Central Role of α-Synuclein Oligomers in Neurodegeneration in Parkinson Disease

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Since the original clinical description of “shaking palsy” by James Parkinson almost 200 years ago, substantial progress has been achieved in the treatment of symptoms of Parkinson disease (PD), the most common movement disorder. In about 95% of cases, symptoms of PD result from sporadic disease of unknown etiology. In addition, similar symptoms can be caused by certain infections and intoxication with some chemicals; in these cases, the condition is called secondary Parkinsonism.

Modern treatment of PD and Parkinsonism is based on the discovery that PD is caused by selective death of dopaminergic neurons in the substantia nigra pars compacta, which is responsible for initiation of intentional movements. As a result of this selective loss of neurons, an acute shortage of a major neurotransmitter dopamine (DA) develops. For partial compensation of this shortage, DA precursor levodopa is administered to patients, resulting in major improvement in symptoms. Over time, however, the loss of dopaminergic neurons continues, corresponding neural circuits become compromised, and the drug becomes ineffective. Advanced stages of PD are treated by implantation of electrodes to compensate for the damaged circuits by means of electrical stimulation.

The molecular mechanism or mechanisms of neurodegeneration in PD, the original cause of the selective dopaminergic neuronal loss, are mostly unknown. Many important findings have been reported; however, no unifying and universal theory has been proposed and accepted. The most plausible explanation is that these dopaminergic neurons contain the highest concentration of DA, which, it is presumed, increases their vulnerability. The DA itself is not toxic at physiologic concentrations. However, it is actively metabolized, primarily by monoamine oxidase, producing toxic derivatives, of which the most damaging are aldehydes. Normally, these derivatives are polymerized to form dark nontoxic neuromelanin, which is deposited in the cells. The earliest pathologic finding related to PD was the depigmentation of the substantia nigra in patients with Parkinsonism of infectious origin. In the early 1920s, it was considered improbable that a change in such a tiny area of the brain could cause the dramatic symptoms; as we now know, there are only about $5 \times 10^9$ pigmented dopaminergic neurons in normal substantia nigra. The depigmentation probably indicates the decrease in the concentration of DA or the inability of the cells to polymerize its toxic derivatives, or both.

A condition similar to PD can be imitated by administration of MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine), a synthetic compound that disrupts the work of the mitochondria. The
action of MPTP can be prevented by simultaneous administration of monoamine oxidase inhibitors; therefore, not the MPTP but its oxidized metabolites are toxic, similarly to DA. Administration of MPTP inhibits the mitochondrial complex I, and this inhibition results in increased generation of reactive oxygen species (ROS). Other inhibitors of complex I, for example, chronic manganese intoxication or chronic administration of rotenone in experimental animals, also may cause parkinsonism and selective death of dopaminergic neurons. These basic data suggest that ROS have a special function in the loss of dopaminergic neurons, although this connection has not been proved unequivocally and the major disturbance in the generation of energy and maintenance of proper redox potential of the pairs NADH/NAD+ (NADH dehydrogenase/nicotinamide adenine dinucleotide) and reduced or oxidized glutathione could be no less important.

Another conspicuous pathologic feature of neuronal damage in PD, formation of dense protein inclusions known as Lewy bodies (LBs), in affected cells has been known for some time. Lewy bodies consist primarily of α-synuclein (α-Syn) protein and components of proteasome machinery, chaperones, and α-tubulin. The exact role(s) of the inclusion bodies in disease pathogenesis, whether harmful, benign, or protective, was unknown for many years.

The important role of the major LB component, α-Syn protein, in PD pathogenesis was first elucidated in genetic studies. In familial PD, the most common causative mutations have been found in α-Syn gene product.

