $\Delta yfh1$ cells as a result of transcriptional repression of several genes encoding critical antioxidant enzymes (SODs, catalases, glutaredoxins, and thioredoxins) and of decrease of total glutathione levels [58, 70]. Glutathione is a major antioxidant molecule in eukaryotic cells. Furthermore, reversible protein S-glutathionylation is a post translational modification that provides protection of protein cysteines from irreversible oxidation and also functions in the transduction of redox signals [71]. Several studies show that frataxin deficiency leads to the impairment of glutathione homeostasis [52, 53, 72–75]. A significant increase in the glutathione pool bound to proteins was observed in blood samples, fibroblasts, and lymphoblasts from FRDA patients and yeast $\Delta yfh1$ cells [72, 74, 75]. In addition, Pastore et al. found that in patient fibroblasts actin was glutathionylated, which caused disassembly of the filaments [74]. The analysis of autopsy samples from the spinal cord of FRDA patients also showed abnormal microfilament polymerization [76].

The discovery of actin glutathionylation and the demonstration that the Nrf2-dependent Phase II antioxidant pathway is defective in patient fibroblasts provided the first mechanism for the reduction of antioxidant defenses in human frataxin-deficient cells [57]. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor that responds to oxidants by inducing expression of antioxidant enzymes to restore redox homeostasis in the cell. The Nrf2 activity is regulated by the actin-associated Keap1 (Kelch-like ECH-associated protein 1), which is an adaptor protein for the Cul3-dependent E3 ubiquitin ligase complex. Under normal conditions, Keap1 sequesters Nrf2 in the cytoplasm and promotes its rapid degradation via ubiquitination [77]. Under oxidative stress conditions, the cysteines of the Keap1 protein became oxidized, and the activity of the Cul3-Keap1 ubiquitin

E3 ligase complex is reduced. As a consequence, Nrf2 is stabilized and translocated to the nucleus where it binds to DNA sequences of the *cis*-acting antioxidant responsive element (ARE), activating the expression of genes encoding antioxidant enzymes like SODs, catalase, glutathione S-transferase, and NADH quinone oxidoreductase [77]. In fibroblasts from FRDA patients, Keap1 is not associated with actin, and Nrf2 is distributed diffusely in the cell [57]. In addition, in FRDA fibroblasts treated with oligomycin or tert-butylhydroquinone, Nrf2 fails to translocate to the nucleus and the antioxidant Phase II genes are not induced [57]. This phenotype can be reversed by 24 h treatment with the catalase mimetic Euk134; the actin stress fibers were reorganized, and Nrf2-signalling was restored, highlighting the role of H_2O_2 in FRDA pathophysiology. Recently, it was shown that the *PIP5K1B* gene that is located upstream of the *FXN* gene is also silenced in FRDA patient lymphocytes and fibroblasts [48]. The pip5k1β protein is a key regulatory factor of actin cytoskeleton dynamics, and its down-regulation in fibroblasts causes actin network destabilization [48]. Therefore, it is very likely that in FRDA patient cells, *PIP5K1B* gene silencing contributes to actin disassembly, but a direct evidence of actin glutathionylation in cells deficient for pip5k1β protein is missing.

Microarray analysis of dorsal root ganglia from YG8R frataxin-deficient mice and controls revealed significant differences in genes belonging to the thiol antioxidant, the myelination, and the axon transport functional categories [52]. In addition, the authors found not only a decreased expression of antioxidant genes that are regulated by Nrf2, but also Nrf2 (at mRNA and protein levels) in YG8R dorsal root ganglia compared to controls. However, their observations using different cell lines (HeLa, fibroblast, and ND7/23 dorsal root ganglion neuron cell lines and patient lymphoblasts) did not support deficient translocation of Nrf2 to the nucleus upon oxidant insult; Nrf2 is being mainly localized in the nucleus in frataxin-deficient cells. Consistent with these data, D'Oria et al. found no increase in Nrf2 expression in frataxin-silenced NSC34 cells (derived from the fusion of neuroblastoma cells and spinal cord motor neurons) compared to controls upon treatment with oxidized glutathione neither an increase in the Nrf2 nuclear fraction [78]. Neurons and fibroblasts are completely different cells types, and it is possible that in neurons antioxidant defense regulation depends primarily on Nrf2 levels while in fibroblasts it is the actin-driven translocation to the nucleus the major regulator.

Chronic inflammation and activity of glial cells are important factors that lead to neurodegeneration in other diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis [79]. In FRDA, degeneration affects different types of neurons in the peripheral and central nervous systems, and it is likely that other cell types are also affected by frataxin deficiency. Several reports suggest that Schwann cells (SCs) could be specifically affected and that peripheral neuron loss would be a secondary event [9, 80]. Schwann cells are glial cells specialized in the myelination of axons in the peripheral nervous system. Signaling between neurons and SC plays an essential role in SC proliferation, survival, migration, and myelination [81]. On the opposite, SC also contributes to the preservation of axon integrity [82]. Analysis of the dorsal roots in patient autopsy samples shows differences in the myelination of thin fibers and reduced number of SC [9]. In addition, patient sural nerve autopsies also suggest participation of SC in neurodegeneration since although the large myelinated fibers are significantly reduced the total number of axons per unit area is similar to that of controls [83]. In agreement with these observations, *in vitro* studies using human SC lines showed frataxin knock-down by siRNA blocks cell cycle progression at G_2M, and this is followed by an inflammatory response and an increase in cell death [80]. Treatment of these cells with antiinflammatory and antiapoptotic drugs rescued the death phenotype. Altogether, these results suggest that defects in SC could be at the origin of hypomyelination or demyelination and axon degeneration in FRDA patients.

5. Mitochondrial and Nuclear DNA Damage

Oxidative DNA damage is a natural consequence of aerobic metabolism that is exacerbated when cells are in oxidative stress conditions. ROS induce more than 20 lesions in the DNA, such as oxidized bases, apurinic/apyrimidinic (AP) sites, base deamination products, oxidized sugar fragments, and DNA strand breaks [84]. Damaged DNA bases, if not repaired, may have miscoding properties leading to mutation upon replication or by blocking progression of the replication fork [85, 86]. The most studied DNA lesion is the 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a product of oxidation of guanine. Conflicting results have been obtained for the levels of urinary 8-oxodG between FRDA patients and controls, since a difference has not always been found [87, 88]. However, mitochondrial and nuclear DNA damage have been reported in human, mouse, and yeast frataxin-deficient cells [51, 89–92].

Mitochondrial DNA (mtDNA) loss was one of the first phenotypes reported for the Δ*yfh1* yeast cells [93–95] and for FRDA patient cells [96]. Karthikeyan et al. showed that strong frataxin depletion in yeast cells resulted in complete loss of mtDNA after 15 generations and that reduced frataxin levels resulted in only partial loss of mtDNA after 22 generations [91]. These results indicate that mtDNA loss is dependent on the frataxin levels in the cell. Recently, it was shown that in Δ*yfh1* yeast cells, mtDNA loss is oxygen exposition-dependent, with complete retention in anaerobic conditions [51]. Mitochondrial DNA lesions and significant loss in the heart, cerebellum, and dorsal root ganglia have been observed in FRDA patients [92, 96–98].

Decades ago, two reports showed evidence of nuclear DNA damage in skin fibroblasts and blood lymphocytes of FRDA patients in response to ionizing radiation and mutagens, respectively [99, 100]. These studies suggested that in these patients, an increased susceptibility to DNA damage and/or defective DNA repair pathways were present. In agreement with this hypothesis, the transcriptome profiling of total blood from 28 children with FRDA revealed the molecular signature of cell response to DNA damage [92]. Using quantitative PCR, the same authors showed increased number of mitochondrial and nuclear DNA lesions

in blood cells from FRDA patients compared to controls. A link between frataxin expression, DNA-repair, and tumor initiation was observed in murine liver [89]. In addition, this work showed that overexpression of human frataxin in hamster fibroblasts was associated with a decrease in the nuclear mutation frequency.

Detailed studies on nuclear DNA damage and repair pathways have been performed in yeast. Analysis of diploid frataxin-deficient yeast cells showed evidence of chromosomal instability with higher levels of illegitimate mating, higher rate of spontaneous mutation, and increased sensitivity to the DNA-alkylating methyl methanesulfonate and to the replication inhibitor hydroxyurea than controls [90]. Furthermore, deletion of the glutathione peroxidase encoding gene *GPX1* in frataxin-deficient cells resulted in a marked increase in the nuclear mutation rate. These results led the authors to suggest that the increased spontaneous nuclear damage in Δ*yfh1* cells was caused by H_2O_2 generated in the mitochondria [90]. Consistent with this hypothesis, the analysis of the exposure of anaerobically grown Δ*yfh1* cells to oxygen showed that frataxin-deficiency in yeast cells leads to increased nuclear DNA damage [51]. The effect of oxygen was very rapid, 15 min after Δ*yfh1* cell exposition to oxygen, antioxidant levels, were decreased and DNA strand breaks were visible. At 30 min, cell cycle was arrested at G1/S, and mutation frequency was increased in Δ*yfh1* cells. Two hours later, the cells adapted to oxygen since the cell cycle was reinitiated but were in a chronic oxidative stress state with a high spontaneous mutation rate. The nuclear DNA lesions detected in Δ*yfh1* cells were primarily caused by oxidized bases and single-strand breaks, and the Apn1 AP-endonuclease of the base excision repair pathway was essential for the repair these DNA lesions [51]. Altogether, these observations suggest that DNA damage and repair could be important features in FRDA disease progression.

6. Antioxidant Therapy

Different strategies for the discovery of effective treatments for FRDA disease are currently being developed or tested in clinical trials (see http://www.curefa.org/pipeline.html). Several groups have done significant advances in *FXN* gene replacement or frataxin replacement therapies [101, 102]. Other strategies target the expression of the GAA-expanded gene (e.g., the HDAC inhibitor RG2833 is in Phase I clinical trial) or the stabilization of the frataxin protein (e.g., erythropoietin is in Phase II clinical trial). In addition, molecules targeting physiological functions that are defective in patient cells, such as increase in mitochondrial functions and iron-sulfur cluster synthesis or decrease in oxidative stress and iron toxicity, are being developed or in clinical trials. The conclusion of a recent review of the results of all randomized controlled clinical trials with minimal duration of 12 months was that none of the pharmacological drugs tested, including idebenone, had a significant beneficial effect on the neurological symptoms in FRDA patients [103]. Nevertheless, several ongoing clinical trials are testing promising antioxidant molecules. The α-tocopheryl quinone EPI-A0001 is a potent antioxidant molecule that has been tested in a double-blind, randomized, placebocontrolled trial of two doses in 31 adults with FRDA for a short period (four weeks) [104]. Glucose tolerance was tested, and no statistical difference was observed in the Disposition Index, which is a measure of diabetic tendency. However, after four weeks of treatment a dose-dependent improvement in the Friedreich Ataxia Rating Scale score was observed indicating neurologic function improvement in the patients. The EPI-743 is a new drug based on vitamin E that modifies disease progression in patients suffering from inherited mitochondrial respiratory chain disorders [105]. A Phase II clinical trial is currently recruiting FRDA patients. OX1 (indole-3-propionic acid) is a naturally occurring drug compound that prevents oxidative stress by a combination of hydroxyl radical scavenging activity and metal chelation. This molecule is now in preclinical studies for toxicological safety.

7. Conclusion

In the last decade, major achievements have been obtained concerning the primary function of frataxin, and it is almost completely accepted that it is in iron-sulfur cluster synthesis. Also, the mechanisms that regulate *FXN* expression with expanded GAA repeats are well known. However, despite a strong international effort there is no proven therapy for FRDA that stops disease progression. One of the reasons could be that our knowledge of the physiology of frataxin-deficient cells, and in particular the neurons affected by the disease, is still reduced. A large number of studies show that frataxin-deficient cells present numerous pleiotropic secondary phenotypes, which arise from iron-sulfur clusters deficit, such as mitochondrial dysfunction, perturbed iron homeostasis, DNA damage and mutagenesis, and oxidative stress. However, other areas of research that are relevant for FRDA pathology and fundamental for the survival and functioning of neurons are still poorly studied; examples are mitochondria transport and dynamics, calcium homeostasis, nitric oxide signaling, and inflammation. A clearer picture of the effects of frataxin deficiency in neurons and other cell types that might participate in the degeneration process in FRDA disease is necessary.

The major problem until recently was the lack of good *in vitro* cellular and murine models. The mouse model that best recapitulates the disease features is the humanized mouse model (YAC transgenic mice containing the human *FXN* gene with 190 GAA repeats) [63]. However, in these mice the first phenotypes appear after 6 to 12 months of age, and there is a possibility of GAA trinucleotide repeat instability. New technologies that can allow the development of new and relevant models for FRDA disease research from stem cells have been developed. It is now possible to obtain individual types of neurons by patterning and differentiation of embryonic stem cells and induced pluripotent cells (iPS) or also by direct reprogramming of fibroblast or other cell types. A protocol for differentiation of peripheral nerve system neurons from human embryonic stem cells has been published [106]. The first reports of differentiation of neurons and cardiomyocytes from iPS cells from FRDA patients are encouraging, although the GAA trinucleotide

repeats are unstable [107, 108]. Liu et al. differentiated sensory neurons and cardiomyocytes from iPS cells obtained from FRDA patients [107], and Hick et al. described mitochondrial deficits in the differentiated cells [109]. In the next years, the development of these new cell models will greatly contribute to our knowledge of the neurodegeneration process in FRDA disease.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

Research on frataxin at the Gomes Laboratory was financed by the National Ataxia Foundation (USA) and by the ITQB Strategic Grant PEst-OE/EQB/LA0004/2011 (to ITQB-Laboratório Associado, Oeiras).

References

[1] N. Friedreich, "Über degenerative Atrophie der spinalen Hinterstränge," *Virchows Archiv fur Pathologische Anatomie und Physiologie und für klinische Medizin*, vol. 27, pp. 1–26, 1863.

[2] N. Friedreich, "Über degenerative Atrophie der spinalen Hinterstränge," *Virchows Archiv fur Pathologische Anatomie und Physiologie und für klinische Medizin*, vol. 26, pp. 391–419, 1863.

[3] N. Friedreich, "Über degenerative Atrophie der spinalen Hinterstränge," *Virchows Archiv fur Pathologische Anatomie und Physiologie und für klinische Medizin*, vol. 26, pp. 433–459, 1863.

[4] R. Santos, S. Lefevre, D. Sliwa, A. Seguin, J. Camadro, and E. Lesuisse, "Friedreich ataxia: molecular mechanisms, redox considerations, and therapeutic opportunities," *Antioxidants and Redox Signaling*, vol. 13, no. 5, pp. 651–690, 2010.

[5] A. E. Harding, "Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features," *Brain*, vol. 104, no. 3, pp. 589–620, 1981.

[6] A. Dürr, M. Cossee, Y. Agid et al., "Clinical and genetic abnormalities in patients with Friedreich's ataxia," *The New England Journal of Medicine*, vol. 335, no. 16, pp. 1169–1175, 1996.

[7] M. Pandolfo, "Friedreich ataxia: the clinical picture," *Journal of Neurology*, vol. 256, supplement 1, pp. 3–8, 2009.

[8] J. T. Hughes, B. Brownell, and R. L. Hewer, "The peripheral sensory pathway in Friedreich's ataxia: an examination by light and electron microscopy of the posterior nerve roots, posterior root ganglia, and peripheral sensory nerves in cases of Friedreich's ataxia," *Brain*, vol. 91, no. 4, pp. 803–818, 1968.

[9] A. H. Koeppen, J. A. Morral, A. N. Davis et al., "The dorsal root ganglion in Friedreich's ataxia," *Acta Neuropathologica*, vol. 118, no. 6, pp. 763–776, 2009.

[10] V. Campuzano, L. Montermini, M. D. Moltò et al., "Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion," *Science*, vol. 271, no. 5254, pp. 1423–1427, 1996.

[11] C. Gellera, B. Castellotti, C. Mariotti et al., "Frataxin gene point mutations in Italian Friedreich ataxia patients," *Neurogenetics*, vol. 8, no. 4, pp. 289–299, 2007.

[12] S. I. Bidichandani, T. Ashizawa, and P. I. Patel, "The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure," *American Journal of Human Genetics*, vol. 62, no. 1, pp. 111–121, 1998.

[13] E. Grabczyk, M. Mancuso, and M. C. Sammarco, "A persistent RNA·DNA hybrid formed by transcription of the Friedreich ataxia triplet repeat in live bacteria, and by T7 RNAP *in vitro*," *Nucleic Acids Research*, vol. 35, no. 16, pp. 5351–5359, 2007.

[14] S. Al-Mahdawi, R. M. Pinto, O. Ismail et al., "The Friedreich ataxia GAA repeat expansion mutation induces comparable epigenetic changes in human and transgenic mouse brain and heart tissues," *Human Molecular Genetics*, vol. 17, no. 5, pp. 735–746, 2008.

[15] D. Herman, K. Jenssen, R. Burnett, E. Soragni, S. L. Perlman, and J. M. Gottesfeld, "Histone deacetylase inhibitors reverse gene silencing in Friedreich's ataxia," *Nature Chemical Biology*, vol. 2, no. 10, pp. 551–558, 2006.

[16] P. K. Chan, R. Torres, C. Yandim et al., "Heterochromatinization induced by GAA-repeat hyperexpansion in Friedreich's ataxia can be reduced upon HDAC inhibition by vitamin B3," *Human Molecular Genetics*, 2013.

[17] R. A. Vaubel and G. Isaya, "Iron-sulfur cluster synthesis, iron homeostasis and oxidative stress in Friedreich ataxia," *Molecular and Cellular Neuroscience*, vol. 55, pp. 50–61.

[18] T. L. Stemmler, E. Lesuisse, D. Pain, and A. Dancis, "Frataxin and mitochondrial FeS cluster biogenesis," *The Journal of Biological Chemistry*, vol. 285, no. 35, pp. 26737–26743, 2010.

[19] T. A. Rouault, "Biogenesis of iron-sulfur clusters in mammalian cells: new insights and relevance to human disease," *DMM Disease Models and Mechanisms*, vol. 5, no. 2, pp. 155–164, 2012.

[20] R. Lill, B. Hoffmann, S. Molik et al., "The role of mitochondria in cellular iron-sulfur protein biogenesis and iron metabolism," *Biochimica et Biophysica Acta*, vol. 1823, no. 9, pp. 1491–1508, 2012.

[21] I. De Biase, A. Rasmussen, D. Endres et al., "Progressive GAA expansions in dorsal root ganglia of Friedreich's ataxia patients," *Annals of Neurology*, vol. 61, no. 1, pp. 55–60, 2007.

[22] I. De Biase, A. Rasmussen, A. Monticelli et al., "Somatic instability of the expanded GAA triplet-repeat sequence in Friedreich ataxia progresses throughout life," *Genomics*, vol. 90, no. 1, pp. 1–5, 2007.

[23] J. S. Armstrong, O. Khdour, and S. M. Hecht, "Does oxidative stress contribute to the pathology of Friedreich's ataxia? A radical question," *The FASEB Journal*, vol. 24, no. 7, pp. 2152–2163, 2010.

[24] A. Bayot, R. Santos, J. Camadro, and P. Rustin, "Friedreich's ataxia: the vicious circle hypothesis revisited," *BMC Medicine*, vol. 9, article 112, 2011.

[25] S. Cho, M. G. Lee, J. K. Yang, J. Y. Lee, H. K. Song, and S. W. Suh, "Crystal structure of Escherichia coli CyaY protein reveals a previously unidentified fold for the evolutionarily conserved frataxin family," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 16, pp. 8932–8937, 2000.

[26] S. Dhe-Paganon, R. Shigeta, Y. Chi, M. Ristow, and S. E. Shoelson, "Crystal structure of human frataxin," *The Journal of Biological Chemistry*, vol. 275, no. 40, pp. 30753–30756, 2000.

[27] Y. He, S. L. Alam, S. V. Proteasa et al., "Yeast frataxin solution structure, iron binding, and ferrochelatase interaction," *Biochemistry*, vol. 43, no. 51, pp. 16254–16262, 2004.

[28] G. Musco, T. de Tommasi, G. Stier et al., "Assignment of the 1H, 15N, and 13C resonances of the C-terminal domain of frataxin, the protein responsible for Friedreich ataxia," *Journal of Biomolecular NMR*, vol. 15, no. 1, pp. 87–88, 1999.

[29] M. Nair, S. Adinolfi, C. Pastore, G. Kelly, P. Temussi, and A. Pastore, "Solution structure of the bacterial frataxin ortholog, CyaY: mapping the iron binding sites," *Structure*, vol. 12, no. 11, pp. 2037–2048, 2004.

[30] F. Prischi, P. V. Konarev, C. Iannuzzi et al., "Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly," *Nature Communications*, vol. 1, no. 7, article 95, 2010.

[31] H. Li, O. Gakh, D. Y. Smith IV, and G. Isaya, "Oligomeric yeast frataxin drives assembly of core machinery for mitochondrial iron-sulfur cluster synthesis," *The Journal of Biological Chemistry*, vol. 284, no. 33, pp. 21971–21980, 2009.

[32] C. Iannuzzi, S. Adinolfi, B. D. Howes et al., "The role of cyay in iron sulfur cluster assembly on the *E. coli* iscu scaffold protein," *PLoS ONE*, vol. 6, no. 7, Article ID e21992, 2011.

[33] F. Foury, A. Pastore, and M. Trincal, "Acidic residues of yeast frataxin have an essential role in Fe-S cluster assembly," *EMBO Reports*, vol. 8, no. 2, pp. 194–199, 2007.

[34] T. Wang and E. A. Craig, "Binding of yeast frataxin to the scaffold for Fe-S cluster biogenesis, Isu," *The Journal of Biological Chemistry*, vol. 283, no. 18, pp. 12674–12679, 2008.

[35] A. R. Correia, T. Wang, E. A. Craig, and C. M. Gomes, "Iron-binding activity in yeast frataxin entails a trade off with stability in the $\alpha 1/\beta 1$ acidic ridge region," *Biochemical Journal*, vol. 426, no. 2, pp. 197–203, 2010.

[36] P. Cavadini, J. Adamec, F. Taroni, O. Gakh, and G. Isaya, "Two-step processing of human frataxin by mitochondrial processing peptidase: precursor and intermediate forms are cleaved at different rates," *The Journal of Biological Chemistry*, vol. 275, no. 52, pp. 41469–41475, 2000.

[37] S. Schmucker, M. Argentini, N. Carelle-Calmels, A. Martelli, and H. Puccio, "The *in vivo* mitochondrial two-step maturation of human frataxin," *Human Molecular Genetics*, vol. 17, no. 22, pp. 3521–3531, 2008.

[38] I. Condò, N. Ventura, F. Malisan, A. Rufini, B. Tomassini, and R. Testi, "*In vivo* maturation of human frataxin," *Human Molecular Genetics*, vol. 16, no. 13, pp. 1534–1540, 2007.

[39] O. Gakh, T. Bedekovics, S. F. Duncan, D. Y. Smith IV, D. S. Berkholz, and G. Isaya, "Normal and Friedreich ataxia cells express different isoforms of frataxin with complementary roles in iron-sulfur cluster assembly," *The Journal of Biological Chemistry*, vol. 285, no. 49, pp. 38486–38501, 2010.

[40] A. Seguin, A. Bayot, A. Dancis et al., "Overexpression of the yeast frataxin homolog (Yfh1): contrasting effects on iron-sulfur cluster assembly, heme synthesis and resistance to oxidative stress," *Mitochondrion*, vol. 9, no. 2, pp. 130–138, 2009.

[41] O. Gakh, D. Y. Smith IV, and G. Isaya, "Assembly of the iron-binding protein frataxin in *Saccharomyces cerevisiae* responds to dynamic changes in mitochondrial iron influx and stress level," *The Journal of Biological Chemistry*, vol. 283, no. 46, pp. 31500–31510, 2008.

[42] A. R. Correia, S. Y. Ow, P. C. Wright, and C. M. Gomes, "The conserved Trp155 in human frataxin as a hotspot for oxidative stress related chemical modifications," *Biochemical and Biophysical Research Communications*, vol. 390, no. 3, pp. 1007–1011, 2009.

[43] M. Cossee, A. Durr, M. Schmitt et al., "Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes," *Annals of Neurology*, vol. 45, no. 2, pp. 200–206, 1999.

[44] A. R. Correia, S. Adinolfi, A. Pastore, and C. M. Gomes, "Conformational stability of human frataxin and effect of Friedreich's ataxia-related mutations on protein folding," *Biochemical Journal*, vol. 398, no. 3, pp. 605–611, 2006.

[45] A. R. Correia, C. Pastore, S. Adinolfi, A. Pastore, and C. M. Gomes, "Dynamics, stability and iron-binding activity of frataxin clinical mutants," *FEBS Journal*, vol. 275, no. 14, pp. 3680–3690, 2008.

[46] N. Calmels, S. Schmucker, M. Wattenhofer-Donzé et al., "The first cellular models based on frataxin missense mutations that reproduce spontaneously the defects associated with Friedreich ataxia," *PLoS ONE*, vol. 4, no. 7, Article ID e6379, 2009.

[47] H. Li, O. Gakh, D. Y. T. Smith, W. K. Ranatunga, and G. Isaya, "Missense mutations linked to Friedreich ataxia have different but synergistic effects on mitochondrial frataxin isoforms," *The Journal of Biological Chemistry*, vol. 288, no. 6, pp. 4116–4127, 2013.

[48] A. Bayot, S. Reichman, S. Lebon et al., "Cis-silencing of PIP5K1B evidenced in Friedreich's ataxia patient cells results in cytoskeleton anomalies," *Human Molecular Genetics*, 2013.

[49] S. S. Leal, H. M. Botelho, and C. M. Gomes, "Metal ions as modulators of protein conformation and misfolding in neurodegeneration," *Coordination Chemistry Reviews*, vol. 256, no. 19-20, pp. 2253–2270, 2012.

[50] C. M. Gomes, "Protein misfolding in disease and small molecule therapies," *Current Topics in Medicinal Chemistry*, vol. 12, no. 22, pp. 2460–2469, 2012.

[51] S. Lefevre, C. Brossas, F. Auchère, N. Boggetto, J. M. Camadro, and R. Santos, "Apn1 AP-endonuclease is essential for the repair of oxidatively damaged DNA bases in yeast frataxin-deficient cells," *Human Molecular Genetics*, vol. 21, no. 18, pp. 4060–4072, 2012.

[52] Y. Shan, R. A. Schoenfeld, G. Hayashi et al., "Frataxin deficiency leads to defects in expression of antioxidants and Nrf2 expression in dorsal root ganglia of the Friedreich's ataxia YG8R mouse model," *Antioxidants & Redox Signaling*, 2013.

[53] E. Napoli, F. Taroni, and G. A. Cortopassi, "Frataxin, iron-sulfur clusters, heme, ROS, and aging," *Antioxidants and Redox Signaling*, vol. 8, no. 3-4, pp. 506–516, 2006.

[54] V. Irazusta, E. Cabiscol, G. Reverter-Branchat, J. Ros, and J. Tamarit, "Manganese is the link between frataxin and iron-sulfur deficiency in the yeast model of Friedreich ataxia," *The Journal of Biological Chemistry*, vol. 281, no. 18, pp. 12227–12232, 2006.

[55] M. M. Santos, K. Ohshima, and M. Pandolfo, "Frataxin deficiency enhances apoptosis in cells differentiating into neuroectoderm," *Human Molecular Genetics*, vol. 10, no. 18, pp. 1935–1944, 2001.

[56] P. R. Anderson, K. Kirby, W. C. Orr, A. J. Hilliker, and J. P. Phillips, "Hydrogen peroxide scavenging rescues frataxin deficiency in a *Drosophila* model of Friedreich's ataxia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 2, pp. 611–616, 2008.

[57] V. Paupe, E. P. Dassa, S. Goncalves et al., "Impaired nuclear Nrf2 translocation undermines the oxidative stress response in Friedreich ataxia," *PLoS ONE*, vol. 4, no. 1, Article ID e4253, 2009.

[58] S. Lefevre, D. Sliwa, F. Auchre et al., "The yeast metacaspase is implicated in oxidative stress response in frataxin-deficient cells," *FEBS Letters*, vol. 586, no. 2, pp. 143–148, 2012.

[59] R. Santos, N. Buisson, S. A. B. Knight, A. Dancis, J. Camadro, and E. Lesuisse, "Candida albicans lacking the frataxin homologue: a relevant yeast model for studying the role of frataxin," *Molecular Microbiology*, vol. 54, no. 2, pp. 507–519, 2004.

[60] R. P. Vázquez-Manrique, P. González-Cabo, S. Ros, H. Aziz, H. A. Baylis, and F. Palau, "Reduction of *Caenorhabditis elegans* frataxin increases sensitivity to oxidative stress, reduces lifespan, and causes lethality in a mitochondrial complex II mutant," *The FASEB Journal*, vol. 20, no. 1, pp. 172–174, 2006.

[61] J. V. Llorens, J. A. Navarro, M. J. Martínez-Sebastían et al., "Causative role of oxidative stress in a *Drosophila* model of Friedreich ataxia," *The FASEB Journal*, vol. 21, no. 2, pp. 333–344, 2007.

[62] A. P. Runko, A. J. Griswold, and K. Min, "Overexpression of frataxin in the mitochondria increases resistance to oxidative stress and extends lifespan in *Drosophila*," *FEBS Letters*, vol. 582, no. 5, pp. 715–719, 2008.

[63] S. Al-Mahdawi, R. M. Pinto, D. Varshney et al., "GAA repeat expansion mutation mouse models of Friedreich ataxia exhibit oxidative stress leading to progressive neuronal and cardiac pathology," *Genomics*, vol. 88, no. 5, pp. 580–590, 2006.

[64] G. Tozzi, M. Nuccetelli, M. Lo Bello et al., "Antioxidant enzymes in blood of patients with Friedreich's ataxia," *Archives of Disease in Childhood*, vol. 86, no. 5, pp. 376–379, 2002.

[65] S. Jiralerspong, B. Ge, T. J. Hudson, and M. Pandolfo, "Manganese superoxide dismutase induction by iron is impaired in Friedreich ataxia cells," *FEBS Letters*, vol. 509, no. 1, pp. 101–105, 2001.

[66] K. Chantrel-Groussard, V. Geromel, H. Puccio et al., "Disabled early recruitment of antioxidant defenses in Friedreich's ataxia," *Human Molecular Genetics*, vol. 10, no. 19, pp. 2061–2067, 2001.

