

Isolation Strategies and Proteomic Characterization of Extracellular Vesicles

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentligens försvaras i Arvid Carlsson, Medicinaregatan 3, Göteborg, den 12 April 2019, klockan 13:00

av **Aleksander Cvjetkovic**

Fakultetsopponent:

Professor Aled Clayton

Division of Cancer & Genetics, Cardiff University, Cardiff, Wales

Avhandlingen baseras på följande delarbeten

- I. **The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles.**
Cvjetkovic A, Lötvall J, Lässer C.
J Extracell Vesicles. 2014 Mar 25;3. doi:10.3402/jev.v3.23111
- II. **Detailed Analysis of Protein Topology of Extracellular Vesicles-Evidence of Unconventional Membrane Protein Orientation.**
Cvjetkovic A, Jang SC, Konečná B, Höög JL, Sihlbom C, Lässer C, Lötvall.
Sci Rep. 2016 Nov 8;6:36338. doi:10.1038/srep36338
- III. **Detailed analysis of the plasma extracellular vesicle proteome after separation from lipoproteins.**
Karimi N, Cvjetkovic A, Jang SC, Crescitelli R, Hosseinpour Feizi MA, Nieuwland R, Lötvall J, Lässer C.
Cell Mol Life Sci. 2018 Aug;75(15):2873-2886. doi:10.1007/s00018-018-2773-4
- IV. **Proteomic profiling of tumor tissue resident EVs of colon cancer patients.**
Cvjetkovic A, Lässer C, Crescitelli R, Thorsell A, Taflin H, Lötvall J.
In Manuscript

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Aleksander Cvjetkovic

Department of Internal Medicine and Clinical Nutrition, Institute of Medicine,
Sahlgrenska akademien, Göteborgs universitet.

Abstract

“Extracellular vesicles” is the collective term used to describe vesicular entities that are released from cells into the extracellular environment. These vesicles are composed of a delineating lipid membrane and its cargo which can comprise of bioactive molecules such as lipids, RNA, DNA and proteins which can be shuttled between cells and thus function as a means of cell-to-cell communication.

The aims of this thesis were to address how discrepancies in isolation procedure effects the isolate, to distinguish vesicular proteins from co-isolated proteins, to determine the proteome of tissue resident EVs in tumors of colorectal cancer patients and finally to develop a method for high quality vesicle isolates from blood plasma.

We demonstrate that different rotor types will influence not only the yield of isolated vesicles, but also the purity. Furthermore, prolonged ultracentrifugation can up to a point produce higher yields at no apparent cost to purity. Even after purification of vesicles with a density gradient, however, there are proteins in the isolate whose vesicular nature can be questioned as they are susceptible to membrane-impermeable proteolytic digestion. Interestingly, proteolysis of perceived luminal motifs of transmembrane proteins suggests the existence of proteins with unconventional topological orientation within the membrane. We further illustrate that vesicles isolated directly from colorectal tumor tissue greatly differ from vesicles from corresponding healthy tissue in their proteomic makeup. Lastly, we demonstrate the possibility of attaining a highly purified vesicle isolate from blood plasma that is of high enough quality for relevant proteomic evaluation.

In conclusion, we demonstrate how both yield and purity can be optimized in cultured samples as well as in complex biological samples.

Keywords: Colorectal cancer, extracellular vesicles, exosomes, mass spectrometry, plasma, proteomics, ultracentrifugation