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MALDI profiling of cancerous vesicles in culture media: Application to liquid biopsies

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Overview

Previous work in this research group discovered a multitude of potential biomarkers that could be detected[1] but the aim of this work is to streamline the process by developing a fast identification method that not only identifies the presence of the cancerous cells but also how the cells are responding to chemotherapy. With high specificity and low cost it is anticipated this will enable broad adoption and improved success in the cancer therapy.

Here we present the results from a pilot study using a benchtop linear MALDI-TOF platform (Figure 1) for the comparative proteomic profiling of human cancer cells and Circulating extracellular vesicles (EV) in view of liquid biopsy applications (as a potential application for liquid biopsy oncological diagnosis).

Introduction

- Tumours excrete vesicles into the fluids that surround them (e.g. interstitial fluid, blood, etc.) Current biomarker discovery work is focussed on identifying tumour biomarkers from tissue biopsies, however they are invasive and traumatic for the patient.
- Circulating extracellular vesicles (EV) are considered as promising novel diagnostic targets as they contain a multitude of potential biomarker molecules related to their cellular origin.
- The goal of this project is to provide diagnostic information on the presence of a tumour, its drug resistance and the success of treatment non-invasively and directly from body fluids (liquid biopsies).

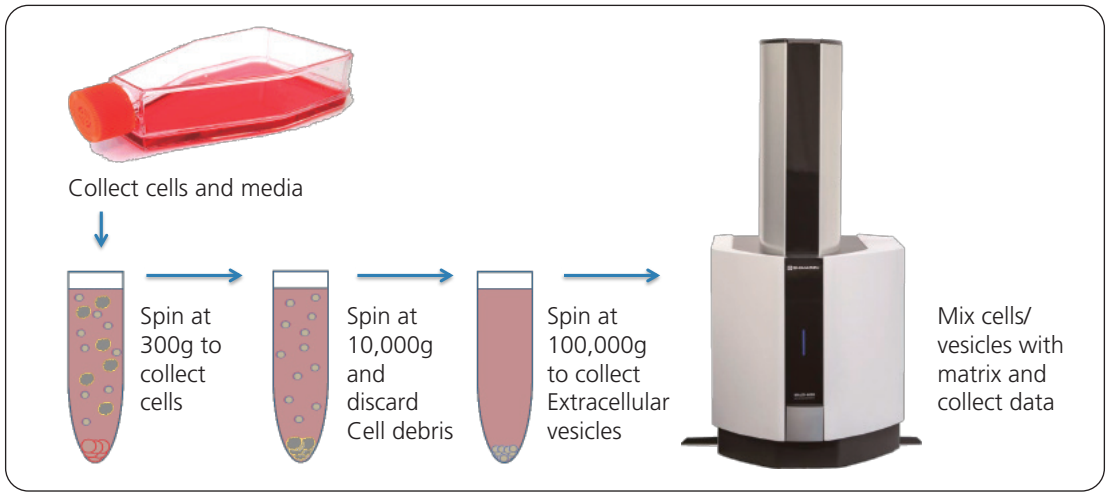

Figure 1 – Overview of sample preparation.

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Methods

- Two colon cancer cell lines corresponding to a primary colon cancer (CCL-228) and colon cancer cells derived from the metastasis cell line (CCL-227) were used in this study. Cell lines were obtained from the ATCC (Rockville, MD, USA).
  Cell lines were cultured in RPMI-1640 (Gibco, Invitrogen, Germany) at 37°C.
- Resistant sub-clones of CCL-227 were generated by continuous exposure of tumour cells to increasing concentrations of 5-fluorouracil (FU) over a time period of more than 2 years. Starting with the addition of 1µM FU under standard cell culture conditions, this concentration was gradually enhanced when the growth rate of the exposed cells was similar to that of FU naive cells. Escalation to the most resistant cell lines included cultivation periods at 5, 10, 25, 75 and 125µM FU; 15–31 cell passages were necessary before proceeding to the next higher-dose level.
- Culture media was collected with the corresponding cells for analysis. After the separation of the cells, cell culture media was subjected to ultracentrifugation and the pellets were retained. Particle measurements showed a sample related size distribution of 100-200 nm. Proteins were obtained by solvent-based extraction from the samples, dried under vacuum and stored at -80°C before analysis.
- Mass spectra were obtained using CHCA in the mass range between 2,000-20,000 m/z. Protein extracts were prepared in 0.1% TFA: acetonitrile 70:30 (v/v) and 0.5 µL was spotted with 0.5 µL of 10mg/ml CHCA matrix. Once dry, the samples were analysed using a MALDI-8020 benchtop MALDI-ToF mass spectrometer (Shimadzu).
- Partial least squares data analysis (PLS-DA) was performed using Mass++ DA (Shimadzu)).