[67] G. Tan, L. Chen, B. Lonnerdal, C. Gellera, F. A. Taroni, and G. A. Cortopassi, "Frataxin expression rescues mitochondrial dysfunctions in FRDA cells," *Human Molecular Genetics*, vol. 10, no. 19, pp. 2099–2107, 2001.

[68] A. Wong, J. Yang, P. Cavadini et al., "The Friedreich's ataxia mutation confers cellular sensitivity to oxidant stress which is rescued by chelators of iron and calcium and inhibitors of apoptosis," *Human Molecular Genetics*, vol. 8, no. 3, pp. 425–430, 1999.

[69] V. Calabrese, R. Lodi, C. Tonon et al., "Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia," *Journal of the Neurological Sciences*, vol. 233, no. 1-2, pp. 145–162, 2005.

[70] A. Bulteau, A. Dancis, M. Gareil, J. Montagne, J. Camadro, and E. Lesuisse, "Oxidative stress and protease dysfunction in the yeast model of Friedreich ataxia," *Free Radical Biology and Medicine*, vol. 42, no. 10, pp. 1561–1570, 2007.

[71] M. M. Gallogly and J. J. Mieyal, "Mechanisms of reversible protein glutathionylation in redox signaling and oxidative stress," *Current Opinion in Pharmacology*, vol. 7, no. 4, pp. 381–391, 2007.

[72] F. Piemonte, A. Pastore, G. Tozzi et al., "Glutathione in blood of patients with Friedreich's ataxia," *European Journal of Clinical Investigation*, vol. 31, no. 11, pp. 1007–1011, 2001.

[73] F. Auchère, R. Santos, S. Planamente, E. Lesuisse, and J. Camadro, "Glutathione-dependent redox status of frataxin-deficient cells in a yeast model of Friedreich's ataxia," *Human Molecular Genetics*, vol. 17, no. 18, pp. 2790–2802, 2008.

[74] A. Pastore, G. Tozzi, L. M. Gaeta et al., "Actin glutathionylation increases in fibroblasts of patients with Friedreich's ataxia: a potential role in the pathogenesis of the disease," *The Journal of Biological Chemistry*, vol. 278, no. 43, pp. 42588–42595, 2003.

[75] A. L. Bulteau, S. Planamente, L. Jornea et al., "Changes in mitochondrial glutathione levels and protein thiol oxidation in Δyfh1 yeast cells and the lymphoblasts of patients with Friedreich's ataxia," *Biochimica et Biophysica Acta*, vol. 1822, no. 2, pp. 212–225, 2012.

[76] M. Sparaco, L. M. Gaeta, F. M. Santorelli et al., "Friedreich's ataxia: oxidative stress and cytoskeletal abnormalities," *Journal of the Neurological Sciences*, vol. 287, no. 1-2, pp. 111–118, 2009.

[77] T. Nguyen, P. Nioi, and C. B. Pickett, "The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress," *The Journal of Biological Chemistry*, vol. 284, no. 20, pp. 13291–13295, 2009.

[78] V. D'Oria, S. Petrini, L. Travaglini et al., "Frataxin deficiency leads to reduced expression and impaired translocation of NF-E2-related factor (Nrf2) in cultured motor neurons," *International Journal of Molecular Sciences*, vol. 10, no. 4, pp. 7853–7865, 2013.

[79] T. Philips and W. Robberecht, "Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease," *The Lancet Neurology*, vol. 10, no. 3, pp. 253–263, 2011.

[80] C. Lu, R. Schoenfeld, Y. Shan, H. Tsai, B. Hammock, and G. Cortopassi, "Frataxin deficiency induces Schwann cell inflammation and death," *Biochimica et Biophysica Acta*, vol. 1792, no. 11, pp. 1052–1061, 2009.

[81] J. Newbern and C. Birchmeier, "Nrg1/ErbB signaling networks in Schwann cell development and myelination," *Seminars in Cell and Developmental Biology*, vol. 21, no. 9, pp. 922–928, 2010.

[82] K. Nave and B. D. Trapp, "Axon-glial signaling and the glial support of axon function," *Annual Review of Neuroscience*, vol. 31, pp. 535–561, 2008.

[83] J. A. Morral, A. N. Davis, J. Qian, B. B. Gelman, and A. H. Koeppen, "Pathology and pathogenesis of sensory neuropathy in Friedreich's ataxia," *Acta Neuropathologica*, vol. 120, no. 1, pp. 97–108, 2010.

[84] M. S. Cooke, M. D. Evans, M. Dizdaroglu, and J. Lunec, "Oxidative DNA damage: mechanisms, mutation, and disease," *The FASEB Journal*, vol. 17, no. 10, pp. 1195–1214, 2003.

[85] D. Svilar, E. M. Goellner, K. H. Almeida, and R. W. Sobol, "Base excision repair and lesion-dependent subpathways for repair of oxidative DNA damage," *Antioxidants and Redox Signaling*, vol. 14, no. 12, pp. 2491–2507, 2011.

[86] L. Gros, M. K. Saparbaev, and J. Laval, "Enzymology of the repair of free radicals-induced DNA damage," *Oncogene*, vol. 21, no. 58, pp. 8905–8925, 2002.

[87] J. B. Schulz, T. Dehmer, L. Schöls et al., "Oxidative stress in patients with Friedreich ataxia," *Neurology*, vol. 55, no. 11, pp. 1719–1721, 2000.

[88] N. A. Di Prospero, A. Baker, N. Jeffries, and K. H. Fischbeck, "Neurological effects of high-dose idebenone in patients with Friedreich's ataxia: a randomised, placebo-controlled trial," *The Lancet Neurology*, vol. 6, no. 10, pp. 878–886, 2007.

[89] R. Thierbach, G. Drewes, M. Fusser et al., "The Friedreich's ataxia protein frataxin modulates DNA base excision repair in prokaryotes and mammals," *Biochemical Journal*, vol. 432, no. 1, pp. 165–172, 2010.

[90] G. Karthikeyan, L. K. Lewis, and M. A. Resnick, "The mitochondrial protein frataxin prevents nuclear damage," *Human Molecular Genetics*, vol. 11, no. 11, pp. 1351–1362, 2002.

[91] G. Karthikeyan, J. H. Santos, M. A. Graziewicz et al., "Reduction in frataxin causes progressive accumulation of mitochondrial damage," *Human Molecular Genetics*, vol. 12, no. 24, pp. 3331–3342, 2003.

[92] A. C. Haugen, N. A. Di Prospero, J. S. Parker et al., "Altered gene expression and DNA damage in peripheral blood cells from Friedreich's ataxia patients: cellular model of pathology," *PLoS Genetics*, vol. 6, no. 1, Article ID e1000812, 2010.

[93] R. B. Wilson and D. M. Roof, "Respiratory deficiency due to loss of mitochondrial dna in yeast lacking the frataxin homologue," *Nature Genetics*, vol. 16, no. 4, pp. 352–357, 1997.

[94] M. Babcock, D. de Silva, R. Oaks et al., "Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin," *Science*, vol. 276, no. 5319, pp. 1709–1712, 1997.

[95] F. Foury and O. Cazzalini, "Deletion of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria," *FEBS Letters*, vol. 411, no. 2-3, pp. 373–377, 1997.

[96] J. L. Bradley, J. C. Blake, S. Chamberlain, P. K. Thomas, J. M. Cooper, and A. H. V. Schapira, "Clinical, biochemical and molecular genetic correlations in Friedreich's ataxia," *Human Molecular Genetics*, vol. 9, no. 2, pp. 275–282, 2000.

[97] M. Houshmand, M. S. S. Panahi, S. Nafisi, A. Soltanzadeh, and F. M. Alkandari, "Identification and sizing of GAA trinucleotide repeat expansion, investigation for D-loop variations and mitochondrial deletions in Iranian patients with Friedreich's ataxia," *Mitochondrion*, vol. 6, no. 2, pp. 82–88, 2006.

[98] M. M. Heidari, M. Houshmand, S. Hosseinkhani, S. Nafissi, B. Scheiber-Mojdehkar, and M. Khatami, "A novel mitochondrial heteroplasmic C13806A point mutation associated with iranian Friedreich's ataxia," *Cellular and Molecular Neurobiology*, vol. 29, no. 2, pp. 225–233, 2009.

[99] H. J. Evans, V. Vijayalaxmi, B. Pentland, and M. S. Newton, "Mutagen hypersensitivity in Friedreich's ataxia," *Annals of Human Genetics*, vol. 47, no. 3, pp. 193–204, 1983.

[100] P. D. Lewis, J. B. Corr, C. F. Arlett, and S. A. Harcourt, "Increased sensitivity to gamma irradiation of skin fibroblasts in Friedreich's ataxia," *The Lancet*, vol. 2, no. 8140, pp. 474–475, 1979.

[101] A. Gimenez-Cassina, R. Wade-Martins, S. Gomez-Sebastian, J.-C. Corona, F. Lim, and J. Diaz-Nido, "Infectious delivery and long-term persistence of transgene expression in the brain by a 135-kb iBAC-FXN genomic DNA expression vector," *Gene Therapy*, vol. 18, no. 10, pp. 1015–1019, 2011.

[102] P. M. Vyas, W. J. Tomamichel, P. M. Pride et al., "A TAT-frataxin fusion protein increases lifespan and cardiac function in a conditional Friedreich's ataxia mouse model," *Human Molecular Genetics*, vol. 21, no. 6, Article ID ddr554, pp. 1230–1247, 2012.

[103] M. Kearney, R. W. Orrell, M. Fahey, and M. Pandolfo, "Antioxidants and other pharmacological treatments for Friedreich ataxia," *Cochrane Database of Systematic Reviews*, no. 4, Article ID CD007791, 2009.

[104] D. R. Lynch, S. M. Willi, R. B. Wilson et al., "A0001 in Friedreich ataxia: biochemical characterization and effects in a clinical trial," *Movement Disorders*, vol. 27, no. 8, pp. 1026–1033, 2012.

[105] G. M. Enns, S. L. Kinsman, S. L. Perlman et al., "Initial experience in the treatment of inherited mitochondrial disease with EPI-743," *Molecular Genetics and Metabolism*, vol. 105, no. 1, pp. 91–102, 2012.

[106] M. Valensi-Kurtz, S. Lefler, M. A. Cohen et al., "Enriched population of PNS neurons derived from human embryonic stem cells as a platform for studying peripheral neuropathies," *PLoS ONE*, vol. 5, no. 2, Article ID e9290, 2010.

[107] J. Liu, P. J. Verma, M. V. Evans-Galea et al., "Generation of induced pluripotent stem cell lines from Friedreich ataxia patients," *Stem Cell Reviews and Reports*, vol. 7, no. 3, pp. 703–713, 2011.

[108] S. Ku, E. Soragni, E. Campau et al., "Friedreich's ataxia induced pluripotent stem cells model intergenerational GAATTC triplet repeat instability," *Cell Stem Cell*, vol. 7, no. 5, pp. 631–637, 2010.

[109] A. Hick, M. Wattenhofer-Donzé, S. Chintawar et al., "Neurons and cardiomyocytes derived from induced pluripotent stem cells as a model for mitochondrial defects in Friedreich's ataxia," *Disease Models & Mechanisms*, vol. 6, no. 3, pp. 608–621, 2013.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 162152, 13 pages
http://dx.doi.org/10.1155/2013/162152

Review Article

Deconstructing Mitochondrial Dysfunction in Alzheimer Disease

Vega García-Escudero,[1,2] Patricia Martín-Maestro,[1,2] George Perry,[3] and Jesús Avila[1,2]

[1] *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), 28049 Madrid, Spain*
[2] *Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), 28031 Madrid, Spain*
[3] *University of Texas at San Antonio, San Antonio, TX 78249, USA*

Correspondence should be addressed to Jesús Avila; javila@cbm.uam.es

Received 18 April 2013; Accepted 23 May 2013

Academic Editor: Cláudio M. Gomes

There is mounting evidence showing that mitochondrial damage plays an important role in Alzheimer disease. Increased oxygen species generation and deficient mitochondrial dynamic balance have been suggested to be the reason as well as the consequence of Alzheimer-related pathology. Mitochondrial damage has been related to amyloid-beta or tau pathology or to the presence of specific presenilin-1 mutations. The contribution of these factors to mitochondrial dysfunction is reviewed in this paper. Due to the relevance of mitochondrial alterations in Alzheimer disease, recent works have suggested the therapeutic potential of mitochondrial-targeted antioxidant. On the other hand, autophagy has been demonstrated to play a fundamental role in Alzheimer-related protein stress, and increasing data shows that this pathway is altered in the disease. Moreover, mitochondrial alterations have been related to an insufficient clearance of dysfunctional mitochondria by autophagy. Consequently, different approaches for the removal of damaged mitochondria or to decrease the related oxidative stress in Alzheimer disease have been described. To understand the role of mitochondrial function in Alzheimer disease it is necessary to generate human cellular models which involve living neurons. We have summarized the novel protocols for the generation of neurons by reprogramming or direct transdifferentiation, which offer useful tools to achieve this result.

1. Introduction

Alzheimer disease (AD) is characterized by the presence of two aberrant structures in the brain of the patients, senile plaques and neurofibrillary tangles, together with marked neuronal death [1]. Senile plaques are filamentous aggregates of amyloid-beta peptide (Aβ) [2], whereas the main component of neurofibrillary tangles is the microtubule-associated protein tau [3]. This picture of the patients' brain showing plaques and tangles can be found at the end of the disease, usually at autopsy; however, using imaging techniques like positron emission tomography these aggregates can be detected *in vivo* in Alzheimer's patients by using compounds like the Pittsburg compound B for amyloid [4] or 18F-THK23 for Tau aggregates [5].

It is suggested that Alzheimer disease is a silent neurodegeneration where neuronal damage occurs before the diagnosis of the disease. Thus, plaques and tangles may appear before the onset of the disease symptoms [6]. On the other hand, mitochondrial dysfunction is one of the earliest and most prominent features in vulnerable neurons in the brain of AD patients [7] and likely in other neurodegenerative disorders [8].

There are at least two types of Alzheimer disease: familial Alzheimer disease (FAD) and sporadic Alzheimer disease (SAD). In FAD, the causes of the disease are the presence of specific mutations in at least one of the three genes identified as amyloid precursor protein (APP) and presenilin-1 and -2 (ps-1 and ps-2) [9]. However, little is known about the cause of the onset of SAD. It is known that the main risk for AD is aging related to oxidative damage [10]. It has been found that oxidative damage may facilitate the expression of beta-secretase (BACE1), a protein involved in the generation of Aβ [11, 12]. Moreover, it has been shown that oxidative stress induces a pathogenic PS1 conformational change in neurons *in vitro*, increasing Aβ42/40 ratio [13]. In FAD, increased Aβ may result in mitochondrial dysfunction and augmented ROS levels [14]. In SAD, the reverse has been suggested.

Mitochondria-derived reactive oxygen species result in an enhanced amyloid-beta formation [15] and the Aβ increase may lead to further mitochondrial dysfunction, resulting in even higher ROS levels [16]. Then, the cycle repeats and degeneration increases. Also, oxidative stress may facilitate tau phosphorylation at some of the sites found to be modified in AD patients [17, 18]. On the other hand, tau accumulation causes mitochondrial distribution deficits in a mouse model for AD [19].

2. Mitochondrial Damage in AD

2.1. Oxidative Stress and Energy Production. Oxidative stress is a primary event in the development of AD [10]. This oxidative stress may be due to the presence of dysfunctional mitochondria resulting in generation of reactive oxygen species [20]. Mitochondria generate cell energy as electrons flow through mitochondrial complex I to IV of the electron transport chain, from donors with lower redox potential to acceptors with higher redox potential. The final acceptor is oxygen that is reduced to water and the generated energy drives the phosphorylation of ADP to ATP by the mitochondrial complex V (or ATP-synthase) (Figure 1). Although the transport of the electrons through mitochondrial complexes is an efficient process, some reactive oxygen species (ROS) may be produced. Dysfunctional mitochondria generate high levels of ROS that may be toxic for cells with a long life span and a deficiency in antioxidant defenses, such as neurons [21]. Additionally, mitochondria are at the same time a target of ROS causing the oxidation of their components such as mtDNA, lipids, and proteins increasing mitochondrial deterioration. Mitochondrial dysfunction is one of the earliest and most prominent features of AD [22, 23]. In fact, a decreased expression of either nuclear or mitochondrial genes of the oxidative phosphorylation in the neocortex of AD patients has been shown to correlate with progressive reductions in brain glucose metabolism that can be visualized by positron emission tomography [24, 25]. Testing mitochondrial function in a triple transgenic mouse model for AD, a clear deregulation of oxidative phosphorylation proteins was found [26]. Deregulation of complex I was related to tau toxicity as found in other animal models [27] whereas deregulation of complex IV has been described to be Aβ dependent [28, 29] (Figure 1).

Alterations of several enzymes involved in the tricarboxylic acid cycle such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase have been reported in Alzheimer disease (Figure 1). Postmortem brains showed a reduction of pyruvate dehydrogenase, ATP-citrate lyase, and acetoacetyl-CoA thiolase correlating with decreased production of acetylcoenzyme A and the subsequent cholinergic defects observed in these patients [30]. Reduced activity of α-ketoglutarate dehydrogenase was also observed in brain tissue as well as in peripheral cells from AD patients [31, 32]. Additionally in AD brain, there is a loss of α-ketoglutarate-enriched cells, therefore causing the degeneration of α-ketoglutarate-enriched areas (cortical layers II and IV) which are the ones that are selectively degenerated in AD [33].

On the other hand, products of the toxic action of ROS, like hydroxynonenal (HNE), or the presence of quinones (like coenzyme Qo) may facilitate the self-assembly of Tau protein into fibrillar polymers similar to those paired helical filaments present in the brain of AD patients [34]. These findings suggest another possible link between oxidative stress, neuronal dysfunction, and AD.

It is possible that mitochondrial dysfunction in AD patients not only takes place in the central nervous system but also in cells from peripheral tissues. Increased oxidative stress levels and reduced antioxidant defenses have been observed in AD fibroblasts [35]. Regarding this, it has been described that lipoic acid and N-acetylcysteine may decrease the mitochondrial-related oxidative stress in Alzheimer disease patients [36].

2.2. Mitochondrial Dynamics Alteration. Mitochondria are highly dynamic organelles, ranging from giant tubular networks to small round entities through rapid and reversible fission and fusion processes. Mitochondria failure may arise from a deficient dynamic balance of mitochondrial fission and fusion that, in AD, is greatly shifted toward fission and it may result in the presence of dysfunctional mitochondria in damaged neurons [37].

The delicate balance of fission and fusion is regulated by several mitochondrial proteins (Figure 2). Fission requires several outer mitochondrial membrane (OMM) proteins such as the GTPase dynamin-like protein 1 (DLP1, also known as dynamin-related protein, Drp1) that is recruited from the cytosol to the OMM for fission [38]. This process depends on its GTPase activity as well as on posttranslational modifications such as phosphorylation, S-nitrosylation, sumoylation, and ubiquitination [39–42]. Other OMM proteins involved in fission are Fis1, which plays a regulatory role, and mitochondrial fission factor, Mff, which is fundamental for the mitochondrial recruitment of DLP1 [43]. On the other hand, mitochondria fusion depends on other GTPases such as mitofusin 1 and 2, responsible for outer membrane fusion, and optic atrophy, Opa1, that carries out fusion of the inner membrane and is also important for cristae formation and mtDNA inheritance [44]. The involvement of mitochondrial elongation factor, MIEF1, in mitochondrial fusion in vertebrates has recently been described. This factor recruits and inactivates DLP1 executing a negative effect on fission and actively promotes fusion in a manner distinct from mitofusins [45].

Mitochondrial dynamics are critical for the maintenance of mitochondrial integrity and functions including energy metabolism, ROS generation, and apoptosis regulation [7]. Fusion permits the proper distribution of mitochondrial components such as lipids membranes, oxidative phosphorylation complexes, and mtDNA. Moreover, fusion is important in maintaining the proper mitochondrial ultrastructure and elongation is a mechanism for mitochondria to escape autophagy-mediated destruction. On the other hand, fission permits the recycling of irreversibly damaged mitochondria by mitophagy and plays an important role in the proper

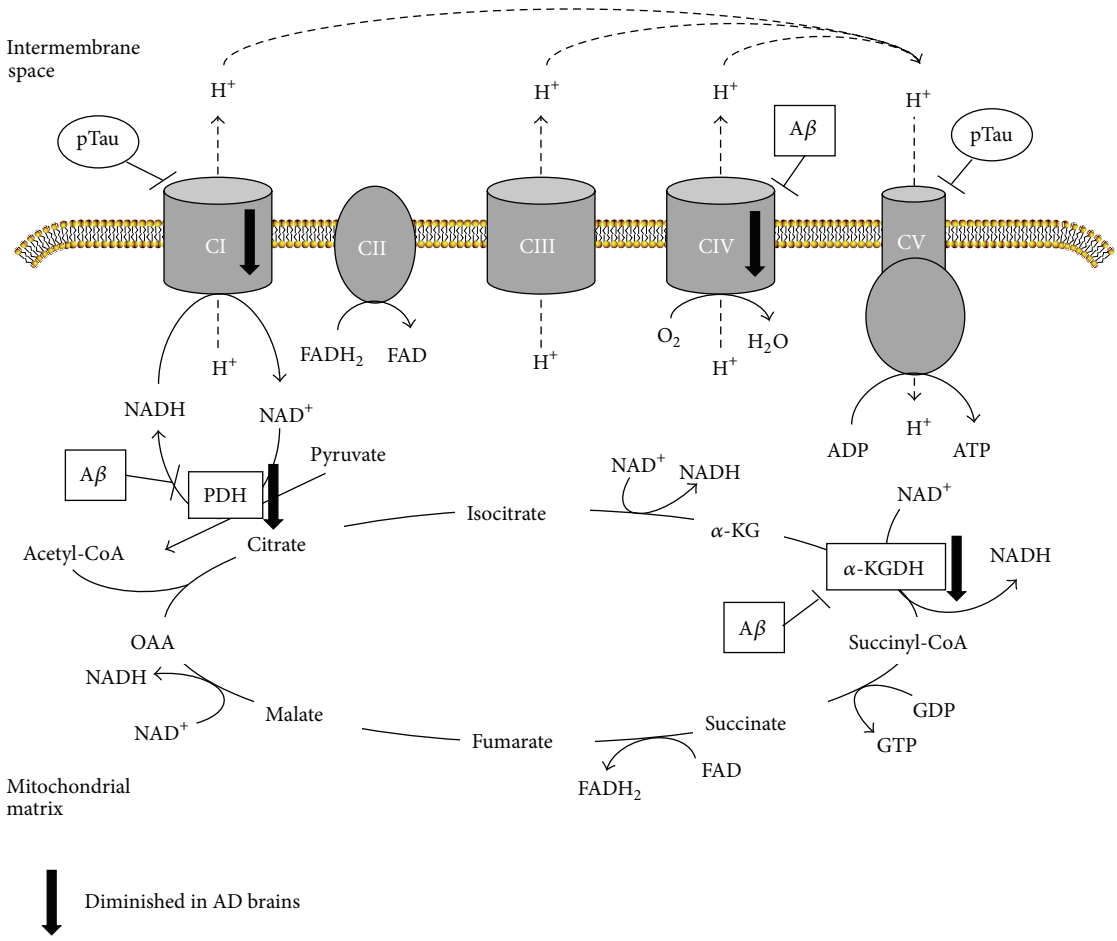


Figure 1: *AD-related alterations of mitochondrial respiratory chain and tricarboxylic acid cycle.* Scheme of alterations in protein levels found in Alzheimer disease brains as well as the targets of amyloid-β (Aβ) and phosphorylated Tau (pTau). Oxidative phosphorylation complexes are labeled as CI to CIV. PDH: pyruvate dehydrogenase, α-KG: α-ketoglutarate, α-KGDH: α-ketoglutarate dehydrogenase, OAA: oxaloacetate.

assembly of mitochondrial electron transport chain complexes. A proper balance of fusion and fission proteins is fundamental for the correct distribution of the mitochondria in the cell [44]. This is particularly important for neurons that may have very long axons and also for the function of synapses, which are subcellular regions with high metabolic requirement [46].

Possible dysfunction of mitochondrial dynamics proteins has been tested in models for neurodegenerative disorders [47, 48], Alzheimer disease being one of them [49]. In fact, abnormal mitochondrial dynamics and synaptic degeneration are considered early events in Alzheimer Disease (extensively discussed in Reddy et al. [50]). Mitochondrial dynamics alteration has been found either in neurons or fibroblasts in AD patients and models [7]. Mitochondria distribution has also been found altered in both cell types characterized by their accumulation into perinuclear areas. However, while neurons exhibit increased fragmentation, fibroblasts show elongated and highly interconnected mitochondrial network [47]. Vulnerable neurons in AD brain exhibit significant reduction in mitochondrial length and increased width with a significant increased overall size consistent with unopposed fission suggesting alterations of mitochondrial dynamics [48]. In agreement with these findings, an abnormal distribution of mitochondria was found in pyramidal neurons of AD-affected individuals where mitochondria were redistributed away from axons in the pyramidal neurons [51]. Accordingly, levels of fusion proteins OPA1, Mfn1, and Mfn2 were significantly reduced whereas levels of Fis1 were significantly increased in AD (Figure 2). In the case of the fission protein DLP1, while some authors have described a reduction in neurons [47, 52] and fibroblasts of sporadic patients [47], others have shown an increase [53]. These differences can be explained because the major pool of DLP1 is cytosolic and its recruitment on mitochondrial membrane to mediate fission events depends on posttranslational modifications [7] (Figure 2). In this sense, higher DLP1 levels in mitochondrial fraction [51] as well as increased Ser616 phosphorylation and S-nitrosylation in AD brains [40] have been described. Primary hippocampal neurons treated with Aβ-derived diffusible ligands (ADDLs) demonstrated shortened mitochondria in neurons and alteration of fission and fusion proteins [51]. Moreover, time-lapse recordings in these neurons showed impairment of both, fission and fusion

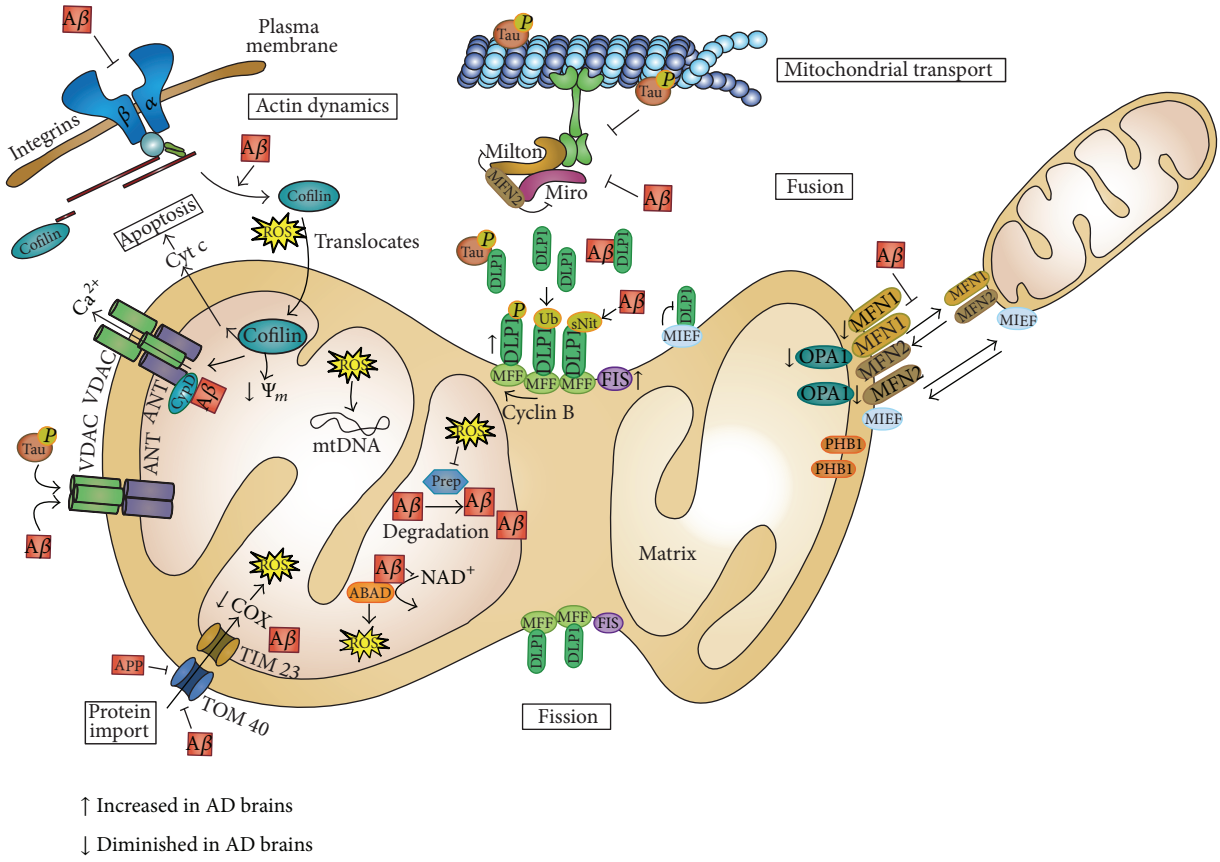


FIGURE 2: *Mitochondrial alterations found in AD.* Scheme of the effect of amyloid-β (Aβ) and phosphorylated-Tau (pTau) over mitochondrial dynamics, transport, protein import, membrane permeabilization, and apoptosis as well as actin dynamics. Alterations of the levels of involved proteins found in AD brains are also summarized. DLP1: dynamin-like protein 1, MFF: mitochondrial fission factor, FIS: fission 1, MIEF: mitochondrial elongation factor, MFN1: mitofusin 1, MFN2: mitofusin 2, OPA1: optic atrophy 1, PHB1: prohibitin 1, ABAD: Aβ-binding alcohol dehydrogenase, Prep: presequence protease, VDAC: voltage-dependent anion channel, ANT: adenine nucleotide translocase, CypD: cyclophilin D, Cyt C: cytochrome *c*, COX: cytochrome *c* oxidase, Ψ_m: mitochondrial membrane potential, and mtDNA: mitochondrial DNA.

processes, with fusion process being more severely affected [51]. Recent evidence has shown an abnormal interaction of Aβ monomers and oligomers with DLP1 that increases with the progression of the disease suggesting a possible cause of abnormal mitochondrial dynamics and synaptic damage [53]. On the other hand, the expression of AD-causing Swedish APP mutation in M17 cells also induced shorter and fatter mitochondria, with a slight but significant increase in size, but a decrease in the total mitochondrial number while the number of damaged mitochondria was increased [48]. Similar observations have been found in transgenic mice (Tg2574) harboring the APP Swedish mutation [54].

