Combating Parkinson's disease-associated toxicity by modulating proteostasis

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The incidence of age-associated neurodegenerative disease is growing rapidly, and it is expected to triple by 2050 (1). Currently, there are no cures or treatments that significantly modify disease progression. As researchers develop an in-depth understanding of the mechanisms of neurodegeneration, common features across different diseases are becoming apparent. Chief among them is the presence of protein aggregates, such as amyloid plaques and tau tangles in Alzheimer's disease (2); α-synuclein–rich Lewy bodies and Lewy neurites in Parkinson's disease (3); huntingtin aggregates in Huntington's disease (4); and aggregates of superoxide dismutase 1 (SOD1), TDP43, and FUS in amyotrophic lateral sclerosis (5). Although the precise mechanisms of protein aggregation-associated toxicity remain unclear, the accumulation of aggregated proteins in the diseased neurons indicates imbalances in protein homeostasis (proteostasis) (proteostasis is reviewed in ref. 6). As such, researchers are studying the potential therapeutic strategy of restoring proteostasis to normal levels by boosting the activity of molecular chaperones. One way to do so is to activate HSF1 (heat shock factor protein 1) by inhibiting HSP90 (heat shock protein 90), thereby stimulating the expression of multiple chaperones. Indeed, this strategy has been shown to be effective in fly models of Parkinson's disease and Huntington's disease (7, 8). However, toxicity associated with HSP90 inhibition remains an ongoing concern. An alternative potential strategy recently proposed is to activate the stress-inducible genes by activating the transcription activator nuclear factor erythroid 2-related factor (Nrf2) (9). Nrf2 controls the expression of multiple stress-inducible genes in response to oxidative stress (10, 11), proteostasis (12, 13), and inflammation (14). It does so by binding to the upstream regulatory DNA sequence known as the antioxidant response element. In PNAS, Skibinski et al. (15) investigate the effects of Nrf2 activation on cellular toxicity induced by two different Parkinson's disease-associated proteins, α-synuclein and leucine-rich repeat kinase 2 (LRRK2).

Parkinson's disease is a common neurodegenerative disease that is characterized by progressive neuronal loss in the substantia nigra pars compacta. Although the precise mechanism of neuronal death remains unknown, genetic studies in the past two decades have identified a number of genes associated with rare inheritable Parkinson's disease, including Parkin/PRKN, DJ-1/Park7, PINK1, SNCA (α-synuclein), and LRRK2 (16). α-Synuclein is a small protein (140 residues) whose mutation is associated with disease pathogenesis, and it is the main protein found in Lewy bodies of Parkinson's disease brains (16). Although the mechanism of α-synuclein–associated toxicity is still unclear, it has been shown that its disease-associated mutants are aggregation-prone and its aggregates are associated with neuronal toxicity (17, 18). LRRK2 is a large (2,527 amino acids) multidomain protein whose mutation is a common cause of Parkinson's disease (19). The mechanism by which LRRK2 causes Parkinson's disease remains unclear; however, the disease-associated LRRK2 mutants have been shown to display aberrant GTPase and kinase activities (20, 21).

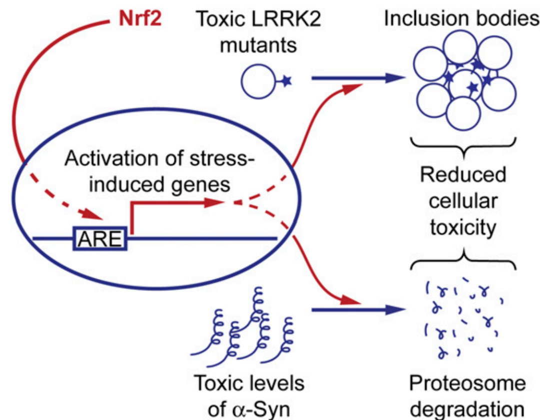

Fig. 1. Cartoon presentation of Nrf2 rescuing LRRK2- and α-synuclein (α-Syn)–induced toxicity as described by Skibinski et al. (15). Nrf2 activation leading to the transcription of stress-inducible genes resulted in a reduction of toxic levels of α-synuclein and sequestration of toxic LRRK2 mutants into inclusion bodies.

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To investigate the effects of Nrf2 activation on α-synuclein– and LRRK2-induced toxicity, Skibinski et al. (15) created expression constructs of α-synuclein and LRRK2 fused with differently colored fluorescent proteins and cotransfected each with an Nrf2 expression vector into neurons. The same set of transfected neurons was observed over a period of 1 wk, and images were taken at 12- to 24-h intervals using a robotic automated microscope the authors built (22). Using this imaging assay system, Skibinski et al. (15) first confirmed that Nrf2 was functional in the cells they used and then that the expression of α-synuclein and LRRK2 mutants was indeed toxic, thereby confirming the fidelity of the assay to be used.

In rat neurons cotransfected with Nrf2 and α-synuclein expression vectors, Skibinski et al. (15) observed that the activation of Nrf2 rescued α-synuclein–induced toxicity. Similarly, activation of Nrf2 also rescued rat neurons expressing two different disease-associated mutants of LRRK2, G2019S and Y1669C. To determine whether these rescue effects of Nrf2 also occur in human neurons, Skibinski et al. (15) repeated the same experiments using human induced pluripotent stem cell-derived neurons, in which they observed similar rescue effects for α-synuclein–induced toxicity.

To gain insights into the mechanism of Nrf2 rescue, Skibinski et al. (15) transfected neurons with α-synuclein fused to a green-Dendra2 fluorescence protein and quantified the green fluorescence level in the presence and absence of Nrf2 expression. The fluorescence levels in neurons expressing Nrf2 were found to be lower than the levels of controls, thus suggesting that Nrf2 activation might modulate α-synuclein turnover. Using an elegant method called optical pulse labeling (which irreversibly turns green Dendra2–α-synuclein red) to determine the lifetime of Dendra2–α-synuclein in neurons, Skibinski et al. (15) observed that activation of Nrf2 resulted in about a 10% increase in the rate of Dendra2–α-synuclein clearance compared with controls, thus suggesting that Nrf2 rescued α-synuclein–induced toxicity by lowering the cellular steady-state levels of α-synuclein (Fig. 1).

Skibinski et al. (15) used the same optical pulse labeling experiment to examine the neurons expressing toxic mutants of LRRK2. Interestingly, they found no reduction in steady-state levels of LRRK2 upon Nrf2 activation. Instead, they observed that Nrf2 activation led to a significantly greater risk of inclusion body formation for the Parkinson's disease-associated LRRK2 mutants, thus suggesting that Nrf2 activation might reduce LRRK2-associated toxicity by sequestering its toxic forms into potentially more inert inclusion bodies (Fig. 1).

It remains to be determined whether Nrf2 activation is an effective strategy for treating Parkinson's disease and its potential application for other neurodegenerative diseases; however, given the increasingly broad function of Nrf2 in different stress response pathways, proteostasis, and immunity, it offers an exciting addition to the arsenal for combating neurodegeneration.

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