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The role of α-synuclein in neurodegenerative diseases

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Abstract

Alpha-synuclein is a 140 amino acid neuronal protein that has been associated with several neurodegenerative diseases. A point mutation in the gene coding for the α-synuclein protein was the first discovery linking this protein to a rare familial form of Parkinson's disease (PD). Subsequently, other mutations in the α-synuclein gene have been identified in familial PD. The aggregated proteinaceous inclusions called Lewy bodies found in PD and cortical Lewy body dementia (LBD) were discovered to be predominantly α-synuclein. Aberrant aggregation of α-synuclein has been detected in an increasing number of neurodegenerative diseases, collectively known as synucleopathies. Alpha-synuclein exists physiologically in both soluble and membrane-bound states, in unstructured and α-helical conformations, respectively. The physiological function of α-synuclein appears to require its translocation between these subcellular compartments and interconversion between the 2 conformations. Abnormal processing of α-synuclein is predicted to lead to pathological changes in its binding properties and function. In this review, genetic and environmental risk factors for α-synuclein pathology are described. Various mechanisms for in vitro and in vivo α-synuclein aggregation and neurotoxicity are summarized, and their relevance to neuropathology is explored.

Keywords: Parkinson's disease; LBD; Synucleopathy; Lewy bodies; Serine phosphorylation; Protein aggregation; Tissue transglutaminase; Oxidative stress; Nitration; Protofibrils; Fibrillation; Dopamine transporter; Tyrosine hydroxylase; Iron; Muscarinic receptors; Endocytosis; Vesicle recycling

Abbreviations: αSp22, *O*-glycosylated α-synuclein; 6-OHDA, 6-hydroxydopamine; Aβ, β-amyloid; AD, Alzheimer's disease; A, adenine; Ala, alanine; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; AR-JP, autosomal recessive juvenile parkinsonism; BS3, bis(sulphosuccinimidyl)suberate; CD, circular dichroism; cDNA, complementary deoxyribonucleic acid; CNS, central nervous system; DAT, dopamine transporter; DCCD, dicyclohexylcarbodiimide; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzymes; E3, ubiquitin ligase; EPR, electron paramagnetic resonance, also known as electron spin resonance (ESR); FTIR, Fourier transform infrared; G, guanine; Gln, glutamine; Glu, glutamate; Gly, glycine; GRK, G-protein-coupled receptor kinase; H_2O_2, hydrogen peroxide; HEK293, human-embryonic kidney-derived cells; JNK, c-Jun N-terminal kinase; LBD, cortical Lewy body dementia; Lys, lysine; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; mM, millimolar; MPP+, methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mRNA, messenger ribonucleic acid; MSA, multiple system atrophy; NAC, non-Aβ component of AD amyloid; NACP, non-Aβ component of AD amyloid precursor protein; NBIA 1, neurodegeneration with brain iron accumulation type 1; nm, nanometer; nM, nanomolar; NMR, nuclear magnetic resonance; PAGE, polyacrylamide gel electrophoresis; PD, Parkinson's disease; PGP 9.5, ubiquitin carboxyl-terminal hydrolase; PI, phosphatidylinositol; PLC, phospholipase C; PLD_2, phospholipase D_2; Pro, proline; PUFA, polyunsaturated fatty acid; PVDF, polyvinylidene fluoride; Ser, serine; S, sulfur; SN*pc*, substantia nigra *pars compacta*; TfR, transferrin receptor; Thr, threonine; tTG, tissue transglutaminase; UV, ultraviolet; Val, valine; WT, wild-type.

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1. Introduction: history of the discovery of the synuclein family of proteins

The synuclein family of proteins was initially identified by expression screening of cDNA clones generated from the electric lobe of *Torpedo californica* that reacted to antiserum against purified cholinergic vesicles (Maroteaux et al., 1988). The original *Torpedo* clone was then used to screen a rat brain cDNA library. Open reading frames coding for 143 and 140 amino acids were detected in *Torpedo* and rat, respectively, with 85% homology. Approximately 100 amino acids of the N-terminus of both proteins contained repeat sequences of 11 amino acids, with a central conserved core of 6 residues consisting of slight variations of the sequence Lys-Thr-Lys-Glu-Gly-Val, followed by a more variable acidic tail of 40–50 residues. The calculated molecular masses of the *Torpedo* and rat proteins were 14.8 and 14.5 kDa, respectively. A primary antibody raised against a fusion protein made from the original *Torpedo* cDNA clone detected the protein subcellularly on portions of the nuclear membrane and in high concentrations in presynaptic terminals of the nervous system, but not in peripheral tissue. From this distribution, the investigators named the novel protein syn (synapse)-nuclein (nucleus). Western blot analysis of *Torpedo*-extracted proteins with synuclein antigenicity revealed 3 bands, with the strongest signal detected corresponding to an apparent molecular mass of 17.5 kDa and weaker signals at 18.5 and 20 kDa. The latter 2 species were speculated to reflect posttranslational modifications of the dominant species.

In subsequent research, Maroteaux and Scheller (1991) screened a rat brain cDNA library using the rat cDNA probe identified previously, now renamed SYN1. In addition to several phages homologous to the original probe, they found 2 other species of cDNA, SYN2 and SYN3. The predicted sizes of the proteins coded from SYN1 and SYN2 were 14.5 and 15.7 kDa, respectively, and were found in approximately equal concentrations. SYN3 coded for a smaller molecule containing only 3 conserved repeats, which was present in lesser concentration. The synucleins were mapped to discrete regions of rat brain, with dense labeling in piriform and frontal cortices, lateral olfactory tract, hippocampal pyramidal cells, dentate granule cells, and some brainstem nuclei, although the antibody used recognized an epitope common to all 3 isoforms and thus did not distinguish among them. Unexpectedly, in addition to the Western blot bands corresponding to the lower molecular mass synucleins, a 45 kDa form was copurified with synaptosomal membranes, using standard fractionation procedures. This higher molecular mass protein could be solubilized from the P2 pellet using alkaline conditions, and preliminary evidence indicated that it could be converted to a lower molecular mass synuclein isoform by phosphatidylinositol (PI)-specific phospholipase C (PLC).

Jakes et al. (1994) purified synucleins from human cerebral cortex and sequenced 2 abundant isoforms of 140 and 134 amino acids, which they named α- and β-synuclein, respectively. The 140 amino acid protein is identical to the non-Aβ component of AD amyloid precursor protein (NACP) molecule (Ueda et al., 1993) is 95% homologous with the SYN1-coded protein isolated from rat brain (Maroteaux & Scheller, 1991), differing from the latter by only 5 residues. The 134 amino acid protein is the human homologue to bovine phosphoneuroprotein 14 (Nakajo et al., 1993). Both proteins were found to be heat-stable and