Abnormal mitochondrial morphology has been found in fibroblasts from sporadic AD patients, where they become significantly elongated and form a highly connected network [47]. This discrepancy in mitochondrial morphology may be due to differences in the expression pattern of proteins involved in dynamics, showing decreased DLP1 and unchanged OPA1. Similar differences between fibroblasts and neurons have been also found in Parkinson's disease [7].

Mitochondrial mobility has also been altered in AD causing a mitochondrial reduction in neurites [7] (Figure 2). Aβ induces a reduction in motile mitochondria [55] and ADDL impairs anterograde and retrograde axonal transport of mitochondria in hippocampal neurons [56]. Primary neurons from Tg2576 APP transgenic mice showed a specific impairment of anterograde mitochondrial transport. These results suggest that mitochondrial fission/fusion and mitochondrial transport can be coupled. In fact, it has been demonstrated that Mfn2 interacts with Miro and Milton, two adaptor proteins involved in the regulation of mitochondrial transport [57], although further work is necessary to clarify this relationship.

The alteration in mitochondrial dynamics leads to severe consequences in the cell such as structural changes in the cristae formation and assembly of electron transport complex compromising bioenergetics and causing calcium dyshomeostasis, increased oxidative stress, mitochondrial DNA damage, and synaptic dysfunction (reviewed in [7]).

3. Relationship between Mitochondrial Dysfunction and AD-Related Pathology

3.1. Amyloid Beta. In FAD, an increase in the level of Aβ may result in oxidative damage [22, 58, 59]. APP and Aβ

accumulate in mitochondrial membranes causing structural and functional damage (reviewed in [60]). Nonglycosylated full-length and C-terminal truncated APP has been found to accumulate in the protein import channels of mitochondria of human AD brains [61] (Figure 2). APP forms stable complexes with the translocase of the outer mitochondrial membrane 40 (TOM40) import channel and the translocase of the inner mitochondrial membrane 23 (TIM23) inhibiting the entry of nuclear-encoded cytochrome *c* oxidase subunits IV and Vb proteins, which was associated with decreased cytochrome *c* oxidase activity and increased ROS production. Additionally, an interaction has been discovered between Aβ and phosphorylated Tau with voltage-dependent anion channel 1 (VDAC1) in the brains of AD patients and from APP, APP/PS1, and 3XTg AD mice which may block the mitochondrial pores leading to mitochondrial dysfunction [62].

Amyloid-beta interacts with the mitochondrial protein ABAD (Aβ-binding alcohol dehydrogenase) which is upregulated in the temporal lobe of AD patients as well as in AβPP transgenic mice [63] (Figure 2). This complex prevents the binding of nicotinamide adenine dinucleotide NAD+ to ABAD, thereby changing mitochondrial membrane permeability and reducing the activities of respiratory enzymes causing elevated ROS.

Moreover, it has been suggested that the oxidative stress induced by Aβ may oxidize and inactivate presequence protease, PreP, one of the proteins involved in Aβ degradation in the mitochondria thus increasing Aβ concentration in mitochondrial matrix and its pathologic effects [64] (Figure 2).

Amyloid-beta has been also involved in alterations of mitochondrial dynamics. In fact, the overexpression of human APP Swedish double mutation in neuroblastoma cell lines induces a higher percentage of highly fragmented and slower mitochondria correlating with an alteration of the levels of the proteins involved in mitochondrial dynamics such as increased fission protein Fis-1 and reduced levels of fusion proteins like OPA1 and DLP-1 [48]. In agreement with these *in vitro* findings, an abnormal distribution of mitochondria was also found in pyramidal neurons of AD-affected individuals.

Additionally, Aβ enhances nitrosative stress-inducing s-nitrosylation of DLP1, which favors mitochondrial fission followed by mitochondrial depletion from axons and dendrites and subsequently synaptic loss [40] (Figure 2).

Mitochondrial Aβ may also interact with cyclophilin D, an integral part of the mitochondrial permeability transition pore (mPTP), which potentiates free radical production, causes synaptic failure, and promotes the opening of the mPTP leading to apoptosis [65] (Figure 2).

On the other hand, Aβ accumulation could result in cytoskeletal aberrations [66] (Figure 2). Aβ oligomers interact with integrins leading to improper control of focal adhesion assembly and signaling, therefore causing the dysregulation of cofilin, which is involved in the regulation of actin dynamics. The inhibition of actin dynamics is associated with increased ROS production and reduced mitochondrial potential. Moreover, cofilin in response to oxidative stress translocates to the mitochondria where it induces swelling, a drop in mitochondrial membrane potential, and cytochrome *c* release promoting the opening of mPTP and apoptosis.

3.2. Presenilin. Presenilins 1 and 2 are multitransmembrane proteins that associate with nicastrin, APH-1, and PEN-2, form high-molecular γ-secretase complex, and are involved in Aβ production byintramembrane cleavage of APP. Ps-1 gene mutations are the most prevalent in FAD, but besides the generation of Aβ, little is known about its implication in mitochondrial dysfunction and oxidative damage. It has been demonstrated that presenilin-1 [67] and presenilin-2 are also located in mitochondria as part of the γ-secretase complex [68]. Noteworthy, presenilin mutations have been shown to sensitize cells to apoptosis by mechanisms suggested to involve impaired mitochondrial function and Ps-2/γ-secretase activity can modify mitochondrial membrane potential [69]. Moreover, ps-2 KO mouse embryonic fibroblasts exhibit lower basal respiratory rate. On the other hand, in at least two transgenic mouse models expressing human tau with AD mutations at presenilin-1, PS1M146L [70] and PS1A246E [71], the existence of mitochondrial abnormalities prior to cognitive deficits has been described. Also, in the case of PS1M146L mice, it was found that the mutation increases mitochondria ROS formation and oxidative damage. Finally, it has been recently shown that presenilins and γ-secretase activities are concentrated in a specialized subcompartment of the endoplasmic reticulum (ER) that is physically and biochemically connected to mitochondria, called mitochondria-associated ER membranes (MAM) which are involved in mitochondrial function and dynamics, among others [72, 73]. Either in presenilin KO mice or fibroblasts from FAD and SAD patients, MAM function is increased correlating with a significantly increased area of apposition between ER and mitochondria.

3.3. Tau. Tau is involved in the axonal transport of organelles such as mitochondria [74]. Hyperphosphorylated Tau may block the transport of mitochondria leading to energy deprivation and oxidative stress at the synapse as well as to neurodegeneration [75] (Figure 2). Analysis of brain proteins from P301L mutant human tau transgenic mice revealed deregulation of mitochondrial respiratory chain complex components such as complex V and reduced complex I activity as well as an impaired mitochondrial respiration with the subsequent ROS accumulation with aging [27] (Figure 1). Accordingly, the overexpression of P301L tau mutation in human neuroblastoma cells has been shown to induce substantial complex I deficit accompanied by decreased ATP levels and increased susceptibility to oxidative stress [76]. This was paralleled by pronounced changes in mitochondrial morphology, decreased fusion and fission rates accompanied by reduced expression of OPA-1 and DLP-1. In contrast, the overexpression of wt tau exhibited protective effects on mitochondrial function and dynamics including enhanced complex I activity. Moreover, an abnormal interaction of hyperphosphorylated Tau and mitochondrial fission protein DLP-1 has been described suggesting a relationship with mitochondrial dynamics alteration [77] (Figure 2). Other

researchers have found that the expression of human tau mutations in both *Drosophila* (R406W) and mouse neurons (P301L) results in elongation of mitochondria, which is accompanied by mitochondrial dysfunction and cell cycle-mediated cell death [78]. We have previously mentioned an interaction of phospho-Tau and VDAC1 that may in turn block the mitochondrial pores leading to mitochondrial dysfunction [62] (Figure 2). On the other hand, increased oxidative stress has been shown to cause Tau hyperphosphorylation in a superoxide dismutase 2 knockout mouse model [79]. Furthermore, the inhibition of complex I with annonacin led to a redistribution of Tau from the axons to the cell body which correlates with a retrograde transport of mitochondria and finally to cell death [80]. Lastly, the downregulation of the proteins involved in the axonal transport of mitochondria such as Miro and Milton in *Drosophila* has shown loss of axonal mitochondria that promotes Tau phosphorylation in Ser262 via partitioning defective-1 (*Drosophila* homolog of mammalian microtubule affinity-regulating kinase) causing late-onset neurodegeneration in the fly [81].

4. The Use of Mitochondria-Targeted Antioxidants in AD

Since, as previously indicated, mitochondrial dysfunction and oxidative stress may play a role in the development of AD, many efforts have been proposed to demonstrate the therapeutic potential of antioxidants in this disease. Different components such as vitamin E [82], curcumin [83], *Gingo biloba* [84], and melatonin [85] have demonstrated their potential to reduce Aβ levels and improve mitochondrial function and cognitive behavior in animal models of AD. However, clinical trials using these antioxidants or others, such as huperzine A, have shown only modest or no effect in cognitive function. The modest effect may be related to unsuccessful cross of the blood-brain barrier, not well-thought-out experimental design of the clinical trials, or the late stage of patients involved [50].

To improve this poor result many efforts have been done to develop mitochondria-targeted antioxidants, such as triphenylphosphonium-based antioxidants (MitoQ, MitoVitE, Mito-α-lipoic acid, MitoPBN) [86], cell-permeable small peptide-based molecules (SS31, SS02, SS19, and SS20) [87], and choline esters of glutathione and N-acetyl-l-cysteine [88]. The first group results from the combination of lipophilic triphenylphosphonium cation with ubiquinol, α-tocopherol, α-lipoic acid, and α-phenyl-N-tert-butylnitrone, respectively. Due to their positive charge they are accumulated several hundredfold within mitochondria driven by the membrane potential, enhancing the protection of mitochondria from oxidative damage [86]. MitoQ accumulates in mitochondria driving the conversion of H_2O_2 to H_2O and O_2, reducing the toxic insult of free radicals.

Szeto-Schiller or SS peptides are a serial of small cell-permeable antioxidant peptides which have a sequence motif that allows them to target mitochondria [87]. They scavenge H_2O_2 and ONOO– and inhibit lipid peroxidation. Their antioxidant activity is attributed to the tyrosine or dimethyltyrosine (Dmt) residue. Dmt has demonstrated to be more effective than tyrosine in scavenging of ROS. SS31 (H-D-Arg-Dmt-Lys-Phe-NH2) has demonstrated its efficacy in rodent models of different diseases.

The antioxidant effect of MitoQ and SS31 has been tested *in vitro* in mouse cell models of AD [89]. Both components were able to prevent the effects of Aβ in mouse neuroblastoma (N2a) cells, such as increased expression of mitochondrial fission genes, decreased expression of fusion genes, peroxiredoxins, and endogenous cytoprotective antioxidant enzymes, and, increased number of intact mitochondria and neurite outgrowth. Additionally, in neurons from a mouse model of AD (Aβ precursor protein transgenic mouse, Tg2576 line) incubated with Aβ, MitoQ and SS31 achieved an increase in neurite outgrowth and a decrease in cyclophilin D expression. Posterior work using primary neurons from Tg2576 mice [54] confirmed the capacity of SS31 to mitigate the effects of oligomeric Aβ, such as decreased anterograde mitochondrial movement, increased mitochondrial fission, decreased fusion and structurally damaged mitochondria, abnormal mitochondrial and synaptic proteins, defective mitochondrial function, and apoptotic neuronal death. SS31 was able to restore mitochondrial transport and synaptic viability and decreased the percentage of defective mitochondria, demonstrating its protective effect from Aβ toxicity.

Moreover, MitoQ has been shown to prevent cognitive decline in 3xTg-AD mice as well as early neuropathology, such as oxidative stress, Aβ accumulation, astrogliosis, synaptic loss, and caspase activation [90].

Another example of mitochondria-targeted antioxidants could be the compound SKQ1 (plastoquinonyl decyltriphenylphosphonium), a membrane-penetrating cation that is specifically accumulated in the inner mitochondrial membrane [91]. SKQ1 lowers the rate of ROS formation in the respiratory chain due to mild uncoupling. SKQ1 reversed the appearance of a typical behavioral trait of aging in rats [92].

Further evidence suggesting the therapeutic capacity of mitochondria-targeted antioxidants was recently obtained from the *in vivo* studies using APP transgenic mice that carried the human mitochondria-targeted catalase (MCAT) gene [93]. These mice lived 5 months longer than did APP mice. Their brain sections showed a reduction in the levels of full-length APP, C-terminal fragment 99, BACE1, Aβ levels (40 and 42), Aβ deposits, and oxidative DNA damage relative to the brain sections from the APP mice. Additionally, significant increased levels of soluble APPα and C-terminal fragment 83 were found in the MCAT/APP mice, suggesting that oxidative stress plays a primary role in AD etiopathology.

All these findings indicate that mitochondria-targeted molecules may be an effective therapeutic approach to treat patients with AD.

5. Removal of Defective Mitochondria

Autophagy is a normal cellular recycling process that involves degradation of intracellular components including proteins,

protein complexes, and organelles through lysosomal degradation. Mitochondrial function is regulated by autophagy in a process named mitophagy, in which dysfunctional mitochondria are recycled by engulfment into autophagosomes that then fuse with lysosomes for their content degradation. The segregation of damaged mitochondria depends on fission and fusion events that, as we have previously discussed, are altered in AD. Although increased mitochondrial autophagy in AD has been described [94], further studies will be necessary to clarify if this is a protective process because they may not be properly recycled by fusion with lysosomes or it may not be selective for damaged mitochondria.

One of the mechanisms described for the regulation of mitochondrial recycling by autophagy involves the E3 ubiquitin ligase Parkin. After mitochondrial damage, PTEN-induced kinase 1 (PINK1) is stabilized in mitochondria inducing the recruitment of Parkin [95]. Parkin-mediated ubiquitination recruits autophagy adapter proteins, such as p62, which interacts with LC3 mediating the cargo engulfment into autophagosomes. Parkin ubiquitinates several mitochondrial proteins, such as VDAC1 [96] and mitofusins that may be involved in the segregation of damaged mitochondria via an inhibition of mitochondrial fusion events [97]. Therefore the alterations described in mitochondrial dynamics-related proteins and autophagy in AD may affect the mitophagy increasing mitochondrial damage and ROS accumulation.

In AD, Aβ, and Tau aggregation has been associated with mitochondrial damage, oxidative stress, and cytoskeletal alteration of neurons. Autophagy plays a fundamental role in neuronal function and is intensively involved in AD-related protein aggregation [98]. Indeed, it has been demonstrated that autophagy is the major degradational pathway following unfolded protein response activation in neuronal cells, an early event in AD brain, suggesting a connection between its activation and the observed autophagic pathology [99]. Accordingly, an accumulation of autophagic vesicles in the cortex of AD patients compared to nondemented ones has been shown [98]. Moreover, an increase of autophagic vesicles containing mitochondria in pyramidal neurons from AD patients has been found, suggesting a mitophagy alteration [94, 100]. According to this, Parkin, one of the proteins involved in the target of mitochondria to be degraded by mitophagy, has been shown to be reduced in the cortex of AD brains [101]. Additionally, autophagy alterations have been described in AD brain and animal models. Beclin 1, a protein that plays a key role in autophagy, has been shown to be diminished in the affected brain regions in AD patients early in the disease process [102]. In the same work, in an APP transgenic mouse model, the downregulation or overexpression of beclin1 increased or diminished, respectively, the Aβ accumulation, extracellular Aβ deposition, and neurodegeneration, highlighting the relevance of autophagy in AD-related pathology. Moreover, a link between FAD and autophagy has been recently indicated, showing that autophagy requires functional Ps-1 for lysosomal maturation and that is impaired by Alzheimer-related ps-1 mutations [103]. Thus, ps-1 mutations could indirectly affect mitochondrial function by impairing its recycling by mitophagy.

On the other hand, autophagy has been proposed to play an active role in AD pathogenesis. In this regard, autophagic vesicles have been demonstrated to be an active compartment for Aβ generation and their abnormal accumulation in affected neurons of the AD brain contributes to Aβ deposition [104].

Due to the crucial role of autophagy in AD, the Moussa group proposed the induction of autophagy by overexpression of Parkin as a therapeutic strategy. Parkin could ubiquitinate and decrease intracellular Aβ levels and plaque deposition by a synergistic activation of proteasomal degradation [105] and Beclin1-dependent autophagic clearance [106]. Additionally, Parkin-induced autophagy facilitated clearance of vesicles containing debris and defective mitochondria counteracting oxidative stress and preventing mitochondrial dysfunction [106]. Parkin reverses intracellular Aβ accumulation and its negative effects on proteasome function [101].

Other strategies for the induction of autophagy as a therapeutic strategy in AD have been tested in animal models for the disease. With this aim several molecules have been tested such as rapamycin [107, 108], cystatin B [109], trehalose [110], *scyllo*-Inositol [111], and latrepirdine [112], although effects on improving mitochondrial recycling were not studied in these works.

6. Novel Models for the Study of AD

Hitherto, the majority of observations about mitochondrial failure come from the study of animal models of familial AD, patient-derived nonneuronal cells, and postmortem analysis of the patient's brain. As we have previously discussed, mitochondria are highly dynamic organelles which coordinate a vast amount of cellular functions; therefore, although the analysis of the brain can give us clues about alterations in the amount of involved proteins and in the structural changes by image analysis, for the proper study of mitochondrial function the use of living cells is necessary. In this direction, the use of patient-derived fibroblasts can be very helpful but we should not forget that these are not the cells that degenerate in the disease, so they might be subject to some kind of compensatory mechanism, making them very different from neurons. Additionally, fibroblast environment, energy requirements, and protein expression pattern and morphology are very different from those of neurons, so the extrapolation of the results obtained in this kind of cells should be taken cautiously. FAD animal models or the expression of AD human mutations in human neuroprecursor cells offers us the possibility of studying mitochondrial function in a cellular model related to the disease. However, we should take into consideration that the majority of AD cases are sporadic or are not related to any known mutation associated with the disease. For these reasons, there is an increasing effort in generating neuronal cells derived from SAD patients.

A revolutionary work in 2006 by Takahashi and Yamanaka demonstrated for the first time that adult differentiated cells such as fibroblasts are able to be retrodifferentiated to generate stem cells [113]. Four transcriptions factors, Oct3/4,

Sox2, c-Myc, and Klf4, were enough to induce pluripotency in primary fibroblast indicating that cell programming could be reversed. This innovative concept opens the possibility of generating neuronal cells from patient-derived fibroblasts having a deep impact on the study of neurodegenerative diseases. Noteworthy, Yamanaka obtained the Nobel Prize in 2012 in recognition to his discovery. After this pioneer work many efforts have been done to successfully reproduce this result in human fibroblasts [114] as well as optimize the protocol by changing the transcriptional factors [115], as well as substituting them by small molecules [116–118] or miRNAs [119–121] for transcription factors.

An increasing number of papers have come out in the last few years using fibroblasts for the generation of induced pluripotent stem cells (iPS), some of which are focused in the generation of neurons from these iPS for the study of Alzheimer disease [122, 123]. Using this approach of generating neurons from presenilin-associated FAD patient's fibroblasts, increased Aβ42 production and secretion was found [122]. Similar studies using sporadic and APP duplication associated AD fibroblasts have also found an increase in Aβ40, phospho-Tau (Thr 231), or in active GSK3 (lacking Ser9 phosphorylation) as well as the accumulation of early endosomes [123].

More recently, direct conversion of fibroblast to functional neurons (induced neurons, iN) by just three transcription factors, Ascl1, Brn2 (also called Pou3f2), and Myt1l has been described [124]. This was subsequently reproduced by using human fibroblasts [125]. Also, microRNA-mediated conversion of human fibroblasts to neurons has been indicated, although its efficiency is increased by the addition of transcription factors [126]. Additionally, a combination of one microRNA and two transcription factors appears to be sufficient to reprogram human fibroblasts to functional neurons [127]. Other authors have reported the direct conversion of fibroblasts to neural progenitor cells [128] or tripotent neural precursor cells (iNSC) [129, 130] which have the advantage of being expandable.

The conversion of human fibroblasts from Alzheimer disease patients directly to functional neurons has been reported [131]. These reprogrammed neurons exhibit some of the hallmarks of the brain of the patients such as an altered processing of amyloid precursor protein and an increased production of Aβ.

Several works have pointed out that there are differences in gene expression patterns between iPS, embryonic stem cells, and somatic cells [132, 133]. These differences involve reprogramming process-dependent genes and those retained from somatic cells due to epigenetic memory. This fact should be taken into consideration to extrapolate the results obtained using these models. However, to better understand the relevance of these differences in the field of neurodegenerative diseases it would be necessary to compare the gene expression pattern of the reprogrammed cells with the somatic original cell as well as neuronal tissue from the same patient.

Although an increasing amount of work now uses either iPS, iN, or iNSC for modeling neurodegenerative diseases, in the case of Alzheimer, no mitochondrial function studies have been done so far in these models. Further efforts will be necessary to improve the efficiency of these protocols to increase neuron generation rate in order to perform this kind of approach.

Mitochondrial function is essential for neuronal differentiation and survival. Taking into consideration all the mitochondrial alterations described for Alzheimer disease, it is possible that the reprogramming of fibroblasts from AD patients into neurons could be more difficult. It has been described that mtDNA integrity is essential for mitochondrial maturation during differentiation of neuronal stem cells [134]; therefore, it is possible that lack of mtDNA integrity due to oxidative damage of nucleic acids may impair the reprogramming from fibroblasts to neurons. Also, a change in protein levels, like DLP1, previously mentioned, or other mitochondrial proteins such as prohibitin [135], could make AD fibroblasts more vulnerable to mitochondrial damage diminishing the efficiency of reprogramming. Therefore, the study of mitochondria during the reprogramming process might give important clues to understand not only the role of mitochondria during neuronal differentiation but also the relevance of AD-associated mitochondrial dysfunction in the neurodegeneration process.

References

[1] A. Alzheimer, "Über eine eigenartige Erkrankung der Hirninde," *Zeitschrift fur Psychiatrie und Psychisch-Gerichtliche Medizin*, vol. 64, pp. 146–148, 1907.

[2] C. L. Masters, G. Simms, and N. A. Weinman, "Amyloid plaque core protein in Alzheimer disease and Down syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 82, no. 12, pp. 4245–4249, 1985.

[3] I. Grundke-Iqbal, K. Iqbal, and M. Quinlan, "Microtubule-associated protein tau. A component of Alzheimer paired helical filaments," *Journal of Biological Chemistry*, vol. 261, no. 13, pp. 6084–6089, 1986.

[4] W. E. Klunk, H. Engler, A. Nordberg et al., "Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B," *Annals of Neurology*, vol. 55, no. 3, pp. 306–319, 2004.

[5] M. T. Fodero-Tavoletti, N. Okamura, S. Furumoto et al., "^{18}F-THK523: a novel in vivo tau imaging ligand for Alzheimer's disease," *Brain*, vol. 134, no. 4, pp. 1089–1100, 2011.

[6] G. M. Savva, S. B. Wharton, P. G. Ince, G. Forster, F. E. Matthews, and C. Brayne, "Age, neuropathology, and dementia," *The New England Journal of Medicine*, vol. 360, no. 22, pp. 2302–2309, 2009.

[7] X. Zhu, G. Perry, M. A. Smith, and X. Wang, "Abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 33, supplement 1, pp. S253–S262, 2013.

[8] J. Avila, "Common mechanisms in neurodegeneration," *Nature Medicine*, vol. 16, no. 12, p. 1372, 2010.

[9] D. L. Price, R. E. Tanzi, D. R. Borchelt, and S. S. Sisodia, "Alzheimer's disease: genetic studies and transgenic models," *Annual Review of Genetics*, vol. 32, pp. 461–493, 1998.

[10] D. J. Bonda, X. Wang, G. Perry et al., "Oxidative stress in Alzheimer disease: a possibility for prevention," *Neuropharmacology*, vol. 59, no. 4-5, pp. 290–294, 2010.

[11] K. Xiong, H. Cai, X.-G. Luo, R. G. Struble, R. W. Clough, and X.-X. Yan, "Mitochondrial respiratory inhibition and oxidative

stress elevate β-secretase (BACE1) proteins and activity in vivo in the rat retina," *Experimental Brain Research*, vol. 181, no. 3, pp. 435–446, 2007.

[12] E. Tamagno, M. Parola, P. Bardini et al., "β-site APP cleaving enzyme up-regulation induced by 4-hydroxynonenal is mediated by stress-activated protein kinases pathways," *Journal of Neurochemistry*, vol. 92, no. 3, pp. 628–636, 2005.

[13] L. Wahlster, M. Arimon, N. Nasser-Ghodsi et al., "Presenilin-1 adopts pathogenic conformation in normal aging and in sporadic Alzheimer's disease," *Acta Neuropathologica*, vol. 125, no. 2, pp. 187–199, 2013.

[14] A. Belkacemi and C. Ramassamy, "Time sequence of oxidative stress in the brain from transgenic mouse models of Alzheimer's disease related to the amyloid-β cascade," *Free Radical Biology and Medicine*, vol. 52, no. 3, pp. 593–600, 2012.

[15] K. Leuner, T. Schütt, C. Kurz et al., "Mitochondrion-derived reactive oxygen species lead to enhanced amyloid beta formation," *Antioxidants and Redox Signaling*, vol. 16, no. 12, pp. 1421–1433, 2012.

[16] F. M. LaFerla, K. N. Green, and S. Oddo, "Intracellular amyloid-β in Alzheimer's disease," *Nature Reviews Neuroscience*, vol. 8, no. 7, pp. 499–509, 2007.

[17] M. Taga, F. Mouton-Liger, C. Paquet, and J. Hugon, "Modulation of oxidative stress and tau phosphorylation by the mTOR activator phosphatidic acid in SH-SY5Y cells," *FEBS Letters*, vol. 585, no. 12, pp. 1801–1806, 2011.

[18] D. J. Bonda, R. J. Castellani, X. Zhu et al., "A novel perspective on tau in alzheimer's disease," *Current Alzheimer Research*, vol. 8, no. 6, pp. 639–642, 2011.

[19] K. J. Kopeikina, G. A. Carlson, R. Pitstick et al., "Tau accumulation causes mitochondrial distribution deficits in neurons in a mouse model of tauopathy and in human Alzheimer's disease brain," *American Journal of Pathology*, vol. 179, no. 4, pp. 2071–2082, 2011.

[20] R. X. Santos, S. C. Correia, X. Wang et al., "A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 20, supplement 2, pp. S401–S412, 2010.

[21] P. I. Moreira, C. Carvalho, X. Zhu, M. A. Smith, and G. Perry, "Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology," *Biochimica et Biophysica Acta*, vol. 1802, no. 1, pp. 2–10, 2010.

[22] K. Schmitt, A. Grimm, A. Kazmierczak, J. B. Strosznajder, J. Götz, and A. Eckert, "Insights into mitochondrial dysfunction: aging, amyloid-β, and tau-A deleterious trio," *Antioxidants and Redox Signaling*, vol. 16, no. 12, pp. 1456–1466, 2012.

[23] S. Hauptmann, I. Scherping, S. Dröse et al., "Mitochondrial dysfunction: an early event in Alzheimer pathology accumulates with age in AD transgenic mice," *Neurobiology of Aging*, vol. 30, no. 10, pp. 1574–1586, 2009.

[24] K. Chandrasekaran, K. Hatanpää, D. R. Brady, and S. I. Rapoport, "Evidence for physiological down-regulation of brain oxidative phosphorylation in Alzheimer's disease," *Experimental Neurology*, vol. 142, no. 1, pp. 80–88, 1996.

[25] K. Chandrasekaran, K. Hatanpää, S. I. Rapoport, and D. R. Brady, "Decreased expression of nuclear and mitochondrial DNA-encoded genes of oxidative phosphorylation in association neocortex in Alzheimer disease," *Molecular Brain Research*, vol. 44, no. 1, pp. 99–104, 1997.

[26] V. Rhein, X. Song, A. Wiesner et al., "Amyloid-β and tau synergistically impair the oxidative phosphorylation system in triple transgenic Alzheimer's disease mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 47, pp. 20057–20062, 2009.

[27] D. C. David, S. Hauptmann, I. Scherping et al., "Proteomic and functional analyses reveal a mitochondrial dysfunction in P301L tau transgenic mice," *Journal of Biological Chemistry*, vol. 280, no. 25, pp. 23802–23814, 2005.

[28] A. Eckert, K. L. Schulz, V. Rhein, and J. Götz, "Convergence of amyloid-β and tau pathologies on mitochondria in vivo," *Molecular Neurobiology*, vol. 41, no. 2-3, pp. 107–114, 2010.

[29] C. Caspersen, N. Wang, J. Yao et al., "Mitochondrial Aβ: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease," *The FASEB Journal*, vol. 19, no. 14, pp. 2040–2041, 2005.

[30] E. K. Perry, R. H. Perry, and B. E. Tomlinson, "Coenzyme A-acetylating enzymes in Alzheimer's disease: possible cholinergic "compartment" of pyruvate dehydrogenase," *Neuroscience Letters*, vol. 18, no. 1, pp. 105–110, 1980.

[31] G. E. Gibson, K.-F. R. Sheu, J. P. Blass et al., "Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease," *Archives of Neurology*, vol. 45, no. 8, pp. 836–840, 1988.

[32] K.-F. R. Sheu, A. J. L. Cooper, K. Koike, M. Koike, J. G. Lindsay, and J. P. Blass, "Abnormality of the α-ketoglutarate dehydrogenase complex in fibroblasts from familial Alzheimer's disease," *Annals of Neurology*, vol. 35, no. 3, pp. 312–318, 1994.

[33] L.-W. Ko, K.-F. R. Sheu, H. T. Thaler, W. R. Markesbery, and J. P. Blass, "Selective loss of KGDHC-enriched neurons in Alzheimer temporal cortex: does mitochondrial variation contribute to selective vulnerability?" *Journal of Molecular Neuroscience*, vol. 17, no. 3, pp. 361–369, 2001.

