IPD Project Details

Project ID: IPD8192

Project Title: Proteomic Alterations in Multiple Myeloma: A Comprehensive Study Using Bone Marrow Interstitial Fluid and Serum Samples

Description: Multiple myeloma (MM) is a plasma cell associated cancer and the second most common hematological malignancies. Although researchers have been working on MM, comprehensive quantitative proteomic analysis of Bone Marrow Interstitial Fluid (BMIF) and Blood Serum is not yet reported. Being a proximal biofluid for the hematological malignancies, BMIF could serve as a potential source for identifying diagnostic, prognostic and therapeutic markers for various blood cancers including MM. Moreover, serum, being a minimal invasive biofluid, serves as a potential source for discovering diagnostic and prognostic markers for various cancers including MM as well. We employed multipronged quantitative proteomic approaches like iTRAQ and SWATH-MS for MM BMIF and serum where we identified 279 and 116 non-redundant differentially expressed proteins in MM BMIF and serum respectively. Additionally, the probable biological and functional roles of the differentially abundant proteins were probed in the manifestation of MM disease pathology by using various bioinformatic tools. Furthermore, a selected panel of statistically significant proteins was verified for their differential abundance by immunoblotting as well as MS-based SRM assays. The significant discrimination efficiency of the models generated through multivariate statistical analysis was decent to distinguish between the MM and controls. Moreover, potential candidate proteins were also validated in a fresh independent cohort of serum samples using Enzyme-linked immunosorbent assay (ELISA), as it is a minimal invasive fluid that could easily be used for the diagnostics applications. The significance of this study remains in the fact that the proteins which are observed in the BMIF and also reflected in the serum with similar expression profile are proposed as a potential candidate biomarker panel for MM diagnosis

Principal Investigator: Dr Srikanth Rapole

PI Affiliation: Proteomics Lab, National Centre for Cell Science, Pune, Maharashtra, India

Sample Preparation: Sample Processing Protocol for iTRAQ Hundred microgram protein from MM BMIF and serum [three samples pooled (one set), total four sets] and respective controls [three samples pooled (one set), total four sets] were subjected to in-

solution digestion using trypsin.

Peptide Separation: The 4 plex iTRAQ labeling of digested peptides was performed as per the manufacturer's protocol (AB Sciex). Briefly, iTRAQ reagents dissolved in ethanol were added to the respective protein sample i.e. 114-controls, 115-MM samples, 116-MM samples and 117-controls and incubated at RT for 1 h. The labeled samples were pooled and concentrated using SpeedVac (Savant- SPD 1010, Thermo Electron Corporation). Labeled peptide samples were fractionated by SCX chromatography (Poly-SULFOETHYL A column 100 x 4.6mm, 5 mm, 300°A, PolyLC, Columbia, MD) using a Shimadzu HPLC. The fractionated peptide samples were again concentrated and desalted usingC18 ZipTips (Millipore) before performing the LC-MS/MS analysis. Sample Processing Protocol for SWATH The label-free SWATH-MS analysis was performed on BMIF and serum samples (12 MM and 12 controls), which were depleted for Albumin and IgG. An equal amount of BMIF and serum proteins were subjected to trypsin digestion, and the peptides were analyzed using a Triple TOF 5600 mass spectrometer (SCIEX, USA) equipped with Eksigent Nano 2D Ultra 2D plus (Eksigent, Canada) having an Eksigent Nano LC 3C18 CL reverse phase column (75 ?m × 15 mm, 3 ?m, 120 Å) along with NanoLC Trap Chrom XP C-18-CL (3 ?m, 120 Å, 350 ?m × 0.5 mm) column.

Protein Characterization: Data Processing Protocol for iTRAQ The peptide fractions collected from the SCX chromatography were further subjected to desalting through C18 ziptip (Millipore). EksigentMicroLC 200 system (Eksigent) coupled to the Triple TOF 5600 mass-spectrometer (AB Sciex) was employed to reveal the identity of the desalted iTRAQ labelled proteins. The proteins were separated on the microLC through the Eksigent C18-reverse phase column (100 × 0.3 mm, 3 ?m, 120° Å). ProteinPilot software (version 4.0; AB Sciex) was employed for searching the proteins through the SwissProt database with 1 missed cleavage and 1% FDR as input parameters. Data Processing Protocol for SWATH For SWATH MS experiments, the instrument was specifically tuned to optimize the quadrupole settings towards the selection of precursor ion window of 25 m/z width. An isolation width of 26 m/z (containing 1 m/z for the window overlap) was utilized and a set of 34 overlapping windows was constructed covering precursor mass range of 400–1,250 m/z. The SWATH MS/MS spectra were obtained from 100 to 2,000 m/z. The Ions were fragmented in the collision cell using rolling collision energy with an additional CE spread of ± 15 eV. An accumulation time (dwell time) of 96 ms was used for all fragment-ion scans in high-sensitivity mode, and for each SWATH-MS cycle a survey scan in high-resolution mode was acquired for 100 ms, resulting in a duty cycle of 3.33 s. The dual source parameters were as follows: ion source gases GS1, GS2, curtain gas at 25 psi, temperature 200 °C, and ion spray voltage floating at 5,500 V. High-quality spectral ion libraries were generated for SWATH analysis through data dependent analysis of individual samples. The Peakview software (version 2.2.0; AB Sciex) was employed for peak extraction and spectral alignment with the parameters mentioned henceforth: no. of peptides = 2, no. of transitions = 10, peptide confidence = 99%, XIC extraction window = 3 min, XIC width = 30 ppm. The

data was further subjected to Markerview software V 1.3.1(AB Sciex) to get statistical data interpretation. In MarkerView, the results were shown as three output files containing AUC of the ions, the summed intensity of peptides for protein and the summed intensity of ions for the peptide. The summed intensity of peptides was used for the further relative quantitative and multivariate statistical analysis using Metaboanalyst 3.0 and SIMCA 14.1 platforms.

Experiment Type: SWATH MS, SRM/MRM, Shotgun proteomics

Species: Homo sapiens - 9606

Tissue: Bone marrow (bto:0000141), Blood serum

Cell Type: Bone marrow, Blood serum

Disease: Multiple myeloma (doid:9538)

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: monohydroxylated residue, iTRAQ4plex-116 reporter+balance reagent acylated residue

PubMed ID: 33585190