Title (Current 12 words) – Limit 20 words

Determination of Synphilin-1 interaction networks using isotopically tagged cross-linking and mass spectrometry

Introduction (Current 114 words) – Limit 120 words

Parkinson disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons and the presence of inclusion Lewy bodies. Synphilin-1 is one of the major components of these inclusions and is implicated in the pathogenesis of PD. Upon proteasome inhibition, synphilin-1 readily forms cytoplasmic protein aggregates, which are transported to aggresome. Capturing aggregates of abnormal proteins in the aggresome removes their toxicity and, therefore, clarifying mechanisms of their formation is essential for understanding the origin of many protein misfolding disorders, including PD. Previously, we identified and quantified Synphilin-1 interacting proteins using tandem affinity purification and LC-MS/MS. Here, we cross-linked Synphilin-1 protein complexes and used LC-MS/MS to further characterize the Synphilin-1 interaction network.

Methods (Current 118 words) – Limit 120 words

Synphilin-1 was constructed to carry a His-tag. A plasmid containing this recombinant synphinlin-1 was produced and transfected into HEK293T cells. The cell extracts were loaded onto cobalt beads. After washing off non-specific binders, the cross-linking reaction was performed on the cobalt beads by incubation with isotopically tagged d0/d12-BS^3 cross-linkers at a ratio of 1:1. Cross-linked Synphilin-1 protein complexes were purified and digested by trypsin and analyzed by nanocapillary reversed-phase LC-MS/MS on an LTQ-Oribitrap mass spectrometer (Thermo). The proteins were identified using Proteome Discoverer (Thermo) integrated with MASCOT (Matrix Science). Isotopically labeled cross-linked peak pairs were extracted using Progenesis LCMS (Nonlinear Dynamics) to generate inclusion lists for performing targeted MS/MS. The cross-linked peptides were assigned using xQuest (ETH Zurich).

Preliminary data (current 292 words) – Limit 300 words

Isotopically tagged cross-linked tryptic peptides have two characteristics that enable their targeted selection and fragmentation within a complex peptide mixture. First, cross-linked peptides have two basic tryptic C termini and thereby tend to carry higher charge states (≥3+).

Second, peptides cross-linked with d0 (Light) and d12 (Heavy) BS^3 cross-linkers at a ratio of 1:1, are observed as peak pairs having a mass difference of 12.07573 u and equal signal abundance in the MS spectra. Two LTQ-Oribitrap instrument methods were used for targeted fragmentation of cross-linked peptides: targeted mass tag and a master inclusion list. The mass tag method allows fragmentation of peak pairs with a specific mass difference found in pre-scans. An inclusion list which contained all cross-linked peak pairs was used to target fragmentation.

One hundred sixty-one protein groups were found in the cross-linked Synphilin-1 protein complex. These were used to generate a fasta database for the xQuest search. Progenesis LCMS extracted 8586 peptide peaks (charge state≥3+) from the LCMS run, of which 262 isotopic peak pairs were extracted using an custom algorithm programmed in-house.

Thirteen peak pairs were fragmented using the mass tag method, among which 6 cross-linked modified peptides were assigned. Twenty peak pairs were fragmented using the inclusion list method, among which 6 cross-linked modified peptides were assigned. The two methods yielded complementary results. As an example, the transcription factor GATA-6 (at position K485) was observed to be cross-linked with elongation factor 1-alpha 1 (at position K443).

In summary, the synphilin-1 interacting network was determined by cross-linking on affinity beads followed by mass spectrometry analysis, which leads to better understanding of the formation of aggresomes and protein misfolding disorders.

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Novel aspect (Current 18 words) – Limit 20 words

Synphilin-1 protein interacting network was determined by isotopically tagged cross-linking on affinity beads followed by mass spectrometry analysis.

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