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Isolation and characterization of extracellular vesicles from different types of mesenchymal stromal/ stem cells for future translation research

I.M. Gordiienko[1,2], M.V. Shamshur[2,3,4], N.M. Petryk[2], A.V. Zlatska[2,3,4]

[1] R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine
45, Vasyl′kivs′ka Str., Kyiv, Ukraine, 03022

[2] Biotechnology Laboratory, Medical Company "Good Cells"
9, Ivana Kramskoho Str., Kyiv, Ukraine, 03115

[3] Institute of Genetic and Regenerative Medicine, NAMS of Ukraine
67, Vyshhorodska Str., Kyiv, Ukraine, 04114

[4] M.D. Strazhesko National Scientific Center of Cardiology, Clinical and Regenerative Medicine, NAMS of Ukraine
5, Sviatoslava Khorobroho Str., Kyiv, Ukraine, 03151
imgordiienko@gmail.com

Background. Extracellular vesicles (EVs) derived from mesenchymal stromal/stem cells (MSCs) hold great promise as novel therapeutic agents for stimulating tissue regeneration and immunomodulation. The molecular profile and functional properties of EVs depend on the type of parent MSCs and should be considered when developing EV-based products. The **aim** of our study was to isolate and characterize EVs from umbilical cord-derived MSCs (UC-MSCs), adipose tissue-derived MSCs (ADSCs) and dental pulp stem cells (DPSCs). **Methods.** Cell culture, flow cytometry, directed multiline differentiation assay, differential ultracentrifugation, nanoparticle tracking analysis (NTA), BCA protein assay, and ELISA methods were used. **Results.** All types of examined MSCs cultures exhibited fibroblastic morphology, $CD73^+$, $CD90^+$, $CD105^+$, $CD34^-$, $CD45^-$, $HLA\text{-}DR^-$ phenotypes, and were able to differentiate in adipogenic and osteogenic lineage. DPSCs expressed Nestin and CD271 at high levels and were able to differentiate into neuronal and glial lineages compared to UC-MSCs and ADSCs. The growth medium was changed to serum-free medium (SFM) for EVs harvesting when MSCs reached 80–90% confluence. The quantities of EVs isolated were 191±4.75 billion from UC-MSCs, 161.8±6.2 billion from DPSCs, and 15.5±6.5 billion from ADSCs. The highest level of EVs purity was observed in UC-MSC and DPSC-derived EVs, but not in ADSC-derived EVs. The average sizes of UC-MSCs, DPSCs, and ADSCs-derived EVs were 166.95±4.56 nm, 170.81±3.42 nm and 232.01±5.23 nm, respectively. EVs isolated all studied MSC types were positive for CD63 and CD81. UC-MSC-derived EVs contained higher levels of bFGF (100.4±5.3 pg/ml), EGF (2.2±0.5 pg/ml), VEGF (773±15 pg/ml), GDNF (14.1±3 pg/ml), IL-6 (343±7 pg/ml), and IL-1RA (60±7 pg/ml) compared to DPSCs and ADSCs. However, the high level of IL-1RA were specific for DPSCs-derived EVs. The EVs particle concentration, average particle size and total protein concentration remained stable for at least six months of storage at –80 °C. **Conclusions.** UC-MSCs and DPSCs are the most suitable cell types for large-scale EVs isolation. Based on size distribution and CD63/CD81 expression, EVs isolated from UC-MSCs, DPSCs, and ADSCs can be defined as exosomes. UC-MSC-derived EVs contain higher levels of growth factors and cytokines compared to DPSC- and ADSC-derived EVs, which may mediate stronger tissue regeneration through regulation of cell proliferation, immunosuppression, and vasculogenesis.

Keywords: extracellular vesicles, mesenchymal stromal/stem cells, exosomes, growth factors, cytokines.