

Therapeutic Potential of Mesenchymal Stem Cell-derived Exosomes Isolated with the PS Affinity Method

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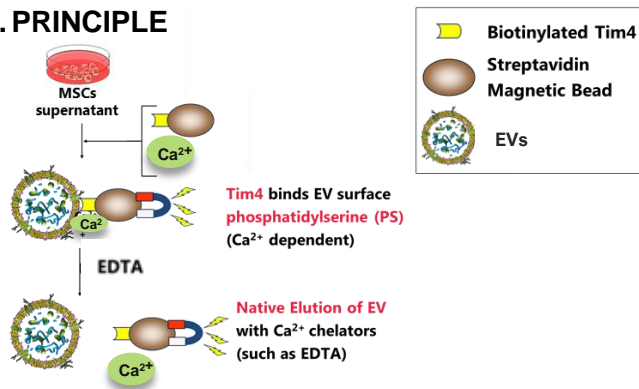
I. INTRODUCTION

Mesenchymal -derived Stem Cells (MSCs) have attracted much attention as a potential cellular therapeutic tool due to their regenerative and immunomodulatory capabilities. Their therapeutic effects are largely mediated by paracrine factors including Extracellular Vesicles (EVs) that are nano-sized lipid bilayer particles. EVs derived from mesenchymal stem cells (MSC) carry lipids, proteins, and nucleic acids derived from their producing cells are emerging as a promising therapeutic strategy in regenerative medicine.

II. OBJECTIVE

To examine whether MSC-derived EVs isolated by the PS method have similar functions as those isolated by ultracentrifugation, we isolated EVs from the supernatant of bone marrow-derived MSCs and analyzed the effects of anti-inflammatory and anti-fibrosis.

III. PRINCIPLE



Product Features

- ◆ Simple and easy method using **magnetic beads**
- ◆ Capable of purifying exosomes from **various samples** such as cell cultures and body fluids (plasma, serum, etc.).
- ◆ Maximize recovery of **intact exosomes** by native elution buffer

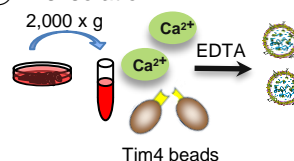
IV. MATERIALS AND METHODS

① Cell culture



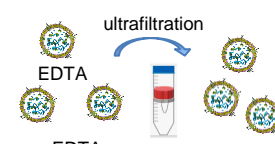
Human BM-derived MSCs are cultured to 90% confluence, replaced with DMEM/10% EV-depleted FBS, and cultured for 120 hours.

② EVs isolation



After centrifuging the cell culture supernatant at 2,000 x g, and then EVs are isolated.

③ Buffer exchange



To remove EDTA, the buffer is exchanged with D-PBS using a 100K ultrafiltration column.

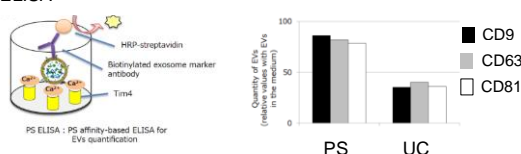
④ EVs treatment and qRT-PCR

Human PBMC-derived monocytes or human fetal lung diploid fibroblast cells (TIG3 cells) are treated with EVs. Determined a gene expression amount of anti-inflammatory or fibrosis markers using RT-PCR.

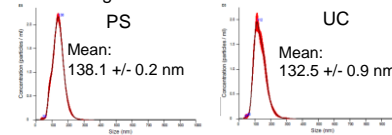
V. RESULTS

1. Characterization of BM-MSC-derived EVs

A. ELISA



B. NanoSight



C. Western blot

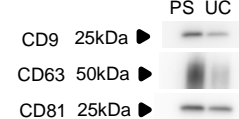


Figure 1. (A) Principle of PS sandwich ELISA and Exosome concentrations determined by CD9, CD63 or CD81 antibodies. (B) Particle size distribution analysis using NanoSight. (C) Western blot analysis on the expression of the exosome markers CD9, CD63, and CD81.

2. BM-MSC-derived EVs decreases the expression of pro-inflammatory cytokines and increases the anti-inflammatory cytokines in LPS-treated human PBMC-monocyte.

3. BM-MSC-derived EVs decreases the expression of fibrosis markers in TGFβ-treated lung fibroblastic cells, TIG3.

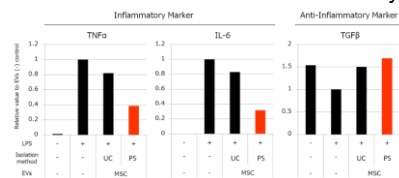


Figure 2. The relative expression levels of the inflammatory-associated genes were evaluated by qRT-PCR after stimulation by LPS and then subsequently after MSC-EVs treatment with LPS: TNF-α, IL-6, and TGFβ.

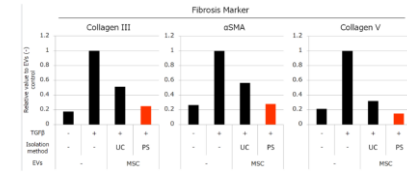


Figure 3. The relative expression levels of the fibrocytic genes were evaluated by qRT-PCR after stimulation by TGFβ and then subsequently after MSC-EVs treatment with TGFβ: Collagen III, αSMA, and Collagen V.

VI. CONCLUSION AND DISCUSSION

We have developed the Exosome Isolation Kit using the PS affinity. Our study showed that the PS affinity method can isolate MSC-derived EVs with high recovery efficiency while maintain the high activity of them. We hope the PS affinity method would be a powerful tool for the development of future MSC-derived EVs therapies.

MagCapture™ Exosome Isolation Kit PS



#299-77603 2 tests & #293-77601 10 tests

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