Chapter 4
Involvement of S-Nitrosylation in Neurodegeneration

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4.1 Introduction

Nitric oxide (NO) is a diffusible signaling molecule that regulates various physiological functions in the biological system. For example, nitric oxide signals vasodilation through the activation of soluble guanylate cyclase (sGC) by binding to the enzyme's heme group directly. Activation of sGC increases the production of cyclic guanosine monophosphate (cGMP) which signals the relaxation of smooth muscle in the blood vessels for vasodilation. Another signal mechanism mediated by NO is through the attachment of NO covalently to the cysteine residues in proteins to modulate their activities directly. This modification, designated as S-nitrosylation, is now recognized as an important post-translational modification that is comparable to phosphorylation in regulating physiological responses such as vasodilation, neurotransmission, vesicle trafficking and apoptosis (Fig. 4.1). However dysregulation of S-nitrosylation is emerging as a contributor in the development of a number of diseases such as neurodegeneration.

Neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) are characterized by progressive degeneration of specific groups of neurons. The pathogenic mechanisms of these disorders are not completely understood, but NO induced nitrosative stress has long been regarded as an important contributing factor. It is believed that elevated nitrosative stress through free radicals can damage protein, lipid and DNA which can ultimately lead to neurodegeneration. However, recent studies suggest that NO induced S-nitrosylation of neuroprotective proteins can also contribute to the process of neurodegeneration.

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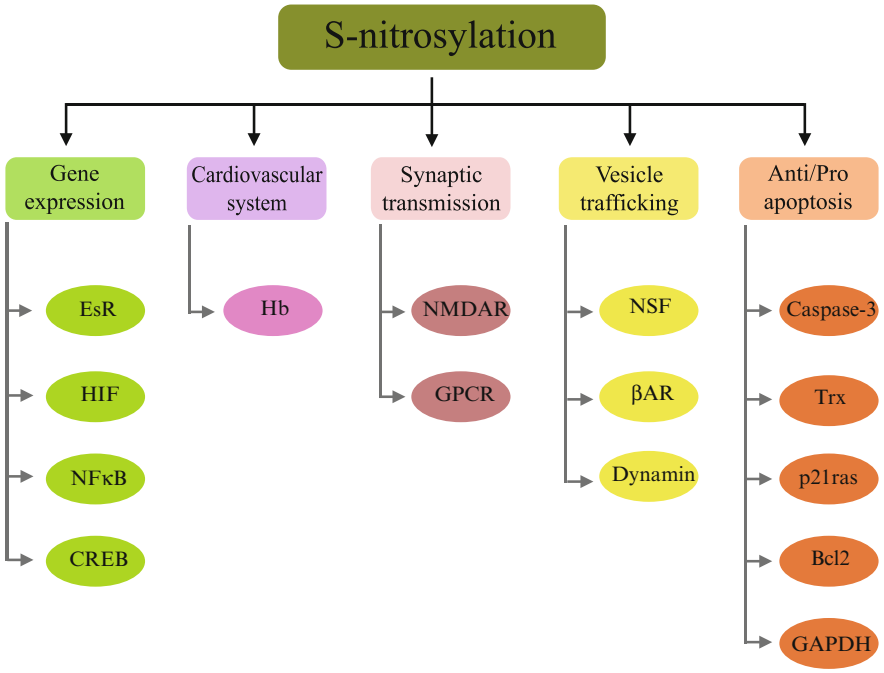

Fig. 4.1 Physiological functions of S-nitrosylation. S-nitrosylation is involved in the regulation of several important cellular systems including gene expression, cardiovascular system, synaptic transmission, vesicle trafficking and regulation of apoptosis. *EsR* estrogen receptor, *HIF* hypoxia inducible factor, *NFκB* nuclear factor kappa B, *CREB* cAMP responsive element binding, *Hb* hemoglobin, *NMDAR* N-methyl D-aspartate receptor, *βAR* beta-adrenergic receptor, *GPCR* G-protein-coupled receptor, *NSF* *N*-ethylmaleimide-sensitive factor, *Trx* thioredoxin, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase. See text for the details

4.2 Biochemistry of S-Nitrosylation

S-nitrosylation occurs when a NO molecule is covalently attached to the thiol group of cysteine residues in proteins. In the biological system, proteins can be S-nitrosylated through a number of mechanisms which include transnitrosylation, reaction with electrophilic nitrogen oxide species, or through metalloprotein catalyzed reactions. Transnitrosylation, also called transnitrosation, is a process by which a NO group is exchanged for a hydrogen group between cysteine residues. This reaction is largely depending on the pH, in which the reaction rate slows dramatically when pH decreases (Arnelle and Stamler 1995). The generation of nitrosothiols directly from NO under physiological condition requires an electron acceptor to act as an electrophilic nitrosating intermediate before the formation of nitrosothiols. In the biological system, oxygen is a common electron acceptor that reacts with NO to generate the N_2O_3 intermediate which then reacts with cysteine resides in proteins forming the nitrosothiols (Gaston et al. 2003). Apart from N_2O_3, a metal nitrosyl can

also act as an intermediate in the process of nitrosothiol formation. For example, Cu^{+} or Fe^{2+} in metalloproteins can serve as electron acceptors to facilitate the generation of nitrosothiols (Gaston et al. 2003). In summary, S-nitrosylation can be carried out by transnitrosylation or through the oxidation of a thiol group by NO via intermediates such as N_2O_3 and metal nitrosyls.