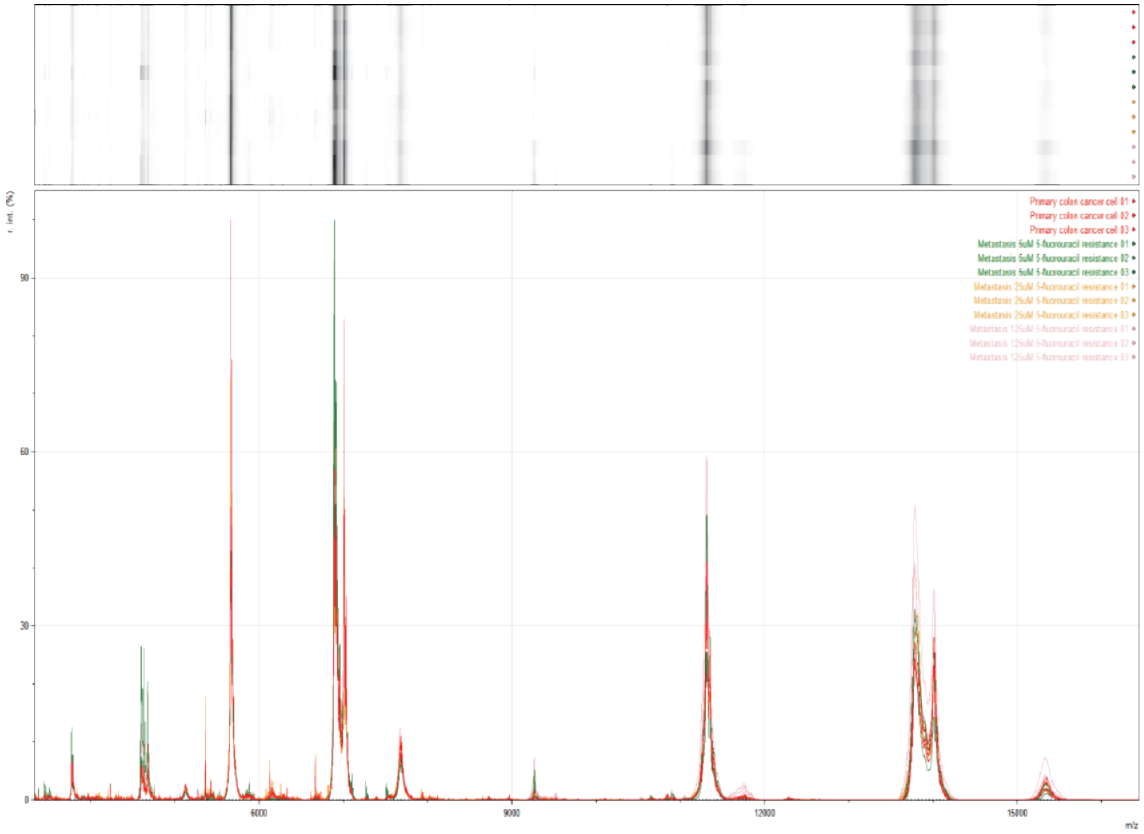

Figure 3 - Normalised spectra of the cancerous cells. No obvious differences observed.

Results

Overlaying the MALDI-MS analysis of the cells, there are no obvious differences between the different cell populations. Overlaying the spectra of the EVs reveals patterns of differentially expressed protein peaks (Figure 4). The protein patterns show distinctive

