Nrf2: a modulator of Parkinson's disease?

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Abstract

Parkinson's disease (PD) is a complex multifactorial disorder that has been associated with the processes of oxidative stress. In the absence of curative therapies, modification of the neurodegenerative process - including the manipulation of endogenous antioxidant pathways - is the focus of intensive research. Recently, genetic and pharmacological accretion of the transcription factor, and phase II antioxidant 'master regulator' Nrf2, has shown to demonstrably mitigate the toxic neuronal effects of parkinsonian agents such as MPP^+, rotenone, and hydrogen peroxide *in vitro* and *in vivo*. Furthermore, baseline genetic variability in Nrf2-dependant pathways may promote neuronal susceptibility to exogenous agents and correlate with PD onset within certain populations. While contemporary evidence directly implicating Nrf2 in the pathogenesis of PD is not conclusive and likely contingent upon the evaluation of complex interacting factors - including genetic variation and a history of environmental exposures - it remains a promising target for therapeutic benefit in the modulation of oxidative stress.

Introduction

Parkinson's disease is a common neurodegenerative disorder influenced by a complex interaction of environmental factors on a background of genetic variation. It is the second most common neurodegenerative disease and the most common movement disorder, being described in all human populations irrespective of ethnicity and location (Mellick 2013). The current clinical diagnosis of PD is based on the presence of one or more cardinal signs: bradykinesia, resting tremor, postural instability, and by a positive and sustained response to L-Dopa treatment (Wood-Kaczmar, Gandhi et al. 2008). Explicit degeneration of nigrostriatal dopaminergic neurons underscores the motor symptoms of the disease. Unfortunately, by the time motor features are clinically recognised, up to 70% of nigrostriatal dopaminergic neurons have been lost; this further contributes to the complexity already associated with treatment (Fearnley and Lees 1991, Dauer and Przedborski 2003). Another key hallmark of PD is the presence of α-synuclein containing Lewy bodies and Lewy neurites (Chandra, Fornai et al. 2004, Fournier, Vitte et al. 2009, Markesbery, Jicha et al. 2009). Lewy bodies, commonly recognised as the aggregates that underpin idiopathic PD, were first observed in 1912 by Fritz Jakob Heinrich Lewy. Over 80 years later, the protein α-synuclein was discovered to be the integral component of Lewy bodies, and its encoding gene, *SNCA*, a cause of autosomal dominant forms of the disease (Polymeropoulos, Lavedan et al. 1997). Interestingly, this common pathology is not found in all forms of Parkinsonism (Miyakawa, Ogino et al. 2013) and the functional significance of Lewy bodies in PD pathogenesis remains largely unknown.

Current therapies utilise dopamine (DA) replacement to alleviate perturbed motor function caused by nigrostriatal-specific dopaminergic degeneration (Poewe 2010). While this form of treatment provides most patients with relief from the majority of the symptoms of Parkinsonism over a considerable period, it does not halt or alter underlying disease progression. With time,

treatment response becomes subject to fluctuation and the emergence of additional symptoms that are not directly related to dopaminergic deficiency present. These symptoms reflect the more global nervous system disorder, with degeneration occurring throughout the central and peripheral nervous system. Indeed, the Braak hypothesis suggests that many non-dopaminergic areas including the hypothalamus, cortical regions, and dorsal motor nucleus of the vagus contain significant synucleinopathy early and throughout the disease course (Braak, Del Tredici et al. 2003). Dysfunction of these non-dopaminergic regions can be considerably disabling because current therapies do not address these symptoms.