4.3 Specificity of S-Nitrosylation In Vivo

Up to date, more than 100 proteins have been demonstrated to be S-nitrosylated. The site of S-nitrosylation is usually depending on a consensus acid-base motif in the target protein (Stamler et al. 1997a). For example, in the beta-subunit of hemoglobin, the S-nitrosylated cysteine (Cys 93) is flanked by an acidic and a basic amino acid (His92-Cys93-Asp94) (Stamler et al. 1997a, b; Chung et al. 2005a). Interestingly, later studies found that this acid-base motif is not necessarily based on the primary amino acid sequence, but it can be formed by the secondary or tertiary protein structure (Hess et al. 2001; Chung 2007). For example, the enzymatic activity of methionine adenosyltransferase (MAT) is inhibited by S-nitrosylation at Cys121 (Perez-Mato et al. 1999). However, Cys121 in MAT is not flanked by acidic and basic amino acids in the primary sequence. However, replacement of the acidic (Asp355) or basic (Arg357 and Arg363) amino acids located in the vicinity of Cys121 due to protein folding reduces S-nitrosylation of MAT (Perez-Mato et al. 1999), indicating the importance of the three-dimensional structure in the formation of the acid-base motif for S-nitrosylation.

4.4 Detection of Protein S-Nitrosylation

Because of the labile nature of S-nitrosylation and the lack of sensitive method in detecting this modification, it is still difficult to identify and study how S-nitrosylation can regulate the function of proteins in different cellular pathways. A number of methods have been developed to detect S-nitrosylation in vitro and in vivo (Table 4.1) (Chung et al. 2005b; Torta et al. 2008). The most sensitive method is the photolysis or the chemical-induced release of NO from nitrosothiols, which is coupled with chemiluminescence to detect the amount of NO released from S-nitrosylated proteins (Chung et al. 2005b). This approach is sensitive but it requires expensive instrumental setup and it also lacks the ability to distinguish which protein is S-nitrosylated. A less expensive approach is to use a colorimetric or fluorometric method instead of chemiluminescence to detect the NO released from nitrosothiols, but the sensitivity of this approach is much lower (Chung et al. 2005b). A recently developed biotin switch assay provides an inexpensive biochemical method to detect protein S-nitrosylation in a reasonably sensitive approach to characterize protein S-nitrosylation (Chung et al. 2005b). In this assay, the

Table 4.1 Principles, advantages and limitations of methods in studying S-nitrosylation

Methods	Principles	Advantages	Limitations
Chemiluminescence, colorimetric and fluorometric methods	Photolysis or chemical induced release of NO from nitrosothiols	Sensitive and quantitative	Non-specific and indirect measurement
Anti-nitrosothiol antibodies	Antibodies against S-nitrosylated proteins	Convenient for Western blot or immuno-histochemistry	Non-specific reactivity to cysteine residues in proteins
Biotin switch assay	S-nitrosylated cysteine is converted to biotinylated cysteine	Convenient to be detected by biochemical methods	Low sensitivity and complicated procedure
Mass spectrometry	Detection of S-nitrosylation based on changes in mass	Identification of the site of modification	Purified proteins required

S-nitrosylated cysteine residue is switch to a biotin group, which can then be detected by biochemical methods. Although the biotin switch is still not as sensitive as the chemiluminescence approach, it has greatly assisted the study and identification of proteins that are S-nitrosylated in different cellular pathways. Lastly, antibodies have also been developed to detect S-nitrosylated proteins by Western blot or immunohistochemistry. However, these antibodies usually suffer from non-specific reactivity to cysteine residues in proteins (Chung et al. 2005b).

4.5 Physiological Function of S-Nitrosylation

4.5.1 S-Nitrosylation and Gene Expression

S-nitrosylation has been shown to affect gene transcription by modulating the activity of a number of transcriptional factors such as hypoxia inducible factor-1 (HIF-1), estrogen receptor (EsR), nuclear factor kappa B (NFκB) and cAMP responsive element binding (CREB) (Garban et al. 2005; Li et al. 2007; Marshall et al. 2004; Reynaert et al. 2004; Riccio et al. 2006; Nott et al. 2008). For instance, HIF-1 is a heterodimeric transcriptional factor that is composed of HIF-1α and HIF-1β with HIF-1α functions as the regulatory subunit (Yee-Koh et al. 2008). Under normoxic condition, HIF-1α is hydroxylated at conserved proline residues (Pro402, Pro564) at the oxygen-dependent degradation (ODD) domain by prolyl hydroxylases (PHDs). This modified HIF-1α will be targeted by the von Hippel-Lindau protein (pVHL), an E3 ligase in the ubiquitin proteasomal system (UPS), for degradation (Yee-Koh et al. 2008). However, a recent study also showed that HIF-1α can be S-nitrosylated at Cys533 within the ODD domain and

this prevents its degradation by the UPS which is independent of the PHD pathway (Li et al. 2007). This stabilization of HIF-1α by S-nitrosylation has been suggested to be the mechanism of how tumor cells can survival after radiation treatment (Li et al. 2007).

