Nano-flow 2D-HPLC MALDI-TOF MS/MS for differentially expressed protein analysis in breast cancer patient samples

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Proteomics has emerged as a key methodology in the diagnosis and treatment of various diseases, including cancer, and has evolved to encompass reliable, robust and high-throughput technologies based largely on mass spectrometry (MS). MS analysis of expressed proteins can determine protein structure and expression levels with unparalleled sensitivity and quantitative MS allows comparative proteomic analysis between malignant and non-malignant samples to identify disease-relevant protein biomarkers. In this study, malignant and non-malignant breast tissues from 10 patients diagnosed with breast cancer were analyzed to identify differentially expressed proteins between normal and disease state that could serve as diagnostic, prognostic and treatment target biomarkers. Patient samples were prepared for proteomic analysis according to the iTRAQ™ method (Applied Biosystems) where peptides from non-malignant and malignant tissues were labeled with the iTRAQ™ Reagent isobaric tags (114 and 117, respectively). The samples were combined for separation and fractionation by nano-flow 2-D HPLC (UltiMate® 3000, Dionex) and samples were spotted on a MALDI plate by the Probot™ (Dionex) before MALDI-TOF MS/MS analysis (AB 4800 MALDI-TOF/TOF Plus, Applied Biosystems). Mass spectrometry analysis was able to identify as many as 650 proteins in a given sample based on a 2-peptide and 3-peptide matching systems for protein identification. Up- and down-regulated proteins varied by patient however major markers for breast cancer, including BRCA1, ESR1 modification and Her2, were present for all samples. Further analysis of each patient will be presented.