AB013. OS03.03. Quantitative proteomic analysis of type B thymoma using data independent acquisition mass spectrometry

Qiangling Sun[1], Xin Ku[2], Wentao Fang[3], Wei Yan[2]

[1]Central Laboratory, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, China; [2]Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China; [3]Department of Thoracic Surgery, Shanghai Chest Hospital, Shanghai 200030, China

Background: Type B thymoma is a complex heterogeneous disease with diverse pathological features and clinical outcomes. While it is among the most common tumors of the anterior mediastinum, there is so far no comprehensive proteomic study to understand the clinical behavior of these tumors. Data-independent acquisition (DIA) using high resolution mass spectrometer (MS) has emerged as a novel and powerful approach to quantify highly multiplexed proteins in clinical biopsy samples with high accuracy and reproducibility. Here, we report a large scale DIA-MS study to investigate the mechanism of Type B Thymomas across 34 clinical samples. The present study aimed to discover multiple proteins related to different subtypes of type B thymoma samples using label-free DIA-MS.

Methods: Type B thymoma samples and adjacent normal tissues were obtained from Shanghai Chest Hospital tissue biobank. Informed consents were obtained from all participants. Fresh frozen tissues were lysed and subjected to reduction, alkylation and tryptic digestion. Peptides were then subjected to desalt using C18 SPE 96-well plates prior to LC-MS/MS analysis using Orbitrap Fusion. One µg peptides was loaded onto a nano-C18 column and separated at a flow rate of 300 nL/min. Data independent high-resolution MS/MS spectra were acquired by sequential 25 amu window. Protein identification and quantification were processed by Spectronaut software. Differential expressed proteins were subjected to bioinformatics analysis.

Results: DIA-based LC-MS/MS quantification identified 2,300 proteins in thymoma and 1,500 proteins in adjacent normal tissues. Heatmap analysis of the label-free proteomics data resulted in three distinct clusters, namely B1, B2 + B3 and normal tissue. Further data analysis revealed 108 proteins which were differentially expressed among these three groups. Gene ontology (GO) analysis showed that most of the expressed proteins were cytosolic proteins, mitochondrial proteins, cytoskeleton protein and proteins from extracellular region. Further functional annotation by biological process showed that most of the proteins were involved in protein ubiquitination, cell cycle and DNA replication, complement activation, redox oxygen. David analysis also suggested the important pathways including carbon metabolism and TCA cycle, apoptosis, adhesion and migration, immune related Rap1 and Erb B pathway. Seventeen unique protein expressions were observed in B2 + B3 thymoma samples, e.g., protein ABHD11, Alanine aminotransferase 2, KIF1-binding protein, Rhotekin.

Conclusions: We performed a comprehensive global proteomic study using DIA-MS to characterize differential protein express patterns among type B thymomas, leading to discovery of potential molecular fingerprints for type B thymoma. Data presented here also indicated a useful technical utility of DIA-MS approach for developing novel and effective clinical biomarkers. Validation in an independent cohort of patients of a large scale is in progress and will be discussed.

Keywords: Type B thymoma; label-free proteomics; data-independent acquisition (DIA); mass spectrometry