

# Exosomes – characterisation of the RNA and protein cargo

Akademisk avhandling

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av

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Avhandlingen baseras på följande delarbeten:

- I. **Human saliva, plasma and breast milk exosomes contain RNA: uptake by macrophages.**  
Lässer C, Alikhani VS, Ekström K, Eldh M, Paredes PT, Bossios A, Sjöstrand M, Gabrielsson S, Lötvall J and Valadi H.  
*Journal of Translational Medicine*. 2011, 9:9
  
- II. **RNA-containing exosomes in human nasal secretions.**  
Lässer C, O'Neil SE, Ekerljung L, Ekström K, Sjöstrand M and Lötvall J.  
*American Journal of Rhinology and Allergy*. 2011, 25(2):89-93
  
- III. **Identification and quantification of immune-related proteins in nasal exosomes using exclusion list-based proteomics.**  
Lässer C\*, O'Neil SE\*, Sihlbom C, Hansson S, Lundbäck B and Lötvall J.  
*In manuscript*  
\* These authors contributed equally
  
- IV. **Two types of exosomes-like vesicles with different RNA and protein content.**  
Lässer C, Shelke G, Crescitelli R, Sjöstrand M and Lötvall J.  
*In manuscript*



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# Exosomes – characterisation of the RNA and protein cargo

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## ABSTRACT

Exosomes are nano-sized vesicles originating from the multivesicular bodies of cells, and are released into the extracellular environment. Exosomes contain functional proteins, lipids and RNA species, which can be shuttled between cells. Exosomes derived from *in vitro* cultures have been widely investigated, while less is known about *in vivo* exosomes. The aims of this thesis therefore were; to increase the understanding of the RNA and protein cargo of body fluid exosomes and to characterise the cargo of different exosome populations.

Exosomes from saliva, plasma, breast milk and nasal lavage were characterised with electron microscopy, flow cytometry and Western blot and were shown to contain RNA with the typical size distribution for exosomes. Primary macrophages could internalise exosomes isolated from breast milk and saliva. Our results argue that RNA-containing exosomes are frequently secreted *in vivo*, can be taken up by human cells, and may potentially be transferred from mother to infant.

Proteomic analysis showed that the application of exclusion lists during LC-MS/MS analysis resulted in a more thorough exploration of the protein content of exosomes, with this approach identifying over 600 proteins from nasal exosomes. Several of these proteins were associated with immune-related functions, which could argue that exosomes can participate in cell signalling as part of the nasal immune response. Furthermore, a quantitative analysis showed that the expression of several proteins was altered in nasal exosomes from subjects with respiratory diseases compared to healthy controls.

The RNA profiles of human mast cell line, HMC-1, exosomes differed when exosomes were isolated with a 0.2 µm filtration step or without. Furthermore, the presence of two types of exosomes was established in the conditional media from HMC-1 cells. These vesicles were of similar size, but with different densities and extensively different protein and RNA content. These findings could explain some of the differences observed in RNA profiles in previously published exosome studies.

It can be concluded that RNA-containing exosomes are present *in vivo* under healthy condition in humans and the nasal exosomal proteome is associated with immune-related functions, which is altered during inflammation. Methodological conclusions were that exclusion lists can be used to improve exosomal proteomic studies and the elimination of the filtration step during exosome isolation affects the RNA profile of the isolated exosome enriched fraction. The existence of distinct subpopulations of exosomes was demonstrated, each with different RNA and protein cargo, highlighting the diversity of exosomes and the need for further investigations.

**Keywords:** Asthma, body fluid, breast milk, chronic rhinosinusitis, exosomes, extracellular vesicles, nasal lavage, plasma, RNA, saliva

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