Top-down MS/MS Hemoglobinopathy Screening of Neonatal Samples

View Presentation Detail

Authors

Roger Theberge[1]; Carolyn Hoppe[2]; Christian Heckendorf[1]; David H. K. Chui[1]; Catherine E. Costello[1]; Mark E. Mccomb[1]

Institutes

[1]Boston University School of Medicine, Boston, MA; [2]Children's Hospital & Research Center Oakland, Oakland, CA

Introduction

The epidemiology of hemoglobinopathies in the United States reflects the immigration of high risk populations. Newborn screening for hemoglobin disorders is thus becoming more important. Current methods are centered on chromatography and/or electrophoresis. Top-down mass spectrometry is a fast and efficient method to identify and characterize hemoglobin variants. Our Mass Spectrometry center has a long history of variant analysis and, in collaboration with the Hemoglobinopathy Reference Laboratory at Children's Hospital & Research Center Oakland, we have extended our application of top-down MS techniques to include the identification of hemoglobinopathies through analysis of samples from newborns.

Methods

Feasibility studies focused on sequencing hemoglobin variants from adult and newborn samples. Whole blood was diluted 1:300 in water and added in a 1:1 ratio to acetonitrile containing 0.2% formic acid. Selected charge states of the Hb ?-chain were fragmented by HCD in separate *m/z* windows and in a 'multiplex' fashion with multiple charge states *via* accumulation in the HCD collision cell and subsequent HCD fragmentation of the accumulated ions. All data were acquired on a Q Exactive mass spectrometer (Thermo-Fisher Corp., Waltham,MA) with 140,000 resolution @ *m/z* 400. Fragment ion tandem mass spectra were deconvoluted using Xtract software (Thermo) and the resulting fragment masses were analyzed using BUPID-Topdown (Boston University Protein Identifier-Topdown), a custom-programmed software algorithm written in-house.

Preliminary Results/Abstract

Analyses of newborn samples indicate that the hemoglobin variants, Hb S [?6 Glu-Val], Hb C [?6 Glu-Lys], and Hb E [?26 Glu-Lys] can be detected and characterized in a very short time using the top-down approach on the Q Exactive. These variants, representing the most common and clinically relevant hemoglobinopathies, are the target of most newborn screening strategies. Whilst the above goal could be achieved by acquiring a molecular weight profile through scanning from *m/z* 600-1800 and obtaining sequence data by mass selecting a single charge state for MS/MS, better results could be obtained by modifying the experimental workflow. For example, using the selected ion monitoring (SIM) mode for *m/z* 928-950 to detect the variant resulted in shorter acquisition times and improved sensitivity. Also, the Q Exactive mass spectrometer offers the possibility to perform automated simultaneous collisional activation on multiple charge states of precursors. 'Multiplexing' the charge states led to considerable savings in time, as well as more complete sequence coverage than obtained with the data recorded by using a single charge state. Also, sampling a multiplicity of charge states mitigated the possibility that a single charge state selected for MS/MS is not the most abundant in the charge envelope of the ?-chain. Top-Down analysis of neonatal samples provided rapid and unequivocal identification of clinically important hemoglobin variants with minimal sample preparation. We are optimizing the current workflow to reduce analysis time to 1 minute/sample and are exploring the development of automated analysis and data interpretation. The goal is to offer a fast, high-throughput method with minimal sample preparation for screening newborn hemoglobinopathies.

Acknowledgements: This project was funded by NIH grants P41 GM104603 and S10 OD010724 and NIH-NHLBI contract HHSN268201000031C.

Novel Aspect

Q Exactive Top-Down hemoglobinopathy neonatal screening platform