[34] I. Santa-María, F. Hernández, C. Pérez Martín, J. Avila, and F. J. Moreno, "Quinones facilitate the self-assembly of the phosphorylated tubulin binding region of tau into fibrillar polymers," *Biochemistry*, vol. 43, no. 10, pp. 2888–2897, 2004.

[35] C. Cecchi, C. Fiorillo, S. Sorbi et al., "Oxidative stress and reduced antioxidant defenses in peripheral cells from familial Alzheimer's patients," *Free Radical Biology and Medicine*, vol. 33, no. 10, pp. 1372–1379, 2002.

[36] P. I. Moreira, P. L. R. Harris, X. Zhu et al., "Lipoic acid and N-acetyl cysteine decrease mitochondrial-related oxidative stress in Alzheimer disease patient fibroblasts," *Journal of Alzheimer's Disease*, vol. 12, no. 2, pp. 195–206, 2007.

[37] D. J. Bonda, X. Wang, G. Perry, M. A. Smith, and X. Zhu, "Mitochondrial dynamics in alzheimers disease: opportunities for future treatment strategies," *Drugs and Aging*, vol. 27, no. 3, pp. 181–192, 2010.

[38] E. Smirnova, L. Griparic, D.-L. Shurland, and A. M. Van der Bliek, "Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells," *Molecular Biology of the Cell*, vol. 12, no. 8, pp. 2245–2256, 2001.

[39] N. Taguchi, N. Ishihara, A. Jofuku, T. Oka, and K. Mihara, "Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission," *Journal of Biological Chemistry*, vol. 282, no. 15, pp. 11521–11529, 2007.

[40] D. H. Cho, J. Fang, P. Cieplak, A. Godzik, Z. Gu, and S. A. Lipton, "S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury," *Science*, vol. 324, no. 5923, pp. 102–105, 2009.

[41] C. Figueroa-Romero, J. A. Iñiguez-Lluhí, J. Stadler et al., "SUMOylation of the mitochondrial fission protein Drp1 occurs

at multiple nonconsensus sites within the B domain and is linked to its activity cycle," *The FASEB Journal*, vol. 23, no. 11, pp. 3917–3927, 2009.

[42] M. Karbowski, A. Neutzner, and R. J. Youle, "The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division," *Journal of Cell Biology*, vol. 178, no. 1, pp. 71–84, 2007.

[43] H. Otera, C. Wang, M. M. Cleland et al., "Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells," *Journal of Cell Biology*, vol. 191, no. 6, pp. 1141–1158, 2010.

[44] S. A. Detmer and D. C. Chan, "Functions and dysfunctions of mitochondrial dynamics," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 11, pp. 870–879, 2007.

[45] J. Zhao, T. Liu, S. Jin et al., "Human MIEF1 recruits Drp1 to mitochondrial outer membranes and promotes mitochondrial fusion rather than fission," *The EMBO Journal*, vol. 30, no. 14, pp. 2762–2778, 2011.

[46] A. E. Frazier, C. Kiu, D. Stojanovski, N. J. Hoogenraad, and M. T. Ryan, "Mitochondrial morphology and distribution in mammalian cells," *Biological Chemistry*, vol. 387, no. 12, pp. 1551–1558, 2006.

[47] X. Wang, B. Su, H. Fujioka, and X. Zhu, "Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients," *American Journal of Pathology*, vol. 173, no. 2, pp. 470–482, 2008.

[48] X. Wang, B. Su, S. L. Siedlak et al., "Amyloid-β overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 49, pp. 19318–19323, 2008.

[49] B. Oettinghaus, M. Licci, L. Scorrano, and S. Frank, "Less than perfect divorces: dysregulated mitochondrial fission and neurodegeneration," *Acta Neuropathologica*, vol. 123, no. 2, pp. 189–203, 2012.

[50] P. H. Reddy, R. Tripathi, Q. Troung et al., "Abnormal mitochondrial dynamics and synaptic degeneration as early events in Alzheimer's disease: implications to mitochondria-targeted antioxidant therapeutics," *Biochimica et Biophysica Acta*, vol. 1822, no. 5, pp. 639–649, 2012.

[51] X. Wang, B. Su, H.-G. Lee et al., "Impaired balance of mitochondrial fission and fusion in Alzheimer's disease," *Journal of Neuroscience*, vol. 29, no. 28, pp. 9090–9103, 2009.

[52] B. Bossy, A. Petrilli, E. Klinglmayr et al., "S-nitrosylation of DRP1 does not affect enzymatic activity and is not specific to Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 20, supplement 2, pp. S513–S526, 2010.

[53] M. Manczak, M. J. Calkins, and P. H. Reddy, "Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage," *Human Molecular Genetics*, vol. 20, no. 13, pp. 2495–2509, 2011.

[54] M. J. Calkins, M. Manczak, P. Mao, U. Shirendeb, and P. H. Reddy, "Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease," *Human Molecular Genetics*, vol. 20, no. 23, pp. 4515–4529, 2011.

[55] Y. Rui, P. Tiwari, Z. Xie, and J. Q. Zheng, "Acute impairment of mitochondrial trafficking by β-amyloid peptides in hippocampal neurons," *Journal of Neuroscience*, vol. 26, no. 41, pp. 10480–10487, 2006.

[56] X. Wang, G. Perry, M. A. Smith, and X. Zhu, "Amyloid-β-derived diffusible ligands cause impaired axonal transport of mitochondria in neurons," *Neurodegenerative Diseases*, vol. 7, no. 1–3, pp. 56–59, 2010.

[57] A. Misko, S. Jiang, I. Wegorzewska, J. Milbrandt, and R. H. Baloh, "Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex," *Journal of Neuroscience*, vol. 30, no. 12, pp. 4232–4240, 2010.

[58] A. Belkacemi and C. Ramassamy, "Time sequence of oxidative stress in the brain from transgenic mouse models of Alzheimer's disease related to the amyloid-β cascade," *Free Radical Biology and Medicine*, vol. 52, no. 3, pp. 593–600, 2012.

[59] D. A. Butterfield, "Amyloid β-peptide (1–42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review," *Free Radical Research*, vol. 36, no. 12, pp. 1307–1313, 2002.

[60] L. Pagani and A. Eckert, "Amyloid-beta interaction with mitochondria," *International Journal of Alzheimer's Disease*, vol. 2011, Article ID 925050, 12 pages, 2011.

[61] L. Devi, B. M. Prabhu, D. F. Galati, N. G. Avadhani, and H. K. Anandatheerthavarada, "Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction," *Journal of Neuroscience*, vol. 26, no. 35, pp. 9057–9068, 2006.

[62] M. Manczak and P. H. Reddy, "Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease," *Human Molecular Genetics*, vol. 21, no. 23, pp. 5131–5146, 2012.

[63] J. W. Lustbader, M. Cirilli, C. Lin et al., "ABAD directly links Aβ to mitochondrial toxicity in Alzheimer's disease," *Science*, vol. 304, no. 5669, pp. 448–452, 2004.

[64] N. Alikhani, M. Ankarcrona, and E. Glaser, "Mitochondria and Alzheimer's disease: amyloid-β peptide uptake and degradation by the presequence protease, hPreP," *Journal of Bioenergetics and Biomembranes*, vol. 41, no. 5, pp. 447–451, 2009.

[65] H. Du, L. Guo, F. Fang et al., "Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease," *Nature Medicine*, vol. 14, no. 10, pp. 1097–1105, 2008.

[66] D. E. Kang, S. E. Roh, J. A. Woo et al., "The interface between cytoskeletal aberrations and mitochondrial dysfunction in Alzheimer's disease and related disorders," *Experimental Neurobiology*, vol. 20, no. 2, pp. 67–80, 2011.

[67] M. Ankarcrona and K. Hultenby, "Presenilin-1 is located in rat mitochondria," *Biochemical and Biophysical Research Communications*, vol. 295, no. 3, pp. 766–770, 2002.

[68] C. A. Hansson, S. Frykman, M. R. Farmery et al., "Nicastrin, presenilin, APH-1, and PEN-2 form active γ-secretase complexes in mitochondria," *Journal of Biological Chemistry*, vol. 279, no. 49, pp. 51654–51660, 2004.

[69] H. Behbahani, I. G. Shabalina, B. Wiehager et al., "Differential role of presenilin-1 and -2 on mitochondrial membrane potential and oxygen consumption in mouse embryonic fibroblasts," *Journal of Neuroscience Research*, vol. 84, no. 4, pp. 891–902, 2006.

[70] K. Schuessel, C. Frey, C. Jourdan et al., "Aging sensitizes toward ROS formation and lipid peroxidation in PS1M146L transgenic

mice," *Free Radical Biology and Medicine*, vol. 40, no. 5, pp. 850–862, 2006.

[71] C. Strazielle, R. Jazi, Y. Verdier, S. Qian, and R. Lalonde, "Regional brain metabolism with cytochrome c oxidase histochemistry in a PS1/A246E mouse model of autosomal dominant Alzheimer's disease: correlations with behavior and oxidative stress," *Neurochemistry International*, vol. 55, no. 8, pp. 806–814, 2009.

[72] E. Area-Gomez, M. del Carmen Lara Castillo, M. D. Tambini et al., "Upregulated function of mitochondria-associated ER membranes in Alzheimer disease," *The EMBO Journal*, vol. 31, no. 21, pp. 4106–4123, 2012.

[73] E. A. Schon and E. Area-Gomez, "Is Alzheimer's disease a disorder of mitochondria-associated membranes?" *Journal of Alzheimer's Disease*, vol. 20, supplement 2, pp. S281–S292, 2010.

[74] B. Trinczek, A. Ebneth, E.-M. Mandelkow, and E. Mandelkow, "Tau regulates the attachment/detachment but not the speed of motors in microtubule-dependent transport of single vesicles and organelles," *Journal of Cell Science*, vol. 112, no. 14, pp. 2355–2367, 1999.

[75] K. Stamer, R. Vogel, E. Thies, E. Mandelkow, and E.-M. Mandelkow, "Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress," *Journal of Cell Biology*, vol. 156, no. 6, pp. 1051–1063, 2002.

[76] K. L. Schulz, A. Eckert, V. Rhein et al., "A new link to mitochondrial impairment in tauopathies," *Molecular Neurobiology*, vol. 46, no. 1, pp. 205–216, 2012.

[77] M. Manczak, M. J. Calkins, and P. H. Reddy, "Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage," *Human Molecular Genetics*, vol. 20, no. 13, pp. 2495–2509, 2011.

[78] B. DuBoff, J. Gotz, and M. B. Feany, "Tau promotes neurodegeneration via DRP1 mislocalization in vivo," *Neuron*, vol. 75, no. 4, pp. 618–632, 2012.

[79] S. Melov, P. A. Adlard, K. Morten et al., "Mitochondrial oxidative stress causes hyperphosphorylation of tau," *PLoS ONE*, vol. 2, no. 6, article e536, 2007.

[80] M. Escobar-Khondiker, M. Höllerhage, M.-P. Muriel et al., "Annonacin, a natural mitochondrial complex I inhibitor, causes tau pathology in cultured neurons," *Journal of Neuroscience*, vol. 27, no. 29, pp. 7827–7837, 2007.

[81] K. Iijima-Ando, M. Sekiya, A. Maruko-Otake et al., "Loss of axonal mitochondria promotes tau-mediated neurodegeneration and Alzheimer's disease-related tau phosphorylation via PAR-1," *PLOS Genetics*, vol. 8, no. 8, Article ID e1002918, 2012.

[82] V. Conte, K. Uryu, S. Fujimoto et al., "Vitamin E reduces amyloidosis and improves cognitive function in Tg2576 mice following repetitive concussive brain injury," *Journal of Neurochemistry*, vol. 90, no. 3, pp. 758–764, 2004.

[83] F. Yang, G. P. Lim, A. N. Begum et al., "Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid in vivo," *Journal of Biological Chemistry*, vol. 280, no. 7, pp. 5892–5901, 2005.

[84] R. W. Stackman, F. Eckenstein, B. Frei, D. Kulhanek, J. Nowlin, and J. F. Quinn, "Prevention of age-related spatial memory deficits in a transgenic mouse model of Alzheimer's disease by chronic Ginkgo biloba treatment," *Experimental Neurology*, vol. 184, no. 1, pp. 510–520, 2003.

[85] E. Matsubara, T. Bryant-Thomas, J. P. Quinto et al., "Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease," *Journal of Neurochemistry*, vol. 85, no. 5, pp. 1101–1108, 2003.

[86] M. P. Murphy and R. A. J. Smith, "Targeting antioxidants to mitochondria by conjugation to lipophilic cations," *Annual Review of Pharmacology and Toxicology*, vol. 47, pp. 629–656, 2007.

[87] H. H. Szeto, "Mitochondria-targeted peptide antioxidants: novel neuroprotective agents," *AAPS Journal*, vol. 8, no. 3, pp. 521–531, 2006.

[88] S.-S. Sheu, D. Nauduri, and M. W. Anders, "Targeting antioxidants to mitochondria: a new therapeutic direction," *Biochimica et Biophysica Acta*, vol. 1762, no. 2, pp. 256–265, 2006.

[89] M. Manczak, P. Mao, M. J. Calkins et al., "Mitochondria-targeted antioxidants protect against amyloid-β toxicity in Alzheimer's disease neurons," *Journal of Alzheimer's Disease*, vol. 20, no. 2, pp. S609–S631, 2010.

[90] M. J. Mcmanus, M. P. Murphy, and J. L. Franklin, "The mitochondria-targeted antioxidant mitoq prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease," *Journal of Neuroscience*, vol. 31, no. 44, pp. 15703–15715, 2011.

[91] V. P. Skulachev, "Mitochondria-targeted antioxidants as promising drugs for treatment of age-related brain diseases," *Journal of Alzheimer's Disease*, vol. 28, no. 2, pp. 283–289, 2012.

[92] N. A. Stefanova, A. Z. Fursova, and N. G. Kolosova, "Behavioral effects induced by mitochondria-targeted antioxidant SkQ1 in wistar and senescence-accelerated OXYS rats," *Journal of Alzheimer's Disease*, vol. 21, no. 2, pp. 479–491, 2010.

[93] 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," *Human Molecular Genetics*, vol. 21, no. 13, pp. 2973–2990, 2012.

[94] P. I. Moreira, S. L. Siedlak, X. Wang et al., "Autophagocytosis of mitochondria is prominent in Alzheimer disease," *Journal of Neuropathology and Experimental Neurology*, vol. 66, no. 6, pp. 525–532, 2007.

[95] D. P. Narendra, S. M. Jin, A. Tanaka et al., "PINK1 is selectively stabilized on impaired mitochondria to activate Parkin," *PLoS Biology*, vol. 8, no. 1, Article ID e1000298, 2010.

[96] S. Geisler, K. M. Holmström, D. Skujat et al., "PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1," *Nature Cell Biology*, vol. 12, no. 2, pp. 119–131, 2010.

[97] M. E. Gegg, J. M. Cooper, K.-Y. Chau, M. Rojo, A. H. V. Schapira, and J.-W. Taanman, "Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy," *Human Molecular Genetics*, vol. 19, no. 24, pp. 4861–4870, 2010.

[98] R. A. Nixon, J. Wegiel, A. Kumar et al., "Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study," *Journal of Neuropathology and Experimental Neurology*, vol. 64, no. 2, pp. 113–122, 2005.

[99] W. Scheper, D. A. T. Nijholt, and J. J. M. Hoozemans, "The unfolded protein response and proteostasis in Alzheimer disease: preferential activation of autophagy by endoplasmic reticulum stress," *Autophagy*, vol. 7, no. 8, pp. 910–911, 2011.

[100] P. I. Moreira, S. L. Siedlak, X. Wang et al., "Increased autophagic degradation of mitochondria in Alzheimer disease (Autophagy)," *Autophagy*, vol. 3, no. 6, pp. 614–615, 2007.

[101] K. M. Rosen, C. E.-H. Moussa, H.-K. Lee et al., "Parkin reverses intracellular β-amyloid accumulation and its negative effects on proteasome function," *Journal of Neuroscience Research*, vol. 88, no. 1, pp. 167–178, 2010.

[102] F. Pickford, E. Masliah, M. Britschgi et al., "The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid β accumulation in mice," *Journal of Clinical Investigation*, vol. 118, no. 6, pp. 2190–2199, 2008.

[103] J.-H. Lee, W. H. Yu, A. Kumar et al., "Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations," *Cell*, vol. 141, no. 7, pp. 1146–1158, 2010.

[104] W. H. Yu, A. Kumar, C. Peterhoff et al., "Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for β-amyloid peptide over-production and localization in Alzheimer's disease," *International Journal of Biochemistry and Cell Biology*, vol. 36, no. 12, pp. 2531–2540, 2004.

[105] M. P. Burns, L. Zhang, G. W. Rebeck, H. W. Querfurth, and C. E. Moussa, "Parkin promotes intracellular Abeta1-42 clearance," *Human Molecular Genetics*, vol. 18, no. 17, pp. 3206–3216, 2009.

[106] P. J. Khandelwal, A. M. Herman, H.-S. Hoe, G. W. Rebeck, and C. E.-H. Moussa, "Parkin mediates beclin-dependent autophagic clearance of defective mitochondria and ubiquitinated Aβ in AD models," *Human Molecular Genetics*, vol. 20, no. 11, pp. 2091–2102, 2011.

[107] A. Caccamo, S. Majumder, A. Richardson, R. Strong, and S. Oddo, "Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-β, and Tau: effects on cognitive impairments," *Journal of Biological Chemistry*, vol. 285, no. 17, pp. 13107–13120, 2010.

[108] P. Spilman, N. Podlutskaya, M. J. Hart et al., "Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-β levels in a mouse model of alzheimer's disease," *PLoS ONE*, vol. 5, no. 4, Article ID e9979, 2010.

[109] D.-S. Yang, P. Stavrides, P. S. Mohan et al., "Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits," *Brain*, vol. 134, no. 1, pp. 258–277, 2011.

[110] V. Schaeffer, I. Lavenir, S. Ozcelik, M. Tolnay, D. T. Winkler, and M. Goedert, "Stimulation of autophagy reduces neurodegeneration in a mouse model of human tauopathy," *Brain*, vol. 135, no. 7, pp. 2169–2177, 2012.

[111] A. Y. Lai and J. McLaurin, "Inhibition of amyloid-beta peptide aggregation rescues the autophagic deficits in the TgCRND8 mouse model of Alzheimer disease," *Biochimica et Biophysica Acta*, vol. 1822, no. 10, pp. 1629–1637, 2012.

[112] J. W. Steele and S. Gandy, "Latrepirdine (Dimebon ((R))), a potential Alzheimer therapeutic, regulates autophagy and neuropathology in an Alzheimer mouse model," *Autophagy*, vol. 9, no. 4, 2013.

[113] K. Takahashi and S. Yamanaka, "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors," *Cell*, vol. 126, no. 4, pp. 663–676, 2006.

[114] K. Takahashi, K. Tanabe, M. Ohnuki et al., "Induction of pluripotent stem cells from adult human fibroblasts by defined factors," *Cell*, vol. 131, no. 5, pp. 861–872, 2007.

[115] J. Yu, M. A. Vodyanik, K. Smuga-Otto et al., "Induced pluripotent stem cell lines derived from human somatic cells," *Science*, vol. 318, no. 5858, pp. 1917–1920, 2007.

[116] D. Huangfu, R. Maehr, W. Guo et al., "Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds," *Nature Biotechnology*, vol. 26, no. 7, pp. 795–797, 2008.

[117] J. K. Ichida, J. Blanchard, K. Lam et al., "A small-molecule inhibitor of Tgf-β signaling replaces *Sox2* in reprogramming by inducing nanog," *Cell Stem Cell*, vol. 5, no. 5, pp. 491–503, 2009.

[118] Y. Li, Q. Zhang, X. Yin et al., "Generation of iPSCs from mouse fibroblasts with a single gene, *Oct4*, and small molecules," *Cell Research*, vol. 21, no. 1, pp. 196–204, 2011.

[119] R. L. Judson, J. E. Babiarz, M. Venere, and R. Blelloch, "Embryonic stem cell-specific microRNAs promote induced pluripotency," *Nature Biotechnology*, vol. 27, no. 5, pp. 459–461, 2009.

[120] S.-L. Lin, D. C. Chang, C.-H. Lin, S.-Y. Ying, D. Leu, and D. T. S. Wu, "Regulation of somatic cell reprogramming through inducible mir-302 expression," *Nucleic Acids Research*, vol. 39, no. 3, pp. 1054–1065, 2011.

[121] F. Anokye-Danso, C. M. Trivedi, D. Juhr et al., "Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency," *Cell Stem Cell*, vol. 8, no. 4, pp. 376–388, 2011.

[122] T. Yagi, D. Ito, Y. Okada et al., "Modeling familial Alzheimer's disease with induced pluripotent stem cells," *Human Molecular Genetics*, vol. 20, no. 23, pp. 4530–4539, 2011.

[123] M. A. Israel, S. H. Yuan, C. Bardy et al., "Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells," *Nature*, vol. 482, no. 7384, pp. 216–220, 2012.

[124] T. Vierbuchen, A. Ostermeier, Z. P. Pang, Y. Kokubu, T. C. Südhof, and M. Wernig, "Direct conversion of fibroblasts to functional neurons by defined factors," *Nature*, vol. 463, no. 7284, pp. 1035–1041, 2010.

[125] Z. P. Pang, N. Yang, T. Vierbuchen et al., "Induction of human neuronal cells by defined transcription factors," *Nature*, vol. 476, no. 7359, pp. 220–223, 2011.

[126] A. S. Yoo, A. X. Sun, L. Li et al., "MicroRNA-mediated conversion of human fibroblasts to neurons," *Nature*, vol. 476, no. 7359, pp. 228–231, 2011.

[127] R. Ambasudhan, M. Talantova, R. Coleman et al., "Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions," *Cell Stem Cell*, vol. 9, no. 2, pp. 113–118, 2011.

[128] J. Kim, J. A. Efe, S. Zhu et al., "Direct reprogramming of mouse fibroblasts to neural progenitors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 19, pp. 7838–7843, 2011.

[129] E. Lujan, S. Chanda, H. Ahlenius, T. C. Südhof, and M. Wernig, "Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 7, pp. 2527–2532, 2012.

[130] M. Thier, P. Wörsdörfer, Y. B. Lakes et al., "Direct conversion of fibroblasts into stably expandable neural stem cells," *Cell Stem Cell*, vol. 10, no. 4, pp. 473–479, 2012.

[131] L. Qiang, R. Fujita, T. Yamashita et al., "Directed conversion of Alzheimer's disease patient skin fibroblasts into functional neurons," *Cell*, vol. 146, no. 3, pp. 359–371, 2011.

[132] M. H. Chin, M. J. Mason, W. Xie et al., "Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures," *Cell Stem Cell*, vol. 5, no. 1, pp. 111–123, 2009.

[133] N. Polouliakh, "Reprogramming resistant genes: in-depth comparison of gene expressions among iPS, ES, and somatic cells," *Frontiers in Physiology*, vol. 4, article 7, 2013.

[134] W. Wang, P. Osenbroch, R. Skinnes, Y. Esbensen, M. Bjørås, and L. Eide, "Mitochondrial DNA integrity is essential for mitochondrial maturation during differentiation of neural stem cells," *Stem Cells*, vol. 28, no. 12, pp. 2195–2204, 2010.

[135] P. Zhou, L. Qian, M. D'Aurelio et al., "Prohibitin reduces mitochondrial free radical production and protects brain cells from different injury modalities," *Journal of Neuroscience*, vol. 32, no. 2, pp. 583–592, 2012.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 743938, 7 pages
http://dx.doi.org/10.1155/2013/743938

Research Article

Neuroprotective Effect of Tea Polyphenols on Oxyhemoglobin Induced Subarachnoid Hemorrhage in Mice

Haizhen Mo,[1] Ying Chen,[2] Liyong Huang,[3] Hao Zhang,[1] Juxiang Li,[3] and Wenke Zhou[3]

[1] *Department of Food Science, Henan Institute of Science and Technology, Xinxiang, Henan 453003, China*
[2] *School of Life Science and Technology, Henan Institute of Science and Technology, Xinxiang, Henan 453003, China*
[3] *Department of Neurosurgery, The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan 453100, China*

Correspondence should be addressed to Ying Chen; yingch@yahoo.cn

Received 4 May 2013; Accepted 15 May 2013

Academic Editor: Renata Santos

Tea polyphenols are of great benefit to the treatment of several neurodegenerative diseases. In order to explore the neuroprotective effects of tea polyphenols and their potential mechanisms, an established *in vivo* subarachnoid hemorrhage (SAH) model was used and alterations of mitochondrial function, ATP content, and cytochrome *c* (cyt *c*) in cerebral cortex were detected. This study showed that the alteration of mitochondrial membrane potential was an early event in SAH progression. The trend of ATP production was similar to that of mitochondrial membrane potential, indicating that the lower the mitochondrial membrane potential, lesser the ATP produced. Due to mitochondrial dysfunction, more cyt *c* was released in the SAH group. Interestingly, the preadministration of tea polyphenols significantly rescued the mitochondrial membrane potential to basal level, as well as the ATP content and the cyt *c* level in the brain cortex 12 h after SAH. After pretreatment with tea polyphenols, the neurological outcome was also improved. The results provide strong evidence that tea polyphenols enhance neuroprotective effects by inhibiting polarization of mitochondrial membrane potential, increasing ATP content, and blocking cyt *c* release.

1. Introduction

Polyphenols, the most abundant components in fruits, tea, wine, and vegetables, have attracted attention in recent years due to their healthy effects. It has long been known that tea polyphenols have neuroprotective effects in various pathological states of the nervous system, for example, by lowering the cognitive impairment in most neurodegenerative diseases and by reducing the risk of mortality after stroke [1–3]. Human epidemiological data shows that the risk of a fatal versus nonfatal stroke is significantly reduced by approximately 21% in people that drink 3 cups of tea per day when compared with nontea drinkers. In fact, high consumption of tea, particularly green tea, contributes to the lower rates of cardiovascular disease observed in the Asian population, especially in China, indicating that tea drinking may be a benefiting lifestyle. However, other authors indicate that tea consumption has no correlation with hemorrhagic stroke [4].

Stroke is a leading cause of morbidity and mortality worldwide. Subarachnoid hemorrhage (SAH) accounts for only 5%–10% of all strokes but is a major devastating subtype of stroke affecting 30,000 people in North America yearly [5]. SAH is caused by the rupture of a brain aneurysm and can be divided in two stages, the first 72 h correspond to the early brain injury (EBI) and the period after 72 h corresponds to the delayed vasospasm. About 21% of SAH survivors do not experience delayed vasospasm indicating that EBI may be an important stage predicting the outcome of SAH [6, 7]. In fact, all the factors related to the pathological mechanisms of EBI after SAH may eventually induce irreversible neuronal death, which is associated with neurological deficits and poor outcome [8–10].

A growing body of evidence from animal models and clinical studies indicate that mitochondrial dysfunction is a common event in brain injury, including in SAH [11]. Several lines of research suggest that mitochondrial dysfunction,

induced by oxidative stress and inflammation, results in mitochondrial membrane potential ($\Delta\psi_m$) reduction, cytochrome *c* (cyt *c*) release, and then activation of mitochondrial-dependent cell death, providing evidence that mitochondrial impairment is deleterious [12, 13]. Therefore, mitochondrial function is a potential therapeutic target [14].

Recent results suggest that inhibition of a signal pathway may not be required for the treatment of SAH [15]. In such a scenario, tea polyphenols may be of special interest due to their multiple functions, such as antioxidants, antimutagenic, iron chelators, and in glutamate release [16]. However, tea polyphenols' effects in the time course after SAH are still unknown. The aim of this study was to investigate whether tea polyphenols have neurological and neurobehavioural effects or not, and the pathway involved in these effects in the *in vivo* SAH model. This study contributes to a better understanding of the role of tea polyphenols in SAH prevention, which are nontoxic and inexpensive dietary components.

2. Materials and Methods

2.1. Preparation Oxyhemoglobin (OxyHb). Arterial blood collected from Kunming mouse with heparin was centrifuged at 2,500 ×g for 15 min. The supernatant was discarded. Erythrocytes were washed 3 times with saline solution, lysed with methylbenzene, and centrifuged at 15,000 ×g for 20 min; the supernatant was collected. Using filter membrane, the OxyHb solution was collected, adjusted to 3 μmol/L using measured optical density (OD) at 540 nm and 576 nm, and stored at −80°C [17].

2.2. Animals. The animal use and care protocols were approved by Institutional Animal Care and Use Committee (IACUC) of Xinxiang Medical University**.** One hundred and eighty adult male Kunming mice weighing from 18 to 20 g were purchased from Xinxiang Medical University. All animals were required to undergo institutional quarantine for 7 days prior to use. The environment for animal housing was equipped with controlled temperature (22 ± 3°C), humidity (40%–70%), and a 12 h light/dark alternation. The mice were divided into three groups: sham group (n = 60), SAH group (n = 60), and preadministration tea polyphenols SAH group (n = 60). Subsequently, each group was subdivided into 3 h, 6 h, 12 h, 24 h, and 72 h subgroup (n = 12), respectively. SAH group was injected with OxyHb, and sham group was given isotonic saline for the same period. Pretreatment group was administered intragastrically with water containing tea polyphenols at a dose of 450 mg/kg/d for 7 days before OxyHb injection [18, 19].

2.3. Mouse SAH Model. SAH was performed using the model reported by Shi et al. [20]. Under general anesthesia with 1% pentobarbital (50 mg/kg, intraperitoneally), animals were placed in prone position. The posterior cervical muscles were dissected through a suboccipital midline skin incision and retracted laterally. The exposed transparent atlantooccipital membrane was penetrated by a 30 gauge needle. Under spontaneous breathing, a 23-gauge needle without point was inserted percutaneously into the skull at a controlling depth of 1.5 mm in the cross position at the sagittal suture 2 mm and sutura coronaria 1 mm. Then, 50 μL (150 μmol/L) of OxyHb was injected through this hole into subarachnoid space. After the neurological assessment, the animals were decapitated at different time points after SAH. Prior to decapitation, one part (n = 6) was perfused through the left cardiac ventricle with isotonic saline, followed by 4% paraformaldehyde in phosphate-buffered saline (PBS). The brain tissue was removed and fixed in 4% paraformaldehyde for 48 h and then embedded in paraffin. Sections of 4 mm thickness were cut using a microtome for histologic studies. The others (n = 6) were sacrificed with 0.9% saline solution perfusion through the left cardiac ventricle. The fresh brain was immediately removed and cortex sections selected and then stored at −80°C.

