Optimizing selective reaction monitoring-mass spectrometry (SRM-MS) to measure proteins of interest in schizophrenia

Guilherme Reis-de-Oliveira*, Juliana Silva Cassoli, Daniel Martins-de-Souza

Abstract

Selective reaction monitoring (SRM) is a mass spectrometry-based technique employed to accurately quantify analytes such as proteins. As schizophrenia presents disturbances in protein expression and their pathways, as previously observed by proteomic studies, SRM is a suitable tool to study this disease. This project aims to use SRM to quantify in a targeted fashion proteins associated to schizophrenia, allowing the identification of potential biomarkers for the disease. Here we will show the first steps of optimization of SRM technique.

Key words: *Quantitative Proteomics, Schizophrenia, SRM*

Introduction

Schizophrenia (SCZ) is a chronic psychiatric disorder that affects 1% of population worldwide. Also, SCZ is a multifactorial disease whose etiology can be triggered by genetics and environments factors¹. Studies have shown that such factors are able to change molecular pathways, through disturbances in protein expression. Thus, the quantification of these proteins in SCZ patients enable the identification of key players in the disease, which will allow a better comprehension about the pathophysiological mechanisms of SCZ². Selective Reaction Monitoring (SRM) is most suitable mass spectrometry-based technique for protein quantification. Frequently, this technique is performed in a triple quadrupole mass spectrometer (QQQ) system. The first (Q1) and third (Q3) quadrupoles operate as mass filters. The second quadrupole (Q2) serves as a collision cell. For an SRM experiment, the mass spectrometer is pre-programmed such that the first quadrupole, at a given time, selectively transmits precursor peptide ions of a given m/z value to the collision cell. After collision-induced dissociation (CID) in Q2, one or more specific product ions are selectively transmitted through the third quadrupole, reaching the detector over time (transition)³. This results in an MS peak that is associated with a specific chromatographic retention time and an intensity value. The aim of this project is to establish SRM assays to quantify differentially expressed proteins in SCZ postmortem brain tissue or pre-clinical models.

Results

Here we show the optimization of all parameters concerning SRM assay for proteins of interest to schizophrenia. Initially, proteotypic peptides are chosen to each protein of interest for their quantification in postmortem brains and cell culture samples. As we employ liquid chromatography prior to SRM-MS, Another important parameter to be established is the retention time, which is obtained by scheduled SRM acquisition. Next, the collision energies are optimized and the relative intensities are determined to each peptide measured. The match between retention time and relative intensities are used to quantify the protein of interest. At least three transitions will be recorded for a given peptide and at least two peptides per protein are monitored. Furthermore, we intend to setup the absolute quantitative analysis in the proteins of interest using standard peptides with known concentration.

Conclusions

This work is still in progress. We hope to establish and share the SRM assays to lend support to all projects in the laboratory. The quantification of these proteins will contribute to understand the biochemical basis of schizophrenia. In addition, we may be suggesting potential diagnostic biomarkers and those used for identifying a successful treatment.

¹ Freedman R. Schizophrenia. N Engl J Med [Internet]. 2003 Oct 30;349(18):1738–49. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14585943>

² Nascimento JM, Martins-de-Souza D. The proteome of schizophrenia. npj Schizophr [Internet]. 2015 Mar 4;1(October 2014):14003. Available from: <http://www.nature.com/articles/npjshz20143>

³ Picotti P, Aebersold R. Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods [Internet]. 2012 Jun;9(6):555–66. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22669653>