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Identification of proteins and lipids found in varying abundances in pre- and post-operative cancer patients using a MALDI-TOF-MSMS approach

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1. Overview

In this study, a MALDI-TOF-MSMS based screening approach was used to identify differences in peptide abundance between pre- and post-operative cancer patient groups. For this purpose, protein extract digests of whole and ultra-centrifuged plasma were investigated by multivariate statistics software (eMSTAT). MSMS-based identification of candidate peaks was performed on selected differentially expressed marker peaks. Our previous work had shown that peptide profiles of extracellular vesicles (EVs) can resolve differences in the resistance of cancerous cells to commonly used chemotherapeutic agents. This work intends to investigate the differences of protein abundances in plasma samples of different patient groups with the aim of stepping closer to identifying measurable factors of cancer and to provide potential insight into the biochemistry of the disease.

2. Introduction

We previously demonstrated we could rapidly distinguish fluorouracil resistant cancer sample groups based on protein profiling of extracellular vesicles using a linear benchtop MALDI-TOF instrument [1] and that we could distinguish patient groups with colon cancer (pre- and post-operation), patients with inflammatory bowel disease (IBD) and the healthy control group [2]. The aim of this follow up work is to identify proteins that are differentially expressed in the different sample groups in order to better understand the disease processes and to support the rapid screening approach developed previously.

Here we present the results from this study using a high performance reflectron MSMS MALDI-TOF platform (**Fig. 1**) for the comparative proteomic profiling and identification of proteins derived from circulating EV extracted from plasma samples of patients with colorectal cancer (CRC-Pre), post-operative colorectal cancer patients (CRC-Post), inflammatory bowel disease (IBD) patients and a healthy control group (Control) in view of liquid biopsy applications (as a potential application for liquid biopsy oncological diagnosis).

3. Methods

Whole plasma and EV-enriched fractions were collected. EV enrichment was performed by ultra-centrifugation, non-enriched samples were collected prior to ultracentrifugation (**Fig. 1**). 10 patient samples were prepared for each group: CRC-Pre) / CRC-Post, IBD and the control group. Samples were analysed on a MALDI-7090 MALDI-TOF-TOF mass spectrometer (Shimadzu). The entire sample processing and subsequent MS-analysis was performed using MALDI-nanochip targets (Tethis). After the identification of consistent peaks in the non-enriched and EV-enriched samples, the exact mass was used as a lock mass for the next stage of MSMS candidate selection. Peak lists were imported into eMSTAT (Shimadzu) software to look for statistical differences in the abundance of peptides in the different patient samples using Partial Least-Squares Discriminant Analysis (PLS-DA). These were used as candidates for MSMS to identify their protein origins. With the discovery of a common marker in all samples, MSMS spectra were acquired and subjected to Mascot database search (SwissProt) for protein identification.

4. Results

40 plasma samples from patients with colon cancer (CRC pre / CRC post), inflammatory bowel disease (IBD) and healthy controls were used for evaluation. Protein extracts were analysed by MALDI-MS in the range of *m/z* 2000-20000 which has previously been shown to contain most informative peaks of exosomes [3]. A comparison of the mass spectra showed distinct differences particularly in the range above *m/z* 8000 after exosome isolation. PLS-DA of the whole dataset recorded on the MALDI-nanochip showed a good clustering and separation of the samples belonging to the four study groups (**Fig. 2**).

MS data acquired from all the non-enriched tryptic digests was submitted to Mascot for peptide mass fingerprinting (PMF). In this search, 5 proteins were identified including albumin. Albumin was identified in the control and IBD group while apolipoprotein-A1 was identified in all groups (**Fig. 3**).

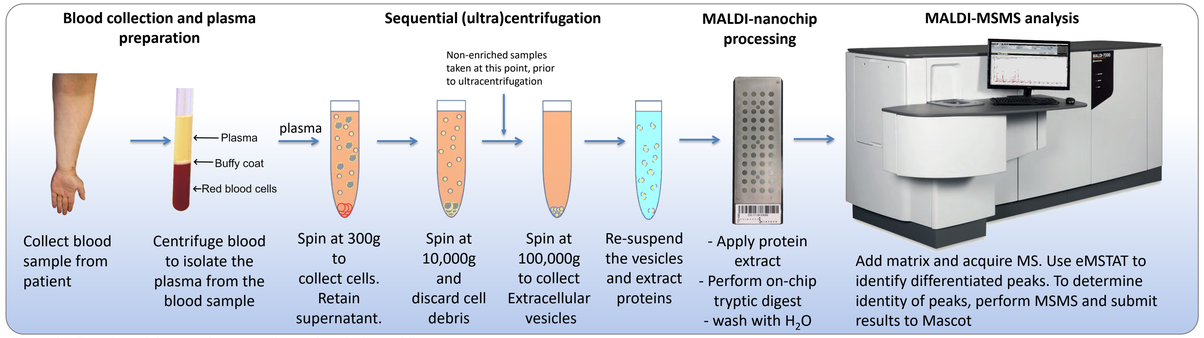

Figure 1 – Overview of the sample preparation and analysis workflow.