EsR is an important transcriptional factor that mediates cellular changes in response to the hormone estrogen. It is known that EsR is redox-sensitive, potentially because of the cysteine residues within the two highly conserved zinc finger domains. In fact, it was reported that the cysteine residues within EsR can be S-nitrosylated and S-nitrosylation of EsR inhibits the binding of EsR to specific estrogen-responsive elements (EREs) (Garban et al. 2005). This provides another example of how direct S-nitrosylation of transcriptional factor can affect gene transcription at the cellular level.

In contrast to HIF-1 and EsR, S-nitrosylation modulates the activity of NFκB indirectly. Under basal condition, NFκB is sequestered to the cytoplasm through its interaction with inhibitory κB (IκB) (Marshall et al. 2004; Reynaert et al. 2004). When the NFκB is activated, IκB kinase (IKK) phosphorylates IκB which leads to its degradation by the UPS. Without binding to IκB, NFκB translocates to the nucleus and initiates gene transcription (Marshall et al. 2004; Reynaert et al. 2004). S-nitrosylation of IKK decreases its phosphorylation activity towards IkB, thus preventing NFκB's translocation into the nucleus for gene transcriptional activation (Marshall et al. 2004; Reynaert et al. 2004).

CREB is a well-known transcriptional factor that regulates important cellular processes in neurons such as synaptic plasticity, axonal growth, and survival (Lonze and Ginty 2002). For example, brain-derived neurotrophic factor (BDNF) is a growth factor that activates CREB-mediated gene transcription and promotes neuronal survival. It was initially reported that the activation of CREB by BDNF was dependent on the S-nitrosylation of components in the CREB DNA-binding complex (Riccio et al. 2006). A more recent study showed that BDNF-induced S-nitrosylation of histone deacetylase 2 (HDAC2) at Cys262 and Cys274 increases acetylation of histones and promotes CREB dependent gene transcription which promotes dendritic growth and neuronal survival (Nott et al. 2008).

4.5.2 S-Nitrosylation and Cardiovascular System

NO was first identified as the vasodilation signaling molecule produced by eNOS in the endothelium to induce smooth muscle relaxation by the activation of sGC. However, recent studies suggest that NO plays a more important role in modulating the cardiovascular system. Studies suggest that red blood cells carry NO at the Cys93 of the beta subunit of hemoglobin (Luchsinger et al. 2003). Under low O_2 tension, the release of O_2 from the hemoglobin in tissues with high metabolic rate will trigger a conformational change that also promotes the release of NO (Pawloski et al. 2001; McMahon et al. 2002). The released NO can induce local vasodilation so as to facilitate increased blood flow to active tissues (Pawloski et al. 2001;

McMahon et al. 2002). In agreement with the hypothesis that nitrosothiols are important in blood flow regulation, mice that are defective in S-nitrosogluthione (GSNO) metabolism exhibit an increased level of nitrosothiols in the blood and a higher blood pressure under anesthesia (Liu et al. 2004). Interestingly, in another report, it was found that infusion of deoxygenated blood or nitrosothiols to the nucleus tractus solitarius (NTS) can induce hyperventilatory response as observed in animals exposed to hypoxic condition (Lipton et al. 2001). These results suggest that although the role of NO in cardiovascular system is not completely clear, NO through S-nitrosylation is an important component in the regulation of the cardiovascular system.

4.5.3 S-Nitrosylation and Neurotransmission

In the central nervous system, N-methyl D-aspartate receptor (NMDAR) is a well-characterized target for S-nitrosylation. S-nitrosylation of NMDAR subunits has been identified at Cys744 and Cys798 in NR1, and Cys87, Cys320, and Cys399 in NR2 (Nakamura and Lipton 2008). Because nNOS is linked to the NMDAR through PSD-95, and activation of NMDAR activates nNOS, the S-nitrosylation of NMDAR due to increased NO production serves as a negative feedback mechanism for NMDAR activation (Nakamura and Lipton 2008). Downstream of NMDAR, several components are activated through S-nitrosylation. For instance, H-ras and Dexras1 are S-nitrosylated and activated upon NMDAR activation to facilitate downstream signaling (Fang et al. 2000). Activation of ras family proteins by S-nitrosylation has been implicated in tumorigenesis (Lim et al. 2008), whereas S-nitrosylation of Dexras1 has been linked to increased iron uptake in neurons (Cheah et al. 2006). Although the long-term consequence of how the activation of these molecules by S-nitrosylation can modulate the synaptic plasticity is not completely clear, it is generally agreed that S-nitrosylation is an important component in the normal synaptic function.

