

Conference Paper

The Size of Vesicles Produced By Different Stem Cells

Alchinova I.B.^{1,2}, Vyalkina M.V.¹, Karganov M.Yu.¹, and Saburina I.N.¹

¹Institute of General Pathology and Pathophysiology, Baltiyskaya str., 8, Moscow, 125315, Russian Federation

²Research Institute for Space Medicine, Federal Biomedical Agency of Russia, Orekhovyy Boulevard, 28, Moscow, 115682, Russian Federation

Abstract

Extracellular vesicles (EVs) are now intensively studied in the context of their action on the irradiated organism. Suspensions of EVs isolated from culture media of two different cell lines were analyzed by transmission electron microscopy and nanoparticle tracking analysis (NTA) to evaluate size and concentration of contained particles. The results obtained indicate the difference in analyzed parameters depending on the cell type.

Keywords: stem cells, extracellular vesicles, nanoparticle tracking analysis

Corresponding Author:

Margarita Vyalkina
 nedzumy@bk.ru

Received: 17 January 2018

Accepted: 25 March 2018

Published: 17 April 2018

Publishing services provided by
Knowledge E

© Alchinova I.B. et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the PhysBioSymp17 Conference Committee.

1. Introduction

We have previously demonstrated [1] that intravenous injection of human bone marrow multipotent mesenchymal stromal cells (BM-MMSC) to irradiated mice promoted partial recovery of their physiological parameters. Extracellular vesicles (EVs) secreted by practically all cells are now intensively studied as effectors of the paracrine mechanism of the therapeutic effect of MMSC on the irradiated organism [2, 3]. These particles are supposed to participate actively in cell-to-cell communication and cell interaction with the microenvironment. Our aim was to isolate and characterize extracellular vesicles produced by various types of stem cells.

2. Materials and methods

Suspensions of extracellular vesicles isolated from culture media (CM) of passage 2 human BM-MMSC (cells previously used in the therapy of irradiated animals; CM-1) and passage 4 rat adipose tissue-derived MMSC (AT-MMSC; CM-2) were analyzed by transmission electron microscopy and nanoparticle tracking analysis (NTA).

 **OPEN ACCESS**

2.1. Cell cultures preparation

Isolation and culturing of primary human BM MMSC was carried out as described in [1].

For isolation of the stromal vascular fraction cells, fragments of subcutaneous fat were taken during surgery and the cells were isolated according to the standard protocol [4]. The obtained tissue fragments were minced under sterile conditions, small fragments were incubated with collagenase 1 (0.07%; "PanEco", Russia) and dispase (0.025%; "PanEco", Russia) over 25-30 min. After incubation, growth medium was added to the solution with enzymes and tissue fragments and the samples were centrifuged for 5 min at 1000 rpm. The supernatant was discarded; the pellet was resuspended in growth medium and filtered through a nylon filter to remove large tissue fragments. The cell suspension was seeded on Petri dishes and cultured for 7-10 days in full growth medium (DMEM/F12 with glutamine (PanEco), 1% penicillin-streptomycin (PanEco), and 10% FCS). The cultures were passaged after attaining 80-90% confluence, and passage 4 culture was used in the experiments.

2.2. Characterization of stem cell cultures

Immunophenotyping of the obtained cells was carried out using the following markers: CD90 FITC, CD105 PE, CD29 PE, CD133 PE, CD19 PE, CD45 PE, CD34 PE, CD11b PE (BD Biosciences, USA). Cells were washed with Versen solution, treated with 0.25% trypsin, pipetted, and centrifuged (300g, 5 min). The pellet was resuspended in phosphate buffered saline (PBS) (pH = 7.4) and aliquoted. Each sample was incubated with antibodies for 15 min in the dark at 25°C. The results were analyzed on a "Cytomics FC 500" flow cytometer ("Beckman Coulter", USA).

Both analyzed cultures had all the properties of MMSC culture: the cells rapidly adhered to the culture plastic, formed dense rapidly proliferating colonies, expressed typical surface markers of MMSC culture and did not express markers of hemopoietic and lymphocyte cells (see Tab.1).

2.3. Isolation of extracellular vesicles

EVs were isolated by differential centrifugation at +4°C in accordance with modified protocol [5]. All used dishes and tools were washed for 2 hours in specially purified water ("Mediana", MSU). The purity of water was assessed by the resistance value,

TABLE 1: The immunophenotypic profile of analyzed cultures.

