

Cytochalasin B-induced membrane vesicles convey angiogenic activity of parental cells

Gomzikova M., Zhuravleva M., Miftakhova R., Arkhipova S., Evtugin V., Khaiboullina S., Kiyasov A., Persson J., Mongan N., Pestell R., Rizvanov A.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© Gomzikova et al. Naturally occurring extracellular vesicles (EVs) play essential roles in intracellular communication and delivery of bioactive molecules. Therefore it has been suggested that EVs could be used for delivery of therapeutics. However, to date the therapeutic application of EVs has been limited by number of factors, including limited yield and full understanding of their biological activities. To address these issues, we analyzed the morphology, molecular composition, fusion capacity and biological activity of Cytochalasin B-induced membrane vesicles (CIMVs). The size of these vesicles was comparable to that of naturally occurring EVs. In addition, we have shown that CIMVs from human SH-SY5Y cells contain elevated levels of VEGF as compared to the parental cells, and stimulate angiogenesis in vitro and in vivo.

<http://dx.doi.org/10.18632/oncotarget.19723>

Keywords

Angiogenesis, Cell-free therapy, Cytochalasin B-induced membrane vesicles, Extracellular vesicles, Membrane vesicles

References

- [1] Battistella V, de Freitas GR, da Fonseca LM, Mercante D, Gutfilen B, Goldenberg RC, Dias JV, Kasai-Brunswick TH, Wajnberg E, Rosado-de-Castro PH, Alves-Leon SV, Mendez-Otero R, Andre C. Safety of autologous bone marrow mononuclear cell transplantation in patients with nonacute ischemic stroke. *Regen Med.* 2011; 6: 45-52. <https://doi.org/10.2217/rme.10.97>.
- [2] Cox CS, Jr., Baumgartner JE, Harting MT, Worth LL, Walker PA, Shah SK, Ewing-Cobbs L, Hasan KM, Day MC, Lee D, Jimenez F, Gee A. Autologous bone marrow mononuclear cell therapy for severe traumatic brain injury in children. *Neurosurgery.* 2011; 68: 588-600. <https://doi.org/10.1227/NEU.0b013e318207734c>.
- [3] Osawa H, Orii K, Terunuma H, Abraham SJ. Combining autologous peripheral blood mononuclear cells with fibroblast growth factor therapy along with stringent infection control leading to successful limb salvage in diabetic patient with chronic renal failure and severe toe gangrene. *Int J Stem Cells.* 2014; 7: 158-61. <https://doi.org/10.15283/ijsc.2014.7.2.158> [pii].
- [4] Jungebluth P, Holzgraefe B, Lim ML, Duru AD, Lundin V, Heldring N, Wiklander OP, Nordin JZ, Chrobok M, Roderburg C, Sjoqvist S, Anderstam B, Beltran Rodriguez A, et al. Autologous Peripheral Blood Mononuclear Cells as Treatment in Refractory Acute Respiratory Distress Syndrome. *Respiration.* 2015; 90: 481-92. doi: 000441799 [pii] <https://doi.org/10.1159/000441799>.
- [5] Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest.* 2005; 115: 1743-55. <https://doi.org/10.1172/JCI22593>.

