Isolation and proteomic characterisation of microvesicles by mass spectrometry

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Introduction

Microvesicles belongs to so-called extracellular vesicles group. They are heterogeneous membrane-bound sacs that are shed from the surface of cells into the extracellular environment in a highly regulated process. Because they carry a proteomic signature of their cells of origin, their proteomic and glycomic analysis can represent the innovative source of biomarkers, not only cancer but also inflammation, metabolic and other various diseases. Thus, microvesicles play an important role in future biomedical research and in diagnostic and therapeutic strategies.

Results and discussion

According to peptide concentration in fractions and literature, we analyze fractions 4 to 11 where vesicles were expected. Proteomic analysis of microvesicles isolated from pooled human serum resulted in the discovery of 77 unique proteins, from which 75 was confirmed by the Extracellular Vesicle Database [3] and 14 of them are also present in list of top 100 extracellular vesicle proteins. All identified proteins were compared with list of candidate cancer biomarkers [4] and we confirmed 7 proteins to be potential cancer biomarkers (Tab. 1).

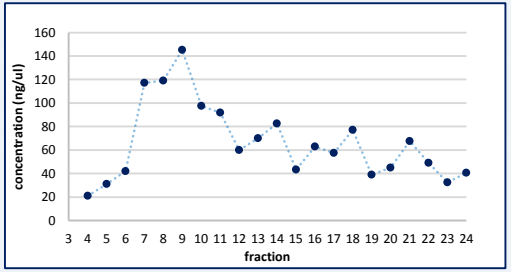

Fig. 2. Peptide concentration of the 24 SEC fractions.

Tab. 1. Table of 7 potential cancer biomarkers.

Protein name	UniProt ID	Gene name	Potential biomarker of
Haptoglobin	P00738	HP	leukemia, lung cancer
Apolipoprotein C-III	P02656	APOC3	myeloid leukemia
Alpha-2-HS-glycoprotein	P02765	AHSG	leukemia
Clusterin	P10909	CLU	leukemia
Apolipoprotein A-I	P02647	APOA1	ovarian cancer
Apolipoprotein A-II	P02652	APOA2	prostate cancer
Serotransferrin	P02787	TF	laryngeal cancer

Materials and methods

400 µl of serum pool from healthy male individuals was centrifuged at 3,300 x g for 20 minutes, supernatant was carefully removed and mixed with four times the volume of cold acetone [1]. Mixture was vortexed and centrifuged at 3000 x g for 1 minute. Vesicles containing supernatant was concentrated to 500 µl and applied to a 10 ml Sepharose CL-4B column [2]. Using 80 % cold acetone with 100 mM ammonium acetate as eluent, 24 fractions of 500 µl were collected. For proteomic analysis, fractions 4-24 were dried and pellets were denatured, reduced, alkylated and digested with trypsin. Enzymatic digestion was stopped with trifluoroacetic acid, peptides were purified by reverse phase SPE and for each fraction was determined its concentration (Fig. 2).
Proteome was analyzed by nanospray LC-MS/MS Orbitrap Elite™ Hybrid Ion Trap-Orbitrap Mass Spectrometer coupled with UltiMate™ 3000 RSLCnano System.

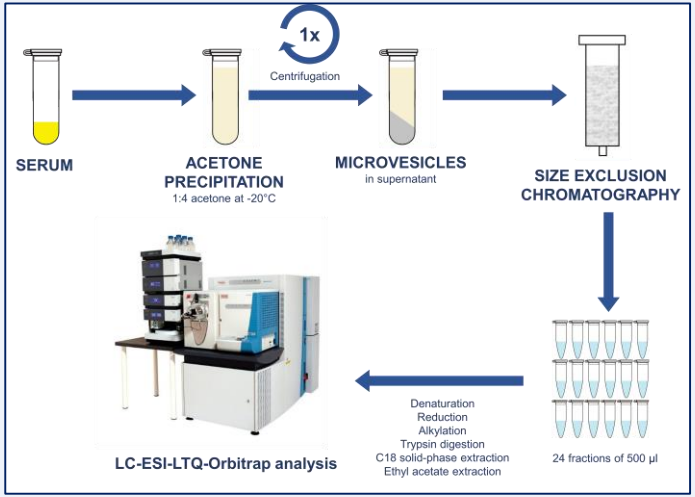

Fig. 1. Schematic diagram showing the steps of isolation of vesicles.

Conclusion

Coupling organic precipitation and SEC, we were able to identify 77 proteins, 7 of which are in the list of potential biomarkers.

Plans for future

- improve isolation protocol
- analysis of the glycan composition of the microvesicles proteins

Acknowledgement

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