WP-031

MALDI-nanochip based Screening of Exosomal Biomarkers: Application to Cancer Diagnostics

Michael D. Nairn[1]; Michael Wuczkowski[2]; Jesús Jiménez[3]; Iris Prinz[4]; Marco Rissoglio[5]; Emanuele Barborini[5,6]; Gerald Stübiger[2, 7]

[1]Shimadzu, Manchester, United Kingdom; [2]Medical University of Vienna, Vienna, Austria; [3]Clover Bioanalytical Software, Granda, Spain; [4]Stratec Consumables, Salzburg, Austria; [5]Tethis, Milan, Italy; [6]Luxembourg Institute of Science and Technology; [7]Comprehensive Cancer Center, Vienna, Austria

1. Overview

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]. The aim of this follow up work is to identify the 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 MS/MS MALDI-TOF platform (**Fig. 1**) for the comparative proteomic profiling of circulating extracellular vesicles (EV) extracted from plasma samples of patients with colorectal cancer, postoperative colorectal cancer patients, IBD patients and a healthy control group in view of liquid biopsy applications (as a potential application for liquid biopsy oncological diagnosis).

2. Introduction

Exosomes are small cell-derived vesicles (50-150 nm) which are increasingly recognised as a promising source of circulating biomarkers for non-invasive diagnostics from body fluids (liquid biopsy). MALDI-MS profiling of exosomal proteins was demonstrated as being capable to detect cancer-cell specific molecular signatures which can be used to differentiate between cancer types and stages as well as different grades of chemoresistance of cancer cells [1, 2]. This distinguishes MALDI-MS as promising tool for application in liquid biopsy based cancer diagnostics. However, the identification of the disease-related exosomal biomarkers represents a challenging task. Here we present a MALDI-nanochip platform in combination with bioinformatics data analysis for the comparative profiling and detection of exosomal proteins as potential cancer biomarkers.

3. Methods

Blood samples were prepared according to standard methods and exosomes were isolated using sequential (ultra)centrifugation. Proteins were solvent-extracted, dried under vacuum and stored at -80°C before analysis. Proteins were directly analysed and subsequently subjected to tryptic digestion after application to the MALDI-nanochips (Tethis) (**Fig. 1**). On-chip digests were dried, washed and covered with 0.5 µL CHCA in ACN:2.5%TFA = 70:30 (v/v). Alternatively, samples were digested in-solution, desalted using C18-ZipTips and applied to FlexiMass-DS targets (Shimadzu). For protein profiling the AXIMA-Performance (Shimadzu) instrument was used. Protein identification was performed using the MALDI-7090 MALDI-TOF/TOF mass spectrometer (Shimadzu) with Mascot protein database search (Swiss/Uniprot). Statistical analysis was performed using Clover MS Data Analysis (Clover Biosoft) and eMSTAT (Shimadzu) software.

4. Results

40 plasma samples from patients with colon cancer (CRC pre/post operative), inflammatory bowel disease (IBD) and healthy controls were used for evaluation. First, 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 [2]. 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**).

Next, tryptic digests of the individual samples were subjected to peptide mass fingerprinting (PMF) in order to identify the discriminatory peptide peaks between the study groups based on multivariate data analysis (**Fig. 3**) and MS/MS analysis was then performed on the discriminant peaks to identify the digested proteins using Mascot. From in-solution digests peaks of more abundant plasma proteins (e.g. alpha-1-antitrypsin, immunoglobulin heavy constant alpha 1, haptoglobin, fibrinogen gamma chain, etc.) were identified in selected samples of the study groups. These proteins were also found in the MALDI-nanochip processed samples but they showed no differentiation between the study groups. In contrast, several less abundant peaks that gave lower Mascot scores were found to be differentially expressed between CRC, IBD and healthy subjects.

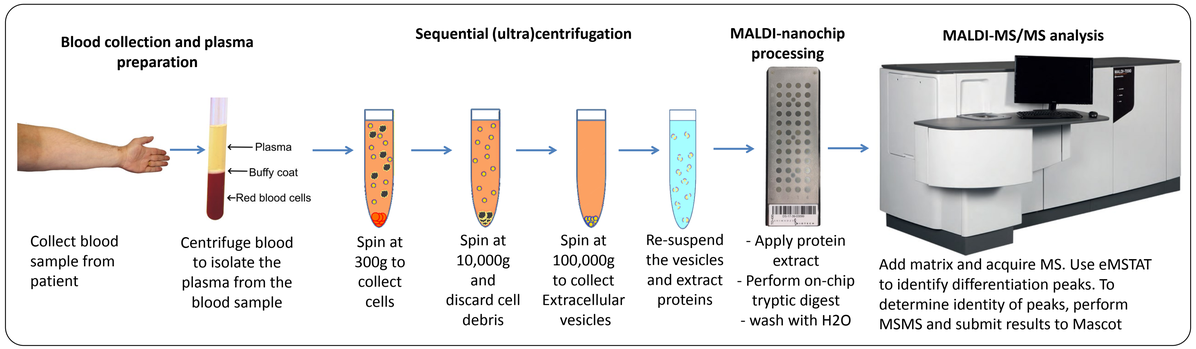

Figure 1 – Overview of the sample preparation and analysis workflow.

