Developing PRM on the timsTOF Pro for biomarker studies in cerebrospinal fluid

Jarrod Marto

Dept. of Cancer Biology, Blais Proteomics Center and Dana-Farber Cancer Institute, Dept. of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, U.S.A.

Parkinson's disease (PD) is the second most common neurodegenerative disorder, afflicting more than 4 million people worldwide. The prevalence of PD is expected to rise in future years with the general trend toward increased lifespan. PD progresses slowly and typically goes undetected until clinical diagnosis based on overt symptoms of motor dysfunction, at which point a majority of dopaminergic neurons have been destroyed. PD symptoms vary across individuals, and a prodromal phase may precede clinical symptoms by several years. Hence there is an urgent need for robust PD biomarkers to facilitate early detection and all aspects of disease management.

Our biomarker study is designed around analysis of cerebrospinal fluid (CSF) from independent discovery and validation patient cohorts. For the discovery stage we used stable isotope labeling and extensive peptide fractionation to characterize more than 6,000 proteins across ~200 CSF samples from PD and healthy control (HC) subjects. We used regression analysis encompassing the proteomic data as well as clinical covariates to identify ~60 proteins whose abundance in the CSF was associated with PD. These results included previously reported proteins such as VGF and SCG2, along with many others not previously identified in the CSF proteome, or associated with PD. We used an independent cohort of patient and control CSF samples for the validation phase. To circumvent the need for antibodies and improve validation throughput and performance, we developed label-free PASEF-PRM acquisition on a Bruker timsTOF Pro mass spectrometer. The combination of PRM and ion mobility separation simultaneously maximizes peptide selectivity and detection, as well as the number of peptides quantified per unit acquisition time. We used protein standards spiked into CSF to establish quantification accuracy as a function of peptide abundance, number of fragments, and other parameters. Our results indicate CVs for quantification of approximately 20% for peptides spanning a wide range of abundance in a CSF matrix. Data for analytical characterization of PASEF-PRM in addition to data for CSF proteins will be presented.