INTERACTIONS OF ALPHA-SYNUCLEIN WITH MITOCHONDRIA BY WIDEFIELD, STED AND STORM MICROSCOPY

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KEY WORDS: α-Synuclein, Mitochondria, Widefield, STED, STORM, FRET

ABSTRACT

Parkinson’s disease (PD) is the second most common neurodegenerative disorder. Its pathologic hallmark is the functional loss of dopaminergic neurons and the appearance of Lewy Bodies, intracellular amyloid aggregates constituted mainly by the protein α-Synuclein (aSyn). Several reports associate mitochondrial (oxidative) stress as one of the putative promoters of PD pathology, leading to the functional loss and ultimately death of dopaminergic neurons in. aSyn is an intrinsically disordered protein that binds readily to phospholipid membranes, and its increased expression promotes PD pathology. This investigation was focused on the characterization of aSyn interactions with isolated mitochondria and their possible relationship to increased mitochondrial stress, operating as a key mechanism in PD.

Isolated mitochondria were obtained from rat brains or from human SH-SY5Y neuroblastoma cells and import assays were performed with fluorescently labeled aSyn within the next 4 hours after their purification. The mitochondria were incubated with different concentrations of aSyn (0-100 µM) to assess dose-dependent effects. Samples were fixed in PFA and the mitochondrial TOM20 outer membrane (OM) marker was stained with specific antibodies. 3D image stacks were recorded by widefield microscopy with a 150× objective, STED images were recorded in a commercial SP5-STED with a 100× objective, and STORM images were acquired in a custom-built setup with a 120× system magnification.

The binding and import of aSyn to mitochondria was detected with all microscopy techniques employed. Deconvolution of widefield 3D stacks with the SVI Huygens software revealed a uniform distribution of TOM20 on the mitochondrial surface. aSyn was also localized to the OM at low concentration, but was imported into the mitochondria at higher concentrations. At low concentrations, STED images showed a non-uniform surface distribution of aSyn clusters. Possible aggregates of aSyn were visible by STORM and STED inside the mitochondria at higher concentrations. A close association of aSyn and TOM20 was revealed by FRET imaging; a high FRET efficiency of 0.5 diminished to 0.05 when unstained aSyn was added to the reaction.

These results and the observation that binding of aSyn to the mitochondria perturbs the respiratory chain suggest that elevated aSyn expression may indeed lead to increased mitochondrial stress with consequent impairment of cellular functions.