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Parkinson's disease

Viral vector delivery of parkin generates model results in rats

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In the December 14, 2004 issue of the *Proceedings of National Academy of Sciences*, Christophe Lo Bianco *et al*[1] published the first *in vivo* demonstration of a novel gene therapy for Parkinson's disease (PD). Using a recombinant lentiviral vector that encoded the parkin gene, the authors were able to block dopamine neurodegeneration in a rat model of PD.

PD is characterized by progressive degeneration of the dopamine-producing neurons of the substantia nigra, that is, the pigmented region of the midbrain that gives rise to the nigrostriatal pathway. In most PD cases, which are nonfamilial, the etiology of the disease is unknown, and is thus referred to as idiopathic PD. However, in the last decade, mutations that lead to various inherited forms of the disease have been identified in several genes, two of which have gained particular attention: mutations or duplications in the α-synuclein (*α-syn*) gene were found to lead to PD in an autosomal dominant manner, and loss of function mutations in a gene named parkin has been found to be associated with an autosomal recessive juvenile form of PD.

Interestingly, parkin is one of the several E3 ligases that mediate ubiquitination of mutated or damaged proteins and facilitate the proteosome's removal of these proteins from the cells. Initial *in vitro* evidence indicated that a glycosylated (22 kDa) form of α-syn might be a substrate for parkin.[2] According to this model, parkin would ubiquitinate and facilitate degradation of *α-syn* (left side of Figure 1), and loss-of-function mutations in this gene would thus lead to accumulation of the 22 kDa synuclein protein species. Under this model, the parkin and α-syn mutations that lead to different forms of familial PD converge at the functional level. Conversely, then, enhancement of parkin function should lead to beneficial effects in PD cases.

Indeed, transgenic fly models have provided data that indicate that overexpression of parkin could counteract the degenerative changes that the overexpression of α-syn in these models induces.[3] However, the rescue of dopamine neurons in the parkin-α-syn double transgenic flies was seen in the absence of any changes in α-syn protein levels. By contrast, co-expression of parkin and Pael-R – another substrate for parkin – was associated with a profound reduction in detectable Pael-R protein. This difference argues in favor of two different mechanisms of action of parkin. While in the case of Pael-R, the ability of parkin to associate with the proteosomal degradation pathway is the likely explanation for rescue of dopamine neurons, another function of parkin independent of this pathway might protect these neurons from α-syn-mediated toxicity.

Lo Bianco *et al*[1] show that co-expression of the A30P-mutated form of human a-syn protein and wild-type parkin in the rat brain, using viral-vector-mediated gene transfer, leads to protection of the transduced dopamine neurons from a-syn toxicity. Interestingly, however, the number of inclusions, as assessed with an antibody recognizing the α-syn protein phosphorylated at serine residue in position 129 (the form found in pathological Lewy body inclusions in PD patients[4]), was increased rather than decreased in these rats.

So, if parkin is not facilitating the degradation of α-synuclein, how does it mediate its protective effects? The authors hypothesize that parkin enhances the sequestration of toxic soluble prefibrillar oligomers into mature hyperphosphorylated inclusions and so promotes dopamine neuron survival (right side in Figure 1). This hypothesis would be in line with the view that the soluble protofibrillar form of α-syn, rather than the aggregated form present in the inclusions, is the toxic species.[5] As the observations reported in the Lo Bianco paper are limited to only 6 weeks survival, it remains unclear if these inclusions could become toxic to a cell over a more protracted time course. This brings up a second question: How would this aggregated α-syn be removed from the cell? Cuervo *et al*[6] have recently provided evidence that native wild-type α-syn is selectively translocated to lysosomes and normally degraded. It is not known whether the mature hyperphosphorylated inclusions are turning over in the affected cells, and to what extent they might be targeted to the lysosomal compartment for degradation. It is indeed an intriguing question what would happen if the cells were unable to degrade the mutated α-syn. Would the affected dopamine neurons eventually die at a later time, or can the cells function well over a long term in the presence of inclusions that might continuously increase in size and number?

Another interesting piece of information comes from another paper by Lo Bianco *et al*[7] published a few months ago, where they tested the ability of glial cell line-derived neurotrophic factor (GDNF) in the same α-syn overexpression model. Surprisingly, although GDNF has been documented extensively as one of the most potent survival-promoting factors for DA neurons, it did not have any effect in the α-syn overexpression model. This discrepancy probably highlights the fact that our ability to show efficacy in a given model is dependent on (and is biased towards) the mechanism of action of the toxic insult. Nearly all protective or regenerative effects of GDNF had been shown in neurotoxic lesions that are based on oxidative damage triggered, for example, by inhibition of mitochondrial enzymes. Thus, the present data obtained with overexpression of parkin is highlighting a new pathway to tackle the degenerative process in PD, with actions different from and possibly complementary to GDNF's neuroprotective effects.