2.4. Mortality and Neurological Functions Assessment. Recently, the Garcia scoring system has been developed to evaluate the animal neurological behavior and function in a blinded fashion [21]. The results showed that the higher the neurological score, the better the outcome. Briefly, the neurobehavioral examination was performed at 6 h, 12 h, 24 h, and 72 h. An 18-point scoring system was used based on (1) spontaneous activity, (2) symmetry of limb movement, (3) climbing, (4) body proprioception, (5) movement of forelimbs, and (6) response to vibrissae touch (score scale: 0–3 each).

2.5. Lactate Dehydrogenase (LDH) Assay from Brain Cortex. The supernatant of all the samples was collected after homogenate and the LDH content was determined using an LDH assay kit according to the manufacturer's instructions (Nanjing Institute of Jiancheng Biological Engineering, China) [22]. LDH cytotoxicity was calculated using OD as LDH cytotoxicity (U/g protein) = (OD sample − OD blank)/(OD standard solution − OD blank standard solution) × standard solution concentration/sample protein concentration.

2.6. Isolation of Mitochondria from Cerebral Cortex. Intact mitochondria were isolated from fresh brain cortex layer using a tissue mitochondria isolation kit (Beyotime Institute of Biotechnology, China). In brief, after homogenization of 0.5 g cortical tissue in ice-cold MSH buffer (10 mM HEPES, pH 7.5, containing 200 mM mannitol, 70 mM sucrose, 1.0 mM EGTA, and 2.0 mg/mL serum albumin), the homogenate was centrifuged at 1,000 ×g at 4°C for 10 min. The collected supernatant was then centrifuged at 3,500 ×g at 4°C for 10 min to obtain a mitochondrial pellet [23].

2.7. Assay of Mitochondrial Membrane Potential. Changes in mitochondrial membrane potential ($\Delta\psi_m$) were measured using a JC-1 (5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanide iodide) staining (mitochondrial membrane potential assay kit, Beyotime Institute of Biotechnology, China) according to the manufacturer's instructions. Briefly, isolated mitochondria were suspended in 0.5 mL

medium containing 5 mM JC-1. Samples were analyzed using an automatic microplate reader (Thermo Scientific, USA) at time scan method. The intensities of green (excitation/emission wavelength = 485/538 nm) and red (excitation/emission wavelength = 485/590 nm) fluorescence were analyzed in each sample and represented a surrogate marker of loss of mitochondrial $\Delta\psi_m$ [24].

2.8. Assay for Cellular ATP Levels from Brain Cortex. After being thawed, samples were homogenized in boiling double distilled water in order to denature endogenous ATPase present in the tissue. The supernatant fraction of the homogenate was collected after centrifugation at 10,000 ×g for 10 min. ATP levels were measured using ATP colorimetric assay kit according to manufacturer's instructions (Nanjing Institute of Jiancheng Biological Engineering, China) [25].

2.9. Immunohistochemistry for Cyt c Antigen. Deparaffinized sections were treated with 0.3% hydrogen peroxide in methanol for 15 min at room temperature to block endogenous peroxidase activity. The sections were incubated in 0.01 M, pH 6.5 sodium citrate buffer for 10 min at 121°C, and cooled to room temperature. After being blocked with 10% normal goat serum for 1 h at room temperature, the slides were subsequently incubated overnight with anti-cyt *c* antibody (BA0781, Boster Bio-Engineering Limited Company, China) at a dilution of 1 : 100. After being extensively washed with PBS, the slides were incubated with Histostain-Plus kit (SP-9001, Zymed, USA). The sections were then counterstained with DAB (ZLI-9032, Zhongshan Golden Bridge Biotechnology Co., LTD, China). Quantitative evaluation was measured using IDA-2000 software (Beijing Konghai Technology Company, China). At least 10 visual fields were captured and more than 500 cells were counted [26, 27].

2.10. Statistical Analysis. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) program. All data were reported as means ± SD of three independent experiments. The physiological variables were analyzed by one-way ANOVA followed by LSD multiple comparison post hoc analysis. The neurological scores were compared by Kruskal-Wallis nonparametric test followed by multiple comparison procedures by Duncan's method. For all comparisons, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Mortality and Neurological Scores. Of a total of 180 mice, 8 (4.4%) died over the course of the experiment, 5 (8.3%) in the SAH group, 3 (5.0%) in the tea polyphenols pretreated animals, and 0 in the sham group. This suggests that tea polyphenols pretreatment reduced the mortality in consequence of SAH.

The neurological scores obtained for sham, SAH, and tea polyphenols + SAH groups are depicted in Figure 1. The neurological score observed for mice with SAH was significantly lower than that of the sham group from 6 h to 72 h after SAH ($P < 0.01$ versus sham), whereas the improved neurological scores were observed after 12 h of SAH in animals pretreated with tea polyphenols pretreatment group ($P < 0.01$ versus SAH), indicating that tea polyphenols rescued neuronal injury.

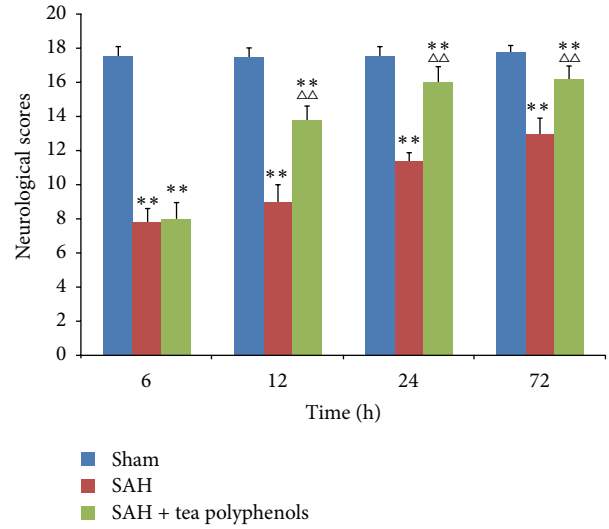


Figure 1: Neurological deficits in OxyHb-induced SAH mice. Values are expressed as mean ± SD of triplicate samples. $^{**}P < 0.01$ versus sham; $^{\triangle\triangle}P < 0.01$ versus SAH.

3.2. The Neuroprotective Role of Tea Polyphenols in Early Brain Injury after SAH. LDH activity is the most widely used marker in cytotoxic studies. Using this assay, we detected a neuroprotective role for tea polyphenols in the EBI stage after SAH. LDH activity was stable in the cortical tissue of the sham group (Figure 2). After SAH, LDH levels were significantly increased in the cortex along with the SAH progress ($P < 0.01$ versus sham), but the peak level was observed 12 h after SAH, indicating that LDH activity might be time dependent. However, pretreatment with tea polyphenols led to a significant decrease in LDH activity compared to the SAH group ($P < 0.01$ versus SAH). A significant difference was observed between groups pretreated with tea polyphenols and sham ($P < 0.01$ versus sham), except at 12 h. LDH levels after pretreatment with tea polyphenols were variable compared to those detected in the sham group.

3.3. The Preventive Effect of Tea Polyphenols on Mitochondrial Depolarization in the Cortex after SAH. Mitochondrial membrane potential ($\Delta\psi_m$), a widely recognized biomarker of mitochondrial function, can be measured using a cationic lipophilic dye, JC-1. OxyHb caused significant mitochondrial membrane depolarization in the SAH group, which was expressed as an increase in JC-1 green/red fluorescence ratios ($P < 0.01$ versus sham) (Figure 3). However, the preadministration of tea polyphenols prevented the loss of mitochondrial membrane potential ($P < 0.05$ versus sham); 12 h after SAH, no significant difference was observed compared with the sham group ($P > 0.05$ versus sham). The

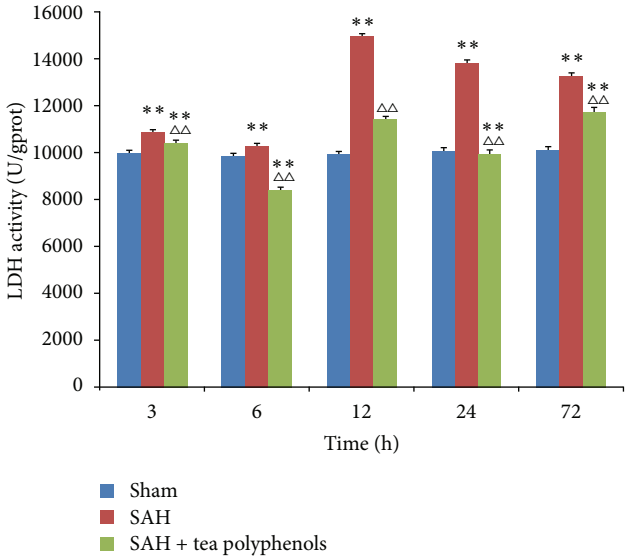


Figure 2: Effect of tea polyphenols on LDH activity in OxyHb-induced SAH. Data are expressed as the mean ± SD of three independent experiments. $^{**}P < 0.01$ versus sham; $^{\triangle\triangle}P < 0.01$ versus SAH.

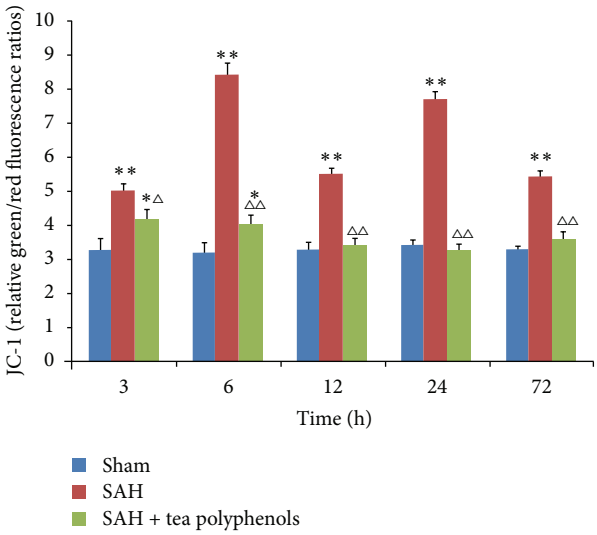


Figure 3: Effect of tea polyphenols on the mitochondrial membrane potential. Data are expressed as the mean ± SD of three independent experiments. $^{*}P < 0.05$ versus sham; $^{**}P < 0.01$ versus sham; $^{\triangle}P < 0.05$ versus SAH; $^{\triangle\triangle}P < 0.01$ versus SAH.

decrease of mitochondrial membrane potential in SAH group was greatly alleviated by tea polyphenols pretreatment with a time-dependent effect ($P < 0.05$ versus SAH).

3.4. Tea Polyphenols Increasing ATP Content in the Development of SAH. After SAH, ATP content was determined using a validated ATP detection assay in the cortex (Figure 4). Within the cortical region, the steady-state ATP level was stable in the sham group at different time points. However,

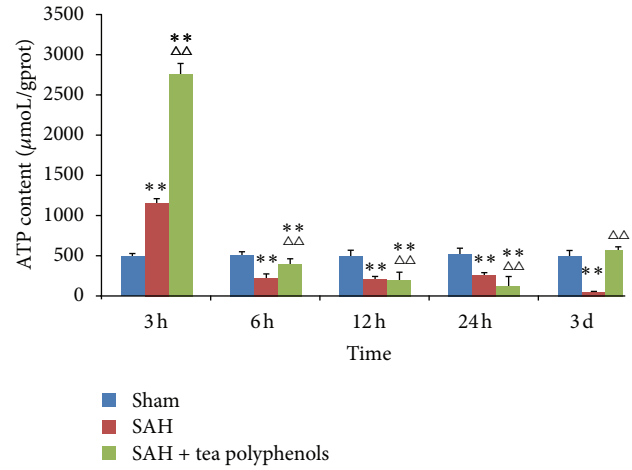
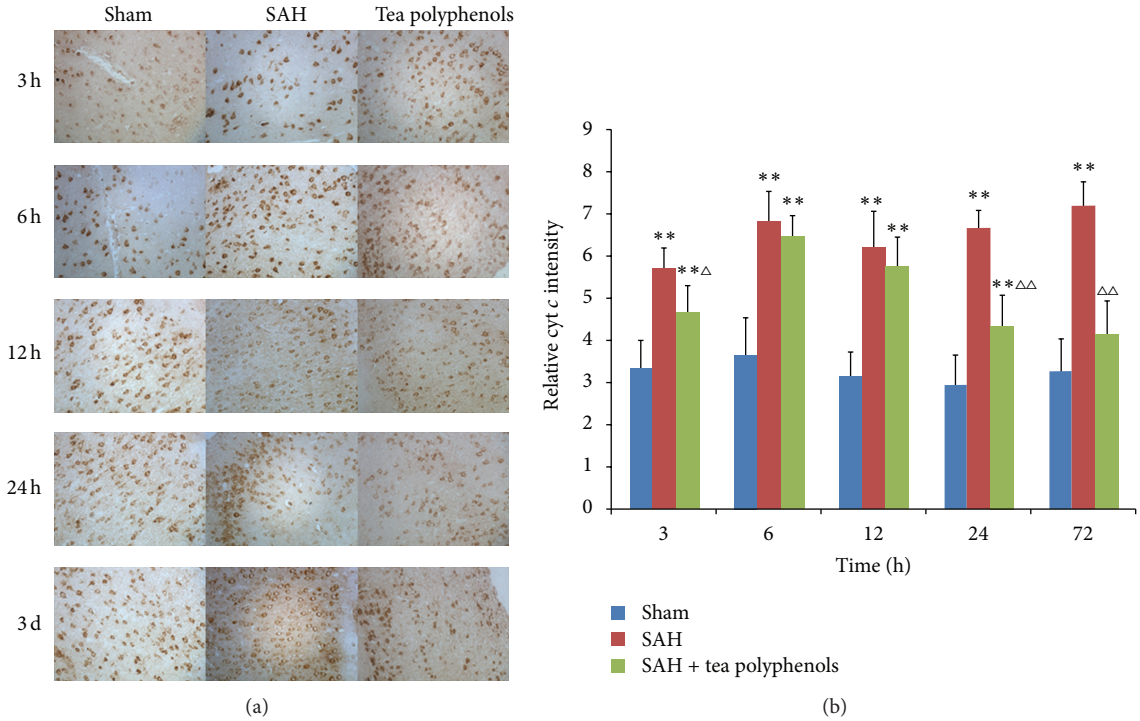


Figure 4: Alteration of ATP content after SAH. All the experiments were performed as described in Section 2. Data are expressed as the mean ± SD of three independent experiments. $^{**}P < 0.01$ versus sham; $^{\triangle\triangle}P < 0.01$ versus SAH.

ATP levels were dramatically altered both in the SAH and the tea polyphenols pretreatment groups with a distinct pattern. Interestingly, the ATP content increased rapidly, showing the highest values 3 h after SAH both in the SAH and the tea polyphenols pretreatment groups ($P < 0.01$ versus sham). After 3 h of SAH, ATP levels dramatically declined and the lowest levels were observed at 3 days after SAH, suggesting a time-dependent ATP depletion ($P < 0.01$ versus sham). In the tea polyphenols treatment group, a fluctuation in the ATP level was observed. ATP levels were gradually reduced from 6 h to 24 h, whereas a significant increase in ATP content was observed 3 days after SAH ($P > 0.05$ versus sham, $P < 0.01$ versus SAH). In conclusion, tea polyphenols significantly increased ATP content after SAH.

3.5. Effect of Tea Polyphenols on the Cyt c in EBI after SAH. Considering that cytoplastic cyt *c* level is increased along with depolarization of mitochondrial membrane potential, cyt *c* content was measured in the different experimental models using immunohistology. As shown in Figure 5(a), a moderate and stable immunoreactivity was observed in the sham group, whereas robust cyt *c* levels were observed at all time points after SAH. However, after pre-administration of tea polyphenols, cyt *c* levels were gradually decreased to basal level. The cyt *c* levels were significantly increased in the SAH group compared to the sham group ($P < 0.01$ versus sham) (Figure 5(b)). Interestingly, cyt *c* values after pre-administration of tea polyphenols were higher than that of sham, but lower than that of SAH group. Significant difference was observed from 3 h to 24 h of sham ($P < 0.01$ versus sham), as well as 3 h, 24 h and 3 days of SAH ($P < 0.05$ versus SAH), indicating that tea polyphenols can block cyt *c* release after SAH.



(a) (b)

FIGURE 5: Tea polyphenols prevented cyt *c* release. (a) Identification of cyt *c* by immunohistochemical assay (×400). (b) Quantification of cyt *c* level by densitometry. At least 10 visual fields were captured and more than 500 cells were counted. The quantification represents means and standard deviations of results from three independent experiments. $^{**}P < 0.01$ versus sham; $^{\triangle}P < 0.05$ versus SAH; $^{\triangle\triangle}P < 0.01$ versus SAH.

4. Discussion

Mitochondria contribute to many cellular events involving intracellular calcium homeostasis, reduction-oxidation potential, cell cycle regulation, and synaptic plasticity [28]. There is a current awareness that mitochondria are highly likely subjected to insults; therefore, mitochondrial dysfunction may act as one of the main trigger events in central nervous system disease, such as Parkinson's disease, Alzheimer's disease, and ischemic stroke, leading to cell death, and ultimately diseased brain [29–31]. The depolarization of the mitochondrial membrane potential ($\Delta\psi_m$) is a common event in mitochondrial dysfunction through which apoptosis, necrosis, and autophagy can be driven [32, 33]. Our results show a significant decrease in $\Delta\psi_m$ in the SAH group compared with sham group. Several studies have shown that tea polyphenols significantly prevent cell swelling and the decline in the $\Delta\psi_m$ [34]. In line with these reports, we observed that in the pretreated tea polyphenols group, the $\Delta\psi_m$ gradually increased to basal level 12 h after SAH indicating that one mechanism by which tea polyphenols exert their protective effects is possibly by inhibition of the depolarization of the inner membrane potential. Due to lack of glycolytic capacity, more mitochondria are required to produce the necessary energy in the brain than that in other organs [35]. In pathological conditions, loss of $\Delta\psi_m$ leads to the mitochondrial permeability transition (MPT) pore opening and to osmotic swelling of the mitochondrial matrix and to defective oxidative phosphorylation, thus impairing ATP synthesis. After SAH, the ATP levels were decreased compared to sham controls but could be restored by tea polyphenols pretreatment, indicating that tea polyphenols may block mitochondrial dysfunction followed by increased ATP content, eventually leading to neuronal cell survival.

Since the brain is highly sensitive to changes in mitochondrial respiration due to its higher consumption of oxygen and fewer free radicals scavenger ability, changes in $\Delta\psi_m$ may also ultimately lead to apoptosis through downregulation of antiapoptotic proteins, as well as activation of proapoptotic pathways [36]. More specifically, mitochondrial dysfunction would inhibit cytochrome *c* oxidase activity and lead to release of cyt *c* into the cytosol, which initiates the caspase cascade. There is no doubt that mitochondrial respiratory dysfunctions leading to low $\Delta\psi_m$ might result in the release of pro-apoptotic proteins, such as the apoptosis inducing factor (AIF) and cyt *c* [37]. The latter activates caspase-9, thus activating caspase-3-dependent apoptotic pathway. In addition, in low $\Delta\psi_m$ conditions, PINK1 accumulates on the surface of the mitochondria and recruits Parkin, an ubiquitin ligase, which ubiquitinylates Bcl-2, further inducing cyt *c* related and apoptosis [38, 39]. It has been shown that increased cyt *c* mediates DNA fragmentation and apoptosis in mouse brains in subarachnoid hemolysate [40]. In other words, with SAH progression, neuronal cells enter the apoptotic pathway in the cortex. However, tea polyphenols can significantly inhibit cyt *c* release by blocking mitochondrial dysfunction at the EBI stage after SAH. This finding is supported by previous results that showed that the number of apoptotic cells was reduced

after using green tea extract for pre-treatment of ischemia in gerbils [41].

5. Conclusion

In the present study, we found that neuroprotective effects of tea polyphenols may rely on their mitochondrial protection behavior. Our studies showed that tea polyphenols pretreatment reduces mitochondrial dysfunction markers and increases neurological scores after SAH [42, 43]. This suggests that dietary supplementation with tea polyphenols could be a potential candidate for prevention of SAH. As tea polyphenols represent a class of natural, dietary components, further research will be necessary to better identify which polyphenols play the roles in SAH, and which signaling pathways are involved in these neuroprotective effects.

Conflict of Interests

The authors have declared no conflict of interests.

Acknowledgments

This research was supported by the National Nature Science Foundation of China (no. 31200519), the Program for New Century Excellent Talents in University (NCET), the Program for Science and Technology Innovation Talents in Universities of Henan Province (HASTIT), and the Science and Technology Key Projects of Henan Province (no. 122102110116).

References

[1] O. Weinreb, S. Mandel, T. Amit, and M. B. H. Youdim, "Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases," *Journal of Nutritional Biochemistry*, vol. 15, no. 9, pp. 506–516, 2004.

[2] B. A. Sutherland, R. M. A. Rahman, and I. Appleton, "Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration," *Journal of Nutritional Biochemistry*, vol. 17, no. 5, pp. 291–306, 2006.

[3] S. Kuriyama, A. Hozawa, K. Ohmori et al., "Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project," *American Journal of Clinical Nutrition*, vol. 83, no. 2, pp. 355–361, 2006.

[4] B. E. Sumpio, A. C. Cordova, D. W. Berke-Schlessel, F. Qin, and Q. H. Chen, "Green tea, the "Asian Paradox," and cardiovascular disease," *Journal of the American College of Surgeons*, vol. 202, no. 5, pp. 813–825, 2006.

[5] J. B. Bederson, E. S. Connolly Jr., H. H. Batjer et al., "Guidelines for the management of aneurismal subarachnoid hemorrhage: a statement for healthcare professionals from a special writing group of the stroke council, American Heart Association," *Stroke*, vol. 40, no. 3, pp. 994–1025, 2009.

[6] A. Alaraj, F. T. Charbel, and S. Amin-Hanjani, "Peri-operative measures for treatment and prevention of cerebral vasospasm following subarachnoid hemorrhage," *Neurological Research*, vol. 31, no. 6, pp. 651–659, 2009.

[7] W. J. Cahill, J. H. Calvert, and J. H. Zhang, "Mechanisms of early brain injury after subarachnoid hemorrhage," *Journal of Cerebral Blood Flow and Metabolism*, vol. 26, no. 11, pp. 1341–1353, 2006.

[8] H. Yatsushige, R. P. Ostrowski, T. Tsubokawa, A. Colohan, and J. H. Zhang, "Role of c-Jun N-terminal kinase in early brain injury after subarachnoid hemorrhage," *Journal of Neuroscience Research*, vol. 85, no. 7, pp. 1436–1448, 2007.

[9] S. Park, M. Yamaguchi, C. Zhou, J. W. Calvert, J. Tang, and J. H. Zhang, "Neurovascular protection reduces early brain injury after subarachnoid hemorrhage," *Stroke*, vol. 35, no. 10, pp. 2412–2417, 2004.

[10] J. Cahill, J. W. Calvert, S. Marcantonio, and J. H. Zhang, "p53 may play an orchestrating role in apoptotic cell death after experimental subarachnoid hemorrhage," *Neurosurgery*, vol. 60, no. 3, pp. 531–545, 2007.

[11] G. Fiskum, R. E. Rosenthal, V. Vereczki et al., "Protection against ischemic brain injury by inhibition of mitochondrial oxidative stress," *Journal of Bioenergetics and Biomembranes*, vol. 36, no. 4, pp. 347–352, 2004.

[12] S. Marchi, C. Giorgi, and J. M. Suski, "Mitochondria-ros crosstalk in the control of cell death and aging," *Journal of Signal Transduction*, vol. 2012, Article ID 329635, 17 pages, 2012.

[13] C. Mammucari and R. Rizzuto, "Signaling pathways in mitochondrial dysfunction and aging," *Mechanisms of Ageing and Development*, vol. 131, no. 7-8, pp. 536–543, 2010.

[14] P. H. Reddy and T. P. Reddy, "Mitochondria as a therapeutic target for aging and neurodegenerative diseases," *Current Alzheimer Research*, vol. 8, no. 4, pp. 393–409, 2011.

[15] F. A. Sehba, R. M. Pluta, and J. H. Zhang, "Metamorphosis of subarachnoid hemorrhage research: from delayed vasospasm to early brain injury," *Molecular Neurobiology*, vol. 43, no. 1, pp. 27–40, 2011.

[16] J. V. Higdon and B. Frei, "Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions," *Critical Reviews in Food Science and Nutrition*, vol. 43, no. 1, pp. 89–143, 2003.

[17] T. Sasaki, H. Kasuya, H. Onda et al., "Role of p38 mitogen-activated protein kinase on cerebral vasospasm after subarachnoid hemorrhage," *Stroke*, vol. 35, no. 6, pp. 1466–1470, 2004.

[18] B. Scolaro, D. Delwing-De Lima, J. G. P. Da Cruz, and D. Delwing-Dal Magro, "Mate tea prevents oxidative stress in the blood and hippocampus of rats with acute or chronic ethanol administration," *Oxidative Medicine and Cellular Longevity*, Article ID 314758, 2012.

[19] S. Guo, J. Q. Yan, T. B. Yang, X. Q. Yang, E. Bezard, and B. L. Zhao, "Protective effects of green tea polyphenols in the 6-OHDA rat model of Parkinson's disease through inhibition of ROS-NO pathway," *Biological Psychiatry*, vol. 62, no. 12, pp. 1353–1362, 2007.

[20] W. Shi, L. Y. Huang, R. Z. Wang et al., "Time course of oxyhemoglobin induces apoptosis in mice brain cells *in vivo*," *Acta Neurochirurgica*, no. 104, pp. 23–26, 2008.

[21] X. Zhu, C. Chen, D. Ye et al., "Diammonium glycyrrhizinate upregulates PGC-1α and protects against Aβ1-42-induced neurotoxicity," *PLoS ONE*, vol. 7, no. 4, Article ID e35823, 2012.

[22] B. K. Singh, M. Tripathi, B. P. Chaudhari, P. K. Pandey, and P. Kakkar, "Natural terpenes prevent mitochondrial dysfunction, oxidative stress and release of apoptotic proteins during nimesulide-hepatotoxicity in rats," *PLoS ONE*, vol. 7, no. 4, Article ID e34200, 2012.

[23] K. Chen, Q. Zhang, J. Wang et al., "Taurine protects transformed rat retinal ganglion cells from hypoxia-induced apoptosis by preventing mitochondrial dysfunction," *Brain Research*, vol. 1279, pp. 131–138, 2009.

[24] D. Xinmin, D. Yunyou, P. Chaosheng et al., "Dexamethasone treatment attenuates early seawater instillation-induced acute lung injury in rabbits," *Pharmacological Research*, vol. 53, no. 4, pp. 372–379, 2006.

[25] Y. Liu, D. B. Kintner, G. Begum et al., "Endoplasmic reticulum Ca^{2+} signaling and mitochondrial Cyt c release in astrocytes following oxygen and glucose deprivation," *Journal of Neurochemistry*, vol. 114, no. 5, pp. 1436–1446, 2010.

[26] Y. Chen and Q. Kong, "Evaluation of centrosome abnormalities and p53 inactivation in chemical induced hepatocellular carcinogenesis," *Neoplasma*, vol. 56, no. 2, pp. 169–176, 2009.

[27] J. H. Garcia, S. Wagner, K. F. Liu, X. J. Hu, and J. P. Mohr, "Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats: statistical validation," *Stroke*, vol. 26, no. 4, pp. 627–635, 1995.

[28] M. P. Mattson, M. Gleichmann, and A. Cheng, "Mitochondria in Neuroplasticity and Neurological Disorders," *Neuron*, vol. 60, no. 5, pp. 748–766, 2008.

[29] R. X. Santos, S. C. Correia, X. Wang et al., "Alzheimer's disease: diverse aspects of mitochondrial malfunctioning," *International Journal of Clinical and Experimental Pathology*, vol. 3, no. 6, pp. 570–581, 2010.

[30] R. A. Vaishnav, I. N. Singh, D. M. Miller, and E. D. Hall, "Lipid peroxidation-derived reactive aldehydes directly and differentially impair spinal cord and brain mitochondrial function," *Journal of Neurotrauma*, vol. 27, no. 7, pp. 1311–1320, 2010.

[31] H. Chen and D. C. Chan, "Mitochondrial dynamics—fusion, fission, movement, and mitophagy—in neurodegenerative diseases," *Human molecular genetics*, vol. 18, no. 2, pp. R169–R176, 2009.

[32] C. H. Jing, L. Wang, P. P. Liu, C. Wu, D. Ruan, and G. Chen, "Autophagy activation is associated with neuroprotection against apoptosis via a mitochondrial pathway in a rat model of subarachnoid hemorrhage," *Neuroscience*, vol. 213, pp. 144–153, 2012.

[33] N. Hail and R. Lotan, "Cancer chemoprevention and mitochondria: targeting apoptosis in transformed cells via the disruption of mitochondrial bioenergetics/redox state," *Molecular Nutrition and Food Research*, vol. 53, no. 1, pp. 49–67, 2009.

[34] F. Gomez-Pinilla and T. T. Nguyen, "Natural mood foods: the actions of polyphenols against psychiatric and cognitive disorders," *Nutritional Neuroscience*, vol. 15, no. 3, pp. 127–133, 2012.

[35] P. H. Reddy, "Mitochondrial dysfunction in aging and Alzheimer's disease: strategies to protect neurons," *Antioxidants and Redox Signaling*, vol. 9, no. 10, pp. 1647–1658, 2007.

[36] D. R. Green and G. Kroemer, "The pathophysiology of mitochondrial cell death," *Science*, vol. 305, no. 5684, pp. 626–629, 2004.

[37] G. Cheng, R. H. Kong, L. M. Zhang, and J. N. Zhang, "Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies," *British Journal of Pharmacology*, vol. 167, no. 4, pp. 699–719, 2012.

[38] D. Chen, F. Gao, B. Li et al., "Parkin mono-ubiquitinates Bcl-2 and regulates autophagy," *Journal of Biological Chemistry*, vol. 285, no. 49, pp. 38214–38223, 2010.

[39] C. Zhu, X. Wang, F. Xu et al., "The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia-ischemia," *Cell Death and Differentiation*, vol. 12, no. 2, pp. 162–176, 2005.

