Optimisation and characterisation of different strategies for siRNA loading into exosome

P-35-035

L. Germelli[I], R. Piccarducci[I], L. Luisotti[I], A. Falleni[II], C. Giacomelli[I], L. Marchetti[I], C. Martini[I]

[I]Department of Pharmacy University of Pisa, Pisa, Italy, [II]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

The use of therapeutic RNAs has opened up a new avenue in drug discovery; however, their application is limited by the lack of appropriate systems ensuring efficient delivery and tissue specificity. Exosomes are extracellular vesicles produced by the majority of eukaryotic cells and appear to naturally host endogenous RNAs [1]. Given their biocompatibility, nanosized scale, low immunogenicity, and surface engineering feasibility, exosomes represent interesting biological carriers to deliver therapeutic RNAs. However, RNA loading into exosomes remains challenging, with poor loading efficiency and lack of technology standardization [2]. Here, electroporation, sonication, and transfection were evaluated as methods to improve loading efficiency of a siRNA against GFP transcript into HEK293T cells-derived exosomes, compared to passive loading. 10 µg of exosomes (~7.5x1010) were mixed with 100 pmol of siRNA. Each loading method was carried out, followed by a clearing step to remove unloaded siRNA. All methods showed improvement of siRNA loading into exosomes compared to passive loading, with differences that are currently being investigated by gel electrophoresis and digital-PCR. None of the techniques induced physical alterations of exosomes, evaluated by tuneable resistive pulse sensing (TRPS) and TEM analysis, or in the amount of exosome markers, measured by western blot. Notably, ~50% of GFP silencing was observed after treatment of stably GFP-expressing HEK293T cells with selected loaded exosomes, confirming their ability to efficiently embed and deliver siRNA. Further studies on the effects of loading on the endogenous RNA content of exosomes by transcriptomic analysis are in progress. Overall, this study paves the way to the development and validation of a loading technology for using exosomes as RNA delivery systems.

[1]Previously published in:‘O'Brien K et al.(2020),Nat Rev Mol Cell Biol.,21:585-606.’[2]Previously published in:‘Zeng H et al.(2023),Cells,12:1416.’