IPD Project Details

Project ID: IPD5583

Project Title: Multipronged quantitative proteomics reveals serum proteome alterations in breast cancer intrinsic subtypes

Description: Being molecularly heterogeneous, breast cancer tends to be a complicated oncological disease with high incidence rates throughout the world. The primary aim of this study was to identify the set of serum proteins with discriminatory capabilities towards the four major subtypes of breast cancer. We employed multipronged quantitative proteomic approaches like 2D-DIGE, iTRAQ and SWATH-MS and identified 307 differentially regulated proteins. Luminal A subtype consisted of 24, Luminal B subtype 38, HER2 Enriched subtype 17 and Triple negative breast cancer subtype 10 differentially regulated subtype specific proteins. These specific proteins were further subjected to bioinformatic analysis viz. PANTHER, DAVID and LENS which revealed the involvement in platelet degranulation, fibrinolysis, lipid metabolism, immune response, complement activation, blood coagulation, immune cell activation, glycolysis, amino acid biosynthesis and cancer signaling pathways in the subtypes of the breast cancer. The significant discrimination efficiency of the models generated through multivariate statistical analysis was decent to distinguish each of the four subtypes from controls. Further, some of the statistically significant differentially regulated proteins were verified and validated by immunoblotting and mass spectrometry based selected reaction monitoring (SRM) approach. Our Multipronged proteomics approaches revealed panel of serum proteins specifically altered for individual subtypes of breast cancer.

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Sample Preparation: Serum was first thawed and depletion of IgG and albumin was performed using the IgG and Albumin depletion kit (GE Healthcare). The depleted serum was further desalted using 2D-cleanup kit (GE Healthcare). Finally, the protein samples were dissolved in 50 mM ammonium bicarbonate buffer and the protein concentration was estimated using the 2D Quant Kit (GE Healthcare). The Samples were reduced with 10 mM dithiothreitol (Sigma) at 60 °C for 1h, and then was subjected to alkylation with 50 mM iodoacetamide (Sigma) at room temperature for 1h in the dark.

Peptide Separation: The samples were further subjected to protein digestion using MS grade trypsin (Sigma). The digested peptides were desalted using C18 ziptips (Millipore), dried and then reconstituted in 0.1% formic acid. These reconstituted peptides were injected in to the Eksigent MicroLC 200 system (Eksigent) having Eksigent C18 reverse phase column (100×0.3 mm, 3 µm, 120 Å) coupled with a Triple TOF 5600 mass spectrometer (AB Sciex) to analyse the desalted peptides.

Protein Characterization: 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 = 5, no. of transitions = 10, peptide confidence = 95%, XIC extraction window = 3 min, XIC width = 30 ppm. SWATH file was further exported to MarkerView software (version 1.3.1; AB Sciex) which performed quantitative analysis of proteins, peptides and ions in different samples and provided the summed intensity of ions for the peptide, summed intensity of the peptides for protein and Area under Curve (AUC) of the ions as output results.

Experiment Type: SWATH MS, Shotgun proteomics

Species: Homo sapiens -9606

Tissue: Blood serum (bto:0000133)

Cell Type: Blood serum

Disease: Breast cancer (doid:1612)

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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