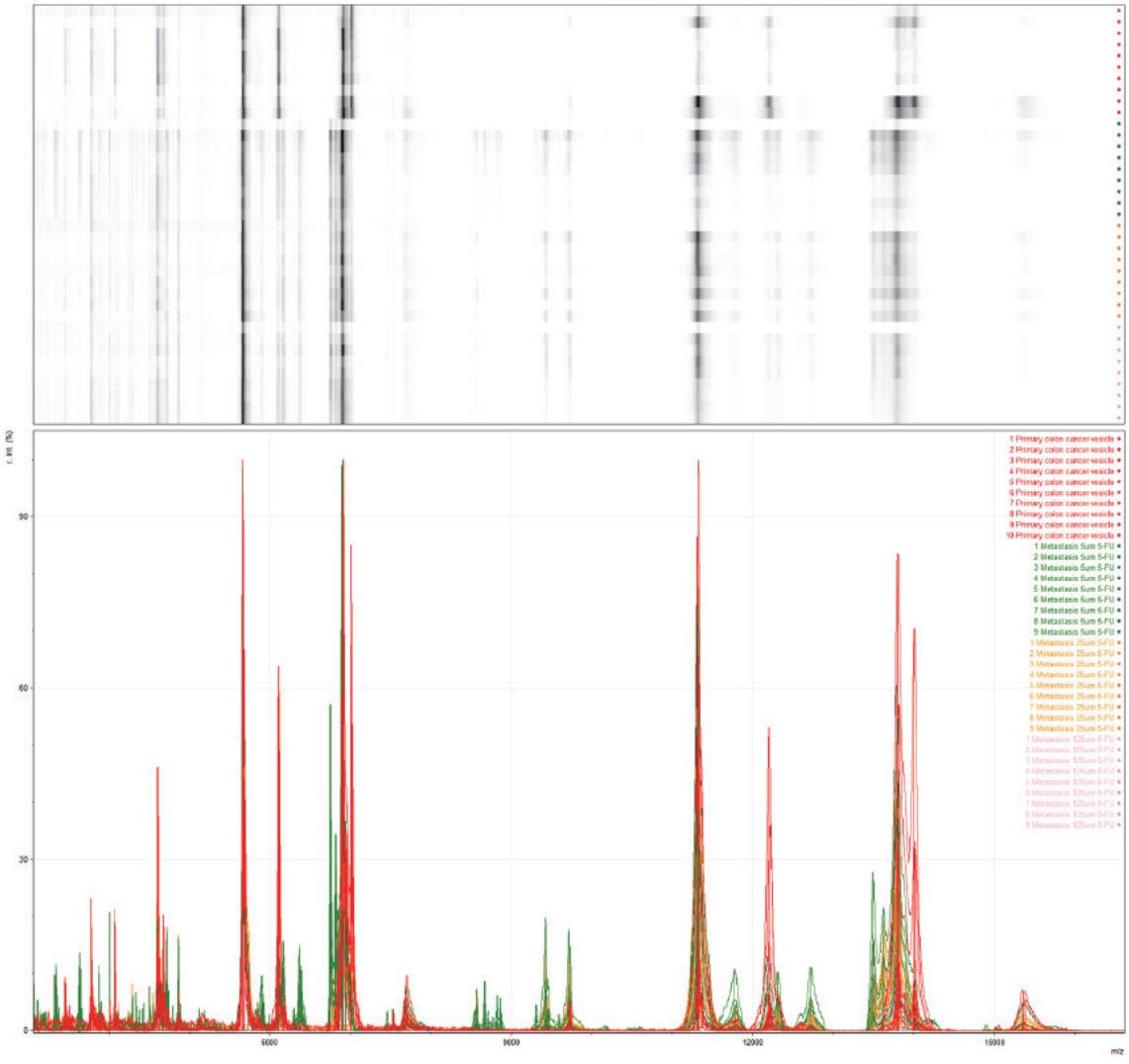

Figure 4 - Normalised spectra illustrating the different expression profiles associated with the vesicle groups. Differential protein expression can be observed.

differences between cells and EV.
Figure 5 shows the results of the PLS-DA . When examining the cell data, despite the visual similarity of the spectra, the different cell populations with their varying degrees of 5-fluorouracil resistance can be separated.
Examining the data collected from the EV, there is a greater distinction between the primary cell line and the metastatic cells and this is in part due to the greater number of differentiating peaks present in the EV samples. What is the most interesting feature of this analysis is the linear grouping of the data derived from the 5-fluorouracil resistant vesicles.