While there are no preventative or curative therapies, modification of the neurodegenerative process is the subject of intensive research. Evidence supporting the oxidative stress hypothesis has prompted investigation into the efficacy of non-enzymatic exogenous antioxidants, including; vitamin C, vitamin E, vitamin D, and coenzyme Q_{10}. While few studies tentatively report efficacy (Etminan, Gill et al. 2005, Spindler, Beal et al. 2009, Knekt, Kilkkinen et al. 2010), others failed to report any statistically significant effect (Etminan, Gill et al. 2005, Parkinson Study Group, Beal et al. 2014). More recently, interest has focused on the pharmacological targeting of antioxidant gene transcription, attempting to utilise the powerful endogenous antioxidant enzymes in order to mitigate oxidative stress and limit neuronal injury (Jazwa, Rojo et al. 2011). One such target is the transcription factor and 'master regulator' Nrf2.

Nrf2-mediated antioxidant response pathway

Nuclear factor erythroid-2-related factor 2 (protein: Nrf2; gene: *NFE2L2*) is a transcription factor in the phase II antioxidant and xenobiotic response pathway and is known as a 'master regulator' of many genes involved in antioxidant, anti-inflammatory, and xenobiotic

detoxification pathways (Alfieri, Srivastava et al. 2011). Under normal resting metabolic conditions, Nrf2 is post-translationally and constitutively regulated in the cytoplasm by its antagonist Keap1. Kelch-like erythroid-cell-derived protein with CNC homology (ECH)-associated protein 1 (Keap1) forms a complex with Cul3-Rbx1 (an E3-ubiquitin ligase) and regulates Nrf2 through targeted ubiquitination. The activity of Nrf2 begins upon exposures of the cell to oxidative stress, xenobiotics, or electrophilic compounds. The resulting modification of Keap1 cysteine 151, and the subsequent stabilisation and translocation of Nrf2 to the cell nucleus, transactivates many target genes (Alfieri, Srivastava et al. 2011, Sykiotis, Habeos et al. 2011). Studies have identified a large number of cytoprotective genes (in excess of 200) under regulation by Nrf2 (Jennings, Limonciel et al. 2013). These genes include antioxidant proteins such as glutathione reductase, NAD(P)H:quinone oxidoreductase 1 (NQO1), and heme-oxygenase 1 (HO-1) (Figure 1). Transcriptional regulation of these xenobiotically responsive genes is mediated by a *cis*-acting antioxidant response element (ARE), predominantly located in the promoter region of the target genes. Alterations in the cellular redox state and/or exposure to electrophilic compounds stabilises Nrf2 and limit its Keap1-mediated degradation to allow for its nuclear translocation and transcriptional activation of antioxidant genes via ARE enhancers.

Figure 1 – Nrf2-mediated phase II antioxidant response pathway

Under resting metabolic conditions, Nrf2 is negatively regulated through targeted ubiquitination mediated by Keap1. Under conditions of stress, Keap1 is oxidised and releases Nrf2, which is stabilised by DJ-1, and translocates to the nucleus where, via ARE enhancers, activates a range of antioxidant enzymes.

Nrf2 and Parkinson's disease

A correlative decline in Nrf2 activity with age (the predominant risk factor for PD) indirectly links Nrf2 with the disease (Suh, Shenvi et al. 2004, Shih and Yen 2007). However, as will be discussed, more direct evidence has been published suggesting Nrf2-mediated antioxidant insufficiency may play an important role in the oxidative stress commonly associated with PD.

Deprenyl (selegiline) is a pharmacological agent historically used as part of the treatment strategy for PD and extensively examined in the well-known DATATOP study (Shoulson 1998). It is a type-B monoamine oxidase inhibitor (MAOI-B) that selectively prevents the breakdown of monoamine neurotransmitters, such as DA, thus, also reducing the reactive oxygen species (ROS) by-products associated with DA metabolism (Birkmayer, Riederer et al. 1977). Interestingly, early studies revealed that treatment with deprenyl induced the expression of antioxidant enzymes (Carrillo, Kanai et al. 1993). More recently, the mechanism of action for this deprenyl-dependent upregulation of cytoprotective genes by was shown to be the result of Nrf2 activation and its subsequent translocation to the nucleus (Xiao, Lv et al. 2011). Furthermore, Nrf2 nuclear recruitment was sufficient to protect against the potent parkinsonian toxin MPP^+.