[40] P. G. Matz, M. Fujimura, A. Lewen, Y. Morita-Fujimura, and P. H. Chan, "Increased cytochrome c-mediated DNA fragmentation and cell death in manganese-superoxide dismutase-deficient mice after exposure to subarachnoid hemolysate," *Stroke*, vol. 32, no. 2, pp. 506–515, 2001.

[41] J. T. Hong, S. R. Ryu, H. J. Kim et al., "Protective effect of green tea extract on ischemia/reperfusion-induced brain injury in Mongolian gerbils," *Brain Research*, vol. 888, no. 1, pp. 11–18, 2001.

[42] C. S. Yang, J. D. Lambert, and S. Sang, "Antioxidative and anti-carcinogenic activities of tea polyphenols," *Archives of Toxicology*, vol. 83, no. 1, pp. 11–21, 2009.

[43] S. Bastianetto, S. Krantic, J. G. Chabot, and R. Quirion, "Possible involvement of programmed cell death pathways in the neuroprotective action of polyphenols," *Current Alzheimer Research*, vol. 8, no. 5, pp. 445–451, 2011.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 683920, 9 pages
http://dx.doi.org/10.1155/2013/683920

Review Article

Neuroprotective Function of DJ-1 in Parkinson's Disease

Hiroyoshi Ariga,[1] Kazuko Takahashi-Niki,[1] Izumi Kato,[1] Hiroshi Maita,[1] Takeshi Niki,[2] and Sanae M. M. Iguchi-Ariga[2]

[1] *Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060-0812, Japan*
[2] *Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan*

Correspondence should be addressed to Hiroyoshi Ariga; hiro@pharm.hokudai.ac.jp

Received 29 March 2013; Revised 24 April 2013; Accepted 29 April 2013

Academic Editor: Anne-Laure Bulteau

Parkinson's disease (PD) is caused by dopaminergic neuronal death in the substantia nigra, resulting in a reduced level of dopamine in the striatum. Oxidative stress and mitochondrial dysfunction are thought to be major causes of neurodegeneration in PD. Although genetic and environmental factors are thought to affect the onset of PD, precise mechanisms at the molecular level have not been elucidated. The *DJ-1 gene* is a causative gene for familial PD (*park7*) and also an oncogene. DJ-1 has various functions, including transcriptional regulation, antioxidative stress reaction, and chaperone, protease, and mitochondrial regulation, and its activity is regulated by its oxidative status, especially that of cysteine 106 (C106) of DJ-1. Excess oxidation of DJ-1, which renders DJ-1 inactive, has been observed in patients with sporadic PD and Alzheimer's disease, suggesting that DJ-1 also participates in the onset and pathogenesis of sporadic PD as well as familial PD. DJ-1 is also a stress sensor and its expression is increased upon various stresses, including oxidative stress. In this review, we describe functions of DJ-1 against oxidative stress and possible roles of DJ-1 in the pathogenesis of PD.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease that occurs in approximately 1% of the population over the age of 65 years. There are two types of PD, familial and sporadic forms of PD. Although familial PD cases account for 10% of total cases of PD, investigations of the functions of familial PD gene products have provided great insights into the molecular mechanisms of the onset of PD, and familial PD gene products are thought to also play roles in the pathogenesis of sporadic PD (see recent reviews [1, 2]).

The *DJ-1* gene has been identified by us as a novel oncogene that transforms mouse NIH3T3 cells in cooperation with activated *ras* in 1997 [3]. In 2003, Bonifati et al. found a large deletion and missense mutation in the *DJ-1* gene in Italian and Dutch PD patients, leading to identification of the *DJ-1* gene as a causative gene for familial PD *park7* with recessive inheritance [4]. Twenty-three pathogenic deletion and point mutations were found in patients with PD (see Parkinson's disease mutation database and references therein, http://www.molgen.ua.ac.be/PDmutDB/default.cfm?MT=0-&ML=0&Page=Home). Compared to *parkin* and *Pink1*, other causative genes of familial PD with recessive inheritance, the number of mutations in the DJ-1 gene is small; numbers of mutations of the three genes are the order of *parkin* > *Pink*1 > *DJ-1*. This might be due to the position of DJ-1 during the course of onset of PD; DJ-1 may be placed upstream of *Pink1* and *parkin* [1, 2].

In this review, we describe functions of DJ-1 against oxidative stress and discuss how loss of function of DJ-1 affects the pathogenesis of PD.

2. Structure, Expression, and Function of DJ-1

DJ-1 is comprised of 189 amino acids with seven β-strands and nine α-helices in total and is present as a dimer [5–9]. Amino acid sequences of DJ-1 are conserved from prokaryotes to eukaryotes and they are now named DJ-1 superfamily [10]. DJ-1 is structurally most similar to the monomer subunit

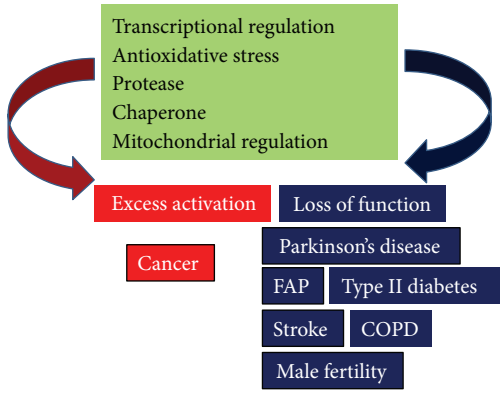


Figure 1: Functions of DJ-1 and its related diseases. DJ-1 is a multifunctional protein. It is thought that excess activation and loss of function of DJ-1 trigger the onset of various diseases, including cancer and Parkinson's disease.

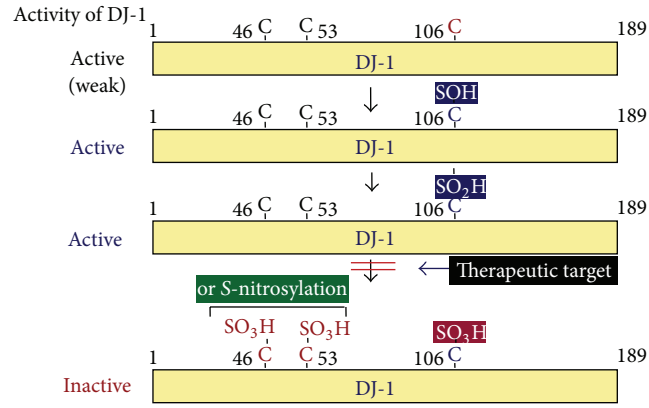
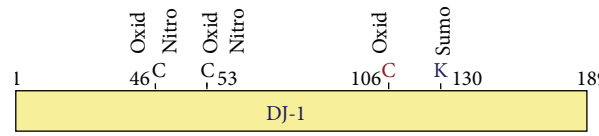


Figure 2: Cysteine oxidation and activation of DJ-1. DJ-1 contains three cysteine residues at amino acid numbers 46, 54, and 106 (C46, C54, and C106, resp.). C106 is sequentially oxidized with SOH, SO_2H, and SO_3H, and then C46 and C54 are oxidized or *S*-nitrosylated.



Oxid: Oxidation
Sumo: Sumoylation
Nitro: *S*-nitrosylation

Figure 3: Posttranslational modifications on DJ-1. DJ-1 is oxidized at amino acid numbers 46, 54, and 106 (C46, C54, and C106, resp.), *S*-nitrosylated at C46 and C54 and sumoylated at K130.

of protease I, the intracellular cysteine protease from *Pyrococcus horikoshii* [5, 11]. DJ-1, however, contains an additional α-helix at the C-terminal region, which blocks the putative catalytic site of DJ-1 [5–7].

DJ-1 is expressed in almost all cells and tissues, including the brain [3]. DJ-1 is expressed in both neurons and glia cells [4, 12, 13]. The expression level of DJ-1 is increased in cells, including reactive astrocytes, under an oxidative stress condition [14], and overexpression of DJ-1 is observed in reactive astrocytes in sporadic PD and other neurodegenerative diseases [12, 15–17]. Knockdown or knockout of DJ-1 expression in astrocytes impairs astrocyte-mediated neuroprotection against oxidative stress through deregulation of mitochondrial complex I and inflammatory responses [17–20].

DJ-1 is a multifunctional protein that participates in transcriptional regulation [21–29], antioxidative stress reaction [14, 30–36], and chaperone [37, 38], protease [39–41], and mitochondrial regulation [33, 42–52] (Figure 1). DJ-1 is located in the cytoplasm, nucleus, and mitochondria in cells, and secreted DJ-1 has been observed in various cultured cells and tissues, including cancer cells and tissues [14, 40, 53–60] and astrocytes [14]. DJ-1 is translocated from the cytoplasm to nucleus upon exposure to growth factors [3], and oxidation of C106 described later is necessary for nuclear translocation of DJ-1 [61]. DJ-1 contains three cysteine residues, C46, C56, and C106. Of the three cysteine residues, C106 is highly susceptible to oxidative stress and is oxidized as SOH, SO_2H, and then SO_3H [30, 33, 34], and mutation of C106 results in loss of all of DJ-1's functions [32, 33, 35] (Figure 2). DJ-1 at C106 with SO_3H is thought to be an inactive form of DJ-1 [38], and excessive oxidized DJ-1 has been observed in brains of patients with PD and Alzheimer's disease [15, 62]. DJ-1 thus possesses quenching activity against reactive oxygen species (ROS) by self-oxidation of its cysteine residues [32, 63]. Phylogenetic analyses showed that, of the DJ-1 superfamily from prokaryotic and eukaryotic representatives, C106 is highly conserved and important for their functions, including enzymatic activities such as thiamin biosynthetic enzymes, protease and isocyanide hydratase, chaperone, and stress response ([10, 64], references therein). DJ-1 is also modified by sumoylation [65], *S*-nitrosylation [66], and phosphorylation [67] (Figure 3). Sumoylation of DJ-1 occurs under an oxidative stress condition in concomitant with acidic shift of DJ-1. Sumoylation of DJ-1 at lysine 130 is necessary for its activity, and excess sumoylation is observed in an L166P pathogenic mutant of DJ-1 [65]. *S*-nitrosylation is observed at cysteines 46 and 53 of DJ-1 under a nitrosative stress condition and affects dimerization of DJ-1, which is necessary for DJ-1 to exert its function [66]. DJ-1 is also phosphorylated in a p53-dependent manner, but phosphorylated amino acid(s) and the effect of phosphorylation on DJ-1 function are not known [67]. From these points, it is thought that DJ-1 also participates in the pathogenesis of sporadic PD as well as familial PD.

3. Transcriptional Regulation of DJ-1 in Response to Oxidative Stress and Dopamine Synthesis

Although DJ-1 does not directly bind to DJ-1 [68], it regulates the activity of DNA-binding transcription factors as a coactivator or corepressor through binding to DJ-1-binding transcription factors. Transcription factors whose

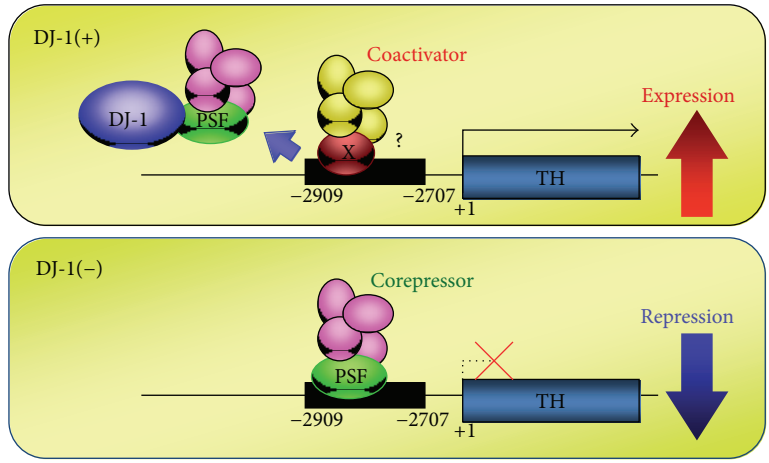


Figure 4: Schematic model of activation of the tyrosine hydroxylase gene by DJ-1. In the absence of DJ-1, PSF binds to the promoter region spanning −2909 to −2707 of the tyrosine hydroxylase (TH) gene to repress its transcription. In the presence of DJ-1, DJ-1 binds to PSF to sequester PSF from the TH gene, resulting in replacement of the corepressor complex with a coactivator complex, thereby activating TH gene transcription.

activity is regulated by DJ-1 include the androgen receptor [21, 22, 27], polypyrimidine tract-binding protein-associated splicing factor (PSF) [25], p53 [23, 28, 69, 70], nuclear factor erythroid-2-related factor 2 (Nrf2) [26], and sterol regulatory element binding protein (SREBP) [68]. Considering oxidative stress response and dopamine synthesis, regulation of Nrf2, p53, and PSF by DJ-1 is important. Nrf2 is a master transcription factor for oxidative stress and detoxication responses. Without such stresses, Nrf2 is localized in the cytoplasm in a complex with Keap1, resulting in degradation by the ubiquitin-proteasome system. Upon oxidative stress, DJ-1 sequesters Keap1, leading to translocation of Nrf2 into the nucleus to activate various antioxidative stress genes, thereby decreasing the ROS level [26]. p53 is a tumor suppressor and plays roles in induction of senescence and apoptosis in cells and in regulation of mitochondrial homeostasis against oxidative stress. DJ-1 directly binds to p53 and regulates p53 activity in various ways; p53 is activated by Topors-mediated sumoylation and inactivated by DJ-1 through inhibition of Topors activity [23], and DJ-1 binds to the DNA-binding region of p53 to inhibit p53 transcriptional activity when affinity of p53 or its mutants to DNA is low, leading to cell cycle progression [70]. It has also been reported that DJ-1 inhibits the induction of apoptosis by p53-induced Bax expression [69]. DJ-1 stimulates the expression of superoxide dismutase (SOD 3) and glutathione ligase genes by an unknown mechanism to reduce ROS level [71, 72].

Dopamine is synthesized from tyrosine by two enzymes: tyrosine hydroxylase (TH) converts tyrosine to L-DOPA and L-DOPA carboxylase (DDC) converts L-DOPA to dopamine. Dopamine is then packed in synaptic vesicles by vesicular monoamine transporter 2 (VMAT2). Although TH level in PD patients is decreased, it is not changed in DJ-1-knockout mice [73–75]. DJ-1 positively regulates human TH gene expression by sequestering transcriptional repressor PSF from the human TH gene promoter [25] (Figure 4). This

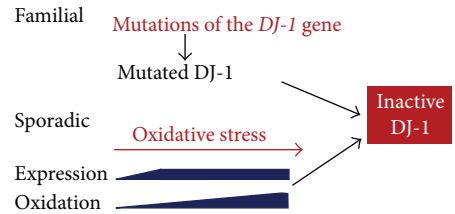


Figure 5: Proposed model of the role of DJ-1 in the onset of Parkinson's disease. In the case of familial Parkinson's disease (PD), the DJ-1 gene is heritably mutated, giving rise to inactive DJ-1 that causes PD. In the case of sporadic PD, DJ-1 expression is induced in cells upon oxidative stress to prevent cell death. During the course of continuous oxidative stress, DJ-1 is highly oxidized, giving rise to inactive DJ-1 that causes PD.

upregulation is only observed in the human TH gene due to lack of the PSF-recognition sequence in the mouse TH gene, and highly oxidized DJ-1 loses this activity [29]. This finding indicates one of the reasons for no change in TH level in DJ-1-knockout mice. DJ-1 also regulates enzymatic activities of TH and DDC [76]. When the sum of SH (reduced) and SOH forms of C106 is more than 50% of total forms of C106, DJ-1 upregulates TH and DDC activities, suggesting that the activity of DJ-1 toward TH and DDC is changed depending on the level of oxidative stress and that it is decreased with aging, which is one of the crucial factors for onset of PD (Figure 5). DJ-1 positively regulates expression of the VMAT2 gene and VMAT2 activity through transcriptional coactivator and protein-protein interaction, respectively [77]. Since VMAT2 re-uptakes excess dopamine into synaptic vesicles to prevent neurons from oxidized dopamine-induced damage, upregulation of VMAT activity by DJ-1 contributes to this reaction. Pathogenic mutations of DJ-1, including both homozygous and heterozygous mutations, have reduced stimulating activity against TH, DDC, and VMAT2 [29, 76, 77].

4. Chaperone and Protease Activity of DJ-1

Structures of DJ-1, *Escherichia coli* chaperone Hsp31 and an Archaea protease are conserved [7]. DJ-1 inhibits the aggregation of α-synuclein under an oxidative condition by its chaperone activity [37, 38]. As stated in Section 2, the structure of DJ-1 is similar to that of cysteine protease from *Pyrococcus horikoshii*, but C-terminal α-helix 9 blocks a catalytic domain of protease [5]. DJ-1 in dopaminergic cells undergoes C-terminal cleavage in response to mild oxidative stress, and a C-terminally cleaved form of DJ-1 with activated protease activity enhances cytoprotective action against oxidative stress-induced apoptosis [41]. Although protease activity is still in debate, low protease activity of DJ-1 has been reported [39–41]. Transthyretin, a causative protein in familial amyloidotic polyneuropathy (FAP), is degraded in cells transfected with full-sized DJ-1 and *in vitro* by recombinant DJ-1 lacking α-helix 9, and mutation of C106 in DJ-1 results in loss of its protease activity [40]. Localizations of DJ-1 and amyloid plaque of transthyretin in FAP patients are mirror images [40], and faint staining of DJ-1 is observed in the outer halo of Lewy bodies in PD patients [15]. These results suggest that α-helix 9 of DJ-1 is opened in cells under oxidative stress conditions in which oxidized protein(s) begins to aggregate and that DJ-1 degrades an aggregated protein(s) that causes neurodegenerative diseases. Identification of the recognition sequence of DJ-1 protease and of the protein(s) that opens α-helix 9 of DJ-1 in cells will lead to elucidation of the physiological role of DJ-1 protease.

5. DJ-1-Mediated Signaling Pathways against Oxidative Stress

There are several pathways against oxidative stress and these pathways prevent cell death, thereby leading to cell growth. The phosphoinositide 3-kinase (PI3 K)/Akt pathway is the major growth signaling pathway. When cells receive growth signals such as epidermal growth factor (EGF) stimulation, PI3 K triggers phosphorylation of Akt/protein kinase B (PKB), leading to activation of continuous phosphorylation cascades, resulting in stimulation of cell growth (see reviews [78, 79] and references therein). Phosphatase and Tensin homolog deleted from chromosome 10 (PTEN) is a lipid phosphatase that inhibits PI3 K and acts as a negative regulator of the PI3 K/Akt pathway. DJ-1 directly binds to PTEN to inhibit its enzymatic activity [80, 81]. After oxidative stress such as that caused by injection of a neurotoxin into mice or by addition of a neurotoxin to cultured cells, the Akt pathway is activated concomitantly with inactivation of PTEN in mouse brains and cultured cells, and the phosphorylation level of Akt is reduced in DJ-1-knockout mice, leading to neuronal cell death [80–82].

Apoptosis signal-regulating kinase 1 (ASK1) is mitogen-activated protein kinase-kinase-kinase 5 (MAP3 K5). It activates c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinases in response to various stresses such as oxidative stress, endoplasmic reticulum stress and calcium influx. TNF-α, LPS, and ischemia also trigger the generation of ROS, resulting in activation of ASK1 (see reviews [83, 84], references therein). ASK1 has been found to be involved in cancer [85, 86], diabetes [87, 88], and cardiovascular diseases [88–90] and neurodegenerative diseases [91–93]. These phenomena are similar to those observed in DJ-1-mediated diseases [3, 4, 40, 94–99]. The generation of ROS is also crucial for TNF-α-induced signaling pathway that leads to apoptosis, and treatment of cells with antioxidants such as N-acetyl-l-cysteine (NAC) inhibits apoptosis induction [83]. Daxx, a death domain-associated protein, associates with ASK1 in the cytoplasm to induce apoptosis after cells are treated with TNF-α [83, 84]. DJ-1 binds to both Daxx and ASK1 to sequester Daxx into the nucleus, preventing Daxx from association with ASK1, thereby inhibiting oxidative stress-induced apoptosis in H_2O_2-treated cultured cells and MPTP-administered-PD model mice [100, 101]. Pathogenic mutants of DJ-1 do not have this activity [102].

The ERK pathway is the main cell-progression pathway starting from Ras, followed by Raf, Mek, and ERK. DJ-1 protects against dopamine toxicity through the Erk kinase pathway in which DJ-1 and Erk are mutually activated upon administration of dopamine into mice or cultured cells [103]. It has been reported that an accelerated loss of substantia nigra cell bodies containing dopamine neurons was observed in aging mice lacking DJ-1 and the glial cell line-derived neurotrophic factor receptor Ret and that DJ-1 interacts with ERK signaling [104]. Furthermore, DJ-1 protects dopaminergic neurons against rotenone-induced apoptosis by enhancing ERK-dependent mitophagy [105]. Thus, DJ-1 prevents cells from oxidative stress-induced death by regulating various signaling pathways.

6. Role of DJ-1 in Mitochondrial Homeostasis

Mitochondrial dysfunction, including reduced mitochondrial complex I activity and mitochondrial membrane potential, is observed in PD patients [106–110] and in DJ-1-knockout mice and flies [47, 111]. Fragmented mitochondria are observed in DJ-1-knockout mice and cells [46, 48, 51]. Although a portion of DJ-1 is present in mitochondria under normal conditions [45, 112] and DJ-1 binds to subunits of mitochondrial complex I to regulate its activity [45], the translocation of DJ-1 into mitochondria is stimulated by oxidative stress, and oxidation of C106 with SO_2H and N-terminal 12 amino acids is necessary for mitochondrial translocation of *DJ-1* [33, 113]. Pathogenic DJ-1 mutants such as L166P and M26I DJ-1 are localized in mitochondria as monomers [113]. DJ-1 ectopically targeted to mitochondria by the addition of an N-terminal mitochondrial targeting sequence has been shown to be more protective against oxidative stress-induced cell death [44]. Considering these findings, it is thought that localization of DJ-1 as a dimer in mitochondria is required for DJ-1 to play a role in antioxidative stress reaction and that DJ-1 localized in mitochondria as a monomer, such as M26I and L166P DJ-1, is, in contrast, harmful to cells.

DJ-1 has no mitochondria-targeting sequence and binds to several chaperones, including Hsp70, CHIP, and

mitochondrial Hsp70/mortalin/Grp75, suggesting that translocation of DJ-1 into mitochondria relies on or depends on other proteins, including mortalin [43]. Mortalin plays a central role in mitochondrial homeostasis through its capacity to direct the import of nuclear-encoded proteins carrying an internal mitochondrial targeting sequence into mitochondria, and mutations of the mortalin gene were found in patients with Parkinson's disease [114].

The role of DJ-1 in autophagy is still in debate, and almost all of the reports focused on mitochondria-specific autophagy, mitophagy. When mitochondrial membrane potential is decreased, DJ-1 is translocated into mitochondria to induce mitophagy, which is clearance of damaged mitochondria [48, 50, 52]. DJ-1 seems to act in parallel to the Pink1/Parkin-mediated mitophagy pathway [50]. Although mitochondrial functions of DJ-1 have been extensively studied, the precise mechanism of mitophagy induction by DJ-1 is still poorly understood.

7. Conclusion and Perspective

DJ-1 has multiple functions and plays a protective role against oxidative stress-induced cell death by using all of its functions. DJ-1 is also a stress sensor and its expression is increased upon various stresses, including oxidative stress. Loss of function and reduced function of DJ-1 trigger the onset of oxidative stress-related diseases, including Parkinson's disease [4, 94, 95], stroke [96, 97], familial amyloidotic polyneuropathy [40], chronic obstructive pulmonary disease (COPD) [98], and type II diabetes [99]. The oxidative status of C106 of DJ-1 determines all of the functions of DJ-1. Excess oxidation of C106 renders DJ-1 inactive, and highly oxidized DJ-1 has been observed in patients with Parkinson's disease and Alzheimer's disease. These results suggest that block of excessive oxidation of DJ-1 is a therapeutic target for the oxidative stress-related diseases stated earlier. Indeed, DJ-1-binding compounds that bind to the C106 region of DJ-1 showed neuroprotective activity against neurodegeneration in Parkinson's disease and stroke animal models through inhibition of excessive oxidation of C106 of DJ-1 [115–117].

Abbreviations

PD:	Parkinson's disease
PSF:	Polypyrimidine tract-binding protein-associated splicing factor
ROS:	Reactive oxygen species
Nrf2:	Nuclear factor erythroid-2 related factor 2
TH:	Tyrosine hydroxylase
DDC:	L-DOPA carboxylase
VMAT2:	Vesicular monoamine transporter 2
PTEN:	Phosphatase and Tensin homolog deleted from chromosome 10
ASK1:	Apoptosis signal-regulating kinase 1.

Conflict of Interests

The authors declare that they have no financial conflict of interests.

Acknowledgments

This work was supported by grants-in-aid from the Ministry of Education, Science, Culture and Sports and by the Program for Promotion of Fundamental Studies in Health Science of the National Institute of Biomedical Innovation (NIBIO) in Japan.

References

[1] D. N. Hauser and T. G. Hastings, "Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism," *Neurobiology of Disease*, vol. 51, pp. 35–42, 2013.

[2] Y. Sai, Z. Zou, K. Peng, and Z. Dong, "The Parkinson's disease-related genes act in mitochondrial homeostasis," *Neuroscience & Biobehavioral Reviews*, vol. 36, no. 9, pp. 2034–2043, 2012.

[3] D. Nagakubo, T. Taira, H. Kitaura et al., "DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras," *Biochemical and Biophysical Research Communications*, vol. 231, no. 2, pp. 509–513, 1997.

[4] V. Bonifati, P. Rizzu, M. J. van Baren et al., "Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism," *Science*, vol. 299, no. 5604, pp. 256–259, 2003.

[5] 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," *The Journal of Biological Chemistry*, vol. 278, no. 33, pp. 31380–31384, 2003.

[6] X. Tao and L. Tong, "Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease," *The Journal of Biological Chemistry*, vol. 278, no. 33, pp. 31372–31379, 2003.

[7] S. J. Lee, S. J. Kim, I. K. Kim et al., "Crystal structures of human DJ-1 and Escherichia coli Hsp31, which share an evolutionarily conserved domain," *The Journal of Biological Chemistry*, vol. 278, no. 45, pp. 44552–44559, 2003.

[8] M. A. Wilson, J. L. Collins, Y. Hod, D. Ringe, and G. A. Petsko, "The 1.1-Å resolution crystal structure of DJ-1, the protein mutated in autosomal recessive early onset Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 16, pp. 9256–9261, 2003.

[9] Q. Huai, Y. Sun, H. Wang et al., "Crystal structure of DJ-1/RS and implication on familial Parkinson's disease," *The FEBS Letters*, vol. 549, no. 1–3, pp. 171–175, 2003.

[10] S. Bandyopadhyay and M. R. Cookson, "Evolutionary and functional relationships within the DJ1 superfamily," *BMC Evolutionary Biology*, vol. 4, article 6, 2004.

[11] X. Du, I. G. Choi, R. Kim et al., "Crystal structure of an intracellular protease from Pyrococcus horikoshii at 2-Å resolution," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 26, pp. 14079–14084, 2000.

[12] P. Rizzu, D. A. Hinkle, V. Zhukareva et al., "DJ-1 colocalizes with tau inclusions: a link between parkinsonism and dementia," *Annals of Neurology*, vol. 55, no. 1, pp. 113–118, 2004.

[13] T. Yanagida, K. Takata, M. Inden et al., "Distribution of DJ-1, Parkinson's disease-related protein PARK7, and its alteration in 6-hydroxydopamine-treated hemiparkinsonian rat brain," *Journal of Pharmacological Sciences*, vol. 102, no. 2, pp. 243–247, 2006.

[14] T. Yanagida, J. Tsushima, Y. Kitamura et al., "Oxidative stress induction of DJ-1 protein in reactive astrocytes scavenges free radicals and reduces cell injury," *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 1, pp. 36–42, 2009.

[15] R. Bandopadhyay, A. E. Kingsbury, M. R. Cookson et al., "The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease," *Brain*, vol. 127, part 2, pp. 420–430, 2004.

[16] M. Neumann, V. Müller, K. Görner, H. A. Kretzschmar, C. Haass, and P. J. Kahle, "Pathological properties of the Parkinson's disease-associated protein DJ-1 in α-synucleinopathies and tauopathies: relevance for multiple system atrophy and Pick's disease," *Acta Neuropathologica*, vol. 107, no. 6, pp. 489–496, 2004.

[17] S. J. Mullett, R. L. Hamilton, and D. A. Hinkle, "DJ-1 immunoreactivity in human brain astrocytes is dependent on infarct presence and infarct age," *Neuropathology*, vol. 29, no. 2, pp. 125–131, 2009.

[18] J. Waak, S. S. Weber, A. Waldenmaier et al., "Regulation of astrocyte inflammatory responses by the Parkinson's disease-associated gene DJ-1," *The FASEB Journal*, vol. 23, no. 8, pp. 2478–2489, 2009.

[19] S. J. Mullett and D. A. Hinkle, "DJ-1 deficiency in astrocytes selectively enhances mitochondrial Complex I inhibitor-induced neurotoxicity," *Journal of Neurochemistry*, vol. 117, no. 3, pp. 375–387, 2011.

[20] N. J. Larsen, G. Ambrosi, S. J. Mullett, S. B. Berman, and D. A. Hinkle, "DJ-1 knock-down impairs astrocyte mitochondrial function," *Neuroscience*, vol. 196, pp. 251–264, 2011.

[21] K. Takahashi, T. Taira, T. Niki, C. Seino, S. M. M. Iguchi-Ariga, and H. Ariga, "DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor," *The Journal of Biological Chemistry*, vol. 276, no. 40, pp. 37556–37563, 2001.

[22] T. Niki, K. Takahashi-Niki, T. Taira, S. M. M. Iguchi-Ariga, and H. Ariga, "DJBP: a novel DJ-1-binding protein, negatively regulates the androgen receptor by recruiting histone deacetylase complex, and DJ-1 antagonizes this inhibition by abrogation of this complex," *Molecular Cancer Research*, vol. 1, no. 4, pp. 247–261, 2003.

[23] Y. Shinbo, T. Taira, T. Niki, S. M. Iguchi-Ariga, and H. Ariga, "DJ-1 restores p53 transcription activity inhibited by Topors/p53BP3," *International Journal of Oncology*, vol. 26, no. 3, pp. 641–648, 2005.