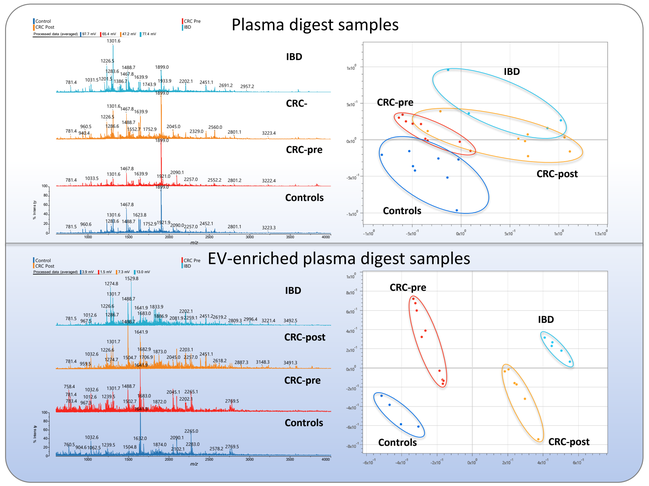

Figure 2 – Spectra from the plasma digest (top) and EV-enriched plasma digest (bottom) analysed on the MALDI-7090. After application of lock mass in both groups, samples were analysed in eMSTAT. There is a greater distinction seen in the EV-enriched samples in comparison to the non-enriched plasma digests.

One peak was conserved across all the non-enriched samples. When MS/MS was performed on this peak, its identity was confirmed as albumin and its exact mass was obtained (*m/z* 1898.9990).

PMF was performed on the MS data from the EV-enriched sample digests. Alpha-1-antitrypsin was identified in these samples. It was later observed that *m/z* 1641.8674 was observed in every sample. The identity of this peptide was obtained through MSMS as alpha-1-antitrypsin. These proteins (albumin and alpha-1-antitrypsin) were initially identified by PMF and confirmed by MSMS. MSMS analysis was then performed on the discriminant peaks to identify the digested proteins using Mascot.

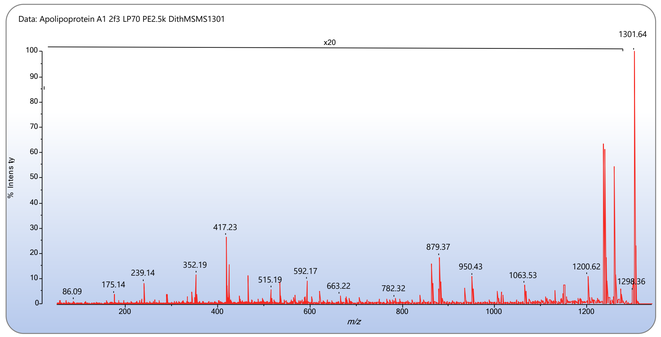

Figure 3 – MS/MS spectrum obtained for *m/z* 1301.64 in one of the IBD non-enriched plasma samples. Spectrum was identified as Apolipoprotein A-1 following Mascot search.

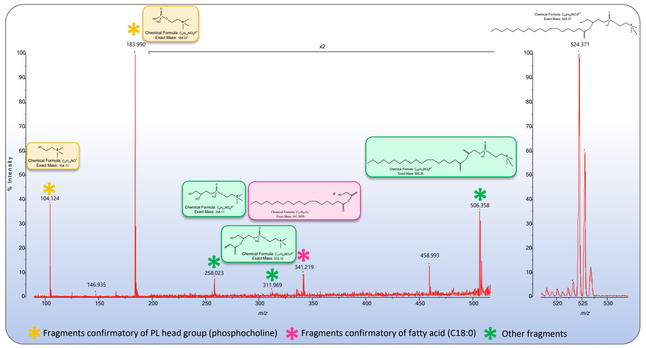

Figure 4 – Annotated MSMS spectrum of a suspected lipid species (*m/z* 524.37) detected in the non-enriched CRC-pre sample. The spectrum has been annotated with the proposed lipid species and the masses/ structures of the proposed fragments.

Alpha-1-antitrypsin peptide peaks were observed in both preparations (EV-enriched/ non-enriched) but showed no differentiation between the study groups. In contrast several peaks were found to be differentially expressed between CRC (pre /post), IBD and healthy subjects therefore these peaks were targeted for MSMS. Apolipoprotein A1, albumin and the three lipids LPC(16:0), LPC(18:1) and LPC(18:0) (**Fig. 4**) were identified from the non-ultracentrifuged samples. Transthyretin and Fibrinogen alpha chain were identified from the ultracentrifuged sample group.

5. Discussion

The work presents a MALDI-nanochip based protein profiling and identification workflow for the analysis of exosomal proteins as potential clinical biomarkers. Through data analysis of mass spectral features using bioinformatics, samples from patients with colon cancer, IBD and healthy subjects could be clearly separated. The peak intensities for the ultracentrifuged samples were found to be approximately an order of magnitude lower than those observed for the non-ultracentrifuged samples but consequently, the relative intensity of certain marker peaks was enhanced . By using the MALDI-nanochip slide we were able to remove some of the more abundant proteins which are usually detected using ZipTip cleanup methods and thereby discover otherwise undetected discriminant peaks. The Shimadzu MALDI-7090 reflection TOF-TOF mass spectrometer was able to use high energy CID to fragment the peptide and lipid species and several identifications were made. With the collection of identified proteins and lipids and the presence of conserved masses in both groups, we have demonstrated a pathway for establishing a high-throughput screening platform for clinical purposes (e.g. cancer diagnostics) in the future.

6. References

1.Serafim, V. *et al.* Classification of cancer cell lines using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and statistical analysis. Int J Mol Med, **40**, 1096-1104 (2017).

2.Nairn M. *et al* MALDI-nanochip based Screening of Exosomal Biomarkers: Application to Cancer Diagnostics. ASMS 2020, WP-031.

3.Stübiger G. *et al.* MALDI-MS Protein Profiling of Chemoresistance in Extracellular Vesicles of Cancer Cells. Anal Chem, **90**, 13178-13182 (2018).