It has been reported that Nrf2 is predominantly localised to the cytosol in nigral dopaminergic neurons taken from normal control subjects, whereas in age-matched PD patients (early Braak staging 1-2), Nrf2 is found within in the cell nucleus; this is suggestive of an attempt (in the PD cells) to reduce oxidative stress (Ramsey, Glass et al. 2007). Paradoxically, Nrf2 activation has been associated with dysregulated downstream gene expression in PD patients (Nakamura, Wang et al. 1997, Cook, Vitale et al. 2011). This apparent disconnect may highlight a disease-specific aberration of the Nr2-mediated pathway. Furthermore, nuclear translocation of Nrf2 was not reported in the hippocampal neurons of Alzheimer's disease (AD) patients (Braak

staging 4-6) (also exhibiting oxidative stress), indicating a cellular and/or disease dependent recruitment that was independent of stimulus type. Cellular differences in Nrf2 expression and activation are also observed between neurons and astrocytes (Bell, Al-Mubarak et al. 2015). Compared to astrocytes, neurons appear to elicit a relatively weaker antioxidant response, measured in part, by reduced glutathione levels (Dringen, Kussmaul et al. 1999). Reportedly, astrocytes control the highly oxidative profile of neurons and confer Nrf2-mediated oxidative neuroprotection in animal models (Dringen, Pfeiffer et al. 1999, Jakel, Townsend et al. 2007). These findings suggest that cell-specific defects in Nrf2-mediated antioxidant response may play a role in neurodegenerative diseases. Further investigations should consider examining the cell-specific neuroprotective mechanisms responsible for mitigating the highly oxidative products of DA metabolism. This may prove clinically relevant when investigating therapeutic drug targets. NQO1, a diverse Nrf2-mediated antioxidant, is an effective metaboliser of DA-derived quinones and is induced by DA (Jia, Zhu et al. 2008). Up regulation and over expression of *NQO1* has been implicated as a neuroprotective mechanism against DA toxicity (Zafar, Inayat-Hussain et al. 2006, Zafar, Siegel et al. 2006) and is reportedly more highly expressed within Parkinsonian astroglial and neuronal cells of the substantia nigra pars compacta compared to controls (van Muiswinkel, de Vos et al. 2004). HO-1, a potent antioxidant regulated by Nrf2, has been found at higher concentrations within PD patient serum compared to controls, a distinction not observed between AD patients and controls (Mateo, Infante et al. 2010); this further supports the suggestion that there may be a PD disease-dependent Nrf2 recruitment. Interestingly, nigral dopaminergic neurons of post-mortem PD patients, compared to controls, show no difference in immunoreactivity to HO-1. However, distinct peripheral Lewy body staining patterns for HO-1 were observed in patients with Lewy body inclusions (Schipper, Liberman et al. 1998). This association has also been observed in cortical Lewy bodies (Castellani, Smith et al. 1996) and is demonstrative of an HO-1 specific

oxidative stress response in Lewy body pathology. Overall, these results are suggestive that Nrf2, and its associated response pathway, is pivotal for nigral cell defence within a Parkinsonian phenotype.

Nrf2 and *PARK* gene products

DJ-1 (gene; *PARK7*) a recessively inherited Parkinson's gene, protects cells from oxidative stress by stabilising Nrf2 and subsequently preventing Keap1-mediated ubiquitination (Clements, McNally et al. 2006). Conversely, a reduction in DJ-1 protein has been associated with a reduction in Nrf2 transcriptional activity (Malhotra, Thimmulappa et al. 2008). Recently, a short, cell penetrating, peptide derivative of DJ-1 was shown to attenuate H_2O_2, 6-OHDA, and DA induced oxidative stress in neuroblastoma cell lines (Lev, Barhum et al. 2015). Activation of the Nrf2-pathway was demonstrated to attenuate the toxin-induced oxidative stress, post-treatment with the short DJ-1 peptide. Pathogenic mutant isoforms, knockdown, and knockout models of DJ-1 have all significantly reduced the expression of the Nrf2-mediated redox signalling molecules, thioredoxin 1 (Im, Lee et al. 2012) and glutathione (Zhou and Freed 2005). To date, no studies have evaluated the role of Nrf2 in PD patients carrying *PARK* mutations.