In the peripheral system, NO has been shown to modulate the beta-adrenergic receptor (βAR) signaling through S-nitrosylation (Whalen et al. 2007). βAR is a G-protein-coupled receptor (GPCR) that is involved in the sympathetic nervous system to regulate autonomic responses such as heart rate and bronchial relaxation. The activity of βAR is in part regulated by consistent internalization from or reinsertion to the cytoplasmic membrane. The internalization of βAR is modulated by (G protein coupled receptors (GPCR) kinases (GRKs) in response to agonist stimulation. For example, GKR2 phosphorylates βAR after agonist stimulation and this will recruit β-arrestin to promote βAR internalization. Previous studies have implicated that NO can affect the GPCR signaling and a recent report showed that this is mediated through S-nitrosylation of GRK2 (Whalen et al. 2007). GRK2 is S-nitrosylated at Cys340 and this modification inhibits its kinase activity, which reduces the agonist induced desensitization and internalization of βAR (Whalen et al. 2007).

4.5.4 S-Nitrosylation and Vesicle Trafficking

Vesicle trafficking in cells is a complex process that involves a number of components. For instance, in exocytosis, the fusion of vesicles to the cytoplasmic membrane is mediated by the formation of a SNARE (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor) complex. Once the process of exocytosis is finished, the SNARE complex is recycled by the *N*-ethylmaleimide-sensitive factor (NSF). NSF is an ATPase that couples the hydrolysis of ATP to the conformational changes that is required for the recycling of SNARE complex. NO is known to affect exocytosis and it was recently found that NSF can be S-nitrosylated at Cys91 and Cys264 and this has been shown to affect both exocytosis and receptor trafficking (Matsushita et al. 2003; Huang et al. 2005). For example, during inflammation, Weibel-Palade bodies are released by exocytosis to mediate vascular thrombosis and inflammation (Matsushita et al. 2003). NO is known to inhibit vascular inflammation, but the exact mechanism was not known. It is now observed that S-nitrosylation of NSF inhibits the release of Weibel-Palade bodies (Matsushita et al. 2003). Interestingly S-nitrosylation of NSF does not affect its ATPase activity, but instead inhibits NSF's normal function in the recycling of SNARE complex (Matsushita et al. 2003).

S-nitrosylation of NSF has also been found to affect trafficking of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor (Huang et al. 2005). It was reported that S-nitrosylation of Cys91 of NSF enhances the interaction between NSF and GluR2 subunit of AMPA receptor (AMPAR) and this facilitates the surface expression of AMPAR (Huang et al. 2005). Since the neurotransmission of NMDAR is associated with the activation of nNOS, the S-nitrosylation of NSF also provides a mechanism of how activation of NMDAR can promote the surface expression of AMPAR (Huang et al. 2005).

Apart from exocytosis and AMPAR trafficking, NO has been found to S-nitrosylate dynamin at the Cys607 and this increases the GTPase activity of dynamin and also its ability to self-assembly (Wang et al. 2006). This explains how NO can enhance endocytosis through S-nitrosylation of dynamin in the regulation of βAR2 and endothelial growth factor receptor (EGFR) (Wang et al. 2006).

4.5.5 S-Nitrosylation and Apoptosis

NO is a well-known regulator for cell survival through a number of mechanisms. For instance, under extensive oxidative stress NO can react with superoxide in mediating damaging effects on protein, lipid and DNA in the cells. For S-nitrosylation, studies have shown that both survival and death inducing factors in the cell can be modulated by S-nitrosylation. This discrepancy may be explained by the sensitivity of different proteins to nitrosative stress. For instance, moderate level of NO may inhibit apoptosis, whereas elevated level of nitrosative stress can induce cell cytotoxicity.

One of the examples that NO enhances cell survival is the S-nitrosylation of caspase-3. Caspase-3 is one of the final executioners in apoptosis. Like the other

members of caspase family, caspase-3 exists as an inactive zymogen under normal cellular condition. Apoptotic signal first triggers the activation of initiator caspases, such as caspase-8, -9 and -10, which in turn activate the effector caspases such as caspase-3, -6 and -7. The activated effector caspases will then cleave other cellular targets, which finally will lead to apoptosis (Thornberry and Lazebnik 1998). Under normal cellular conditions, caspase-3 is S-nitrosylated at the catalytic cysteine residues (Mannick et al. 1999). However, upon stimulation of the cell death inducing factors, such as Fas ligand, caspase-3 is denitrosylated and activated caspase-3 will initiate apoptosis (Mannick et al. 1999, 2001). S-nitrosylation and denitrosylation of cellular or mitochondrial caspase-3 has been shown to be regulated by thioredoxins (Trxs) (Mitchell and Marletta 2005; Benhar et al. 2008). In fact S-nitrosylation of Trx-1 at different cysteine residues have been shown to be important for apoptosis in different models of cell death (Haendeler et al. 2002; Mitchell and Marletta 2005; Mitchell et al. 2007; Benhar et al. 2008).

