Title

Development of a Fit-for-Purpose LC-MRM-MS Assay to Measure Prion Protein in Cerebrospinal Fluid

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Abstract

Prion disease is a fatal and untreatable neurodegenerative disease caused by misfolding of the prion protein (PrP). PrP is a well-validated drug target for prion disease and candidate therapies are being developed to lower PrP levels in the brain. Current assays employ antibodies but they lack sufficient sensitivity to robustly measure PrP levels pre- and post- knockout or drug intervention. Using a 9-plex MRM assay, we were able to detect and quantify human PrP using either a heavy labeled protein standard or heavy labeled peptide standards using nanoelectospray LC-MRM-MS. Experiments were performed to determine the assay limits of detection and quantification, dynamic range, accuracy and precision for each peptide. PrP was measured in CSF samples from patient cohorts by LC-MRM and the results were compared to concentrations determined by a commercial ELISA for human PrP. CV for six peptides from heavy labeled protein was 9 – 23% across a concentration range of 2.4 – 240 ng/mL. Inter-day CV for endogenous PrP peptides in human CSF were less than 23% at 244 ng/mL and the correlation to ELISA was Spearman's rho = 0.40 to 0.69, all $P < 0.0001$. Further development of these assays will include the selection of a single peptide to assay human, mouse or monkey PrP in CSF, transfer of methods to a clinical laboratory and validation in a regulated environment to support future clinical trials.

Bio

Eric Kuhn is a Senior Research Scientist in the Proteomics Platform at the Broad Institute. An expert in peptide quantitation assays using stable isotope labeled standards and LC-MRM-MS, he has designed antibody and aptamer based enrichment (or depletion) strategies to increase the sensitivity and precision of MS-based measurements of proteins in complex biofluids (such as plasma). He continues to design and optimize workflows to increase the detection limits of targeted MS assays in support of a number of biomarker discovery programs. At the Broad Institute, his assays quantify protein changes associated with ovarian cancer, breast cancer, cardiovascular disease and more recently, prion and kidney disease. In collaboration with NIH, he has co-authored guidance documents describing peptide standard handling, specifications and assay best practices. He has also characterized proteins for research and as potential therapeutics at DuPont, AutoImmune, and Millennium Pharmaceuticals and holds a BS in Biochemistry from the University of New Hampshire.