This raises the question whether parkin overexpression might be protective also in PD models where mitochondrial toxins induce dopamine neurodegeneration, such as MPTP and rotenone. Indeed, in idio-

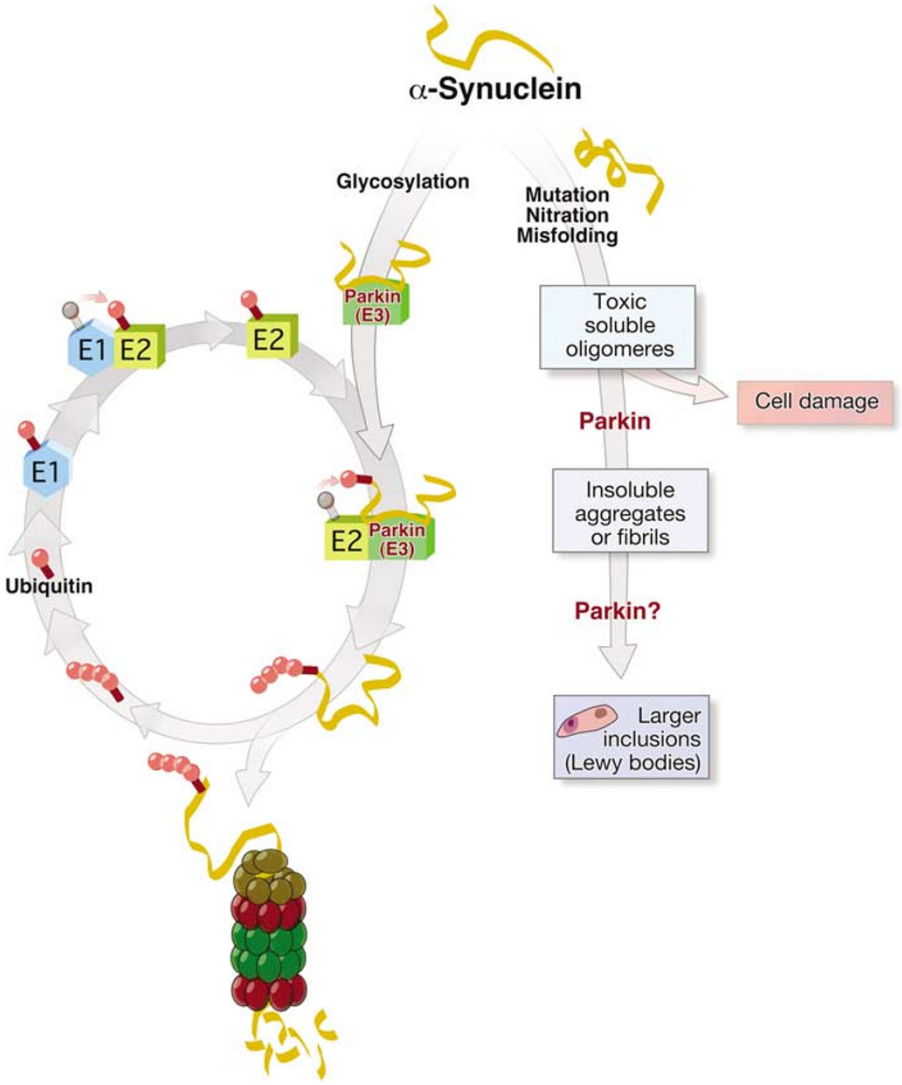

Figure 1 *Roles of α-synuclein and parkin in PD. α-Synuclein is one of the key players in dopamine neuron degeneration in PD. Accumulation of modified forms of this protein by, for example, nitration or misfolding, and point mutations in the gene itself, leads to cell damage through formation of soluble toxic oligomeres (as shown on the right side of the figure). According to current views, the affected cells might aggregate the toxic α-synuclein oligomeres into insoluble fibrillar structures to protect themselves. These structures are, in turn, transformed into larger intracytoplasmic inclusions – so-called Lewy bodies – in the diseased brains. Parkin can prevent the α-synuclein-induced toxicity in two ways: First, when associated with parkin, the glycosylated 22 kDa form of α-synuclein is ubiquitinated and targeted to the proteosomal degradation machinery (as shown on the left side). In this role, parkin acts as a E3 ligase that associates and transfers the ubiquitin moieties from the activated E2 to its substrate. Lo Bianco et al[1] report that overexpression of parkin in a transgenic model of α-synuclein-induced dopamine neuron degeneration can rescue the affected cells from α-synuclein-induced damage. However, the parkin-expressing cells had more, rather than fewer, α-synuclein inclusions. This suggests that parkin, in addition to its role in the proteasome pathway, might act on the soluble toxic α-synuclein oligomeres and increase their sequestration into less toxic aggregates and thus protect the affected cells.*

pathic PD, the primary 'hit' – oxidative damage, mitochondrial dysfunction or protein aggregation – can vary from patient to patient. Parkin gene therapy would seem a rational approach in familiar PD cases where the cause of the disease is a loss of parkin function due to mutations in the parkin gene. In other cases, where the primary cause is unknown, neuroprotective therapies, or modes of disease intervention, might have to be tailored to the individual case, or to defined subgroups of PD patients. α-Syn is clearly one of the culprits in parkinsonian neurodegeneration. However, the extent to which overexpression of parkin to block α-syn-induced toxicity is universally neuroprotective in PD, regardless of the underlying pathogenetic mechanism, remains to be clarified. ■

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