Other proteins that have been found to enhance cell survival after S-nitrosylation include p21ras and Bcl-2. The activation of the ras protein family is known to promote cell survival. However, deregulation of ras activity is also a major component in the process of tumorigenesis. For instance, activation of p21ras leads to activation of downstream signaling in promoting cell survival. S-nitrosylation of p21ras has been associated with pro-survival signal mechanism and this has been linked to the process of tumorigenesis (Raines et al. 2007; Lim et al. 2008). S-nitrosylation of Bcl-2 is another pro-survival modification induced by NO. Under normal cellular condition, Bcl-2 inhibits cell death by binding to proteins such as BAX and BAK in the Bcl-2 protein family (Zhai et al. 2008). However, the protein level of Bcl-2 is controlled by the UPS through proteasomal degradation of Bcl-2 (Zhai et al. 2008). S-nitrosylation inhibits proteasomal degradation of Bcl-2 and thus protects cells against apoptosis induced by BAX and BAK (Azad et al. 2006).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a well-studied cytosolic enzyme that converts glyceradehyde-3-phosphate to glycerate 1,3-bisphosphate in the glycolytic pathway. Apart from the normal metabolic function of GAPDH, recent studies suggest that GAPDH plays an active role in apoptosis. It has been observed that GAPDH is translocated to the nucleus after apoptotic stimuli and the mechanism has been elucidated recently by a number of studies (Hara et al. 2005; Sen et al. 2008). It was initially reported that GAPDH can be S-nitrosylated at Cys150 and this modification enhances its binding to Siah1, an E3 ligase in the UPS (Hara et al. 2005). The translocation of S-nitrosylated GAPDH to the nucleus is mediated by the binding with Siah1 which possesses a nuclear localization signal (Hara et al. 2005). Once in the nucleus, GAPDH stabilizes Siah1 to degrade nuclear proteins that inhibit apoptosis (Hara et al. 2005). GAPDH in the nucleus is also acetylated at Lys160 by p300/CBP and this acetylation promotes the interaction between GAPDH and p300/CBP (Sen et al. 2008). The binding of GAPDH activates the acetyltransferase activity of p300/CBP which facilitates the p53 dependent apoptotic pathways (Fig. 4.2) (Sen et al. 2008). Taken together, these studies show a new role of GAPDH in mediating cell death.

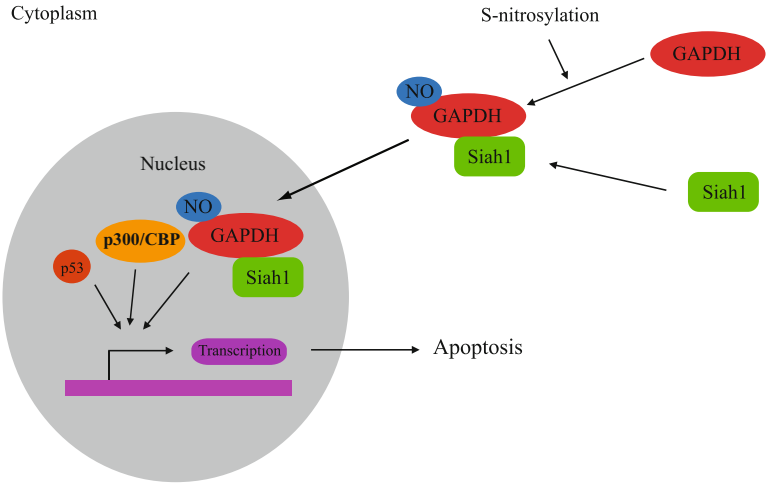

Fig. 4.2 S-nitrosylated GAPDH is translocated to the nucleus during apoptosis. S-nitrosylation of GAPDH enhances its interaction with Siah1, which translocates GAPDH into the nucleus. Nuclear GAPDH interacts with and activates p300/CBP to facilitate the p53 dependent apoptosis

4.6 S-Nitrosylation and Neurodegeneration

NO is produced actively by nNOS in neurons as a signaling molecule especially in the excitatory synapses. Various studies have shown that excessive production of NO is a major source of oxidative stress that contributes to neurodegeneration. This is particularly important as neuronal cell death is usually associated with inflammatory response which can induce the expression of iNOS and further increase the concentration of NO in the affected areas. Excessive amount of NO can react with other free radicals such as superoxide anion to form the more reactive nitrogen species such as peroxynitrite. These free radicals can induce protein nitration, lipid peroxidation, and DNA damage, which ultimately lead to neuronal degeneration (Ischiropoulos and Beckman 2003). This pathogenic mechanism has been demonstrated in a number of neurodegenerative disorders. However, more recent studies suggest that S-nitrosylation of proteins can also contribute to a number of neurodegenerative disorders. For instance, recent studies have found that S-nitrosylation can contribute to the development of ALS, AD and PD.

ALS is the most common motor neuronal disorder marked by degeneration of motor neurons in the spinal cord, brain stem and cortex (Rothstein 2009). Although most of ALS cases are sporadic, mutations of superoxide dismutase 1 (SOD1), have been found in some patients with familial history of ALS (Rothstein 2009). How mutations in SOD1 can cause ALS is not fully understood, but studies found that SOD1 mutants possess increased denitrosylase activity when compared to wild-type (WT) SOD1 (Schonhoff et al. 2006). In cell expressing SOD1 mutants, S-nitrosothiol (SNO) levels are decreased in the mitochondria (Schonhoff et al. 2006). Similarly,

decreased levels of SNO are observed in the spinal cords of mutant SOD1 transgenic mice (Schonhoff et al. 2006). These results suggest that imbalance of SNO levels caused by mutation in SOD1 is one of the mechanisms that lead to motor neuron degeneration in ALS.

