Intercellular communication via exosomes

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ABSTRACT

Exosomes are small membrane bound vesicles between 30-100 nm in diameter of endocytic origin that are secreted into the extracellular environment by many different cell types. They play a role in intercellular communication by transferring proteins, lipids and RNA to recipient cells. The overall aim of this work has been to further investigate the mechanisms by which cells communicate with each other via exosomes.

In Paper I we hypothesized that exosomes from human cells could be used as vectors to provide cells with therapeutic RNA. Herein, exogenous short interfering RNAs were successfully introduced into various kinds of human exosomes using electroporation. Flow cytometry, confocal microscopy and northern blot confirmed the presence of siRNA inside the exosomes. The results showed that exosomes from blood plasma could deliver the siRNA to human monocytes and lymphocytes. The siRNA delivered to the target cells was shown to be functional causing selective gene silencing of mitogen activated protein kinase 1. Our results imply that exosomes from human cells could be used as vectors for delivery of therapeutic exogenous nucleic acids to cells.

In paper II we investigated if exosomes from activated CD3+ T cells could play a role in an immunological response by conveying signals from their secreting cells to recipient resting T cells in an *in vitro* autologous setting. The role of these exosomes was explored in IL-2 mediated T cell proliferation. The results showed that neither exosomes nor IL-2 alone could stimulate proliferation in resting T cells. However, exosomes from stimulated T cells together with IL-2 were able to induce proliferation. T cell cultures stimulated with exosomes and IL-2 showed a higher proportion of CD8+ T cells than cultures without exosomes. Moreover, a cytokine array showed significant changes in the levels of cytokines and chemokines when exosomes were present. The results indicate that activated CD3+ cells communicate with resting autologous T cells via exosomes.

The main focus in paper III was to study the cellular mechanism by which esRNA is selectively packaged into exosome vesicles during their biosynthesis. Using RNA gel mobility shift assay, we showed the presence of RNA-binding proteins (RBPs) in exosomes. Moreover, we developed a method for the identification of exosomal RBPs able to bind to the esRNA and cellular microRNA. Using this method, we could identify 31 different RBPs in exosomes and 78 in cells. To evaluate the possible role of the identified RBPs in the transfer mechanism of RNA into intraluminal vesicles, five gene transcripts from the identified RBPs were silenced. The results revealed that a selective gene silencing of hnRNPA2B1 caused a reduction of RNA present in the extracellular vesicles. Thus, a novel transport mechanism was suggested for the packaging of esRNA into the exosomes.

In conclusion, the studies presented in this thesis have implications for better understanding the RNA and protein transfer mechanism that occurs between cells via exosomes. The described ability of exosomes to deliver exogenous nucleic acids to cells may be of interest in clinical applications e.g. in gene therapy.

Keywords: exosomes, electroporation, RNA, IL-2, RNA binding proteins

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I. Wahlgren J*, Karlson T*, Brisslert M, Sani F, Telemo E, Sunnerhagen P, Valadi H.
Plasma exosomes can deliver exogenous short interfering RNA to monocytes and lymphocytes.
Nucleic Acids Research, 2012 Sep 1; 40 (17).
**These authors contributed equally*

II. Wahlgren J, Karlson T, Glader P, Telemo E, Valadi H.
Activated human T cells secrete exosomes that participate in IL-2 mediated immune response signaling.
PLoS One, 2012; 7 (11).

III. Statello L, Wahlgren J, Ragusa M, Sunnerhagen P, Purello M, Valadi H.
Exosomes contain RNA-binding proteins involved in the transfer mechanism of esRNA
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