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### Deep discovery of biomarkers in proximal fluid of liver cancer patient-derived-xenograft models

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Protein biomarkers are important for early detection, prognosis, and drug response monitoring in cancer treatment. Plasma-based protein markers were sought after because of the easiness of sample collection. However, the potential useful biomarkers are usually masked by the top abundant proteins such as serum albumin and immunoglobulins. In this study, we used the sequential window acquisition of all theoretical MS (SWATH) approach to perform non-labelled semi-quantitative proteomics discovery of proximal fluid of four groups intotally 14 liver cancer xenograft models. The tumor tissues were isolated from the mouse host and then re-suspended in buffer saline briefly to allow the tissue to secrete enough proteins for detection. The secretory proteins in the buffer was digested with trypsin and were fed into the EksigentnanoLCcHiP based C18 column and then identified with ABSCIEX TripleTOF5600 mass spectrometer. A total of 41 runs were performed and each sample was run in duplicates or triplicates. More than 1600 peptides were identified. Principal component analysis was able to segregate the samples into four groups. Significant analysis of the peptides found that 134 peptides were the minimal set of signatures for clear classification of the four groups of samples with highest specificity and sensitivity. The product ion intensities provided a hint for development of Multiple Reaction Monitoring (MRM) assay for absolute quantification of target peptides in the future. In summary, SWATH approach of protein marker discovery can lead not only to the deep discovery of protein markers, but also provide a lead to target peptide selection for the next phase absolute quantification using MRM assays.

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