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News and Commentary

Nitrosative and oxidative stress links dysfunctional ubiquitination to Parkinson's disease

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How can common 'sporadic' forms of neurodegenerative disorders, such as Parkinson's disease (PD) and Alzheimer's disease, mimic very rare genetic mutations to cause a similar phenotype? We show here that abnormal protein accumulation and impaired protein degradation can result from environmental factors causing oxidative and nitrosative stress. On the occasion of this special issue dedicated to the 'discovery of ubiquitin-mediated protein degradation,' we review aberrant protein accumulation via dysfunction of ubiquitination because of nitrosative stress and the resulting clinical significance of this recent discovery to human neurodegenerative disorders, using PD as a model disease.

Pathology of PD

PD is a usually a late-onset, progressive movement disorder characterized by resting tremor, rigidity, and bradykinesia. The pathological features of PD include (1) the selective degeneration of dopaminergic neurons mainly in the substantia nigra pars compacta and (2) the presence of neuronal inclusions named Lewy bodies (LBs), which are widely distributed in the substantia nigra, neocortex, hippocampus, and basal forebrain nuclei, and are especially prevalent in PD cases associated with dementia.[1,2] LBs contain a number of aggregated proteins including α-synuclein, a highly abundant protein found in presynaptic terminals, and synphilin-1, an α-synuclein-interacting protein.[3] Recent studies in mice demonstrate that overexpression of human α-synuclein results in progressive accumulation of α-synuclein- and ubiquitin-immunoreactive inclusions in neurons similar to affected regions in PD brains.[4] Ultrastructural analysis reveals both electron-dense intranuclear deposits and cytoplasmic inclusions. These alterations are associated with loss of dopaminergic terminals in the basal ganglia and motor impairment.

Genetic Mutations Link Dysfunction of Ubiquitination to PD

Although the pathogenic significance of inclusion bodies remains unclear, they are often used as hallmarks of the diagnosis of neurodegenerative diseases. Evidence has emerged suggesting that dysfunction in protein degradation via the ubiquitin–proteasome system (UPS) may contribute to aberrant protein accumulation and pathogenesis in PD as well as other neurodegenerative disorders. The UPS functions primarily to label proteins for subsequent degradation by adding multiple ubiquitin moieties at lysine residues. These proteins are then degraded to their constituent amino acids by the 26S proteasome complex. Other than lysosomes, the UPS represents the major route of intracellular protein degradation in eucaryotes, and it is delicately and specifically regulated, probably at multiple levels. Recent genetic studies have identified various genes that directly link UPS to the pathogenesis of PD.

For example, an important molecule in the pathogenesis of PD is parkin. Parkin is an E3 ubiquitin ligase, the third in a series of enzymes that adds ubiquitin to specific substrates, usually earmarking them for degradation by the proteasome. Mutations in the parkin gene are associated with autosomal recessive juvenile parkinsonism (ARJP), which accounts for many cases of hereditary PD that are manifest in younger patients, as well as some very rare cases of adult PD.[5,6] One group has reported that mutant parkin fails to bind glycosylated α-synuclein for ubiquitination, leading to α-synuclein accumulation.[7] Synphilin-1 is a substrate for parkin ubiquitination, and it promotes the formation of LB-like inclusions in cultured cells when coexpressed with α-synuclein.[8] At least three other proteins have been identified as putative parkin substrates, including parkin-associated endothelin receptor-like receptor (Pael-R), cell division control related protein (CDCrel-1), and probably parkin itself.[1]

For some mutations in parkin, especially those found in adults with PD, increased E3 ligase activity results in abnormal ubiquitination and accumulation of these substrates, which may contribute to inclusion body formation and impairment in UPS activity. The prominence of ubiquitinated protein species in LBs, including parkin substrates, suggests that parkin might be involved in the formation of LBs. Many parkin mutations resulting in ARJP, especially in younger patients, however, are associated with decreased E3 ligase activity, so these cases lack LBs.