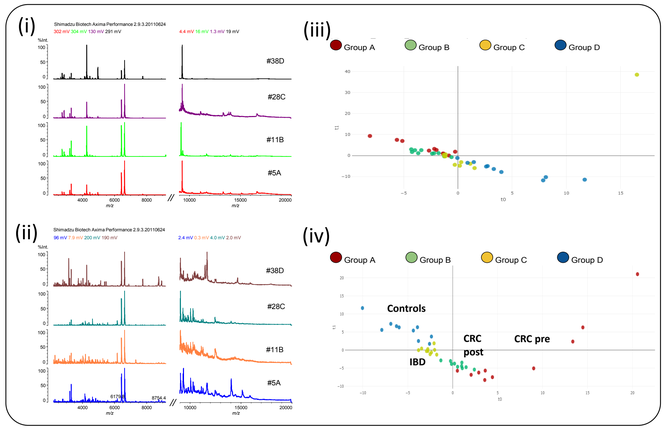

Figure 2 – Representative protein MALDI mass spectra of selected patient samples of the four study groups (A-D) recorded (i) before and (ii) after exosome isolation. PLS-DA plots of all patients of the study groups recorded using (iii) standard target slide and (iv) after MALDI-nanochip sample processing.

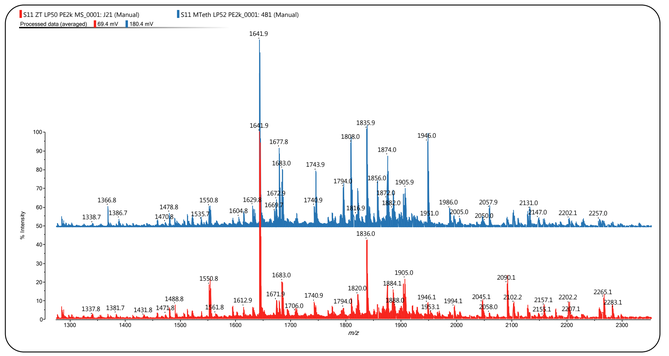

Figure 3 – Comparison of MS spectra illustrating several peaks where the signal intensity has been enhanced when using Tethis slide sample washing (Red) versus Zip-Tip™ sample cleanup (Blue).

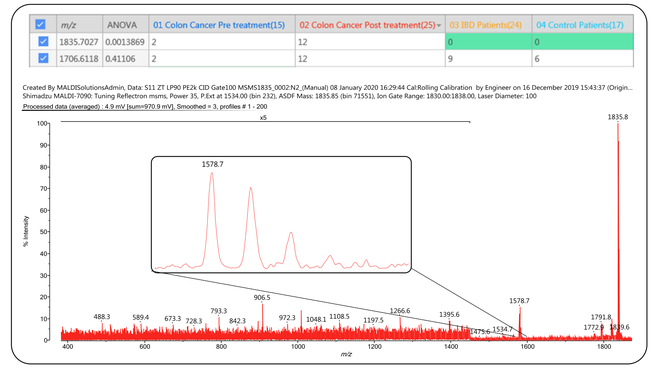

Figure 4 – Using a combination of statistical software (eMSTAT) and high resolution MSMS from HE-CID we are able to discover differences in protein expression in different patient populations. Differentially expressed proteins were then targeted for MSMS identification. This precursor was identified as IGHA1 - Immunoglobulin heavy constant alpha 1.

5. Discussion

The work presents a MALDI-nanochip based protein profiling and identification workflow for the analysis of exosomal proteins as potential clinical biomarkers. Using bioinformatics, data analysis of mass spectral features, samples from patients with colon cancer, IBD and healthy subjects could be clearly separated. The peak intensities were found to vary greatly depending on the method of sample cleanup. By using the Tethis slide we were able to remove some of the more abundant proteins which are usually detected using ZipTip cleanup and discover otherwise undetected discriminant peaks. This looks promising to establish a high-throughput screening platform for clinical purposes (e.g. cancer diagnostics) in the future. We now aim to develop this method further to support patient group differentiation based on putative marker peaks with confident protein identifications.

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