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Editorial

Mesenchymal Stem Cell-Derived Exosomes: New Opportunity in Cell-Free Therapy

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Abstract
Mesenchymal stromal/stem cells (MSCs) are involved in tissue homeostasis through direct cell-to-cell interaction, as well as secretion of soluble factors. Exosomes are the sort of soluble biological mediators that obtained from MSCs cultured media in vitro. MSC-derived exosomes (MSC-DEs) which produced under physiological or pathological conditions are central mediators of intercellular communications by conveying proteins, lipids, mRNAs, siRNA, ribosomal RNAs and miRNAs to the neighbor or distant cells. MSC-DEs have been tested in various disease models, and the results have revealed that their functions are similar to those of MSCs. They have the supportive functions in organisms such as repairing tissue damages, suppressing inflammatory responses, and modulating the immune system. MSC-DEs are of great interest in the scope of regenerative medicine because of their unique capacity to the regeneration of the damaged tissues, and the present paper aims to introduce MSC-DEs as a novel hope in cell-free therapy.

Introduction

Mesenchymal stromal/stem cells (MSCs) as non-hematopoietic stem cells resided in the stroma of the bone marrow[1,2] and comprise 0.001%–0.01% of the total nucleated bone marrow cells.[3,4] Although MSCs are isolated from human adipose tissue, liver, spleen, thymus, umbilical cord blood, placenta, Wharton's jelly, brain, lung, dental pulp, palatine tonsils, peripheral blood and other sources,[5-11] but they are mainly present in the bone marrow.[12,13]

MSCs don't have identical markers due to species diversity, various tissue sources and culture conditions probably.[14] MSCs derived from different sources are similar in phenotype, but are different in functions.[8] The international society for cellular therapy (ISCT) has suggested some criteria for characterizing human MSCs which summarized in 1) Plastic adherence property in standard culture conditions, 2) Expression of CD105, CD90, and CD73, and lack expression of CD34, CD45, CD14 or CD11b, CD79a or CD19 and HLA-DR markers and 3) Differentiation potency to adipocytes, chondroblasts, and osteoblasts *in vitro*.[15]

MSCs exert their roles in the bone marrow via direct cell-to-cell cross-talk as well as secretion of broad-spectrum soluble factors.[16] MSCs can migrate to injured tissues and because of their differentiation capability into various cell lineages and through secretion of soluble molecules can regenerate those injured tissues.[17,18] MSCs augment angiogenesis and inhibit fibrosis via angiogenic and antifibrotic factors, respectively.[19] In addition to, MSCs have neuroprotective and immunosuppression effects.[7,20-24]

MSCs which recognized as main components of stromal cell niches support hematopoietic stem cells (HSCs) homing, proliferation, self-renewal, and differentiation in the bone marrow.[16,25-30] In addition, MSCs suppress HSCs apoptosis.[29,31] Major soluble mediators which produced by MSCs contain cytokines, various growth factors, microRNAs, and exosomes which may affect the differentiation capacities of MSCs and promote tissue repairs.[19,32,33]

The search for MSC-derived exosomes (MSC-DEs) is an attractive scope of the investigation because they are the paracrine effectors of MSCs and involved in cell-to-cell interactions. In this paper, we have summarized characteristics, properties, and isolation of the exosomes secreted by MSCs, also its applications in regenerative medicine as cell-free therapy.

Extracellular vesicles

Extracellular vesicles (EVs) are a general term for different types of membranous components in the 20–1000 nm diameter which released by various cell types in cultured media include stem cells, B and T lymphocytes, dendritic cells, mast cells, adipocytes, neurons, platelets, endothelial and epithelial cells.[34-36] In addition, EVs isolated from many body fluids such as urine, serum, amniotic fluid, saliva, cerebrospinal fluid, breast milk, and nasal secretions.[37-41] Cancer cells secrete exosomes

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in greater levels than normal cells that play a vital role in diagnosis, development, and treatment of some cancers.[40,42-44]

According to the size and source of production, EVs are divided into three general types: 1) Ectosomes or microvesicles with 200–1000 nm that derives from the plasma membrane, 2) Exosomes (40–100 nm) that originate from the inside budding of the late endosomal membrane, and 3) Apoptotic bodies with 50-500 nm that release from apoptotic cells.[34,45-48] Among all of them, exosomes have attracted much interest in the last decades.