Another important molecule that links aberrant UPS activity and PD is ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1), a deubiquitinating enzyme that recycles ubiquitin. Apparently, autosomal dominant mutations of UCH-L1 have been identified in some PD patients.[9] Additional mutations in α-synuclein, DJ-1, and PINK1 may contribute to UPS dysfunction and subsequently lead to PD.[10,11]

Nitrosative and Oxidative Stress Impairs the UPS in Sporadic PD

These rare genetic cases notwithstanding, the vast majority of cases of PD are sporadic, and like many other neurodegen-

erative disorders, are associated with aberrant accumulation of wild-type, ubiquinated proteins. In PD, the LBs encountered in genetic cases of the disease appear similar to LBs found in the sporadic form. Accumulating evidence in both animal models and from human post-mortem material suggests that reactive oxygen/nitrogen species (ROS/RNS) are important in the pathogenesis of sporadic PD,[2] but until recently the relationship of ROS/RNS to LBs remained unknown. Here, we will elaborate on possible causation of UPS dysfunction and LB formation due to ROS/RNS (Figure 1).

A number of epidemiologic studies have suggested that pesticides and other environmental toxins that inhibit mitochondrial complex I result in oxidative and nitrosative stress, and consequent aberrant protein accumulation; dopaminergic neurons appear to be particularly vulnerable to such insults.[12] Administration to animal models of complex I inhibitors, such as MPTP, 6-hydroxydopamine (6-OHDP), rotenone, and paraquat, which result in overproduction of ROS/RNS, reproduces many of the features of sporadic PD, such as dopaminergic neuron degeneration, upregulation and aggregation of α-synuclein, LB-like intraneuronal inclusions, and behavioral impairment.[13]

Until recently, little was known about the role of ROS/RNS in UPS dysfunction. Interestingly, four studies have provided mounting evidence that nitrosative or oxidative stress results in the malfunction of parkin and UCH-L1 via S-nitrosylation or oxidation, and thereby contributes to the pathogenesis of the sporadic form of PD.[14–17]

S-nitrosylation (SNO) is a redox-based modification of specific cysteine thiol groups, whereby nitric oxide (NO) reacts with thiol to form an SNO-protein, and thus regulates protein activity.[18,19] We and others recently reported that the RING-finger E3 ligase, parkin, is S-nitrosylated in the brains of human patients with PD as well as in rodent brains of models of the disease.[14,15] We found that S-nitrosylation of parkin initially stimulated its ubiquitin E3 ligase activity, resulting in an increase in enzyme activity, autoubiquitination, and thereafter a subsequent decrease in activity.[14,20] The initial increase in parkin E3 ligase activity, for example, of synphilin-1, could contribute to LB formation,[20,21] and the subsequent decrease in parkin activity could produce a futile cycle with UPS dysfunction. Additionally, the neuroprotective activity of parkin appears to be compromised when it is S-nitrosylated.[15]

Furthermore, Nishikawa *et al.*[17] found that oxidation of UCH-L1 by 4-hydroxynonenal (a putative endogenous mediator of oxidative stress) results in loss of hydrolase activity. In a complementary study, Choi *et al.*[16] identified an oxidized form of UCH-L1 in sporadic PD brains. Decreased UCH-L1 activity could potentially contribute to UPS dysfunction, accumulation of damaged proteins, and formation of aggregates. Taken together, altered parkin and UCH-L1 activity induced by ROS/RNS may be critically involved in the etiology of sporadic PD, thereby mimicking rare genetic mutations that cause similar phenotypes.