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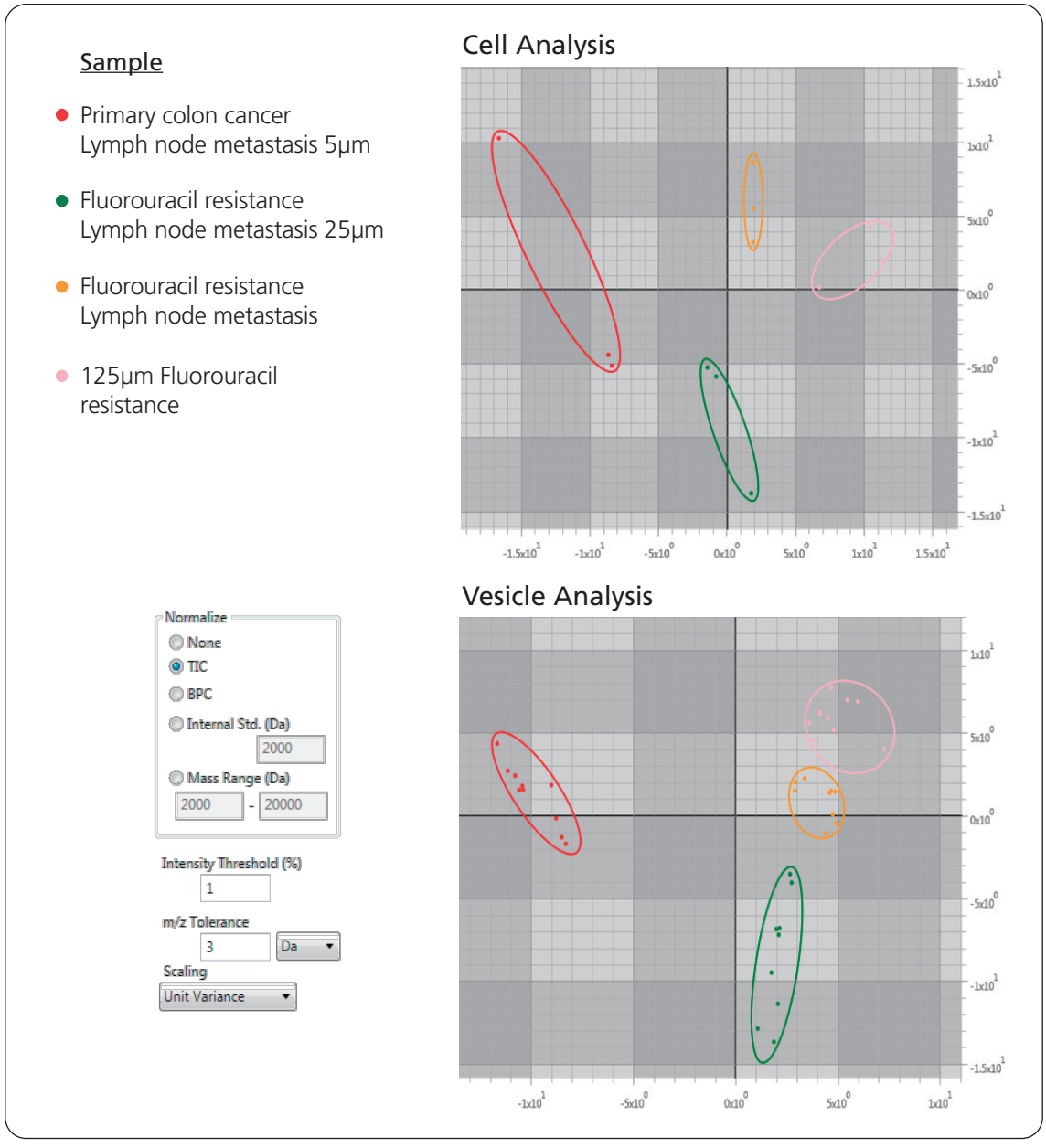

Figure 5 - Score plot of primary colon cancer cells and vesicles (red) against lymph node metastasis with varying degrees of 5-fluorouracil resistance (green and orange and pink). In this display, the peak lists have been normalised to total ion count to reduce variation between expected variations in spectrum signal intensity.

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Conclusions

This work demonstrates proof of concept that MALDI-MS analysis can be used to characterise vesicles from different cancer cell populations and also between those expressing different levels of susceptibility to chemotherapy. Biomarkers indicating the susceptibility of tumours to chemotherapy agents can be analysed and used for guiding treatment regimes. It is anticipated that by using this minimally invasive therapy monitoring technique, cancer therapy can be customised leading to improved cancer survival rates.

1 Schmidt, W. M. S. et al. Dissecting Progressive Stages of 5-Flurouracil Resistance in Vitro Using RNA Expression Profiling. **212**, 200–212 (2004

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