Targeting α-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations

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Progressive neuronal cell loss in a small subset of brainstem and mesencephalic nuclei and widespread aggregation of the α-synuclein protein in the form of Lewy bodies and Lewy neurites are neuropathological hallmarks of Parkinson's disease. Most cases occur sporadically, but mutations in several genes, including *SNCA*, which encodes α-synuclein, are associated with disease development. The discovery and development of therapeutic strategies to block cell death in Parkinson's disease has been limited by a lack of understanding of the mechanisms driving neurodegeneration. However, increasing evidence of multiple pivotal roles of α-synuclein in the pathogenesis of Parkinson's disease has led researchers to consider the therapeutic potential of several strategies aimed at reduction of α-synuclein toxicity. We critically assess the potential of experimental therapies targeting α-synuclein, and discuss steps that need to be taken for target validation and drug development.

Introduction

During the past two decades, a myriad of studies have suggested a substantial pathogenic role for α-synuclein in both familial and sporadic Parkinson's disease. Parkinson's disease belongs to the family of synucleinopathies, which includes dementia with Lewy bodies and multiple system atrophy (MSA). Parkinson's disease is the second most common neurodegenerative disorder, affecting 1–3% of the population older than 50 years[1] and more than 5 million people worldwide.[2] Classic motor signs of Parkinson's disease—including bradykinesia, rigidity, resting tremor, and gait disturbance with postural instability[3]—can be attributed mainly to the substantial loss of dopamine-containing neurons in the substantia nigra pars compacta. In addition to dopaminergic cell loss, another pathological hallmark of Parkinson's disease is the presence of intraneuronal proteinaceous cytoplasmic inclusions, named Lewy bodies, and dystrophic Lewy neurites, both of which contain α-synuclein deposits. The mechanisms leading to the formation and the pathogenic significance of these inclusions remain unknown.

No neuroprotective or neurorestorative therapy exists for treatment of this chronic disorder. However, two key discoveries that were made 17 years ago have had a substantial effect on Parkinson's disease research: the identification of mutations in the gene encoding α-synuclein (*SNCA*) in families with Parkinson's disease[4,5] and the finding that α-synuclein is a major component of Lewy body pathology.[4,5] Despite increasing understanding of Parkinson's disease pathogenesis, the exact mechanisms of progressive dopaminergic cell loss in the substantia nigra pars compacta remain to be unravelled. In this Personal View, we review advances in understanding of the prominent role of α-synuclein in Parkinson's disease, outline major challenges in understanding the clinicopathological value of the several α-synuclein species, address how α-synuclein species could be used as disease biomarkers, and critically assess the potential of experimental therapies targeting α-synuclein. We conclude that therapeutic development should focus on this promising target.

Role of α-synuclein in the disease process

α-Synuclein and Parkinson's disease

In 1912, Lewy[6] first described the intraneuronal proteinaceous cytoplasmic inclusions that became the histopathological hallmark of Parkinson's disease, later referred to as "Lewy bodies" by Tretiakoff.[7] In the early 1990s, α-synuclein was identified as the precursor of the non-amyloid component (NAC) of Alzheimer's disease amyloid plaques.[8–10] The first link between α-synuclein and Parkinson's disease was made in 1997 with the identification of point mutations in the *SNCA* gene in familial forms of Parkinson's disease (*PARK1* locus).[4] *SNCA*, which encodes α-synuclein, was identified on chromosome 4q21-q23 and Ala53Thr was described as the first point mutation causing autosomal dominant Parkinson's disease.[4] Six missense mutations in *SNCA* are now associated with autosomal dominant Parkinson's disease: Ala53Thr, Ala30Pro, Glu46Lys, His50Gln, Gly51Asp, and Ala53Glu[4,11–16] (figure 1). These mutations are extremely rare, with only a few families identified as