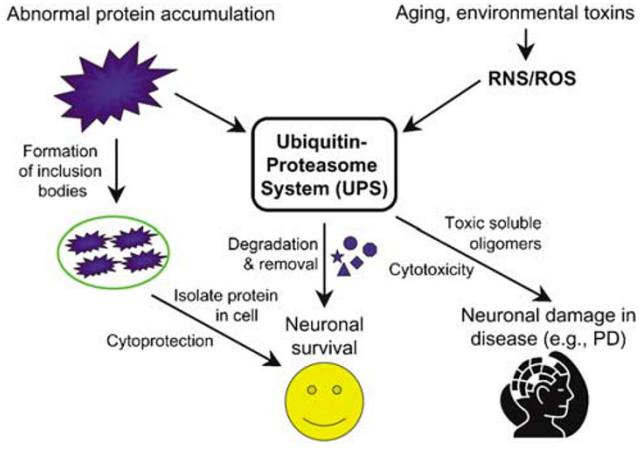

Figure 1 Proposed contribution of UPS dysfunction to PD. UPS is a major pathway that cells utilize to remove and degrade damaged or toxic proteins by tagging the target molecules with polyubiquitin chains through a series of reactions carried out by three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin protein ligase (E3). This precisely regulated pathway can be compromised by ROS/RNS generated during aging or exposure to environmental insults. As a result, accumulation of toxic proteins can contribute to neuronal cell injury and death, and eventually to pathological conditions, such as PD. Interestingly, based on recent work on abnormal protein accumulations in Huntington's disease, it has been proposed that inclusion bodies may be cytoprotective by walling off such proteins as insoluble aggregates or acting as a sink for potentially toxic soluble oligomers of the aberrant protein. In the case of PD, S-nitrosylated parkin (SNO-PARK) may result in a cycle of increased followed by decreased E3 ligase activity, contributing to excessive ubiquitination, LB formation, and UPS dysfunction

Remarks

Excessive nitrosative and oxidative stress may result in UPS dysfunction, thus contributing to abnormal protein accumulation and dopaminergic neuronal demise in sporadic PD. Our elucidation of an NO-mediated pathway to parkin E3 ligase dysfunction provides a mechanistic link between free radical production and UPS deficits in PD. Ultimately, elucidation of this new pathway may lead to the development of new therapeutic approaches for PD associated with aberrant protein accumulation due to nitrosative and oxidative damage of parkin. In addition, the activity of other RING-finger E3 ligases may also be affected by S-nitrosylation, providing a heuristic framework for the pathogenesis of other neurodegenerative and systemic disorders.

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1. Giasson BI and Lee VM (2003) Cell 114: 1–8
2. Jenner P (2003) Ann. Neurol. 53: S26–S38
3. Clayton DF and George JM (1998) Trends Neurosci. 21: 249–254
4. Masliah E *et al.* (2000) Science 287: 1265–1269
5. Kitada T *et al.* (1998) Nature 392: 605–608
6. Shimura H *et al.* (2000) Nat. Genet. 25: 302–305
7. Shimura H *et al.* (2001) Science 293: 263–269
8. Chung KK *et al.* (2001) Nat. Med. 7: 1144–1150
9. Leroy E *et al.* (1998) Nature 395: 451–452
10. Ciechanover A and Brundin P (2003) Neuron 40: 427–446
11. Valente EM *et al.* (2004) Science 304: 1158–1160

12. Betarbet R *et al.* (2000) Nat. Neurosci. 3: 1301–1306
13. Beal MF (2001) Nat. Rev. Neurosci. 2: 325–334
14. Yao D *et al.* (2004) Proc. Natl. Acad. Sci. USA 101: 10810–10814
15. Chung KK *et al.* (2004) Science 304: 1328–1331
16. Choi J *et al.* (2004) J. Biol. Chem. 279: 13256–13264
17. Nishikawa K *et al.* (2003) Biochem. Biophys. Res. Commun. 304: 176–183
18. Hess DT *et al.* (2005) Nat. Rev. Mol. Cell Biol. 6: 150–166
19. Lipton SA *et al.* (1993) Nature 364: 626–632
20. Lipton SA *et al.* (2005) Science, in press
21. Lim KL *et al.* (2002) J. Neurosci. 25: 2002–2009