Analyzed markers	AT-MMSC passage 4 (rat)	BM-MMSC passage 2 (human)
Isotype IgG	0.5%	-
CD11b-	5.7%	-
CD19-	5.7%	-
CD34-	4.4%	1.31%
CD45-	0.1%	0.6%
CD133-	0.4%	-
CD14-	-	1.1%
CD49b-	-	1%
CD29+	95%	-
CD90+	98.9%	79%
CD105+	93 %	98%
CD73+	-	95%

which averaged 15 mega ohms. CM was centrifuged for 10 min at 300g for cells sedimentation. The supernatant was centrifuged at 16,500g for 20 min (Ultracentrifuge Optima L-90K, Beckman coulter angular rotor) for more complete removal of cells and debris, filtered through a 0.2- μ m filter (Millipore Millex-GN Nylon filter, Germany) to remove particles >200 nm, and then centrifuged at 100,000g for 2 h for EVs sedimentation. The pellet was resuspended in 1.5 ml PBS ("Eco-Service", Russia), which was previously passed through a 0.2- μ m filter and centrifuged at 100,000 g for 1 hour at 4°C. Polyallomer (Beckman) tubes with steel caps were used for ultracentrifugation (the covers were washed with specially purified water).

2.4. Transmission electron microscopy (TEM)

The preparations were absorbed on copper formvar-coated (0.5% solution of formaldehyde in dichloroethane was used) meshes (1GC 200, PELCO, USA), contrasted with 2% sodium uranyl acetate for electron microscopy, and examined in a JEM-1400 electron microscope (JEOL, Japan) with a digital camera Quemesa (Olympus, Japan) and software (Olympus Soft Imaging Solutions GmbH, Munster, Germany) at $\times 40,000$. The sizes of the investigated objects were estimated using the ImageJ program (National Institute of Health, USA).

2.5. Nanoparticle Tracking Analysis (NTA)

The mean size and concentration of the particles in the samples were evaluated by the NTA method (Nanosight LM10-HSBF, Great Britain) based on tracing the Brownian motion of individual nanoparticles and measuring their standard mean square shift over a time interval related to particle size by the Stokes-Einstein formula.

3. Results and Discussion

In CM-1, 36-45-nm near-spherical particles were detected during the transmission electron microscopy (Fig. 1A). CM-2 contained two types of particles: 79-106-nm particles with morphological signs of extracellular vesicles and 37-53-nm particles corresponding to very-low-density lipoproteins present in the serum added to the culture medium (Fig. 1B).

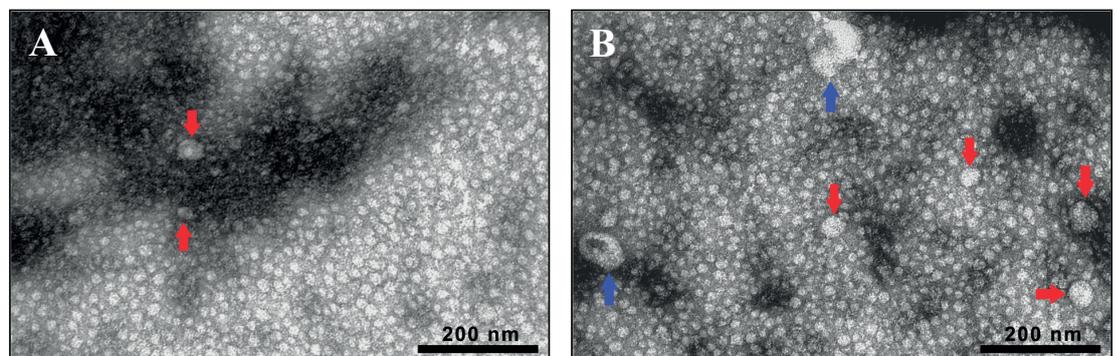


Figure 1: Transmission electron microscopy of analyzed culture media, $\times 40,000$. A – TEM of EVs from human BM-MMSC, 36-45-nm particles are indicated by red arrows. B – TEM of EVs from rat AT-MMSC, 79-106-nm particles are indicated by blue arrows and 37-53-nm particles are indicated by red arrows.

