Farnesyl-GFP as a promising fluorescent tag for exosomes labelling

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Extracellular vesicles (EVs) are cell-derived nanoparticles essential in cell-to-cell communication. Among EVs, exosomes represent membrane vesicles formed by the inward budding of endosomal membrane and secreted by fusion with the cell membrane, thus containing thousands of different macromolecules originating from the parent cell and delivering to the host cell. Therefore, exosome bioengineering represents an appealing approach to mainly sort the cargo as well as modify the exosome surface to target desired recipient cells, thus producing bio-inspired nanovesicles with specific loading and/or targeting features[1]. Consequently, monitoring exosomes' journey by staining with fluorescent dyes is a current appealing approach, although challenging. Therefore, herein HEK293T cell-derived exosomes were isolated by size exclusion chromatography and characterized by dynamic light scattering, tuneable resistive pulse sensing, and transmission electron microscopy for physical properties assessment as well as western blot for biological evaluation; also, micro BCA^{TM} assay was performed to evaluate the protein concentration. The exosome labelling was performed by using different methods. Particularly, transient transfections with a DNA plasmid encoding for green fluorescent protein (GFP), a well-known tag protein, or farnesyl GFP (f-GFP), facilitating the GFP anchoring to the cell membrane, were performed in HEK293T cells. Also, exosomes were labelled post-isolation by the addition of a fluorescent lipophilic dye (Vybrant^{TM} DiD). Then, the tagged exosomes' uptake into recipient cells was monitored by confocal microscopy. The obtained preliminary results highlighted that f-GFP allows to efficiently tag exosomes and track their delivery into recipient cells. Overall, the current work aims to suggest a simple and systematic workflow to optimize a method to track the exosome route from biogenesis to delivery.

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