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Identification of novel synphilin-1 interactors involved in aggresome formation and Parkinson disease pathogenesis using Tandem Affinity Purification and Mass Spectrometry

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Novel Aspect

Potential protein candidates for aggresome formation studies in mammalian cells can be identified using tandem affinity purification and mass spectrometry.

Introduction

Parkinson disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons and the presence of Lewy bodies. Synphilin-1 is one of the major components of these inclusions, and is implicated in the pathogenesis of PD. Upon inhibition of proteasome, synphilin-1 readily forms cytoplasmic aggregates, which are transported to aggresome, a recently discovered storage compartment for abnormal proteins. Capturing aggregates of abnormal proteins in aggresome relieves their toxicity, and therefore clarifying mechanisms of its formation is essential for understanding the origin of many protein misfolding disorders, including PD. To clarify these mechanisms, we seek to identify proteins that associate with synphilin-1 using tandem affinity purification and LC/MSMS, in order to test their role in aggresome formation.

Methods

Synphilin-1 was constructed to carry a His-tag and a GFP-tag at the N- and C- termini, respectively. A plasmid containing this recombinant synphinlin-1 was produced and transfected into HEK293T cells. Synphilin-1 and associated proteins were purified from cells treated with proteasome inhibitor or naïve cells using two consequent affinity steps, cobalt beads followed by anti-GFP antibody beads. Purified synphilin-1 protein complexes were digested by trypsin in solution, analyzed by data-dependent nanocapillary reversed-phase LC/MSMS, identified by automated searching against Swiss-Prot protein database using MASCOT integrated Proteome Discoverer, and annotated by an in-house program--STRAP. To address nonspecific interactors, vector transfected HEK293T cells were treated identically and analyzed in parallel as a negative control.

Preliminary Data: Three samples were prepared. (1) Synphilin-1 transfected cells (S) were used to identify proteins interacting with synphilin-1 in the absence of aggresome formation. (2) Synphilin-1 transfected cells treated with a proteasomal inhibitor (SI) were used to detect additional proteins that may promote formation of aggresomes. (3) Empty vector transfected cells (V) were used to determine non-specific interactors. During SDS-PAGE analysis, synphilin-1 samples exhibited strong protein bands while the vector contained only traces of proteins, depicting the successful pull-down of the synphilin-1 protein complexes as well as high efficiency of the protein affinity purifications. Western blotting determined the protein recovery rates on cobalt beads and anti-GFP beads were about 60% and 40%, respectively. We identified 50, 98, and 94 proteins in V, S, and SI samples, respectively. Thirty-two proteins were present in all three samples, and were therefore considered to be non-specific interactors. S and SI shared 46 common proteins while they contained 20 and 16 unique proteins, respectively. Some identified synphilin-1 interacting proteins were consistent with previous reports whiles novel interactors could be determined after further validation steps. Comparisons of identified proteins between S and SI were performed based on biological process, cellular component, and molecular function. Although S and SI had similar protein groupings in terms of cellular components, differences were found in terms of biological process and molecular function, indicating different proteins participated in the formation of aggregates and aggresomes. Several proteins of interest have been selected for further validation. Ongoing experiments are focused on extending this approach to additional deletion constructs of synphilin-1 to associate its functional domains with their interacting proteins.

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