

Extracellular Vesicles and RNA Interference in Tumors

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ABSTRACT

Extracellular vesicles (EVs) including apoptotic bodies (ABs), microvesicles (MVs), exosomes (EXOs) and cell derived artificial nanovesicles (NVs) are important mediators of cell-to-cell communication, in part by transferring bioactive molecules such as DNA, mRNA, miRNA, siRNA, proteins, and lipids. These EVs are released by many cell types, including melanoma cells, and are found in many body fluids. EVs derived from various cell types differ in their molecular composition making them as important diagnostic and prognostic markers. The overall aim of this thesis was to use small-RNA sequencing techniques to define the molecular RNA cargo in the EV subsets described above as well as to examine the functional relevance of the EV-associated miRNA and siRNA on recipient cells.

Characterization of EVs showed distinct RNA profiles in ABs, MVs, and EXOs, and there were significantly greater amounts of total RNA in EXOs compared to ABs and MVs. Small RNA sequencing revealed distinct repertoires of noncoding RNAs in the EV subsets. EXOs contained unique sets of miRNAs, which were shown to be differentially expressed in melanoma tumors compared with benign naevi in previously published studies, thus making them potentially useful as carriers of therapeutic agents. This study demonstrates that distinct sets of RNA molecules are present in subsets of EVs, and this provides unique insights into the contribution of extracellular RNA in cancer development and progression.

The BRAF^{V600E} inhibitor vemurafenib inhibited the growth of *in vitro* melanoma cell cultures, and EVs isolated from the treated cells had significantly higher RNA and protein contents compared to EVs from non-treated cells. Small RNA sequencing revealed distinct non-coding RNA species with significant alterations in miRNA between treated and non-treated cell-derived EVs. Moreover, treated cells and the EVs derived from them showed significant upregulation of miR-211 *in vitro* and *in vivo*. Furthermore, when vemurafenib-treated cell-derived EXOs were transferred to BRAF^{WT} cells, KCNMA1 and IGF2R, genes that are known to play roles in tumor progression, were down-regulated and this resulted in growth attenuation. Overall, miR-211 could be used as a biomarker of response in patients diagnosed with BRAF-mutant melanoma. This study also provides the framework for further investigations into the function of miR-211 in melanoma cells and EVs as well as in cells that might receive miRNA from EVs.

Artificial EXO-mimetic NVs were developed by serial extrusion, and they showed similar characteristics as EXOs. Exogenous loading of GFP-siRNA in NVs led to down-regulation of GFP in endothelial cells. Cell-derived NVs carrying endogenously expressed Myc-siRNA showed significant down-regulation of human cMyc both transcriptionally as well as translationally in lymphoma cells. These NVs were efficiently loaded with siRNA and were taken up by recipient cells resulting in the reduction of target gene expression. In conclusion, this study suggests that EXO-mimetic NVs can be a platform for delivering siRNA to cells.

Taken together, EVs have significant therapeutic potential. EVs have emerged as a novel and functionally important vehicle of cell-cell communication that can mediate multiple biological effects. In addition, these vesicles might provide unique signatures that can be used as biomarkers of response to drug treatment.

Keywords: Apoptotic bodies, microvesicles, exosomes, melanoma, therapeutics, exosome-mimetic nanovesicles.

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- I. Small RNA deep sequencing discriminates subsets of extracellular vesicles released by melanoma cells – evidence of unique microRNA cargos.
*Lunavat TR**, Cheng L*, Kim DK, Bhadury J, Jang SC, Lässer C, Sharples RA, López MD, Nilsson JA, Gho YS, Hill AF, Lötvall J.
RNA Biology 2015 July; 12:8, 810-823. (PMID: 26176991)
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- II. BRAF-inhibition alters melanoma vesicular secretome microRNA cargo.
Lunavat TR, Cheng L, Einarsdottir B, Olofsson R, Sharples RA, Lässer C, Gho YS, Hill AF, Nilsson JA, Lötvall J.
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- III. siRNA Delivery by Exosome-Mimetic Nanovesicles – Implications for Targeting c-Myc in Cancer.
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