The role of S-nitrosylation is less defined in AD, but a recent report sheds light on how NO can affect mitochondrial fission and induce neuronal injury through the S-nitrosylation of dynamin-related protein 1 (Drp1) in the pathogenesis of AD (Cho et al. 2009). AD is the most common neurodegenerative disorder that affects increasing number of elderly as the lifespan of general population is improved. A number of mechanisms have been proposed for the development of AD and the β-amyloid (Aβ) induced neurodegeneration is widely believed to be a major contributor (Cho et al. 2009). How Aβ can induce neuronal injury has been studied intensively and a number of hypotheses have been suggested which include excitotoxicity, oxidative stress, mitochondrial dysfunction, and apoptosis. In particular, oxidative stress in connection with mitochondrial dysfunction has been proposed to be an important mechanism in causing neurodegeneration in AD. A recent study provides a pathogenic mechanism of how Aβ can induce nitrosative stress that subsequently leads to abnormal mitochondrial fission/fusion that affects neuronal survival (Cho et al. 2009). Increased nitrosative stress is known to be induced by Aβ and this has been linked to mitochondrial dysfunction. The linkage of nitrosative stress and mitochondrial dysfunction is recently found to be caused by S-nitrosylation of Drp1 (Cho et al. 2009). Drp1 is an important molecule that regulates mitochondria fission and dysfunction of Drp1 has been implicated in neurodegenerative disorders (Cho et al. 2009). Aβ induces production of NO and this leads to the S-nitrosylation of Drp1 (Cho et al. 2009). S-nitrosylation of Drp-1 at Cys644 enhances dimerization and increases GTPase activity of Drp-1 (Cho et al. 2009). S-nitrosylation of Drp-1 induces mitochondrial fragmentation in relation to neurodegeneration in AD (Cho et al. 2009). Taken together, these results provide a new mechanism of how nitrosative stress through S-nitrosylation of Drp1 can contribute to the pathogenesis of AD.

In PD, S-nitrosylation has been recently emerging as an important contributor in the pathogenesis of the disease. PD is the second most common neurodegenerative disorders marked by movement impairment. The disease is caused by a selective degeneration of dopaminergic neurons in the substantia nigra (SNc) with the presence of intraneuronal protein aggregates designated as Lewy bodies (LB) (Savitt et al. 2006). The mechanism of neuronal cell death in the SNc is not completely clear, but studies suggest that oxidative stress, mitochondria dysfunction, protein aggregation, and dysfunction of the UPS are the major contributors for neurodegeneration in PD (Savitt et al. 2006; Tsang and Chung 2009). Recent identification of mutations in genes that cause the rare familial form of PD (FPD) has improved our understanding of PD mechanism. For instance, mutations in α-synuclein (α-syn) and LRRK2 cause autosomal dominant form of FPD, whereas mutations in parkin, DJ1 and PINK1 cause recessive form of FPD (Thomas and Beal 2007). The functional studies of these genes agree with the established pathogenic mechanism of PD. For instance, α-syn is a protein that is prone for aggregation and soon after its initial identification as the FPD linked gene product, α-syn was found to be the

major component of LB (Savitt et al. 2006). On the other hand, parkin was found to be an E3 ligase in the UPS, DJ1 was shown to be an oxidative chaperone, and PINK1 was identified as a mitochondrial kinase that is important for the normal function of mitochondria (Savitt et al. 2006; Tsang and Chung 2009). All these findings support the importance of protein aggregation, oxidative stress, mitochondrial or UPS dysfunction in the development of PD.

Nitrosative stress is known to be an important contributor for PD because nitrated protein aggregates are consistently reported to be a prominent feature of brain tissues in PD patients (Giasson et al. 2000). This notion is further supported by studies showing that nitrated α-syn is prone to aggregation, and nitrated α-syn is commonly found in the LB (Giasson et al. 2000; Tsang and Chung 2009). These findings suggested that nitrosative stress can induce the aggregation of α-syn, which can subsequently lead to the formation of LB. However, more recent studies found that NO can also contribute to PD through the S-nitrosylation of different components in neuroprotective pathways.