PTEN-induced putative kinase 1 (PINK1) (gene; *PARK6*) is a nuclear encoded protein with a kinase domain and a mitochondrial targeting motif, localising the protein to the inner and intermembrane space. PINK1 has previously shown to closely interact with the Parkinson's gene product Parkin (gene; *PARK2*) to maintain mitochondrial membrane integrity and target dysfunctional mitochondria for autophagy (Pickrell and Youle 2015). Recent studies have shown that upon exposure to 6-OHDA, Nrf2 transcriptionally up-regulates *PINK1* mRNA and protects the cells from oxidative stress-induced death (Murata, Takamatsu et al. 2015).

Furthermore, activating Nrf2 with sulforaphane (SFN) in PINK1-KO midbrain neurons and astrocytes of mice, re-established mitochondrial respiration (Dinkova-Kostova, Baird et al. 2015). In addition, exogenous Nrf2 stimulation protected PINK-KO and PINK1-WT cell lines from DA-induced toxicity.

Nrf2 genetics and Parkinson's disease

The complex pathophysiology of PD suggests that the underlying genetic architecture subtly influences disease susceptibility via accumulative, interactive, and combinatorial effects.

In recent years, genome-wide association studies (GWAS) have provided a means to investigate these effects through large population-based single nucleotide polymorphism (SNP) arrays. It is commonly touted that GWAS work according to the 'common disease, common variant' hypothesis (Manolio, Collins et al. 2009). These studies search for disease associations amongst the hundreds of thousands of marker variants across the entire genome. Interestingly many disease-associated variants are located within non-coding regions of the genome, highlighting that their inclusion in disease-association studies are of equal importance to those of protein-coding regions (Hindorff, Sethupathy et al. 2009). Two large-scale meta-analyses of GWAS data have been performed for PD. Neither of these analyses reported genome-wide significance for genetic markers around the gene that codes for Nrf2 (*NFE2L2).* However, this does not preclude the possibility that there are genetic contributions to risk associated with genetic variability around this gene, particularly given the likelihood that such a contribution involves interaction between the genetic background and certain environmental stimuli.

To date, three specific candidate gene studies investigating *NFE2L2* and PD have been undertaken; a Taiwanese study, five independent European studies (from Italy, Malta, Poland, Germany, and Sweden), and a large Australian case-control analysis (von Otter, Landgren et al. 2010, Chen, Wu et al. 2013, von Otter, Bergstrom et al. 2014, Todorovic, Newman et al. 2015). Of the 11 common SNPs investigated, none were significantly associated with PD in either the Taiwanese or European meta-analysis study. Within the sufficiently powered Australian study (PD=1338, controls=1379) one promoter SNP (rs2364725) was significantly associated with reduced risk for PD. Associations between haplotype windows and PD were also reported in both the European and Australian case-control groups. Interestingly, one of these haplotype windows includes a previously described 'functional variant' of the promoter and was associated with delayed age at onset (AAO) and reduced risk of PD (Marzec, Christie et al. 2007, von Otter, Bergstrom et al. 2014, Todorovic, Newman et al. 2015).

GWAS and candidate gene studies do not always consider the potentially important interactions between environmental exposures and genotypes that might exist in specific populations or in specific geographical contexts. Therefore, the clinical value of *NFE2L2* genetic data may prove insightful when gene-environment interactions are adequately captured.