[24] J. Xu, N. Zhong, H. Wang et al., "The Parkinson's disease-associated DJ-1 protein is a transcriptional co-activator that protects against neuronal apoptosis," *Human Molecular Genetics*, vol. 14, no. 9, pp. 1231–1241, 2005.

[25] N. Zhong, C. Y. Kim, P. Rizzu et al., "DJ-1 transcriptionally up-regulates the human tyrosine hydroxylase by inhibiting the sumoylation of pyrimidine tract-binding protein-associated splicing factor," *The Journal of Biological Chemistry*, vol. 281, no. 30, pp. 20940–20948, 2006.

[26] C. M. Clements, R. S. McNally, B. J. Conti, T. W. Mak, and J. P. Y. Ting, "DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 41, pp. 15091–15096, 2006.

[27] J. E. Tillman, J. Yuan, G. Gu et al., "DJ-1 binds androgen receptor directly and mediates its activity in hormonally treated prostate cancer cells," *Cancer Research*, vol. 67, no. 10, pp. 4630–4637, 2007.

[28] J. Fan, H. Ren, E. Fei et al., "Sumoylation is critical for DJ-1 to repress p53 transcriptional activity," *The FEBS Letters*, vol. 582, no. 7, pp. 1151–1156, 2008.

[29] S. Ishikawa, T. Taira, K. Takahashi-Niki, T. Niki, H. Ariga, and S. M. M. Iguchi-Ariga, "Human DJ-1-specific transcriptional activation of tyrosine hydroxylase gene," *The Journal of Biological Chemistry*, vol. 285, no. 51, pp. 39718–39731, 2010.

[30] A. Mitsumoto, Y. Nakagawa, A. Takeuchi, K. Okawa, A. Iwamatsu, and Y. Takanezawa, "Oxidized forms of peroxiredoxins and DJ-1 on two-dimensional gels increased in response to sublethal levels of paraquat," *Free Radical Research*, vol. 35, no. 3, pp. 301–310, 2001.

[31] T. Yokota, K. Sugawara, K. Ito, R. Takahashi, H. Ariga, and H. Mizusawa, "Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition," *Biochemical and Biophysical Research Communications*, vol. 312, no. 4, pp. 1342–1348, 2003.

[32] T. Taira, Y. Saito, T. Niki, S. M. M. Iguchi-Ariga, K. Takahashi, and H. Ariga, "DJ-1 has a role in antioxidative stress to prevent cell death," *The EMBO Reports*, vol. 5, no. 2, pp. 213–218, 2004.

[33] 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," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 24, pp. 9103–9108, 2004.

[34] T. Kinumi, J. Kimata, T. Taira, H. Ariga, and E. Niki, "Cysteine-106 of DJ-1 is the most sensitive cysteine residue to hydrogen peroxide-mediated oxidation *in vivo* in human umbilical vein endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 317, no. 3, pp. 722–728, 2004.

[35] C. Martinat, S. Shendelman, A. Jonason et al., "Sensitivity to oxidative stress in DJ-1-deficient dopamine neurons: an ES-derived cell model of primary Parkinsonism," *PLoS Biology*, vol. 2, no. 11, article e327, 2004.

[36] M. Inden, T. Taira, Y. Kitamura et al., "PARK7 DJ-1 protects against degeneration of nigral dopaminergic neurons in Parkinson's disease rat model," *Neurobiology of Disease*, vol. 24, no. 1, pp. 144–158, 2006.

[37] S. Shendelman, A. Jonason, C. Martinat, T. Leete, and A. Abeliovich, "DJ-1 Is a redox-dependent molecular chaperone that inhibits α-synuclein aggregate formation," *PLoS Biology*, vol. 2, no. 11, article e362, 2004.

[38] W. Zhou, M. Zhu, M. A. Wilson, G. A. Petsko, and A. L. Fink, "The oxidation state of DJ-1 regulates its chaperone activity toward α-synuclein," *Journal of Molecular Biology*, vol. 356, no. 4, pp. 1036–1048, 2006.

[39] J. A. Olzmann, K. Brown, K. D. Wilkinson et al., "Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function," *The Journal of Biological Chemistry*, vol. 279, no. 9, pp. 8506–8515, 2004.

[40] S. Koide-Yoshida, T. Niki, M. Ueda et al., "DJ-1 degrades transthyretin and an inactive form of DJ-1 is secreted in familial amyloidotic polyneuropathy," *International Journal of Molecular Medicine*, vol. 19, no. 6, pp. 885–893, 2007.

[41] J. Chen, L. Li, and L. S. Chin, "Parkinson disease protein DJ-1 converts from a zymogen to a protease by carboxyl-terminal cleavage," *Human Molecular Genetics*, vol. 19, no. 12, pp. 2395–2408, 2010.

[42] H. Ooe, T. Taira, S. M. M. Iguchi-Ariga, and H. Ariga, "Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1," *Toxicological Sciences*, vol. 88, no. 1, pp. 114–126, 2005.

[43] H. M. Li, T. Niki, T. Taira, S. M. M. Iguchi-Ariga, and H. Ariga, "Association of DJ-1 with chaperones and enhanced

association and colocalization with mitochondrial Hsp70 by oxidative stress," *Free Radical Research*, vol. 39, no. 10, pp. 1091–1099, 2005.

[44] E. Junn, W. H. Jang, X. Zhao, B. S. Jeong, and M. M. Mouradian, "Mitochondrial localization of DJ-1 leads to enhanced neuroprotection," *Journal of Neuroscience Research*, vol. 87, no. 1, pp. 123–129, 2009.

[45] T. Hayashi, C. Ishimori, K. Takahashi-Niki et al., "DJ-1 binds to mitochondrial complex I and maintains its activity," *Biochemical and Biophysical Research Communications*, vol. 390, no. 3, pp. 667–672, 2009.

[46] I. Irrcher, H. Aleyasin, E. L. Seifert et al., "Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics," *Human Molecular Genetics*, vol. 19, no. 19, pp. 3734–3746, 2010.

[47] L. Y. Hao, B. I. Giasson, and N. M. Bonini, "DJ-1 is critical for mitochondrial function and rescues PINK1 loss of function," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 21, pp. 9747–9752, 2010.

[48] G. Krebiehl, S. Ruckerbauer, L. F. Burbulla et al., "Reduced basal autophagy and impaired mitochondrial dynamics due to loss of Parkinson's disease-associated protein DJ-1," *PLoS ONE*, vol. 5, no. 2, Article ID e9367, 2010.

[49] H. Ren, K. Fu, D. Wang, C. Mu, and G. Wang, "Oxidized DJ-1 interacts with the mitochondrial protein BCL-XL," *The Journal of Biological Chemistry*, vol. 286, no. 40, pp. 35308–35317, 2011.

[50] K. J. Thomas, M. K. McCoy, J. Blackinton et al., "DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy," *Human Molecular Genetics*, vol. 20, no. 1, pp. 40–50, 2011.

[51] X. Wang, T. G. Petrie, Y. Liu, J. Liu, H. Fujioka, and X. Zhu, "Parkinson's disease-associated DJ-1 mutations impair mitochondrial dynamics and cause mitochondrial dysfunction," *Journal of Neurochemistry*, vol. 121, no. 5, pp. 830–839, 2012.

[52] J. Y. Heo, J. H. Park, S. J. Kim et al., "DJ-1 null dopaminergic neuronal cells exhibit defects in mitochondrial function and structure: involvement of mitochondrial complex I assembly," *PLoS One*, vol. 7, no. 3, article e32629, 2012.

[53] A. Wagenfeld, J. Gromoll, and T. G. Cooper, "Molecular cloning and expression of rat contraception associated protein 1 (CAP1), a protein putatively involved in fertilization," *Biochemical and Biophysical Research Communications*, vol. 251, no. 2, pp. 545–549, 1998.

[54] J. E. Welch, R. R. Barbee, N. L. Roberts, J. D. Suarez, and G. R. Klinefelter, "Breakthrough in andrology: Sp22: a novel fertility protein from a highly conserved gene family," *Journal of Andrology*, vol. 19, no. 4, pp. 385–393, 1998.

[55] F. Le Naour, D. E. Misek, M. C. Krause et al., "Proteomics-based identification of RS/DJ-1 as a novel circulating tumor antigen in breast cancer," *Clinical Cancer Research*, vol. 7, no. 11, pp. 3328–3335, 2001.

[56] L. Allard, P. R. Burkhard, P. Lescuyer et al., "PARK7 and nucleoside diphosphate kinase A as plasma markers for the early diagnosis of stroke," *Clinical Chemistry*, vol. 51, no. 11, pp. 2043–2051, 2005.

[57] M. Waragai, J. Wei, M. Fujita et al., "Increased level of DJ-1 in the cerebrospinal fluids of sporadic Parkinson's disease," *Biochemical and Biophysical Research Communications*, vol. 345, no. 3, pp. 967–972, 2006.

[58] M. Waragai, M. Nakai, J. Wei et al., "Plasma levels of DJ-1 as a possible marker for progression of sporadic Parkinson's disease," *Neuroscience Letters*, vol. 425, no. 1, pp. 18–22, 2007.

[59] C. Maita, S. Tsuji, I. Yabe et al., "Secretion of DJ-1 into the serum of patients with Parkinson's disease," *Neuroscience Letters*, vol. 431, no. 1, pp. 86–89, 2008.

[60] Y. Tsuboi, H. Munemoto, S. Ishikawa, K. I. Matsumoto, S. M. M. Iguchi-Ariga, and H. Ariga, "DJ-1, a causative gene product of a familial form of Parkinson's disease, is secreted through microdomains," *The FEBS Letters*, vol. 582, no. 17, pp. 2643–2649, 2008.

[61] S. J. Kim, Y. J. Park, I. Y. Hwang, M. B. Youdim, K. S. Park, and Y. J. Oh, "Nuclear translocation of DJ-1 during oxidative stress-induced neuronal cell death," *Free Radical Biology & Medicine*, vol. 53, no. 4, pp. 936–950, 2012.

[62] J. Choi, M. C. Sullards, J. A. Olzmann et al., "Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases," *The Journal of Biological Chemistry*, vol. 281, no. 16, pp. 10816–10824, 2006.

[63] A. Mitsumoto and Y. Nakagawa, "DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin," *Free Radical Research*, vol. 35, no. 6, pp. 885–893, 2001.

[64] J. I. Lucas and I. Marín, "A new evolutionary paradigm for the Parkinson disease gene DJ-1," *Molecular Biology and Evolution*, vol. 24, no. 2, pp. 551–561, 2007.

[65] Y. Shinbo, T. Niki, T. Taira et al., "Proper SUMO-1 conjugation is essential to DJ-1 to exert its full activities," *Cell Death and Differentiation*, vol. 13, no. 1, pp. 96–108, 2006.

[66] G. Ito, H. Ariga, Y. Nakagawa, and T. Iwatsubo, "Roles of distinct cysteine residues in S-nitrosylation and dimerization of DJ-1," *Biochemical and Biophysical Research Communications*, vol. 339, no. 2, pp. 667–672, 2006.

[67] R. Rahman-Roblick, U. Hellman, S. Becker et al., "Proteomic identification of p53-dependent protein phosphorylation," *Oncogene*, vol. 27, no. 35, pp. 4854–4859, 2008.

[68] S. Yamaguchi, T. Yamane, K. Takahashi-Niki et al., "Transcriptional activation of low-density lipoprotein receptor gene by DJ-1 and effect of DJ-1 on cholesterol homeostasis," *PLoS One*, vol. 7, no. 5, article e38144, 2012.

[69] J. Fan, H. Ren, N. Jia et al., "DJ-1 decreases Bax expression through repressing p53 transcriptional activity," *The Journal of Biological Chemistry*, vol. 283, no. 7, pp. 4022–4030, 2008.

[70] I. Kato, H. Maita, K. Takahashi-Niki et al., "Oxidized DJ-1 inhibits p53 by sequestering p53 from promoters in a DNA-binding affinity-dependent manner," *Molecular and Cellular Biology*, vol. 33, no. 2, pp. 340–359, 2013.

[71] H. Nishinaga, K. Takahashi-Niki, T. Taira, A. Andreadis, S. M. M. Iguchi-Ariga, and H. Ariga, "Expression profiles of genes in DJ-1-knockdown and L166P DJ-1 mutant cells," *Neuroscience Letters*, vol. 390, no. 1, pp. 54–59, 2005.

[72] W. Zhou and C. R. Freed, "DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T α-synuclein toxicity," *The Journal of Biological Chemistry*, vol. 280, no. 52, pp. 43150–43158, 2005.

[73] M. S. Goldberg, A. Pisani, M. Haburcak et al., "Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial parkinsonism-linked gene DJ-1," *Neuron*, vol. 45, no. 4, pp. 489–496, 2005.

[74] R. H. Kim, P. D. Smith, H. Aleyasin et al., "Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6- tetrahydropyrindine (MPTP) and oxidative stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 14, pp. 5215–5220, 2005.

[75] L. Chen, B. Cagniard, T. Mathews et al., "Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice," *The Journal of Biological Chemistry*, vol. 280, no. 22, pp. 21418–21426, 2005.

[76] S. Ishikawa, T. Taira, T. Niki et al., "Oxidative status of DJ-1-dependent activation of dopamine synthesis through interaction of tyrosine hydroxylase and 4-dihydroxy-L-phenylalanine (L-DOPA) decarboxylase with DJ-1," *The Journal of Biological Chemistry*, vol. 284, no. 42, pp. 28832–28844, 2009.

[77] S. Ishikawa, Y. Tanaka, K. Takahashi-Niki, T. Niki, H. Ariga, and S. M. M. Iguchi-Ariga, "Stimulation of vesicular monoamine transporter 2 activity by DJ-1 in SH-SY5Y cells," *Biochemical and Biophysical Research Communications*, vol. 421, no. 4, pp. 813–818, 2012.

[78] F. Yotsumoto, A. Sanui, T. Fukami et al., "Efficacy of ligand-based targeting for the EGF system in cancer," *Anticancer Research*, vol. 29, no. 11, pp. 4879–4885, 2009.

[79] K. Takeuchi and F. Ito, "EGF receptor in relation to tumor development: molecular basis of responsiveness of cancer cells to EGFR-targeting tyrosine kinase inhibitors," *FEBS Journal*, vol. 277, no. 2, pp. 316–326, 2010.

[80] R. H. Kim, M. Peters, Y. Jang et al., "DJ-1, a novel regulator of the tumor suppressor PTEN," *Cancer Cell*, vol. 7, no. 3, pp. 263–273, 2005.

[81] Y. C. Kim, H. Kitaura, T. Taira, S. M. M. Iguchi-Ariga, and H. Ariga, "Oxidation of DJ-1-dependent cell transformation through direct binding of DJ-1 to PTEN," *International Journal of Oncology*, vol. 35, no. 6, pp. 1331–1341, 2009.

[82] B. Tang, H. Xiong, P. Sun et al., "Association of PINK1 and DJ-1 confers digenic inheritance of early-onset Parkinson's disease," *Human Molecular Genetics*, vol. 15, no. 11, pp. 1816–1825, 2006.

[83] K. Hattori, I. Naguro, C. Runchel, and H. Ichijo, "The roles of ASK family proteins in stress responses and diseases," *Cell Communication and Signaling*, vol. 7, article 9, 2009.

[84] S. Shiizaki, I. Naguro, and H. Ichijo, "Activation mechanisms of ASK1 in response to various stresses and its significance in intracellular signaling," *Advances in Biological Regulation*, vol. 53, no. 1, pp. 135–144, 2013.

[85] T. Iriyama, K. Takeda, H. Nakamura et al., "ASK1 and ASK2 differentially regulate the counteracting roles of apoptosis and inflammation in tumorigenesis," *The EMBO Journal*, vol. 28, no. 7, pp. 843–853, 2009.

[86] M. Benhar, D. Engelberg, and A. Levitzki, "ROS, stress-activated kinases and stress signaling in cancer," *The EMBO Reports*, vol. 3, no. 5, pp. 420–425, 2002.

[87] R. A. Thandavarayan, K. Watanabe, M. Ma et al., "14-3-3 protein regulates Ask1 signaling and protects against diabetic cardiomyopathy," *Biochemical Pharmacology*, vol. 75, no. 9, pp. 1797–1806, 2008.

[88] E. Yamamoto, Y. F. Dong, K. Kataoka et al., "Olmesartan prevents cardiovascular injury and hepatic steatosis in obesity and diabetes, accompanied by apoptosis signal regulating Kinase-1 inhibition," *Hypertension*, vol. 52, no. 3, pp. 573–580, 2008.

[89] Y. Izumiya, S. Kim, Y. Izumi et al., "Apoptosis signal-regulating kinase 1 plays a pivotal role in angiotensin II-induced cardiac hypertrophy and remodelling," *Circulation Research*, vol. 93, no. 9, pp. 874–883, 2003.

[90] O. Yamaguchi, Y. Higuchi, S. Hirotani et al., "Targeted deletion of apoptosis signal-regulating kinase 1 attenuates left ventricular remodeling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15883–15888, 2003.

[91] H. Nishitoh, A. Matsuzawa, K. Tobiume et al., "ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats," *Genes & Development*, vol. 16, no. 11, pp. 1345–1355, 2002.

[92] H. Nishitoh, H. Kadowaki, A. Nagai et al., "ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1," *Genes & Development*, vol. 22, no. 11, pp. 1451–1464, 2008.

[93] H. Kadowaki, H. Nishitoh, F. Urano et al., "Amyloid β induces neuronal cell death through ROS-mediated ASK1 activation," *Cell Death and Differentiation*, vol. 12, no. 1, pp. 19–24, 2005.

[94] P. M. Abou-Sleiman, D. G. Healy, N. Quinn, A. J. Lees, and N. W. Wood, "The role of pathogenic DJ-1 mutations in Parkinson's disease," *Annals of Neurology*, vol. 54, no. 3, pp. 283–286, 2003.

[95] R. Hering, K. M. Strauss, X. Tao et al., "Novel homozygous p.E64D mutation in DJ1 in early onset Parkinson disease (PARK7)," *Human Mutation*, vol. 24, no. 4, pp. 321–329, 2004.

[96] H. Aleyasin, M. W. C. Rousseaux, M. Phillips et al., "The Parkinson's disease gene DJ-1 is also a key regulator of stroke-induced damage," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 47, pp. 18748–18753, 2007.

[97] D. Yanagisawa, Y. Kitamura, M. Inden et al., "DJ-1 protects against neurodegeneration caused by focal cerebral ischemia and reperfusion in rats," *Journal of Cerebral Blood Flow and Metabolism*, vol. 28, no. 3, pp. 563–578, 2008.

[98] D. Malhotra, R. Thimmulappa, A. Navas-Acien et al., "Decline in NRF2-regulated antioxidants in chronic obstructive pulmonary disease lungs due to loss of its positive regulator, DJ-1," *American Journal of Respiratory and Critical Care Medicine*, vol. 178, no. 6, pp. 592–604, 2008.

[99] D. Jain, R. Jain, D. Eberhard et al., "Age- and diet-dependent requirement of DJ-1 for glucose homeostasis in mice with implications for human type 2 diabetes," *Journal of Molecular Cell Biology*, vol. 4, no. 4, pp. 221–230, 2012.

[100] E. Junn, H. Taniguchi, B. S. Jeong, X. Zhao, H. Ichijo, and M. M. Mouradian, "Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 27, pp. 9691–9696, 2005.

[101] S. Karunakaran, L. Diwakar, U. Saeed et al., "Activation of apoptosis signal regulating kinase 1 (ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: protection by α-lipoic acid," *The FASEB Journal*, vol. 21, no. 9, pp. 2226–2236, 2007.

[102] J. Waak, S. S. Weber, K. Görner et al., "Oxidizable residues mediating protein stability and cytoprotective interaction of DJ-1 with apoptosis signal-regulating kinase 1," *The Journal of Biological Chemistry*, vol. 284, no. 21, pp. 14245–14257, 2009.

[103] N. Lev, D. Ickowicz, Y. Barhum, S. Lev, E. Melamed, and D. Offen, "DJ-1 protects against dopamine toxicity," *Journal of Neural Transmission*, vol. 116, no. 2, pp. 151–160, 2009.

[104] L. Aron, P. Klein, T. T. Pham, E. R. Kramer, W. Wurst, and R. Klein, "Pro-survival role for Parkinson's associated gene DJ-1 revealed in trophically impaired dopaminergic neurons," *PLoS Biology*, vol. 8, no. 4, article e1000349, 2010.

[105] H. Gao, W. Yang, Z. Qi et al., "DJ-1 protects dopaminergic neurons against rotenone-induced apoptosis by enhancing ERK-dependent mitophagy," *Journal of Molecular Biology*, vol. 423, no. 2, pp. 232–248, 2012.

[106] Y. Mizuno, S. Ohta, M. Tanaka et al., "Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease," *Biochemical and Biophysical Research Communications*, vol. 163, no. 3, pp. 1450–1455, 1989.

[107] W. D. Parker Jr., S. J. Boyson, and J. K. Parks, "Abnormalities of the electron transport chain in idiopathic Parkinson's disease," *Annals of Neurology*, vol. 26, no. 6, pp. 719–723, 1989.

[108] A. H. V. Schapira, J. M. Cooper, D. Dexter, J. B. Clark, P. Jenner, and C. D. Marsden, "Mitochondrial complex I deficiency in Parkinson's disease," *Journal of Neurochemistry*, vol. 54, no. 3, pp. 823–827, 1990.

[109] M. Orth and A. H. V. Schapira, "Mitochondrial involvement in Parkinson's disease," *Neurochemistry International*, vol. 40, no. 6, pp. 533–541, 2002.

[110] Y. Mizuno, H. Yoshino, S. I. Ikebe et al., "Mitochondrial dysfunction in Parkinson's disease," *Annals of Neurology*, vol. 44, no. 3, pp. S99–S109, 1998.

[111] E. Giaime, H. Yamaguchi, C. A. Gautier, T. Kitada, and J. Shen, "Loss of DJ-1 does not affect mitochondrial respiration but increases ROS production and mitochondrial permeability transition pore opening," *PLoS One*, vol. 7, no. 7, aricle e40501, 2012.

[112] L. Zhang, M. Shimoji, B. Thomas et al., "Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis," *Human Molecular Genetics*, vol. 14, no. 14, pp. 2063–2073, 2005.

[113] C. Maita, H. Maita, S. M. M. Iguchi-Ariga, and H. Ariga, "Monomer DJ-1 and its N-terminal sequence are necessary for mitochondrial localization of DJ-1 mutants," *PLoS One*, vol. 8, no. 1, article e54087, 2013.

[114] L. de Mena, E. Coto, E. Sánchez-Ferrero et al., "Mutational screening of the mortalin gene (HSPA9) in Parkinson's disease," *Journal of Neural Transmission*, vol. 116, no. 10, pp. 1289–1293, 2009.

[115] S. Miyazaki, T. Yanagida, K. Nunome et al., "DJ-1-binding compounds prevent oxidative stress-induced cell death and movement defect in Parkinson's disease model rats," *Journal of Neurochemistry*, vol. 105, no. 6, pp. 2418–2434, 2008.

[116] Y. Kitamura, S. Watanabe, M. Taguchi et al., "Neuroprotective effect of a new DJ-1-binding compound against neurodegeneration in Parkinson's disease and stroke model rats," *Molecular Neurodegeneration*, vol. 6, no. 1, article 48, 2011.

[117] M. Inden, Y. Kitamura, K. Takahashi et al., "Protection against dopaminergic neurodegeneration in Parkinson's disease-model animals by a modulator of the oxidized form of DJ-1, a wild-type of familial Parkinson's disease-linked PARK7," *Journal of Pharmacological Sciences*, vol. 117, no. 3, pp. 189–203, 2011.

Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2013, Article ID 719407, 7 pages
http://dx.doi.org/10.1155/2013/719407

Research Article

The P66Shc/Mitochondrial Permeability Transition Pore Pathway Determines Neurodegeneration

Costanza Savino, PierGiuseppe Pelicci, and Marco Giorgio

Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, 20139 Milan, Italy

Correspondence should be addressed to Marco Giorgio; marco.giorgio@ieo.eu

Received 13 February 2013; Revised 19 April 2013; Accepted 24 April 2013

Academic Editor: Renata Santos

Mitochondrial-mediated oxidative stress and apoptosis play a crucial role in neurodegenerative disease and aging. Both mitochondrial permeability transition (PT) and swelling of mitochondria have been involved in neurodegeneration. Indeed, knockout mice for cyclophilin-D (Cyc-D), a key regulatory component of the PT pore (PTP) that triggers mitochondrial swelling, resulted to be protected in preclinical models of multiple sclerosis (MS), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). However, how neuronal stress is transduced into mitochondrial oxidative stress and swelling is unclear. Recently, the aging determinant p66Shc that generates H_2O_2 reacting with cytochrome c and induces oxidation of PTP and mitochondrial swelling was found to be involved in MS and ALS. To investigate the role of p66Shc/PTP pathway in neurodegeneration, we performed experimental autoimmune encephalomyelitis (EAE) experiments in p66Shc knockout mice (p66Shc−/−), knock out mice for cyclophilin-D (Cyc-D−/−), and p66Shc Cyc-D double knock out (p66Shc/Cyc-D−/−) mice. Results confirm that deletion of p66Shc protects from EAE without affecting immune response, whereas it is not epistatic to the Cyc-D mutation. These findings demonstrate that p66Shc contributes to EAE induced neuronal damage most likely through the opening of PTP suggesting that p66Shc/PTP pathway transduces neurodegenerative stresses.

1. Introduction

The p66Shc protein is the largest isoform encoded by the ShcA locus located in the chromosome 1 in the human or chromosome 3 in the mouse genome. The ShcA locus encodes three isoforms through two different promoters, one for the p46 and p52 isoforms and the other one for the p66 isoform. Notably, the p66Shc isoform is peculiar of vertebrates being conserved in *Fucu*, *Xenophus*, *Rattus*, *Mus*, and *Homo* but not in *Saccharomyces*, *Caenorhabditis*, and *Drosophila* [1]. The p52Shc and p46Shc function as adaptor protein in signal transduction pathways linking different activated receptor tyrosine kinases to the Ras pathway. P66Shc is not involved in Ras activation, although p66Shc has the typical domain organization of all members of the Shc family of adaptor proteins. Instead, p66Shc functions in the intracellular pathways that convert oxidative signals into apoptosis [2, 3].

A fraction of p66Shc has been observed within the mitochondrial intermembrane space [4], and *in vitro* experiments revealed that the recombinant p66Shc human protein interacts and oxidizes Fe^{2+} cytochrome c to form H_2O_2 through a redox center that has been mapped to the N-terminus of the p66Shc protein and is missing in the other two ShcA isoforms [5]. Thus, mitochondrial p66Shc sequesters electrons from the respiratory chain to generate reactive oxygen species (ROS).

P66Shc function is tightly regulated at multiple levels. First of all the total amount of p66Shc is regulated by transcriptional [6] and posttranslational mechanisms. In particular, the half-life of p66Shc has been demonstrated to increase upon apoptotic stimulation, notably in a p53-dependent manner [7]. The localization of p66Shc into mitochondrial intermembrane space is regulated by p66Shc posttranslational modifications including serine phosphorylation by stress kinases like Jnk-1 and Pkc-B and prolyl-isomerization by Pin-1, that induce p66Shc translocation within mitochondria [8].

A further level of activation of p66Shc mitochondrial function is represented by the availability of unbound

p66Shc within mitochondrial vesicles. In fact, mitochondrial p66Shc has been observed to associate with a high molecular weight complex of about 670 KDa and to the mitochondrial chaperon mtHsp70. Notably, treatment of cells with proapoptotic stimuli such as Ultraviolet radiation or H_2O_2 induces the dissociation of this complex and thus the release of monomeric p66Shc free to react with cytochrome c [9]. Finally, the oxidation of cysteine residues and oligomerization state of p66Shc have been reported to regulate its redox function within mitochondria [10].

The activation of p66Shc, and the consequent H_2O_2 accumulation in the mitochondria, impacts on the integrity of mitochondrial inner membrane and transmembrane potential [9]. The addition of recombinant p66Shc protein to isolated mouse liver mitochondria is sufficient to induce opening of the PTP and ballooning of the vesicles [5]. The key event leading to the mitochondrial swelling is indeed the opening of the permeability transition pore (PTP), a high-conductance inner membrane channel whose molecular components have not been identified, that triggers the inner membrane permeability to solutes and as consequence to water [11].

These events are central node of cell death. During apoptosis mitochondria undergo structural and functional remodeling leading to the swelling of the organelles with the subsequent release of apoptogenic factors including cytochrome c, Smac/DIABLO, AIF, and Omi/HtrA2 into the cytosol. These factors activate a cascade of proteases responsible for nuclear DNA fragmentation and finally cell death [12]. The PTP open-closed state is regulated by multiple effectors that act on various sites [13]. In particular, it has been shown that reactive oxygen species producing by mitochondrial respiration are key regulators of PTP opening [14].

Consistently with the role proposed for oxidative stress on cell death and aging [15], primary mouse embryonic fibroblasts (MEFs) derived from p66Shc−/− embryos have lower intracellular concentration of ROS, as revealed by the reduced oxidation of ROS-sensitive probes and the reduced accumulation of endogenous markers of oxidative stress (8-oxo-guanosine) [7]. Likewise, p66Shc−/− mice have diminished levels of both systemic isoprostane [16] and intracellular (nitrotyrosines, 8-oxo-guanosine) oxidative stress [7].

Moreover, p66Shc−/− cells were shown to be resistant to apoptosis induced by a variety of different signals, including H_2O_2, UV, staurosporine, taxol, growth factor deprivation, calcium ionophore, osmotic shock, and CD3-CD4 crosslinking [2], and similarly different tissues of the p66Shc−/− mice were found to be resistant to apoptosis induced by paraquat [17], hypercholesterolemia [16], hyperglycemia [18], immunotoxicity [19], and ischemia [20].

Finally it is not surprising that p66Shc−/− mice resulted to be protected from aging-associated diseases, such as metabolic syndrome [20, 21] atherosclerosis [16], diabetes [18], and neurodegeneration [19, 22, 23] and show prolonged life span [17, 21, 22], and p66Shc/PTP pathway appears a crucial pathway of stress response involved in tissue dysfunction.

2. Materials and Methods

2.1. Cells. NG108-15, N2A, and the SH-SY5Y were grown at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) the NG108-15, Modified Eagle's medium (MEM) the N2A, and the SH-SY5Y with F12 1 : 1 and 1% NeAA, supplemented with 10% (or 15% the SH-SY5Y) fetal bovine serum S.A. (Invitrogen, Carlsbad, CA, USA) and penicillin-streptomycin and glutamine (100 units/mL, 100 μg/mL) (Invitrogen).