We initially reported that parkin can be S-nitrosylated and this modification compromises parkin's neuroprotective function (Chung et al. 2004). Parkin is an E3 ligase in the UPS and mutations in parkin were first identified in a group of Japanese patients that developed early-onset autosomal recessive form of FPD (Savitt et al. 2006). Various studies have demonstrated parkin's protective ability against different kinds of cellular insults (Feany and Pallanck 2003). For instance, as an E3 ligase, parkin ubiquitinates its substrates and directs them for UPS-dependent degradation, thus prevents the accumulation of misfolded or aggregated toxic protein species in neurons (Kahle and Haass 2004). We and another group first reported that parkin can be S-nitrosylated both in vitro and in vivo (Chung et al. 2004; Yao et al. 2004). S-nitrosylation of parkin inhibits its E3 ligase activity and compromises its protective function (Chung et al. 2004; Yao et al. 2004). More importantly, we observed increased levels of parkin S-nitrosylation in the brain tissues of PD animal models and PD patients (Chung et al. 2004). Another FPD linked gene that was found to be S-nitrosylated is DJ-1. A study showed that DJ-1 can be S-nitrosylated at Cys46 and Cys53 (Ito et al. 2006). Further study suggested that Cys46 is an important residue for the dimerization of DJ-1, but how DJ-1 S-nitrosylation can affect its dimerization is not clear (Ito et al. 2006). Since studies have shown that DJ-1 dimerization is crucial for its normal function as a chaperone, DJ-1 S-nitrosylation might affect its protective function in dopaminergic neurons. These results suggest that nitrosative stress through the modification of FAD gene products by S-nitrosylation is an important contributor for the PD pathogenesis.

Apart from parkin and DJ-1, a number of proteins with neuroprotective functions have been demonstrated to be the targets of S-nitrosylation. For instance, protein-disulphide isomerase (PDI), an endoplasmic reticulum (ER) protein that catalyzes disulfide bond formation and assists proper protein folding and maturation, was found to be S-nitrosylated in high levels of nitrosative stress (Uehara et al. 2006). S-nitrosylation of PDI suppresses both the chaperone and isomerase activities of the enzyme (Uehara et al. 2006). Under normal condition, PDI inhibits the formation of Lewy body-like aggregates in a cellular model of PD (Chung et al. 2001; Uehara et al. 2006).

S-nitrosylation of PDI attenuates this anti-aggregate formation function of PDI (Uehara et al. 2006). In SH-SY5Y neuroblastoma, S-nitrosylation of PDI abrogates its neuroprotective function against ER stress induced by unfolded proteins or proteasomal inhibition (Uehara et al. 2006). In neurons exposed to excitotoxicity induced by NMDA, S-nitrosylated PDI was increased and accompanied by accumulation of unfolded and polyubiquitinated proteins and the subsequent neuronal cell death (Uehara et al. 2006). In the post-mortem brain tissues of AD and PD patients, increased levels of S-nitrosylated PDI were observed. Taken together, this study established a link between nitrosative stress and protein misfolding in neurodegenerative disorders through the impairment of PDI.

The S-nitrosylation of parkin, DJ-1 and PDI aggravates cellular insults to neurons because these proteins safeguard cells against protein misfolding or aggregation. In addition, studies have also found that NO can also affect other neuroprotective pathways such as anti-oxidative stress protein or protein that has anti-apoptotic function. For instance, peroxiredoxin 2 (Prx2), an intracellular peroxidase, was found to be S-nitrosylated and this affects its normal function as an anti-oxidative stress enzyme (Fang et al. 2007). Prx2 is the most abundant peroxidase in neurons that metabolizes peroxides and protects neurons against oxidative injuries (Fang et al. 2007). Prx2 was found to be S-nitrosylated at Cys51 and Cys172, and these cysteines are critical residues within the catalytic domain of Prx2 (Fang et al. 2007). The peroxidase activity of Prx2 is attenuated after S-nitrosylation and this affects its neuroprotective function against oxidative stress in neurons (Fang et al. 2007). The levels of S-nitrosylated Prx2 are increased in brain tissues of PD patients which suggest that neurons under such condition are more vulnerable to oxidative stress (Fang et al. 2007).

Our recent study has also identified X-linked inhibitor of apoptosis (XIAP) as another protein whose S-nitrosylation is correlated with the neurodegenerative process in PD (Tsang et al. 2009). XIAP belongs to a highly conserved protein family which promotes cell survival through their baculoviral IAP repeat (BIR) domains (Vaux and Silke 2005; Srinivasula and Ashwell 2008). Similar to parkin, XIAP also possesses a RING domain at the C-terminal which enables XIAP to function as an E3 ligase in the UPS to target a number of substrates including XIAP itself (Vaux and Silke 2005; Srinivasula and Ashwell 2008). Studies have shown that the anti-apoptotic activity mediated by XIAP is through the highly conserved BIR domains of XIAP in antagonizing the pro-apoptotic activities of caspases (Vaux and Silke 2005; Srinivasula and Ashwell 2008). Caspases are the executioners of apoptosis that mediate the process of program cell death. Not surprisingly, overexpression of XIAP is commonly observed in tumors, which is one of the major contributors for the process of tumorigenesis. In our study, we found that XIAP can be S-nitrosylated both in vitro and in vivo. In contrast to parkin, XIAP is not S-nitrosylated at the RING domain but is modified at the BIR domains by NO (Tsang et al. 2009). S-nitrosylation of XIAP impairs its ability to inhibit caspase-3 activity and in turn down-regulates its anti-apoptotic functions. Different from parkin, the E3 ligase activity of XIAP is not affected by S-nitrosylation (Tsang et al. 2009). In the striatum of animal model of PD, elevated levels of S-nitrosylated XIAP were observed when compared to the control. In addition, increased XIAP

