Label-free quantitative proteomic analysis of plasma protein fractionation using multiple immunoaffinity chromatography revealed biomarker candidates of patients with colorectal cancer

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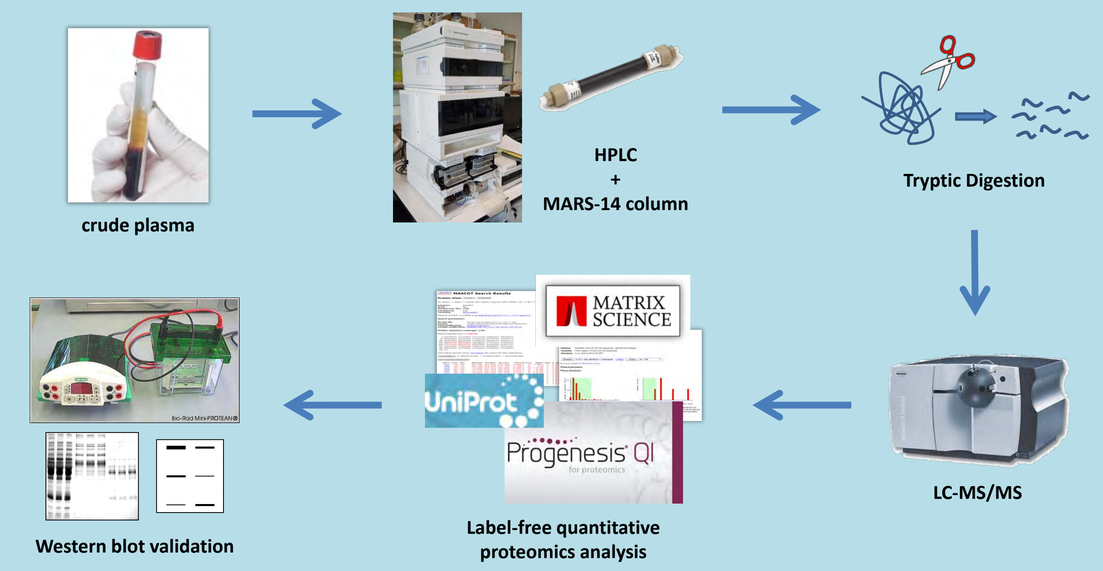
Abstract

Colorectal cancer (CRC) is one of the most common cancer worldwide. Globocan reports in 2020 reveal that it is third ranked incidence (10.0%) and second ranked mortality (9.4%) among all cancers. At present, carcinoembryonic antigen (CEA) is still the only blood-based biomarker approved by the U.S. FDA for colorectal cancer screening when used with other diagnostic means. This is because the CEA level can sometimes be normal in CRC patients and can be abnormal for reasons in other cancers. Nevertheless, searching specific biomarkers from liquid biopsy specimens such as plasma and serum is still an ideal non-invasive approach for primary screening of CRC. However, seeking biomarkers in plasma/serum are challenging because numerous proteins were overshadowed by high abundant proteins such as albumin and immunoglobulins. In our present work, a multiple immunoaffinity chromatography was applied to fractionate pooled plasma samples of healthy control, non-metastasis, and metastasis CRC patients into high abundant and low abundant protein fractions. Each fraction was then analysis by a label-free quantitative proteomics approach using liquid chromatography tandem mass spectrometry (LC-MS/MS) and Progenesis QI software. Among 167 MS/MS-identified proteins, 10 proteins showed accumulated changes in a stage-dependent manner and 5 prominent candidates were validated by western blotting and will be subjected for further validation in an independent cohort study. It is hoped that our techniques will be useful in order to find more specific biomarker candidates of CRC patients.

Methodology



The human 14 multiple affinity removal column (MARS-14), Agilent, is designed to chromatographically remove fourteen high abundant proteins from human plasma samples. Removal of these proteins improves subsequent LC/MS and electrophoretic analysis of the sample by effectively expanding the dynamic range of the analysis. This enables easier isolation and identification of aberrant plasma proteins. In this work, pooled plasma samples from 10 normal controls, 10 non-metastatic CRC patients, and 10 metastatic CRC patients were fractionated by MARS-14 into flow-through (low abundant proteins) and eluted (high abundant proteins) fractions. Each fraction was tryptic digested before subjected to LC-MS/MS. Protein identification and Label-free quantification were performed using Mascot Server v.2.3 and Progenesis QI for proteomics v.4.0. Proteins that have more than 1.5 fold expression compared to normal controls were considered as interesting CRC biomarker candidates. Five candidates were briefly validated by western blot.

Results

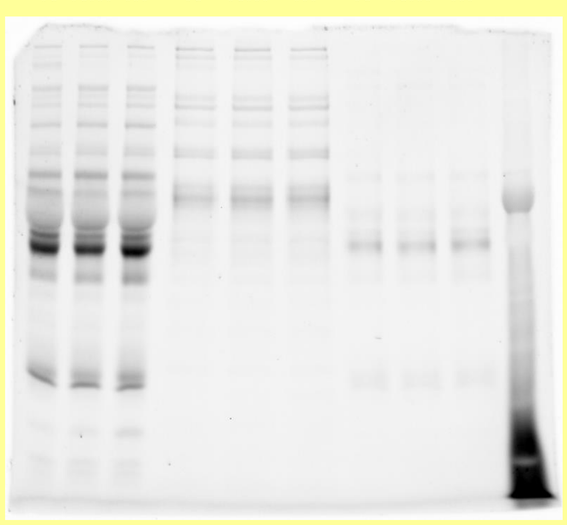


Figure 1. SDS-PAGE of crude plasma and fractionated proteins from MARS-14 immunoaffinity chromatography. Crude plasma, flow-through fraction, and eluted fraction of pooled plasma from healthy normal (N), non-metastatic CRC patients (NM) and metastatic CRC patients (M) were separated by stain-free gel. Different majority of proteins seen in flow-through and elute fractions indicated well fractionation of proteins by MARS-14 column.