Kelly and PC12 were cultured in RPMI 1640 medium (Gibco-BRL, Eggenstein, Germany) supplemented with 10% FBS (and 5% Horse serum the PC12) and penicillin-streptomycin (100 units/mL, 100 μg/mL) (Invitrogen), under 5% CO_2 at 37°C.

2.2. Western Blotting. Cells were washed twice with ice-cold PBS and lysed with 200 μL of lysis buffer. Lysates were vortexed and incubated on ice for 15 min twice and then cleared by spinning at 13.000 rpm for 5 min at 4°C. Proteins were separated by 10% SDS—PAGE gels and immunoblotted according to standard western blotting procedures using primary antibody (polyclonal anti-shc, BD Biosciences).

2.3. Mice. P66Shc−/−, Cyc-D−/−, and p66Shc/Cyc-D−/− mice were bred in the certified IFOM-IEO campus animal facility in accordance with national and institutional guidelines.

Mice were housed in an air-conditioned room (temperature 21 ± 1°C, relative humidity 60 ± 10%) with a white-red light cycle (lights on from 07:00 to 19:00) and with *ad libitum* food availability (2018S Teklad Global 18% Protein Rodent Diet, provided by Harlan Teklad) and drinking water (autoclaved tap water).

All the *in vivo* experiments were performed in accordance with Italian laws and regulations.

2.4. EAE. EAE was induced in p66Shc−/−, Cyc-D−/−, p66Shc/Cyc-D−/−, and C57BL/6 (Wilde type, WT) female mice (6–8 weeks of age). WT mice were obtained from Charles River (Calco, Italy) and housed in specific pathogen-free conditions, allowing access to food and water ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines in compliance with national (D.L. n. 116, G.U., suppl. 40, February 18, 1992) and international laws and policies (EU Council Directive 86/609, OJ L 358, 1, December 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). The protocols for the proposed investigation were reviewed and approved by the Animal of the European Institute of Oncology. EAE was induced by subcutaneous immunization in the flanks with a total of 200 Ag of MOG35–55 (Multiple Peptide Systems, San Diego, CA, USA) in incomplete Freund's adjuvant (Sigma, St. Louis, MO, USA) supplemented with 8 mg/mL of *Mycobacterium tuberculosis* (strain H37RA; Difco, Detroit, MI, USA).

Mice received 500 ng of pertussin toxin (Sigma) i.v. at the time of immunization and 48 h later. Weight and clinical

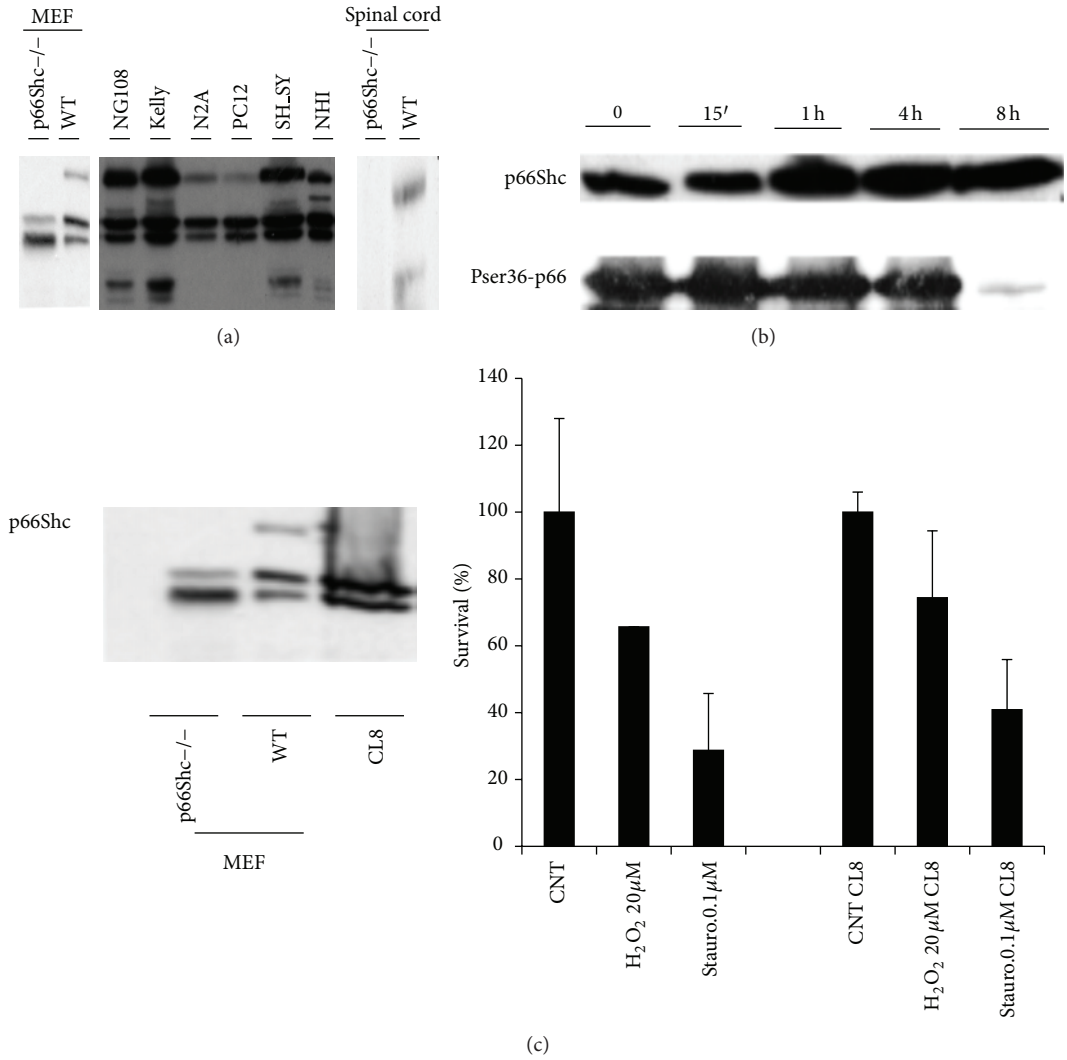


Figure 1: (a) P66Shc expression in different cell lines and spinal cord tissue. Western blot analysis of p66Shc was performed on whole-cell extracts from primary mouse embryonic fibroblasts (MEF), NG108, Kelly (human neuroblastoma), N2A (mouse), PC12(rat), SH-SY5Y (human neuroblastoma), NIH cells lines, and protein extract from mouse spinal cord. (b) Expression of p66Shc (upper panel) after UV treatment and (lower panel) levels of PhopsorylatedSer36-p66Shc in Kelly cells. (c) Expression of p66Shc upon specific p66Shc RNAi Interference on Kelly and response 20 μM H_2O_2 and 0.1 mM Staurosporine.

score were recorded daily (0 = healthy, 1 = flaccid tail, 2 = ataxia, and/or hind-limbs paresis, or slow righting reflex, 3 = paralysis of hind limb and/or paresis of forelimbs, 4 = paraparesis of fore limb, and 5 = moribund or death). The food pellets and the drinking water were placed on Petri plates on the floor of the cage to enable sick mice to eat and drink.

2.5. ELISA. Anti-$MOG_{35–55}$ antibody titers were examined in the sera of the same animal groups as described previously at day 10 after immunization. Blood from these animals was obtained by heart puncture. Sera were collected and applied to $MOG_{35–55}$-coated maxisorb 96-well ELISA plates (Nalge Nunc International, Rochester, NY) in serial dilutions. The plates were incubated for 1 h at room temperature and washed four times, and 60 μL of 1 : 2500 diluted [HRP] α-mouse (Amersham) peroxidase conjugated mouse anti-IgG were applied to the wells. The plates were incubated for 40 min at room temperature and washed three times, and 60 μL of ABTS + H_2O_2 substrate were applied to the wells. The reaction was performed at room temperature for 30 min in the dark. The level of reaction product was assessed as optic density (OD) at 415 nm on a standard plate reader, and the data were presented as mean_SD. OD with $n = 3$ independent measurements per group.

IL-6, IFN-γ, and TNF-α were measured in supernatants of splenocytes (see the following) by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturers' instructions.

2.6. Proliferation Assay. Cells from spleens of p66Shc−/− and WT mice, stimulated or not with MOG, were cocultured for

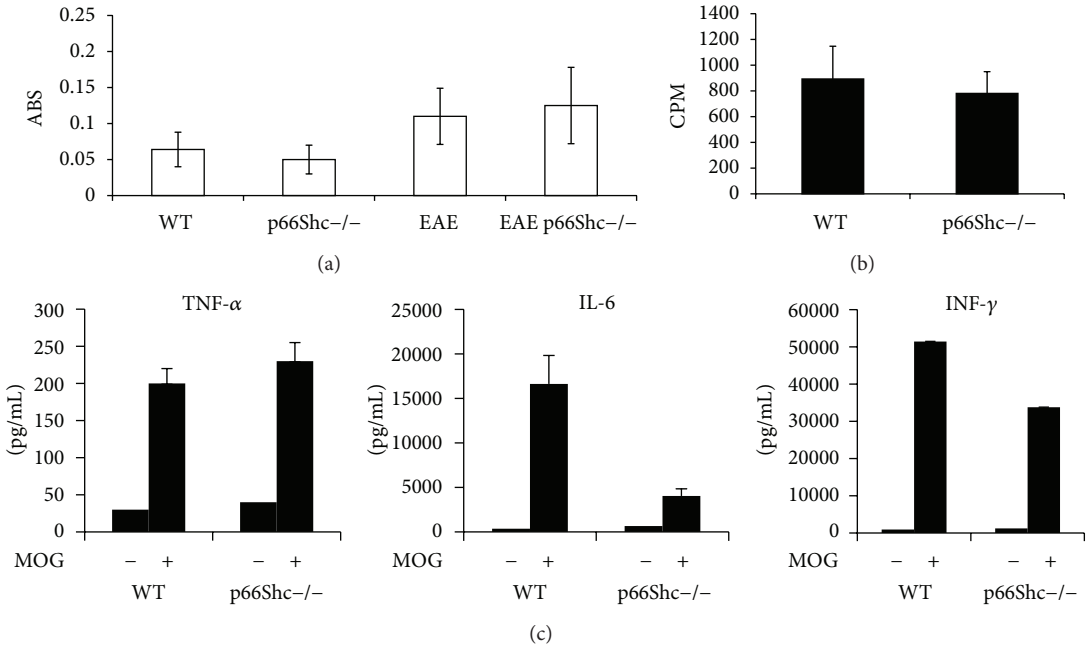


Figure 2: (a) ELISA assay for anti-MOG35–55 antibodies. (b) Proliferation assay. Cells from spleens, stimulated with MOG, were cocultured for 48 h and were radiolabelled with 9,25 kBq well-1 of methyl-3H thymidine in the last 16 h of coculture. The cells are harvested and counted in a liquid scintillation counter. (c) Cytokines production in medium of splenocytes from WT and p66Shc−/− mice. Results are representative of one of two experiments, each with five mice per group.

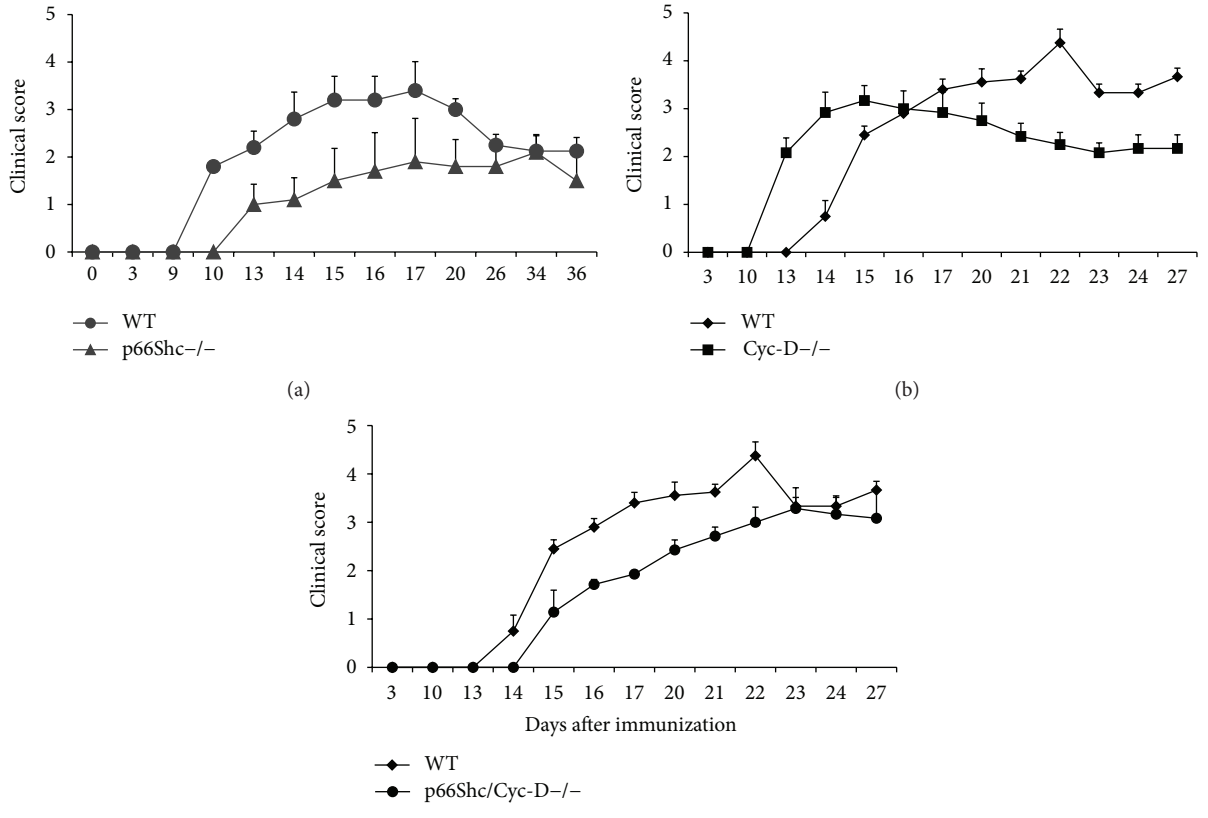


Figure 3: (a) EAE disease signs scores (nonparametric scale) in WT (C57BL/6) and P66Shc−/− mice have a less severe EAE disease course than WT mice. (b) EAE disease signs scores (nonparametric scale) in WT (C57BL/6) and Cyc-D−/− mice. (c) EAE disease signs scores (nonparametric scale) in WT (C57BL/6) and p66Shc/Cyc-D−/− mice.

48 h and were radiolabelled with 9,25 kBq/well of methyl-3H thymidine in the last 16 h of coculture. The cells are harvested and counted by a beta radiation liquid scintillation counter.

3. Results and Discussion

3.1. P66Shc Determines Neuronal Cells Apoptosis. P66Shc expression has been already demonstrated in neuronal cell lines from different mammals [23]. We confirmed by Western Blot (WB) analysis the expression of p66Shc in several neuronal cell lines, in particular Kelly and SH-NY of human origin, and in the mouse spinal cord (Figure 1(a)). The phosphorylation of p66Shc at Serine 36 is the event activating the proapoptotic function of p66Shc [8, 16]. WB analysis revealed p66Shc Ser36 phosphorylation in neuronal cell lines upon different challenges (Figure 1(b), [8]). Then, knocking down the p66Shc expression has been found to increase survival in Kelly cells (Figure 1(c)) as well as in other neuronal cells [2, 23] upon oxidative stress and other pro-apoptotic challenges. In agreement, the overexpression of p66Shc but not of the p66ShcSer36Ala mutant increased cell death in neuronal cells [23].

All these findings indicate that p66shc is present in neuronal cells, including human ones and confers sensitivity to apoptosis.

3.2. P66Shc Does Not Affect Immune Response in EAE. EAE is an acute or chronic-relapsing, demyelinating autoimmune disease model that closely resembles the human MS [24]. During EAE, as a consequence of specific myelin antigen experimental autoimmunization, activated T- and B-lymphocytes infiltrate the central nervous system (CNS) where they produce large amounts of cytokines that together with activated microglia lead to demyelination and axonal degeneration [25, 26]. Several evidences indicate that oligodendrocytes, the myelin-forming glial cells in the CNS, are sensitive to cell death stimuli such as cytotoxic cytokines, antimyelin antibodies, nitric oxide, and oxidative stress. Indeed, apoptosis has been established in glial cells of both human MS patients and EAE animal models, and it has been proposed to be the crucial mechanism of MS and EAE associated dysfunctions [27, 28].

To determine the role of p66Shc in EAE we compared the results of EAE in p66Shc−/− and WT mice. First, we immunized a total number of 9 isogenic WT and 9 p66Shc−/− C57BL/6 mice with myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide following standard protocols [29, 30]. At days 8, 10, and 36 upon MOG immunization, we sacrificed the mice (3 at each time) and extracted the blood and spleens to investigate anti-MOG antibodies titer and MOG-induced splenocyte proliferation, respectively. ELISA test using MOG coated wells revealed that the concentration of antibodies against MOG raised at the same time and extent in both WT and p66Shc−/− mice (Figure 2(a)). Likewise, thymidine incorporation as measure of splenocytes proliferation upon *in vitro* stimulation with MOG resulted to be not significantly altered by the deletion of p66Shc (Figure 2(b)). The secretion of TNF-alpha, IL-6, and interferon gamma by WT and p66Shc−/− splenocytes was comparable as well *in vitro* (Figure 2(c)).

Therefore, p66Shc did not appear to influence humoral and cellular immune response to MOG immunization.

3.3. P66Shc Deletion Delays EAE . Then we performed EAE in WT and p66Shc−/− age matched female mice. Clinical score and weight were recorded daily using a nonparametric scale. Starting from 10 days (approximately depending on the different experiments) after MOG immunization, WT mice developed clinical symptoms, manifested as limb weakness and paralysis (WT score at day 10, 1,80 ± 0,3, see Figure 3(a)) while p66Shc−/− mice did not (p66Shc−/− score at day 10, 0,0 see Figure 3(a)). The onset of the disease in p66Shc−/− mice was significantly delayed (Figure 3(a) and Table 1). All over the experiment, the p66Shc−/− showed milder paralysis than WT and consistently a lower disease severity score than WT mice (Figure 3(a)).

Notably, the 20% EAE WT mice died, whereas no p66Shc−/− died. P66Shc deletion was found to protect significantly from body weight loss as well (day 20 body weight of p66Shc−/− 19,20 ± 1,36 versus WT 16,85 ± 1,13).

3.4. Cyclophilin-D Deletion Did Not Change EAE Expression in p66Shc−/− Mice. To determine whether the protective effect of p66Shc deletion was mediated by PTP, we investigated the genetic interaction between the mutations of p66Shc and of Cyc-D that increases the opening threshold of PTP and was found to ameliorate EAE as well (Figure 3(b), [31]). Indeed, the exact molecular composition of the PTP is still debated [11]. However, some putative components have been indicated, such as the voltage-dependent anion channel (VDAC) located on the outer-mitochondrial membrane, the adenine nucleotide translocase (ANT, located on the inner mitochondrial membrane), and a matrix protein, Cyc-D, but results from knockout mice of these proteins confirmed only for Cyc-D a role in mitochondrial PT, although Cyc-D−/− animals develop normally and undergo apoptosis in response to certain insults [32], suggesting that additional proteins are probably involved.

So, we generated p66Shc/Cyc-D−/− mice by crossing p66Shc−/− and Cyc-D−/− mice both in C57BL/6 background. Then, we performed EAE on the p66Shc/Cyc-D−/− mice, and we evaluated the evolution of their disease over a month. The onset and development of EAE in p66Shc/Cyc-D−/− mice resulted to be identical to those observed for p66Shc−/− mice (Figure 3(c) and Table 2). Notably, the early onset of disease typical of Cyc-D null mice (Figure 3(b), [31]) was lost when also p66Shc was mutated suggesting that p66Shc is necessary for the expression of disease before time by the Cyc-D mutation.

These results indicate that the deletion Cyc-D was not epistatic to the deletion of p66Shc with respect to the to EAE protection.

Table 1: Clinical EAE parameters in WT and p66Shc−/− mice.

Strain	Disease onset (day 10) score	Disease onset (day 13) score	Number of mice with score ≥3	Mean maximum score	AUC (days)
WT	1.80 ± 0.3	2.20 ± 0.34	3/5	3.20 ± 0.50	21.95 ± 3.54
P66Shc−/−	0	1.00 ± 0.43	2/5	2.5 ± 0.62	13.65 ± 4.91

Data are mean of three independent experiments ± SE. (AUC: area under the curve) values are calculated at day 20 from the immunization.

Table 2: Clinical EAE parameters in WT, Cyc-D−/−, and p66Shc/Cyc-D−/− mice.

Strain	Disease onset (day 11) score	Disease onset (day 13) score	Survival at sacrifice Day 27	Number of mice with score ≥3	Day of maximal score	Mean maximum score	AUC (days)
WT	0.75 ± 0.33	2.45 ± 0.18	3/10	10/10	20	4.7 ± 0.21	22.10 ± 2.91
Cyc-D−/−	0	2.08 ± 0.30	6/6	4/6	14	3.17 ± 0.31	26.83 ± 2.55
P66Shc/Cyc-D−/−	0	1.14 ± 0.37	6/7	5/7	21	3.85 ± 0.35	23.50 ± 2.24

Data are mean three independent experiments ± SE. Values are calculated at day 20 from the immunization.

4. Conclusions

Neurodegenerative disease is complex trait disorder that involves several stress response pathways inducing mitochondrial apoptosis. Classical genetics studies, to identify susceptibility genes, are more difficult than for simple mendelian disorders because of the number of loci involved, the incomplete penetrance, and the important role of environmental factors.

Results form *in vitro* experiments showing that p66Shc increases oxidative stress to the mitochondria and the Cyc-D-dependent PTP particularly supported the hypothesis of a genetic interaction of p66Shc and Cyc-D. So, to validate the role of the p66Shc-PTP pathway in neurodegeneration we have studied in mice the epistasis of p66Shc and Cyc-D null mutations on the susceptibility to EAE.

Results from these experiments revealed that both mutations mitigated the disease, but their effects were not additive. These findings indicate that the p66Shc and Cyc-D are epistatic and validate the hypothesis that p66Shc activation by stresses converges to the PTP opening to induce mitochondrial swelling in neurons.

Therefore, early detection of p66Shc activation (expression levels, specific phosphorylation) or its inhibition may represent valid approaches to treat neurodegeneration.

Acknowledgments

The authors thank Mike Forte of the Oregon Health and Science University, USA, and Paolo Bernardi of the University of Padova, Italy, for sharing the Cyc-D−/− mice, Maria Rescigno of the EIO and Iliyan D. Iliev for the experiment on T-cells, Mirella Trinei (EIO) for many helpful discussions, and Massimo Stendardo (EIO) for helping in taking care of the mice. This work was supported by National Institutes of Health Grant no. 1P01AG025532-01A1 awarded to PG.P.

References

[1] L. Luzi, S. Confalonieri, P. P. Di Fiore, and P. G. Pelicci, "Evolution of Shc functions from nematode to human," *Current Opinion in Genetics and Development*, vol. 10, no. 6, pp. 668–674, 2000.

[2] E. Migliaccio, M. Giorgio, and P. G. Pelicci, "Apoptosis and aging: role of p66Shc redox protein," *Antioxidants and Redox Signaling*, vol. 8, no. 3-4, pp. 600–608, 2006.

[3] M. Trinei, I. Berniakovich, E. Beltrami et al., "P66Shc signals to age," *Aging*, vol. 1, no. 6, pp. 503–510, 2009.

[4] F. Orsini, E. Migliaccio, M. Moroni et al., "The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates transmembrane potential," *Journal of Biological Chemistry*, vol. 279, no. 24, pp. 25689–25695, 2004.

[5] M. Giorgio, E. Migliaccio, F. Orsini et al., "Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis," *Cell*, vol. 122, no. 2, pp. 221–233, 2005.

[6] Y. R. Kim, C. S. Kim, A. Naqvi et al., "Epigenetic upregulation of p66shc mediates low-density lipoprotein cholesterol-induced endothelial cell dysfunction," *American Journal of Physiology*, vol. 303, pp. H189–H196, 2012.

[7] M. Trinei, M. Giorgio, A. Cicalese et al., "A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis," *Oncogene*, vol. 21, no. 24, pp. 3872–3878, 2002.

[8] P. Pinton, A. Rimessi, S. Marchi et al., "Protein kinase C β and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc," *Science*, vol. 315, no. 5812, pp. 659–663, 2007.

[9] F. Orsini, M. Moroni, C. Contursi et al., "Regulatory effects of the mitochondrial energetic status on mitochondrial p66Shc," *Biological Chemistry*, vol. 387, no. 10-11, pp. 1405–1410, 2006.

[10] M. Gertz, F. Fischer, D. Wolters, and C. Steegborn, "Activation of the lifespan regulator p66Shc through reversible disulfide bond formation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 15, pp. 5705–5709, 2008.

[11] M. Forte and P. Bernardi, "Genetic dissection of the permeability transition pore," *Journal of Bioenergetics and Biomembranes*, vol. 37, no. 3, pp. 121–128, 2005.

[12] D. R. Green and G. Kroemer, "The pathophysiology of mitochondrial cell death," *Science*, vol. 305, no. 5684, pp. 626–629, 2004.

[13] P. Bernardi, "Mitochondrial transport of cations: channels, exchangers, and permeability transition," *Physiological Reviews*, vol. 79, no. 4, pp. 1127–1155, 1999.

[14] W. Wang, H. Fang, L. Groom et al., "Superoxide flashes in single mitochondria," *Cell*, vol. 134, no. 2, pp. 279–290, 2008.

[15] R. Perez-Campo, M. López-Torres, S. Cadenas, C. Rojas, and G. Barja, "The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach," *Journal of Comparative Physiology B*, vol. 168, no. 3, pp. 149–158, 1998.

[16] C. Napoli, I. Martin-Padura, F. De Nigris et al., "Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 4, pp. 2112–2116, 2003.

[17] E. Migliaccio, M. Giogio, S. Mele et al., "The p66(shc) adaptor protein controls oxidative stress response and life span in mammals," *Nature*, vol. 402, no. 6759, pp. 309–313, 1999.

[18] S. Menini, L. Amadio, G. Oddi et al., "Deletion of p66Shc longevity gene protects against experimental diabetic glomerulopathy by preventing diabetes-induced oxidative stress," *Diabetes*, vol. 55, no. 6, pp. 1642–1650, 2006.

[19] K. G. Su, C. Savino, G. Marracci et al., "Genetic inactivation of the p66 isoform of ShcA is neuroprotective in a murine model of multiple sclerosis," *European Journal of Neuroscience*, vol. 35, pp. 562–571, 2012.

[20] A. Carpi, R. Menabò, N. Kaludercic, P. Pelicci, F. Di Lisa, and M. Giorgio, "The cardioprotective effects elicited by p66Shc ablation demonstrate the crucial role of mitochondrial ROS formation in ischemia/reperfusion injury," *Biochimica et Biophysica Acta*, vol. 1787, no. 7, pp. 774–780, 2009.

[21] I. Berniakovich, M. Trinei, M. Stendardo et al., "p66Shc-generated oxidative signal promotes fat accumulation," *Journal of Biological Chemistry*, vol. 283, no. 49, pp. 34283–34293, 2008.

[22] A. Berry, F. Capone, M. Giorgio et al., "Deletion of the life span determinant p66Shc prevents age-dependent increases in emotionality and pain sensitivity in mice," *Experimental Gerontology*, vol. 42, no. 1-2, pp. 37–45, 2007.

[23] M. Cozzolino, M. G. Pesaresi, V. Gerbino, J. Grosskreutz, and M. T. Carrì, "Amyotrophic lateral sclerosis: new insights into underlying molecular mechanisms and opportunities for therapeutic intervention," *Antioxidants & Redox Signaling*, vol. 17, pp. 1277–1330, 2012.

[24] G. Martino, L. Adorini, P. Rieckmann et al., "Inflammation in multiple sclerosis: the good, the bad, and the complex," *Lancet Neurology*, vol. 1, no. 8, pp. 499–509, 2002.

[25] L. Steinman, "Engineering better cytokines," *Nature Biotechnology*, vol. 21, no. 11, pp. 1293–1294, 2003.

[26] L. Steinman, "Immune therapy for autoimmune diseases," *Science*, vol. 305, no. 5681, pp. 212–216, 2004.

[27] E. Julià, M. C. Edo, A. Horga, X. Montalban, and M. Comabella, "Differential susceptibility to apoptosis of CD4+T cells expressing CCR5 and CXCR3 in patients with MS," *Clinical Immunology*, vol. 133, no. 3, pp. 364–374, 2009.

[28] M. Irony-Tur-Sinai, N. Grigoriadis, D. Tsiantoulas, O. Touloumi, O. Abramsky, and T. Brenner, "Immunomodulation of EAE by alpha-fetoprotein involves elevation of immune cell apoptosis markers and the transcription factor FoxP3," *Journal of the Neurological Sciences*, vol. 279, no. 1-2, pp. 80–87, 2009.

[29] S. Amor, J. K. O'Neill, M. M. Morris et al., "Encephalitogenic epitopes of myelin basic protein, proteolipid protein, and myelin oligodendrocyte glycoprotein for experimental allergic encephalomyelitis induction in Biozzi ABH (H-2Ag7) mice share an amino acid motif," *Journal of Immunology*, vol. 156, no. 8, pp. 3000–3008, 1996.

[30] R. Furlan, E. Brambilla, F. Ruffini et al., "Intrathecal delivery of IFN-γ protects C57BL/6 mice from chronic-progressive experimental autoimmune encephalomyelitis by increasing apoptosis of central nervous system-infiltrating lymphocytes," *Journal of Immunology*, vol. 167, no. 3, pp. 1821–1829, 2001.

[31] M. Forte, B. G. Gold, G. Marracci et al., "Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, pp. 7558–7563, 2007.

[32] E. Basso, L. Fante, J. Fowlkes, V. Petronilli, M. A. Forte, and P. Bernardi, "Properties of the permeability transition pore in mitochondria devoid of cyclophilin D," *Journal of Biological Chemistry*, vol. 280, no. 19, pp. 18558–18561, 2005.