Nrf2 and environmental exposures in Parkinson's disease

It has been established that a number of environmental exposures have an impact on the risk for developing PD. These include heavy metals (e.g. iron, copper, and manganese), pesticides and herbicides (e.g. rotenone and paraquat) and organic solvents (Tanner and Langston 1990, Montgomery 1995). Data shows that certain dosages of these exogenous agents are sufficient, in some cases, to produce a Parkinsonian phenotype in humans (Priyadarshi, Khuder et al.

2000, van der Mark, Brouwer et al. 2012). However, an interaction of environmental influences on a background of genetic susceptibility is often required to reach a threshold of disease manifestation (Menegon, Board et al. 1998, Deng, Newman et al. 2004).

Many of the aforementioned foreign chemical exposures (often termed xenobiotics) trigger the stabilisation of Nrf2 and the subsequent activation of many cytoprotective enzymes that are under Nrf2 regulation. It has been hypothesised that polymorphisms in enzymes involved in xenobiotic metabolism may predispose an individual to PD (Singh, Khan et al. 2008). This hypothesis has been tentatively assessed in a subset of polymorphic Nrf2-mediated gene products. Glutathione *S*-transferases (GSTs) are a group of detoxifying enzymes that act by catalysing the conjugation of glutathione to electrophilic substrates (Hayes, Flanagan et al. 2005). An association of paraquat use with PD risk has been reported to be dependent upon GST genotype (Dick, De Palma et al. 2007, Goldman, Kamel et al. 2012). Moreover, this association has also been observed in patients who have been exposed to general pesticide use (Menegon, Board et al. 1998). Pesticide exposure, associated with PD risk, has also been reported in individuals carrying *NQO1* polymorphisms (Fong, Wu et al. 2007). Interestingly, many of these SNP-disease associations only surpass significance threshold once environmental exposures have been taken into account.

Nrf2 has been extensively investigated as a modifier of a number of pulmonary diseases that have implicated oxidative stress in their pathophysiology. The evidence suggests that animal models deficient in Nrf2 are more prone to cigarette smoke induced apoptosis, oxidative stress, inflammation, and emphysema (Rangasamy, Cho et al. 2004). Cigarette smoke can directly and indirectly activate Nrf2 through xenobiotic exposure and via alterations in redox balance, respectively (Kensler, Wakabayashi et al. 2007). Intuitively, it may be proposed that any genetic aberration, sufficient in altering the metabolism of xenobiotic compounds, such as

cigarette smoke, may modulate these protective pathways. Therefore, in the context of PD, while a number of studies have investigated the interaction between environmental exposures and polymorphisms in cytoprotective genes, only one published study has investigated the interactive effects of exogenous exposures with *NFE2L2* polymorphisms (Todorovic, Newman et al. 2015). This study suggests that common *NFE2L2* polymorphisms may reduce PD susceptibility in pesticide-exposed individuals when compared to wild-type carriers. While not conclusive, this study warrants further investigation into the interactive effects of *NFE2L2* and the environment.

Nrf2 and neuroprotection

The effects of genetically and pharmacologically targeting Nrf2 has been evaluated *in vitro* and *in vivo* (Bosco, Fowler et al. 2006). A number of these studies - directly or indirectly - report a neuroprotective role for Nrf2 against disease or injury.

Human studies

Because of the diverse cytoprotective role of Nrf2, it has become an attractive therapeutic target for modulating diseases that implicate inflammation and oxidative stress. Currently, a number of FDA approved pharmacological agents - that induce Nrf2 activation - such as Tecfidera/BG-12 (dimethyl fumarate), have been used successfully in the treatment of reducing relapse rate in relapsing-remitting Multiple Sclerosis (Gold, Kappos et al. 2012).

Animal studies

Animal models have provided a means for investigating molecular pathways that contribute to the hallmark signs (predominantly dopaminergic degeneration) and motor symptoms of PD.