Acknowledgement

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Results (continue)

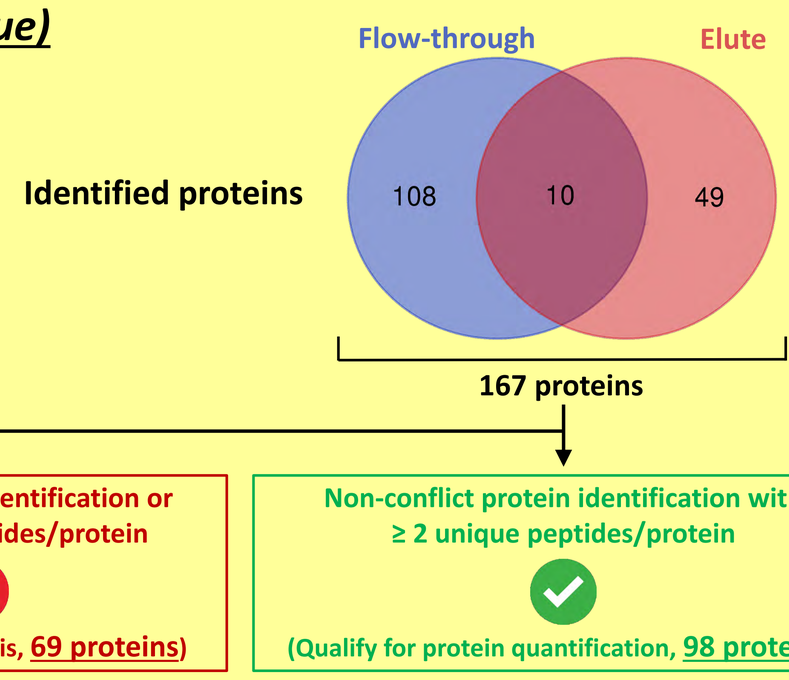

Figure 2. Descriptive analysis of protein identification by LC-MS/MS. Among 167 identified proteins, 108, 49 and 10 were presented in flow-through fraction, elute fraction, and both fractions, respectively. Among all these, 69 proteins were excluded from the analysis due to the identification had either conflict (more than one identification from the same precursor) or uncertain (only one unique peptide/protein) interpretation. Total of 98 proteins were qualified for the label-free protein quantification.

A)

Protein	Peptide count	Unique peptide	Fold change NM/N	Fold change M/N
*Protein 1	7	7	1.266	1.935 ↑
*Protein 2	2	2	0.922	1.874 ↑
Protein 3	2	2	1.834	1.835 ↑
Protein 4	15	14	1.147	1.739 ↑
Protein 5	3	2	1.479	1.501 ↑
Protein 6	26	25	0.626	0.572 ↓
Protein 7	4	2	0.895	0.546 ↓

B)

Protein	Peptide count	Unique peptide	Fold change NM/N	Fold change M/N
*Protein 8	10	8	1.201	1.788 ↑
*Protein 9	6	3	1.231	1.603 ↑
*Protein 10	13	11	1.390	1.530 ↑

Table 1. List of plasma proteins and their expression levels derived from label-free quantitative proteomics analysis of (A) flow-through fraction and (B) elute fraction that showed greater than 1.5 fold different between pooled samples of metastatic CRC patient and healthy normal (M/N). NM/N represents the fold different observed between pooled samples of non-metastatic CRC patients and healthy normal. * represents the protein that was selected for further validation by western blotting.

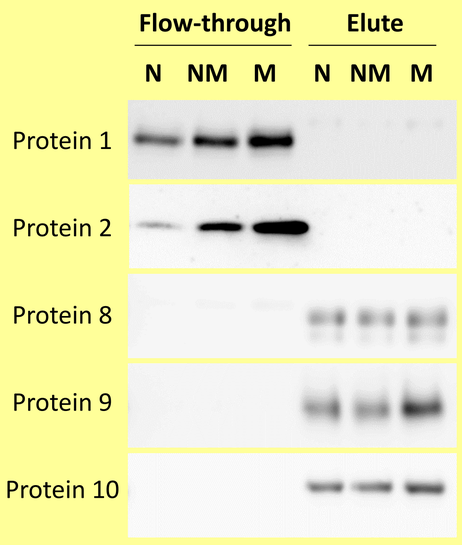

Figure 3. Western blot validation of some prominent candidates that pique our interest using the same sample set for label-free quantitative proteomics. Protein 1 and 2 showed increasing expression in dose-dependent manner from healthy normal group (N), non-metastatic CRC patient group (NM) and metastatic CRC patient group (M) in flow-through fraction, while the expression in elute fraction were barely seen. Protein 8, 9 and 10 were observed in elute fraction but barely seen in flow-through fraction. Protein 8, in contrast to the label-free quantitative proteomics results, showed similar expression level in all sample groups. Protein 9 and 10 showed increasing level in metastatic CRC patient group (M). The western blot results of protein 1,2, 9 and 10 were in accordance with the results from label-free quantitative proteomics approach.

Conclusion

- Immunoaffinity chromatography using MARS-14 column exhibited an efficient isolation of the 14 most-abundant plasma proteins into the elute fraction.
- Label-free quantitative proteomics of the flow-through and elute fractions of pooled plasma samples revealed 10 proteins with greater than 1.5 fold expression different in the metastatic CRC group compared to those of the healthy normal group.
- Four of five western blot-validated proteins showed concordant results with the label-free quantitative proteomics and will be further validated in an independent cohort study