AUSTRALIAN SOCIETY FOR BIOPHYSICS

Abstract: 89O

Hyper-phosphorylation induces structural alterations and exacerbates the cytotoxicity of alpha-synuclein in Parkinson's Disease pathogenicity

Minal Chaturvedi[1, 2,3], Charlotte Conn[4], Celine Valery[3], Smriti Priya[1,2]

[1]*Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow 226001, Uttar Pradesh, India*
[2]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad- 201002, India*
[3]*Translational Immunology & Nanotechnology (TIN) Program, School of Health and Biomedical Sciences, RMIT University, Melbourne, Australia*
[4]*School of Science, RMIT University, Melbourne, Australia*

Alpha-synuclein (α-syn) is an intrinsically disordered protein (IDP) that is known to misfold and aggregate in Parkinson's Disease (PD). A dramatic increase in the phosphorylation of α-syn has been established in PD pathogenicity, but its effect on structure and cytotoxicity is still controversial. We phosphorylated α-syn using an *in vitro* kinase assay. Using high-resolution mass spectrometry we observed multi-serine phosphorylation at Ser42, Ser87 and Ser129 with Polo Like Kinase 2 (PLK2) and at Ser87 and Ser129 with G Protein Coupled Receptor Kinase 4 (GRK4). TEM analysis showed distinct α-syn strain formation post phosphorylation (p-α-syn). Significant structural alterations and peak shift due to phophorylation were confirmed using Nuclear Magnetic Resonance (NMR). The phosphorylated α-syn formed SDS resistant higher molecular weight species. It accelerated the aggregation kinetics of monomeric α-syn and enhanced the nucleation capacity of the strain. The p-α-syn species were cytotoxic as assessed in SH-SY5Y dopaminergic neuronal cells, suggesting diverse pathways of multi-serine phosphorylation induced pathology. Based on these findings, we performed stereotaxic administration of multi-serine phosphorylated α-syn strain aggregates into the Substantia nigra (SN) region of the rat brain. Male wistar rats were used create a PD rat model using Rotenone (1.5mg/Kg body weight, intraperitoneal). The rats were further anaesthetized by injecting ketamine:xylaxine (3:1) and the two strains of alpha-synuclein, α-syn and p-α-syn (2.5mg/10ul) were then administered in the substantia nigra (AP:5.0; L:1.8; V:7.6) of the rat brain. The animals were kept under observation for 1.5 months. The results showed an altered course of pathology spread indicating enhanced spread of the phosphorylated species. Significant increase in dopaminergic neuronal death was observed using phosphorylated α-syn. The proteomic results from high resolution mass spectrometry demonstrate the overall changes in the SN of the rat brain and provide a concrete evidence for phosphorylation in α-syn induced pathology.