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Comparison of size and protein decoration between extracellular vesicles isolated from adipose tissue and hair follicle mesenchymal stem cells

Las Heras Kevin[1,2], Royo Félix[3,4], Garcia Vallicrosa Clara[3], Igartua Manoli[1,2,5], Santos Vizcaino Edorta[1,2,5], Falcon Pérez Juan M[3,4,6], Hernández Rosa María*[1,2,5]

[1] NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy (UPV/EHU).
[2] Bioaraba, NanoBioCel Research Group, Vitoria-Gasteiz, Spain.
[3] Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Exosomes Laboratory, 48160 Derio, Spain
[4] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 28029 Madrid, Spain
[5] Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN).
[6] IKERBASQUE, Basque Foundation for Science, 48013 Bilbao, Spain
*Correspondencia: rosa.hernandez@ehu.eus

1. Introduction

In the current years, extracellular vesicles (EVs) have gained the overall attention in modern regenerative medicine therapeutics. Their demonstrated potential for the treatment of different conditions and their principal role in the efficacy of secretome-based therapies has driven their use [1]. Indeed, mesenchymal stem cells (MSCs)-derived EVs (MSC-EVs) are one the most popular source of EVs due to their largely demonstrated regenerative capacity [1, 2].

Some research groups have performed morphological studies of MSC-EVs by electron microscopy (EM) [3]. However, none of them have compared EVs from adipose tissue-derived MSCs (AT-MSCs) — the gold standard in MSC-EVs research — and hair follicle-derived MSCs (HF-MSCs) by using Cryo-transmission electron microscopy (TEM) imaging on rapidly-frozen samples. With this technique, it could be potentially reduced the sample damaging and artefacts caused by the addition of heavy metals, dehydration, or fixation steps. We therefore used cryo-TEM to compare, in their near-native state, the morphology and membrane protein decoration of EVs isolated from AT-MSCs and HF-MSCs.

2. Materials and methods

2.1. EVs isolation and purification

EVs were isolated and purified from the supernatant of HF-MSCs and AT-MSCs after 3 productions of 72 h each on exos-free DMEM. The collected culture medium was first centrifuged at 2,000 × g for 10 min at 4 ºC to discard cell debris. Then, it was centrifuged serially at 10,000 × g for 30 min to obtain medium/large-sized EVs — pellet 10K (P10K) — and finally centrifuged at 100,000 × g for 90 min — pellet 100K (P100K) — to obtain small-sized EVs. All pellets were re-suspended in ice-cold PBS and another ultracentrifugation process was performed in order to better purify EVs. Finally, both pellets were immediately re-suspended in 100 µL of ice-cold PBS and freeze at -80 ºC until use.

2.2. Cryo-TEM

EVs (3-5 µl) were spotted on glow-discharged lacey grids and cryo-fixed by plunge freezing at -180 º in liquid ethane with a Vibrobot (FEI, The Netherlands). Grids were observed with a JEM-2200FS/CR TEM (JEOL, Japan), operating at 200 kV. Image measurements were performed with Image J software and between 80-120 single EVs were measured on each group.

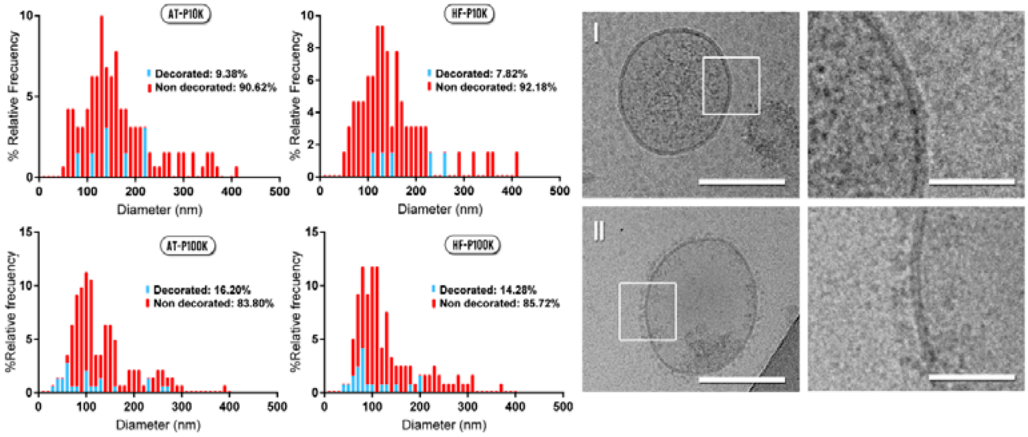

Fig. 1. Protein decoration of EVs and cryo-TEM images. A. Decoration vs relative frequency and diameter of EVs. B(I). Cryo-TEM image of a non-decorated EV. B(II). Cryo-TEM image of a decorated EV. Left images scale bars are 100 nm and right scale bars are 20 nm.

3. Results and discussion

As it can be observed in Fig.1, we obtained a heterogenous population of mostly round-shaped EVs in both cell types. We found a lower amount of decorated EVs than non-decorated in both groups for both P10K and P100K (Fig.1A). The percentage of decoration was similar for AT-EVs and HF-EVs.

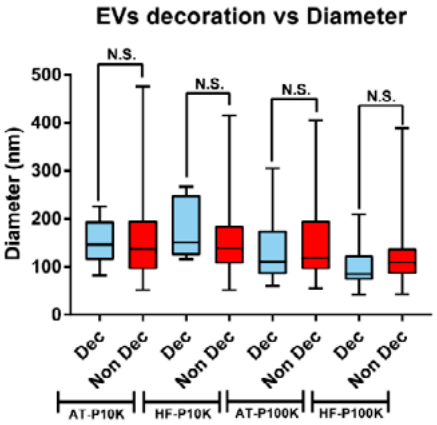

Fig. 2. EVs decoration vs Diameter of EVs. N.S. Non significance.

Furthermore, P100K EVs had more membrane protein decoration than P10K on both cell types — 73 % of increase in AT-EVs and 83 % of increase in HF-EVs —. Finally, we did not observe differences in the size of decorated EVs against non-decorated EVs for both cell types (Fig.2).

4. Conclusions

These results depicted that the percentage of protein-decorated EVs isolated by ultracentrifugation from AT-MSCs and HF-MSCs was small. Furthermore, the decoration ratio was higher in P100K EVs than in P10K EVs, but similar for both cell types. These findings suggest that the morphological and structural profile of the EVs isolated from both AT-MSCs and HF-MSCs is very similar.

Acknowledgments

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