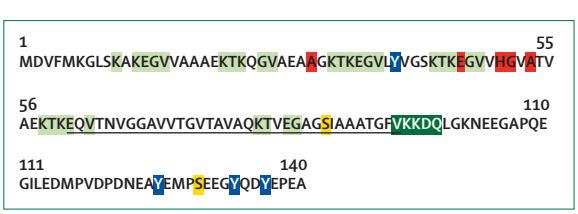

Figure 1: **Primary structure of human α-synuclein**
The aminoacid sequence of human α-synuclein is shown (UniProtKB/Swiss-Prot: P37840), with clinical mutations (Ala53Thr, Ala30Pro, Glu46Lys, His50Gln, Gly51Asp, and Ala53Glu) marked in red. The amphipathic N-terminal region contains six imperfect lysine-rich highly conserved motif repeats (KTKEGV), marked in light green, which are involved in the binding of lipids. The central hydrophobic region contains the non-amyloid β component sequence from residues 61 to 95, underlined. Two major phosphorylation sites (Ser87 and Ser129) are coloured in yellow. Chaperone-mediated autophagy recognition sites are marked in dark green. Nitration sites (Tyr39, Tyr125, Tyr133, and Tyr136) are shown in blue.

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having each mutation. Although the Ala30Pro mutation induces clinicopathological effects similar to those seen in sporadic Parkinson's disease, Ala53Thr, Glu46Lys, His50Gln, and the newly identified Gly51Asp and Ala53Glu mutations are characterised by an earlier onset of parkinsonism with rapid disease progression and additional clinical features, such as hallucinations, dementia, pyramidal tract impairment, and autonomic failure.[4,11–13,16] Neuropathological reports[11,13,17,18] of autopsies of patients with the Ala53Thr, Ala30Pro, Glu46Lys, or Gly51Asp mutation described dopaminergic cell loss with extensive synucleinopathy in several brain regions. The subsequent identification of families with duplication or triplication of the *SNCA* gene (*PARK4* locus) strengthened the link between α-synuclein and Parkinson's disease and indicated that increased concentrations of even the wild-type protein alone can cause the disease.[19,20] The clinical phenotype of patients with *SNCA* triplication (ie, early-onset parkinsonism with dementia) is more severe than in those with *SNCA* duplication (ie, close to that of patients with idiopathic Parkinson's disease), suggesting a dose-dependent association between disease severity and *SNCA* gene dosage. The common genetic variability at the *SNCA* locus is a robust risk factor for disease.[21]

α-Synuclein structure and function

Although its physiological function remains to be fully elucidated, α-synuclein is implicated in modulation of synaptic activity through regulation of synaptic vesicle release.[22] α-Synuclein is a member of a small family of three synuclein proteins: α-synuclein, β-synuclein, and γ-synuclein.[2] Three different domains can be defined in the 14kDa isoform of α-synuclein: an N-terminal domain (residues 1–60), a central NAC domain (residues 61–95), and a C-terminal domain (residues 96–140; figure 1). The N-terminal domain is characterised by repetitions of the six lysine-rich highly conserved motifs KTK(E/Q)GV, similar to lipid-binding motifs in amphipathic helical domains of lipoproteins.[23] All known clinical mutations are present in this region, emphasising the importance of this domain in the aggregation of α-synuclein. The NAC domain has a high content of hydrophobic aminoacids, responsible for the aggregation-prone properties of α-synuclein, whereas the C-terminal end is characterised by a high content of proline, aspartate, and glutamate residues. α-Synuclein and β-synuclein have a high sequence identity (around 90%) in the N-terminal domain, whereas the NAC domain of α-synuclein specifically contains a 12 aminoacid motif (residues 71–82: VTGVTAVAQKTV) that is a key element in the α-synuclein aggregation process, particularly its fibrillation (accumulation of β-sheet-rich aggregates).[24]