These models present with intrinsic limitations (contemporary models are unable to fully recapitulate PD pathogenesis and pathology), however, the use of transgenic and toxin-induced animal models have provided a reductionist's approach to evaluate significant and reproducible pathological hallmarks, including MPTP-induced nigrostriatal lesions resulting in PD-like motor symptoms in treated monkeys (Bezard, Imbert et al. 1997). Interestingly, common neuronal toxins that drive dopaminergic neuron degeneration in animal models cause oxidative stress, these include; MPTP, rotenone, paraquat, and 6-hydroxydopamine (6-OHDA). Nrf2 has been investigated in a number of these studies, with reports tentatively suggesting a role in neuroprotection and modulating neuronal injury. Neuronal susceptibility to hydrogen peroxide (H_2O_2) and glutamate toxicity in Nrf2 knock-out mice is alleviated by Nrf2 overexpression (Kraft, Johnson et al. 2004). In an α-synuclein-PD *Drosophila* model, restoration of decreased locomotor activity and dopaminergic neuron degeneration has been achieved through Nrf2 overexpression and Keap1 knockdown (Nakabeppu, Tsuchimoto et al. 2007, Barone, Sykiotis et al. 2011). Furthermore, activation of Nrf2 through pharmacological interventions (e.g. the administration of sulforaphane (SFN), tert-butylhydroquinone (tBHQ) or dimethyl fumurate) has produced similar protective results. Within the basal ganglia, Nrf2 protein levels, and subsequently, the upregulation of potent antioxidant enzymes – NQO1 and HO-1 – were obtained after intraperitoneal SFN administration (Jazwa, Rojo et al. 2011). Furthermore, these effects were shown to sufficiently protect against MPTP-induced dopaminergic neuronal death. In a separate study, SFN administration not only reduced neuronal cell death, but also contusion volume and neurological dysfunction after traumatic brain injury in rats (Hong, Yan et al. 2010). Another study, examining the effects of the Nrf2 activating agent, tBHQ, reported significant protection against 6-OHDA induced damage to mouse neurons (Jakel, Townsend et al. 2007). It is important to note that the aforementioned studies, evaluating the role of Nrf2 in mitigating toxin-induced neuronal damage, adhered to pre-treatment/s of the Nrf2-activating

compounds prior to toxin administration. This paradigm contrasts with that of the clinical setting, in which patients will present with ongoing disease prior to drug administration. Therefore, while Nrf2 elevation is demonstrably cytoprotective, more studies will need to be conducted that evaluate its effect on established insidious disease models.

Cell lines

Pharmacological activation of the Nrf2 pathway also protects neuroblastoma cell lines from a range of toxic insults. Dose-dependent dopamine-mediated cell death was attenuated with pre-treatment of dimethyl fumarate, further resulting in elevated levels of total intracellular GSH and GST (Nrf2-mediated antioxidants) (Duffy, So et al. 1998). SFN and tBHQ treatment mitigated oxidative stress induced damage and neuronal cell death caused by anti-psychotic medication (Mas, Gasso et al. 2012) and H_2O_2 (Li, Lee et al. 2002, Li, Johnson et al. 2005), respectively. Interestingly, investigation of cellular-specific expression profiles have indicated selective Nrf2 expression in astrocytes compared to neurons (Shih, Shiozawa et al. 2003). Furthermore, it has been suggested that astrocyte-specific Nrf2 pathway activation confers protection to vulnerable neurons (Kraft, Johnson et al. 2004). These studies provide strong evidence supporting the exploitation of Nrf2 and its pathway as part of a neuroprotective strategy. However, while these studies demonstrate their potential for therapeutic efficacy, they have not established any direct pathogenic mechanism/s linking the Nrf2-mediated pathway with PD susceptibility.