Expression of mutant α-Syn protein (or alternatively α-Syn protein) was demonstrated to be selectively toxic for dopaminergic neurons in some models, for example, when expressed from an engineered virus injected in rat brain. It is toxic to dopaminergic neurons of Drosophila when driven by a neuron-specific promoter. At the same time, mutant α-Syn expressed in mice causes various abnormalities in their nervous system, including aggregation and formation of LBs but not loss of dopaminergic neurons. The effect of mutant α-Syn in different species may depend on its specific modifications, processing, or interaction with other proteins, downstream agents of cytotoxicity.

Recently it became clear that LBs may not be the major toxic species of α-Syn and its complexes with other proteins. Lewy body–positive dopaminergic neurons seem to be healthier than their inclusion-free neighbors. Furthermore, the increase in the size of LBs and decreased concentration of soluble α-Syn correlate with reduction of cytotoxic effects. These observations are consistent with the protective role of inclusion formation, a self-defense mechanism to remove neurotoxic soluble α-Syn from a cellular milieu.

The current theory of the origin of PD places it in a large category of neurodegenerative diseases caused by protein misfolding. Proteins implicated in neurodegeneration can be neither efficiently refolded by chaperones to their normal conformation nor degraded by proteasomes, leading to their abnormal turnover, elevated concentration, aggregation, and accumulation of insoluble protein deposits. In PD, the cause is the high level of misfolded α-Syn molecules, which subsequently leads to formation of neurotoxic aggregated intermediates, that is, oligomers and probably small soluble complexes of α-Syn with other proteins, in particular, α-tubulin (Figure 1).

Mutant α-Syn protein tends to acquire abnormal conformation substantially easier than its wild-type counterpart. Gradual accumulation of misfolded α-Syn molecules promotes their oligomerization and formation of toxic species. High concentration of overexpressed normal α-Syn also causes cytotoxicity, which suggests a shift in equilibrium between normal and misfolded conformations and increased rate of oligomerization of the misfolded proteins. In some familial cases of PD, multiplication of the normal α-Syn gene, leading to protein overexpression from several chromosomal gene copies, has been found to be the causative mutation. Negative pleiotropic effects of oligomers on diverse cellular pathways were observed, including binding to cytoskeleton components, α-tubulin and microtubules, and damaging of mitochondrial and cellular membranes.

Although mutations and multiplication of α-Syn genes are the cause of most familial PD, the α-Syn protein is ubiquitously expressed at fairly high levels throughout the brain; therefore, its mere presence cannot be the only reason for selective death of dopaminergic neurons. α-Syn misfolding and oligomerization may be accelerated by several factors in dopaminergic neurons. For example, α-Syn can be modified by highly reactive aldehyde derivatives of DA, and as a result, modified α-Syn readily acquires the misfolded conformation. This hypothesis offers a plausible explanation for the selectivity of neuronal death in the substantia nigra pars compacta and has been reviewed in detail by Galvin.

The most important risk factor for sporadic PD is age. Aging could promote the accumulation of misfolded α-Syn by slowing its turnover, by lowering the level of respiration accompanied by diminishing the amount of energy resources, or by other metabolic changes. It is reasonable to assume that these changes are regulated by the general mechanisms of aging.

After many years of intensive research, caloric restriction was found to be the only environmental factor that can delay aging and substantially extend the lifespan of all studied organisms from yeast to mammals. In mammals, a restricted-calorie regimen provides about 70% of calories compared with ad libitum nutrition. One could expect that, among other diseases associated with aging, caloric restriction should affect the incidence and the course of PD. Caloric restriction alleviates symptoms of parkinsonism in MPTP-induced animal models of PD; however, epidemiologic studies failed to demonstrate a correlation between diet and incidence of PD.