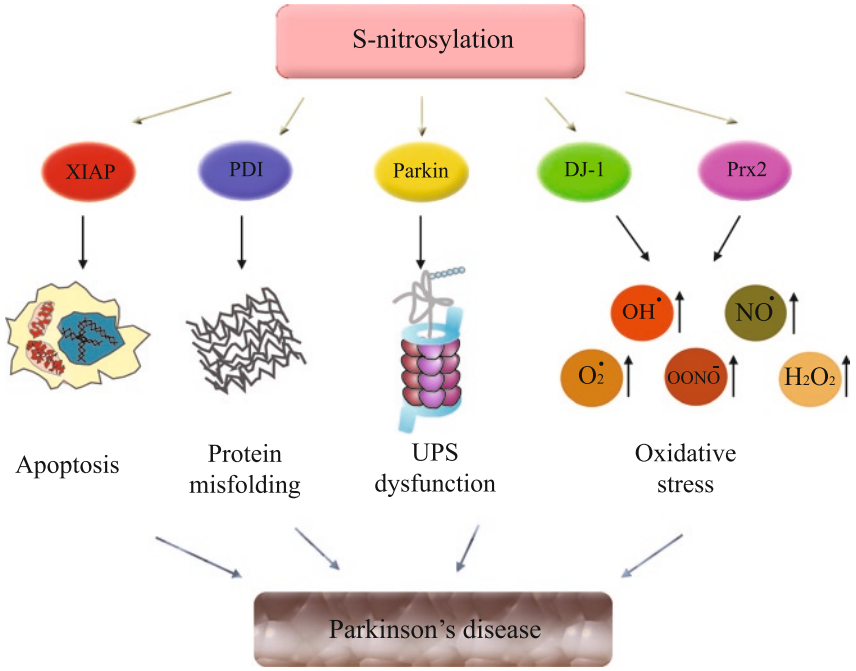

Fig. 4.3 Involvement of S-nitrosylation in the pathogenesis of Parkinson's disease (PD). S-nitrosylation of several proteins including parkin, PDI, DJ-1 and Prx2 and XIAP can lead to UPS dysfunction, protein misfoldong, increased oxidative stress and apoptosis which can contribute to the pathogenesis of PD

S-nitrosylation was also found in the brain tissues of PD patients (Tsang et al. 2009). These results suggested that the pro-survival function of XIAP in neurons is compromised by S-nitrosylation in the development of PD.

In summary, nitrosative stress can contribute to PD via a number of mechanisms. For instance, protein nitration or peroxynitrite-induced damages in lipids and DNA can promote neuronal cell death. In addition, S-nitrosylation of proteins that possess neuroprotective function such as parkin, DJ-1, Prx2 and XIAP can compromise the survival of neurons under various cytotoxic insults (Fig. 4.3), which make neurons more vulnerable to degeneration.

4.7 Conclusion

S-nitrosylation is a post-translation modification that regulates a number of cellular and physiological functions such as gene expression, cell survival, neurotransmission, vesicle trafficking and cardiovascular function. However imbalance of nitrosative stress can also compromise a number of neuroprotective proteins through

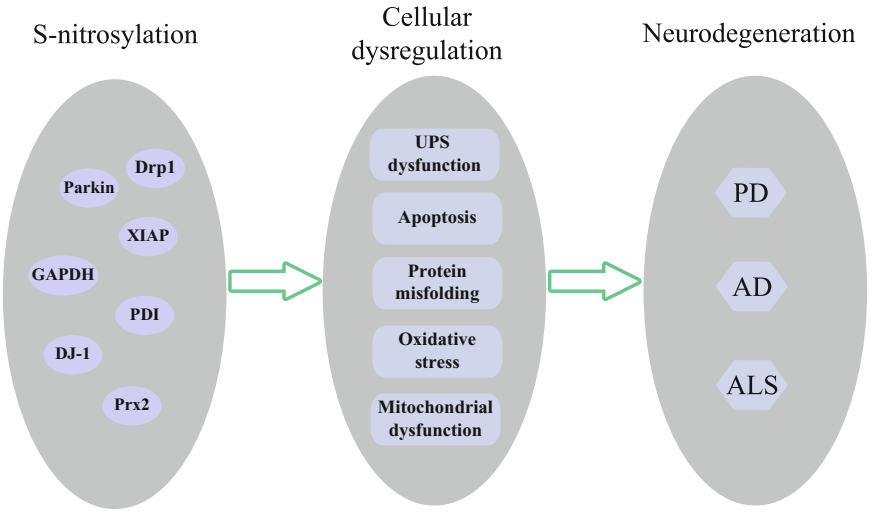

Fig. 4.4 Involvement of S-nitrosylation in neurodegeneration. Dysregulation of S-nitrosylation is involved in the causation of several neurodegenerative diseases including ALS, AD and PD through interconnecting pathways involving UPS dysfunction, protein misfolding, oxidative stress, mitochondrial dysfunction and apoptosis

S-nitrosylation and contribute to the neurodegenerative process in ALS, AD and PD (Fig. 4.4). By more thorough understanding in how S-nitrosylation can affect pathways that contribute to these disorders, new therapeutic targets can be developed for the treatment of these diseases in the future.

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