Parkinson’s Disease Biomarker Development: Immunoprecipitation LC-MS/MS To Quantitatively Detect Phosphorylated alpha-Synuclein in Human CSF

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Abstract
Parkinson’s disease (PD) is a global neurodegenerative disease characterized by progressive deterioration of motor function and cognition. Histopathologically, PD is characterized by the intracellular accumulation of alpha-synuclein (α-syn) in the form of Lewy Bodies and Lewy Neurites. Mutations in α-syn, as well as duplication and triplication of the wild-type locus cause autosomal dominant familial PD. α-Syn is subject to post translational modifications, including phosphorylation and nitration, which results in neurotoxic species. Assessment of phosphorylation status may provide a closer connection to disease progression than total α-syn levels alone. Although primarily an intracellular protein, α-syn is actively excreted by neurons, therefore providing an accessible pool in CSF for biomarker evaluation. Here, we report the development of immunoprecipitation (IP) LC-MS/MS methods to measure low-abundance phosphorylated forms of α-syn in human CSF compared to plasma for biomarker applications. Aims: To assess feasibility of using CSF levels of phosphorylated α-syn as a biomarker for PD. Methods: Phosphorylated species of α-syn (at S87, Y125, S129 and Y133) were immunoprecipitated from non-PD human CSF and plasma with phospho-specific antibodies. The immunoprecipitated protein was subjected to trypsin digestion and a unique signature peptide was analyzed by LCMS/MS. Results: All phospho-epitopes were readily detected in both CSF and plasma. Overall, CSF levels of phosphorylation were ~14% of plasma levels. Conclusions: IP LC-MS/MS methodology of specific α-syn species may provide a novel biomarker approach for PD. This technique is readily applicable to other low abundance CSF analytes and provides sufficiently increased sensitivity for their detection.

Introduction
α-syn Plays a Central Role in PD

Genetics: Mutations (A53T, P30L, E46K) & gene duplication or triplication cause PD
Pathology: Protein is major component of hallmark pathology (Lewy Body, Lewy Neurite)
Modifications: α-syn phosphorylation may play a role in pathogenesis

CSF Phosphorylated α-Syn May Be a Biomarker for PD

Methods
α-Syn and phospho-α-syn were enriched from human CSF and plasma by selective capture with anti-α-syn MAbS or anti-phospho (S87, Y125, S129, and Y133) α-syn MAbs coupled to magnetic beads. The supernatant was aspirated and the beads were suspended in trypsin solution for enzymatic digestion. The α-syn species captured by the MAb/bead complex were digested into peptides directly from the beads and filtered prior to injection into the LC/MS/MS.

Sample Preparation Prior to MS Analysis

LC-MS/MS Analysis

Results
This IP-LC-MS/MS method was successfully applied to the measurement of α-syn and phosphorylated α-syn in human CSF and plasma from non-PD donors. Phosphorylation at S87, Y125, S129, and Y133 were immunoprecipitated and analyzed using a unique peptide via LC-MS/MS. All four phosphorylation sites were measurable and overall phosphorylated levels in CSF were ~14% of plasma levels.

Further Application

This methodology is readily applicable to other rare CSF analytes. This IP-LCMS/MS has been applied to the capture and measurement of LRRK2, using antibodies provided by Michael J. Fox Foundation, as well to the capture and measurement of MAPT (tau). IP and subsequent LC-MS measurements using protein standards are shown below.

Summary
- We have developed methods to detect several phosphorylated forms of α-syn in human CSF.
  - Ours is the first report of measurable levels of phosphorylated α-syn in CSF.
  - We are currently assessing utility of this assay for biomarker applications.
- Our methods are generally applicable to other CSF analytes, such as LRRK2 and tau.
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Human CSF

Phosphorylated alpha-Synuclein in LC-MS/MS To Quantitatively Detect Parkinson's Disease Biomarker

Development: Immunoprecipitation