Caloric restriction is a major factor that determines the concentrations of NADH/NAD+. It stimulates respiration and, by doing so, in-
creases the concentration of NAD\(^+\); it decreases the concentration of oxygen and generation of ROS. In yeast, these changes translate into a longer lifespan through regulatory functions of sirtuins (SIRTs). Sirtuins are NAD\(^+\)-dependent protein deacetylases. In the course of reaction, the acetyl group is transferred from protein to the adenosine diphosphate (ADP)-ribose moiety of NAD\(^+\) and nicotinamide is released. This reaction controls the acetylation of histones, chromatin condensation, and other aspects of global regulation of cell functions. Similar proteins are probably responsible for the effect of caloric restriction on longevity in higher organisms as well, although it remains to be proved in mammals.

During the last decade, several families of proteins similar to yeast proteins responsible for aging were discovered in mammals. These dis-

Figure 1. Role of α-synuclein (α-Syn) in neuronal death in Parkinson disease. Neurotoxic oligomers of α-Syn are shown as the key factors of neurodegeneration. One potential mechanism leading to neuronal death is invasion of α-tubulin polymers, which affects the dynamics and stability of microtubules.
coveries led to the search for potential therapeutic targets among SIRTs for pharmaceutical intervention and development of neuroprotective therapy for PD. The most promising current pharmacologic approaches are stimulation of SIRT1, which imitates caloric restriction conditions, and inhibition of \( \alpha \)-tubulin deacetylase SIRT2, which rescues \( \alpha \)-Syn toxicity, possibly by facilitating \( \alpha \)-Syn inclusion formation or by stabilizing microtubules. 

The finding that SIRT2 activity modulates the toxicity of \( \alpha \)-Syn may shed some light on the central problem in PD research, “the relationship between previously identified factors in PD neurodegeneration (eg, mitochondrial dysfunction or ROS) and the molecular events provoked by disease alleles.”

**Figure 2.** Modulation of \( \alpha \)-synuclein (\( \alpha \)-Syn) neurotoxicity by inhibition of sirtuin-2 (SIRT2) deacetylation activity. A, Sirtuin-2–dependent deacetylation of \( \alpha \)-tubulin promotes formation of a toxic complex with \( \alpha \)-Syn oligomers. B, Sirtuin-2 inhibition leads to reduction of toxic complex formation, promotion of Lewy body formation, and decreased neurotoxicity.
centrion dependent, which suggests a possible link between energy metabolism and α-Syn toxicity. However, whether mammalian SIRTs in general and SIRT2 in particular function as sensors of intracellular NAD$^+$ concentration is unclear (for discussion, see Buck et al$^{14}$). In addition, most NAD$^+$ in the cell seems to be bound with proteins, while experimental determination of free NAD$^+$ concentration is not a trivial task.$^{13}$ Thus, it is uncertain whether fluctuation of intracellular NAD$^+$ would substantially modulate sirtuin activities.

At the same time, epidemiologic studies suggest that another regulator of NAD$^+$, its role as a substrate for poly(ADP-ribose) (PARP) polymerase, is involved in PD. Only a few environmental factors were proved beyond doubt to influence the incidence of PD. Consumption of the 2 most common addictive substances, nicotine and caffeine, both substantially decrease the risk of developing PD. While the mechanism of nicotine action is unclear, the role of caffeine can be explained by the ability of its metabolites to physiologic conditions to inhibit PARP polymerase, the enzyme of first response to genotoxic stress. The substrate of PARP polymerase is NAD$^+$; therefore, the enzyme depends on its availability and rapidly depletes its cellular pool. Inasmuch as the enzyme PARP polymerase is the key trigger of apoptosis, it is conceivable that suppression of PARP polymerase prevents the loss of dopaminergic neurons with their enhanced sensitivity to ROS and other genotoxic agents.

In conclusion, despite substantial progress in recent years, the relationship remains to be clarified between the major components of PD pathogenesis: selective vulnerability and loss of dopaminergic neurons; α-Syn homeostasis and chaperone and proteasome dysfunction; energy depletion; generation of ROS; and aging, which may incorporate some or all of these factors. The SIRTs may be a missing link interconnecting these aspects of the disease, and further investigation of these pathways can provide fruitful insights in PD pathology.

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REFERENCES


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