The native state of α-synuclein is extensively debated. Although some studies have reported that α-synuclein purified from human cells is a helically folded tetramer,[25–27] others have shown that α-synuclein exists predominantly as an unfolded monomer.[8,28] Taken together, these studies[29–31] suggest that α-synuclein exists in various conformations and oligomeric states in a dynamic equilibrium, modulated by factors that either accelerate or inhibit fibrillation (figure 2). Disease-related mutations affect the aggregation dynamics. The identification and characterisation of the toxic α-synuclein species remain incomplete. Two hypotheses have been proposed: toxic species could be amyloid-like insoluble fibrils, notably identified in Lewy bodies, or soluble, prefibrillar intermediates, such as oligomers or protofibrils (figure 2). Several groups have sifted through the different states of α-synuclein aggregation and thoroughly examined the functional consequences of aggregate-associated toxicity. Winner and colleagues[32] generated mutants unable to form fibrils while in an oligomeric state and showed enhanced toxic effects of these mutants in vivo. Increasing evidence from both in-vitro and in-vivo studies has supported the proposal that oligomeric species are the most relevant[28,31–34] to target therapeutically and suggests that Lewy bodies might be protective and represent a form of aggresome.[35] Although the different oligomeric types exist in a dynamic equilibrium and slowly convert into fibrils, Lashuel and colleagues[2] proposed that annular oligomers are part of the pathway leading to amyloid formation and are therefore potentially toxic. This result, if confirmed, suggests that stabilisation of the amyloid pathway might be of therapeutic interest.

α-Synuclein oligomeric species bind to lipids and increase mitochondrial, lysosomal, and vesicular membrane permeability, a common feature of aggregation-prone proteins.[34,36–39] Increasing membrane permeability

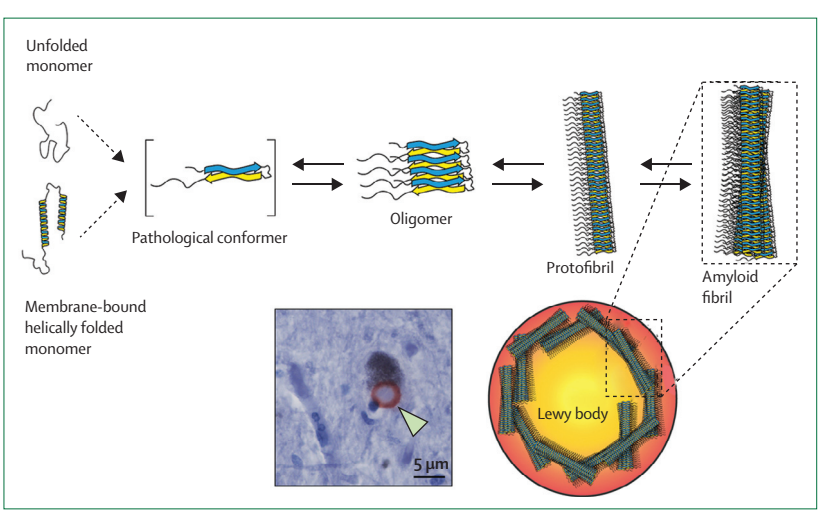

Figure 2: **Schematic diagram of the α-synuclein aggregation pathway**
α-Synuclein exists in various conformations and has at least two structural isoforms: a natively unfolded monomer and a helix-rich membrane-bound form. Both isoforms can undergo substantial structural changes, resulting in the formation of β-sheet-rich assemblies. From in-vitro studies,[29–31] α-synuclein is known to be in a dynamic equilibrium, in which the monomer can aggregate first into several types of small oligomeric species that can be stabilised by β-sheet interactions, and then into higher-molecular-weight insoluble protofibrils, which can polymerise into amyloidogenic fibrils resembling those identified in Lewy bodies. However, the mechanism governing the fundamental conformational change of normal monomeric α-synuclein to a pathological, disease-associated form remains unknown. The photomicrograph shows one synuclein-stained mesencephalic Lewy body (in red) in a neuromelanin-positive neuron from a patient with sporadic Parkinson's disease, indicated by the green arrow.