- [6] Biancone L, Bruno S, Deregibus MC, Tetta C, Camussi G. Therapeutic potential of mesenchymal stem cell-derived microvesicles. *Nephrol Dial Transplant*. 2012; 27: 3037-42. doi: gfs168 [pii] <https://doi.org/10.1093/ndt/gfs168>.
- [7] Takahashi M, Li TS, Suzuki R, Kobayashi T, Ito H, Ikeda Y, Matsuzaki M, Hamano K. Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury. *Am J Physiol Heart Circ Physiol*. 2006; 291: H886-93. doi: 00142.2006 [pii] <https://doi.org/10.1152/ajpheart.00142.2006>.
- [8] den Haan MC, Grauss RW, Smits AM, Winter EM, van Tuyn J, Pijnappels DA, Steendijk P, Gittenberger-De Groot AC, van der Laarse A, Fibbe WE, de Vries AA, Schaliij MJ, Doevendans PA, et al. Cardiomyogenic differentiation-independent improvement of cardiac function by human cardiomyocyte progenitor cell injection in ischaemic mouse hearts. *J Cell Mol Med*. 2012; 16: 1508-21. <https://doi.org/10.1111/j.1582-4934.2011.01468.x>.
- [9] Gneocchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J*. 2006; 20: 661-9. doi: 20/6/661 [pii] <https://doi.org/10.1096/fj.05-5211com>.
- [10] Mirosou M, Jayawardena TM, Schmeckpeper J, Gneocchi M, Dzau VJ. Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *J Mol Cell Cardiol*. 2011; 50: 280-9. doi: S0022-2828(10)00292-0 [pii] <https://doi.org/10.1016/j.yjmcc.2010.08.005>.
- [11] De Jong OG, Van Balkom BW, Schiffelers RM, Bouten CV, Verhaar MC. Extracellular vesicles: potential roles in regenerative medicine. *Front Immunol*. 2014; 5: 608. <https://doi.org/10.3389/fimmu.2014.00608>.
- [12] Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, Morando L, Busca A, Falda M, Bussolati B, Tetta C, Camussi G. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J Am Soc Nephrol*. 2009; 20: 1053-67. doi: ASN.2008070798 [pii] <https://doi.org/10.1681/ASN.2008070798>.
- [13] Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2010; 4: 214-22. doi: S1873-5061(09)00141-X [pii] <https://doi.org/10.1016/j.scr.2009.12.003>.
- [14] Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Agur EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2013; 10: 301-12. doi: S1873-5061(13)00003-2 [pii] <https://doi.org/10.1016/j.scr.2013.01.002>.
- [15] Herrera MB, Fonsato V, Gatti S, Deregibus MC, Sordi A, Cantarella D, Calogero R, Bussolati B, Tetta C, Camussi G. Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomized rats. *J Cell Mol Med*. 2010; 14: 1605-18. doi: JCMM860 [pii] <https://doi.org/10.1111/j.1582-4934.2009.00860.x>.
- [16] Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. *J Cereb Blood Flow Metab*. 2013; 33: 1711-5. doi: jcbfm2013152 [pii] <https://doi.org/10.1038/jcbfm.2013.152>.
- [17] Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med*. 1996; 183: 1161-72.
- [18] Clayton A, Court J, Navabi H, Adams M, Mason MD, Hobot JA, Newman GR, Jasani B. Analysis of antigen presenting cell derived exosomes, based on immunomagnetic isolation and flow cytometry. *J Immunol Methods*. 2001; 247: 163-74. doi: S0022-1759(00)00321-5 [pii].
- [19] Witwer KW, Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, Nolte-t Hoen EN, Piper MG, Sivaraman S, Skog J, Thery C, Wauben MH, Hochberg F. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J Extracell Vesicles*. 2013; 2. <https://doi.org/10.3402/jev.v2i0.20360> 20360 [pii].
- [20] Pick H, Schmid EL, Tairi AP, Ilegems E, Hovius R, Vogel H. Investigating cellular signaling reactions in single attoliter vesicles. *J Am Chem Soc*. 2005; 127: 2908-12. <https://doi.org/10.1021/ja044605x>.
- [21] Mao Z, Cartier R, Hohl A, Farinacci M, Dorhoi A, Nguyen TL, Mulvaney P, Ralston J, Kaufmann SH, Mohwald H, Wang D. Cells as factories for humanized encapsulation. *Nano Lett*. 2011; 11: 2152-6. <https://doi.org/10.1021/nl200801n>.
- [22] Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Res*. 1978; 38: 3751-7.
- [23] Roy Choudhury S, Karmakar S, Banik NL, Ray SK. Targeting angiogenesis for controlling neuroblastoma. *J Oncol*. 2012; 2012: 782020. <https://doi.org/10.1155/2012/782020>.
- [24] Rizvanov AA, Yalvac ME, Shafigullina AK, Salafutdinov, II, Blatt NL, Sahin F, Kiyasov AP, Palotas A. Interaction and self-organization of human mesenchymal stem cells and neuro-blastoma SH-SY5Y cells under co-culture conditions: A novel system for modeling cancer cell microenvironment. *Eur J Pharm Biopharm*. 2010; 76: 253-9. doi: S0939-6411(10)00148-7 [pii] <https://doi.org/10.1016/j.ejpb.2010.05.012>.
- [25] MacLean-Fletcher S, Pollard TD. Mechanism of action of cytochalasin B on actin. *Cell*. 1980; 20: 329-41. doi: 0092-8674(80)90619-4 [pii].

