TMT-proteomic work-flow for tissue-fluid interface biomarkers discovery in amyotrophic lateral sclerosis

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Research question and background

The lack of validated disease biomarkers to be used for disease recognition/monitoring and to evaluate treatment response is the main reason for the lack of success in the establishment of new successful treatments for ALS. It is generally accepted that biomarkers are signals of disease that can be measured in biological fluids; target selection for any biomarkers study is not straight forward as it is not possible to have a comparative analysis of the molecular changes engendered by the disease simultaneously in affected tissue and in biological fluids from patients. Novel methods capable of exploring protein expression in tissue and bio fluids are needed. This may include also the potential for further analysis of circulating white cells from affected individuals, considering the importance of both innate and adaptive immune responses in the progression of ALS. The variable speed of progression in ALS patients may reflect the immune response to the neurodegenerative process.

Methods and tissues used

The aim of our study is to identify soluble biomarkers associated with fast and slow progression of the disease, using an unbiased, sensitive proteomic approach based on Tandem Mass Tag® (TMT®) labeling technology, developed by Proteome Sciences (PS). We have so far profiled affected brain tissues (obtained from NBB) and white cells collected longitudinally from ALS patients and searched for similar fluid-phase biomarkers in matched longitudinal plasma samples. We are also working on the proteomic characterization of progressive bulbar palsy, a rare and fatal subtype of motor neuron disease with a rapid progression to impairment of speech, swallowing and to respiratory failure.

Results and conclusion

We have to this point dissected the protein composition of tissues and plasma from a subset of ALS individuals known to have a fast and a slow progression of the disease. We have identified a broad spectrum of proteins, peptides and of molecular pathways that are linked to the modified regulation of basic cellular functions and appear to be associated to a particular rate of progression. We have also worked on validation assays, combining Selected Reaction Monitoring (SRM) and TMT-calibrator multiplexed workflows to develop orthogonal assays. The validation process also includes commercially available immunoassays for candidate biomarkers. At present, we are undertaking a broad bioinformatics analysis of the proteomic datasets and we are in the process of extending the initial analyses to incorporate a broad range of patients and controls. This study will pre-configure biomarkers assays to be tested in routine clinical practice, strengthening the potential for an early diagnosis and for the accurate monitoring of disease progression.

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