According to NTA results, the mean particle size and their concentration in CM-1 sample were 86 nm and $6.6 \times 10^{10}/\text{ml}$, respectively; for CM-2, the corresponding parameters were 101 nm and $7.9 \times 10^{10}/\text{ml}$ (Fig. 2).

4. Conclusion

The difference in size and concentration of contained particles between two culture media, obtained in our study, can reflect secretion specificity determined by the cell type, which is consistent with literature data [6].

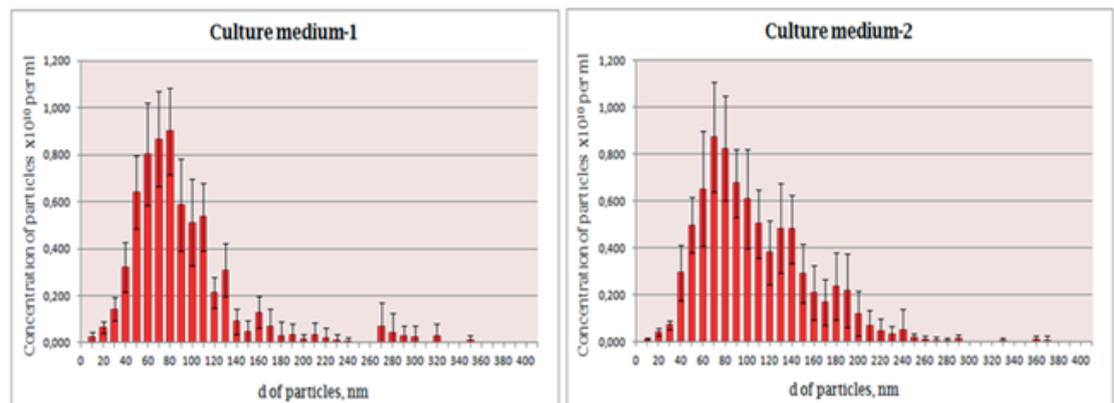


Figure 2: NTA results. The content of particles of different sizes in analyzed suspension of EVs derived from two culture media.

Acknowledgments

The authors are grateful to Lomonosov Moscow State University and personally to E.G. Evtushenko, N.A. Nikitin and E.A. Trifonova.

References

- [1] M.V. Vyalkina, I.B. Alchinova, E.N. Yakovenko, Yu.S. Medvedeva, I.N. Saburina, and M.Yu. Karganov, "Long-Term Effects of Stem Cells on Total-Body Irradiated Mice", *IOP Conf. Series: Journal of Physics: Conf. Series*, vol.784, pp. 012015, 2017, doi:10.1088/1742-6596/784/1/012015
- [2] J.S. Schoefinius, B. Brunswig-Spickenheier, T. Speiseder, S. Krebs, U. Just, and C. Lange, "Mesenchymal Stromal Cell-Derived Extracellular Vesicles Provide Long-term Survival after Total Body Irradiation without additional Hematopoietic Stem Cell Support", *Stem Cells*, doi:10.1002/stem.2716
- [3] S. Wen, M. Dooner, Y. Cheng, E. Papa, M. Del Tatto, M. Pereira,..., and C. Stewart, "Mesenchymal stromal cell derived extracellular vesicles rescue radiation damage to murine marrow hematopoietic cells", *Leukemia*, vol.30(11), pp. 2221-2231, 2016, doi:10.1038/leu.2016.107
- [4] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, and M.H. Hedrick, "Multilineage cells from human adipose tissue: implications for cell-based therapies", *Tissue engineering*, vol.7(2), pp. 211-228, 2004, doi:10.1089/107632701300062859
- [5] C. Lässer, M. Eldh, and J. Lötval, "Isolation and characterization of RNA-containing exosomes", *Journal of visualized experiments: JoVE*, N°59, e3037, 2012, doi:10.3791/3037

- [6] O.N. Shustova, O.A. Antonova, N.V. Golubeva, S.G. Khaspekova, V.V. Ya-kushkin, S.A. Aksuk, I.B. Alchinova, M.Y. Karganov, and A.V. Mazurov, "Differential procoagulant activity of microparticles derived from monocytes, granulocytes, platelets and endothelial cells: impact of active tissue factor", *Blood Coagulation & Fibrinolysis*, vol.28, №5, pp. 373-382, 2017, doi:10.1097/MBC.0000000000000609