To date, gene expression profiling of PD patient-derived substantia nigral neurons have not reported any significant expression differences for *NFE2L2* compared to age-matched controls. One post-mortem study reported that the Nrf2-mediated enzyme, glutathione *S*-transferase 1, was significantly up-regulated in DA-specific neurons isolated from the substantia nigra

(Simunovic, Yi et al. 2009). However, this evidence weakly associates *NFE2L2* expression with PD.

Previously, we reported a PD patient-derived cell model generated from biopsies of the olfactory mucosa (termed human olfactory neurosphere-derived (hONS) cells) that demonstrate disease-specific differences in gene expression when compared to healthy controls (Matigian, Abrahamsen et al. 2010). Geneset enrichment analysis of these disease-specific expression differences identified the 'Nrf2-mediated antioxidant response pathway' as the most significantly altered between PD patients and controls. Functional experiments using these patient-derived hONS cell lines further demonstrated reductions in associated metabolic function, including reduced levels of glutathione and (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS), suggesting deficiencies in Nrf2 function (Cook, Vitale et al. 2011). Moreover, these deficiencies were ameliorated after induction of Nrf2 with SFN. Interestingly, gene expression profiling performed after SFN treatment demonstrated that PD cases had a significantly reduced repertoire of Nrf2-mediated gene response compared to controls. These studies suggest that a base-line aberration in the Nrf2-mediated transcriptional response may play an important role in the pathogenesis of PD. Furthermore, these studies highlight that non-neuronal patient-derived tissue may provide a suitable medium to investigate the genetic and environmental contribution of Nrf2 pathway dysfunction in the progression of idiopathic PD.

Conclusion

While the production of reactive oxygen (and nitrogen) species are physiologically important in the modulation of immunity and cell signalling, the overwhelming evidence suggests that disruption of normal redox mechanisms – resulting in oxidative stress – is unequivocally

associated with neuronal degeneration in PD. It may be suggested that oxidative stress is a result of dying neurons and may prove to be an epiphenomenon of the disease process. However, the tenability of this theory diminishes upon observations of oxidative stress in the early stages of disease progression, and the use of toxins to induce oxidative-mediated dopaminergic neuron injury in animal models of PD (MPTP). It has been reported that the source of oxidative stress has been traced to multiple concomitant processes. Mitochondrial Complex I dysfunction is reportedly responsible for most of the unwarranted neuronal degeneration in PD; while DA synthesis and neuro-inflammation also contribute substantially to the elevated ROS levels. In addition, evidence tentatively suggests that altered antioxidant response mechanisms may also play its part. Nrf2, a transcription factor known as the 'master regulator' of the phase II antioxidant and xenobiotic response pathway, has been evaluated for its role in neuronal cytoprotection and as a modulatory candidate in the pathogenesis of PD.

In vitro and *in vivo* studies demonstrate that both genetic and pharmacological modulation of Nrf2 can provide a successful cytoprotective strategy against neuronal toxic insult. Furthermore, in some cases, Nrf2 activation led to the reversal of cardinal signs in animal models of PD. These studies strengthen the argument supporting Nrf2 accentuation as an important neuroprotective strategy. It is important to note that, while experimentally informative, the total aberration of a chief regulator of xenobiotic metabolism, such as Nrf2, invariably and unsurprisingly, increases the vulnerability of neurons to toxic insult. A more clinically relevant picture may be gleaned from the mitigation of neuronal damage through the restoration, or the pharmacological activation, of Nrf2, from Parkinsonian compounds. The success of dimethyl fumarate, as a strategy against Multiple Sclerosis, testifies to the feasibility of using exogenous Nrf2-activating compounds in the treatment of established neurodegenerative diseases. The proposed theory that Nrf2 dysfunction, or alteration of the Nrf2-mediated antioxidant response pathway, contributes to the pathogenesis of PD is tentative

and likely contingent upon complex interacting factors - including genetic variation and a history of environmental exposures. Regardless, Nrf2 and its associated antioxidant response pathway, remain a promising target for therapeutic benefit in the modulation of oxidative stress.

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