- [26] Honig MG, Hume RI. Fluorescent carbocyanine dyes allow living neurons of identified origin to be studied in long-term cultures. *J Cell Biol.* 1986; 103: 171-87.
- [27] Tetta C, Ghigo E, Silengo L, Deregibus MC, Camussi G. Extracellular vesicles as an emerging mechanism of cell-to-cell communication. *Endocrine.* 2013; 44: 11-9. <https://doi.org/10.1007/s12020-012-9839-0>.
- [28] Gomzikova M.O. RAA. *Current Trends in Regenerative Medicine: From Cell to Cell-Free Therapy.* BioNanoScience. 2016: 1-6. <https://doi.org/10.1007/s12668-016-0348-0>.
- [29] Antonyak MA, Li B, Boroughs LK, Johnson JL, Druso JE, Bryant KL, Holowka DA, Cerione RA. Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. *Proc Natl Acad Sci U S A.* 2011; 108: 4852-7. doi: 1017667108 [pii] <https://doi.org/10.1073/pnas.1017667108>.
- [30] Kawamoto T, Ohga N, Akiyama K, Hirata N, Kitahara S, Maishi N, Osawa T, Yamamoto K, Kondoh M, Shindoh M, Hida Y, Hida K. Tumor-derived microvesicles induce proangiogenic phenotype in endothelial cells via endocytosis. *PLoS One.* 2012; 7: e34045. <https://doi.org/10.1371/journal.pone.0034045> PONE-D-11-21833 [pii].
- [31] Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol.* 2013; 200: 373-83. doi: jcb.201211138 [pii] <https://doi.org/10.1083/jcb.201211138>.
- [32] Choi DS, Kim DK, Kim YK, Gho YS. Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. *Proteomics.* 2013; 13: 1554-71. <https://doi.org/10.1002/pmic.201200329>.
- [33] Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. *Expert Rev Proteomics.* 2009; 6: 267-83. <https://doi.org/10.1586/epr.09.17>.
- [34] Ji H, Chen M, Greening DW, He W, Rai A, Zhang W, Simpson RJ. Deep sequencing of RNA from three different extracellular vesicle (EV) subtypes released from the human LIM1863 colon cancer cell line uncovers distinct miRNA enrichment signatures. *PLoS One.* 2014; 9: e110314. <https://doi.org/10.1371/journal.pone.0110314> PONE-D-14-23898 [pii].
- [35] Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J Neurooncol.* 2013; 113: 1-11. <https://doi.org/10.1007/s11060-013-1084-8>.
- [36] Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. *J Extracell Vesicles.* 2014; 3. <https://doi.org/10.3402/jev.v3.24641> 24641 [pii].
- [37] de Vrij J, Maas SL, Kwappenberg KM, Schnoor R, Kleijn A, Dekker L, Luider TM, de Witte LD, Litjens M, van Strien ME, Hol EM, Kroonen J, Robe PA, et al. Glioblastoma-derived extracellular vesicles modify the phenotype of monocytic cells. *Int J Cancer.* 2015; 137: 1630-42. <https://doi.org/10.1002/ijc.29521>.
- [38] Giusti I, Delle Monache S, Di Francesco M, Sanita P, D'Ascenzo S, Gravina GL, Festuccia C, Dolo V. From glioblastoma to endothelial cells through extracellular vesicles: messages for angiogenesis. *Tumour Biol.* 2016; 37: 12743-53. <https://doi.org/10.1007/s13277-016-5165-0> 10.1007/s13277-016-5165-0 [pii].
- [39] Dieterich L. (2011). *Molecular regulation of inflammation and angiogenesis in the tumor microenvironment.* (Uppsala: Uppsala University).
- [40] Kang T, Jones TM, Naddell C, Bacanamwo M, Calvert JW, Thompson WE, Bond VC, Chen YE, Liu D. Adipose-Derived Stem Cells Induce Angiogenesis via Microvesicle Transport of miRNA-31. *Stem Cells Transl Med.* 2016; 5: 440-50. doi: sctm.2015-0177 [pii] <https://doi.org/10.5966/sctm.2015-0177>.
- [41] Pan Q, He C, Liu H, Liao X, Dai B, Chen Y, Yang Y, Zhao B, Bihl J, Ma X. Microvascular endothelial cells-derived microvesicles imply in ischemic stroke by modulating astrocyte and blood brain barrier function and cerebral blood flow. *Mol Brain.* 2016; 9: 63. <https://doi.org/10.1186/s13041-016-0243-1> 10.1186/s13041-016-0243-1 [pii].
- [42] Cantaluppi V, Gatti S, Medica D, Figliolini F, Bruno S, Deregibus MC, Sordi A, Biancone L, Tetta C, Camussi G. Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia-reperfusion injury by microRNA-dependent reprogramming of resident renal cells. *Kidney Int.* 2012; 82: 412-27. doi: S0085-2538(15)55556-7 [pii] <https://doi.org/10.1038/ki.2012.105>.
- [43] Salafutdinov I. I., SAK, Yalvach M. E., Kudryashova N. V., Lagarkova M. A., Shutova M. V., Kiselev S. P., Masgutov R. F., Zhdanov R. I., Kiyasov A. L., Islamov P. P., Rizvanov A. A. Effect of simultaneous expression of various isoforms of vascular endothelial growth factor VEGF and fibroblast growth factor FGF2 on proliferation of human umbilical cord blood cells HUVEC. *Cellular Transplantation and Tissue Engineering.* 2010; 5: 62-7.