Exosomes

Exosomes have 1.10-1.21 g/mL flotation density in a sucrose gradient that can be precipitated by centrifugation at 100.000 ×g force.[34,49] Because of endosomal origin of the exosomes, all of them include membrane-associated proteins, such as tetraspanins (e.g. CD9, CD63, CD81 and CD82), MHC-I and MHC-II, heat-shock proteins (e.g. Hspa8, Hsp60, Hsp70, Hsp90), GTPases (EEF1A1, EEF2) and proteins involved in multivesicular body biogenesis (Alix and TSG101).[35,37,49,50] Moreover, metabolic enzymes (e.g. GAPDH, LDHA, PGK1, aldolase, PKM), cytoskeletal proteins (e.g. actin, moesin, syntenin), and the carrier proteins such as albumin were identified in exosomes.[49] The certain protein components rely on the origin of exosomes and may undulate according to physiological variations. Apart from the common surface markers of exosomes (CD9 and CD81), MSC-DEs express several molecules of MSCs, such as CD29, CD44, CD90 and CD73.[51,52]

In addition to proteins, exosomes are enriched with a collection of cytokines, certain lipid rafts such as phosphoglycerides, cholesterol, ceramide, fatty-acyl chains as well as mRNAs, miRNAs, non-coding RNAs, tRNAs, rRNAs and rarely DNA.[35,53-55] The comprehensive content of exosomes is accessible freely online at http://exocarta.org as well as http://microvesicles.org (vesiclepedia) databases.

Exosomes originate from the fusion of multivesicular bodies with the plasma membrane. This discharge relies on different chemical, environmental and mechanical stimulants, such as gamma-irradiation, calcium ionophores, heparanase, statins, hypoxia (low O2), acidosis conditions and matrix detachment that all of them increased exosome secretions.[35,53,56] Besides, cross-link activation of TCR/CD3 in T lymphocytes,[57] low O2 in placental MSC culture media,[58] K^+-dependent depolarization of neural cells[59] causes the induction of exosome secretions.

Isolation and Storage of Exosomes

The basic and common method for exosome isolation and purification from cell culture supernatants and in different biological fluids is ultracentrifugation that is often combined with sucrose density gradients.[36] Consecutive centrifuge forces cause to cells and larger particle's removal and exosomes precipitation by centrifugation at least 100,000 ×g force.[60] Other procedures for exosome isolations include high-performance liquid chromatography (HPLC), ultrafiltration, exosome precipitation by volume excluding polymers, e.g. Polyethylene glycols (PEGs), affinity purification with specific antibodies against CD9, CD63, CD81, and CD82.[60,61] In addition, today exosome isolation kits are commercially available that are based on efficient techniques and provide convenient separation. Conditions with low PH increase isolation and existence of exosomes.[62] After separation, exosome identities are determined by at least two of these methods include atomic force microscopy (AFM), scanning electron microscopy (SEM), dynamic light scattering (DLS), flow cytometry (FCM), western blotting, nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) or ELISA.[63-65]

After the isolation and characterization, exosomes for *in vivo* or *in vitro* applications must be frozen because they are unstable at room temperature and 37 °C. Exosomes can be stored for 6 months at -20 °C without cryopreservative agents.[66] Sokolova et al. have examined the stability of exosomes during storage at -20 °C, 4 °C and 37 °C. They reported that at 4 °C and 37 °C the size of the exosomes decreased and also degradations or structural changes occurre. Several freeze and thawing cycles (up to -20 °C) and ultracentrifugation did not change the size of exosomes.[65] Hence, -20 °C or lower temperatures are suitable for exosome storage without changes in the size and structure of the exosomes.

Therapeutic effects of MSC-Derived Exosomes

Mesenchymal stem cells improve repair of injured tissues, also modulation of immune responses. These effects of mesenchymal stem cells are widely mediated by differentiations of MSCs, paracrine signals, and several secreted molecules such as microvesicles.[67,68] MSC-DEs investigated largely in many activities of these cells and its effects on other cells. These exosomes probably to participate in many physiological and pathological processes because they carry trophic factors, which can be delivered to recipient cells.[35,69] Therefore, the isolation and identification of exosomes from MSCs cultured media have made them a popular choice for cell-free therapy in research and clinical trials that could have clinical applications in the near future.

The intravenous injection of exosomes secreted from the human umbilical cord-MSC (huc-MSC) is tolerable in animal models because they had supportive effects on weight loss and had no harmful effects on renal or liver function.[70] MSC-DEs through recovery, repair, and regeneration of the tissue play an important role in maintaining tissue homeostasis[71] and have cardioprotective effects through exciting proliferation, apoptosis prevention, angiogenesis induction, and oxidative stress suppression.[4] These exosomes (MSC-DEs) also have anti-apoptosis and anti-inflammatory effects, anti-cardiac remodeling, cardiac regeneration,

neovascularization and anti-vascular remodeling effects in cardiovascular system.[72] MSC-EVs keep cardiac tissue from ischemic injury through angiogenesis-promoting effects.[73] In addition, MSC-derived exosomes decrease myocardial ischemia/reperfusion (MI/R) injury in mouse models.[74,75]

MSC-derived exosomes by activation of PI3K/Akt pathway, increase in ATP levels, reduce oxidative stress promote the myocardial viability and cardiac function MI/R injury; therefore, MSC-DEs can be a potential adjuvant for reperfusion.[76]

The exosomes derived from BM-MSC keeps kidney against ischemia reperfusion damages with diminished inflammatory responses and apoptosis in rats.[51] In addition, exosomes increase renal epithelial cell proliferation *in vitro*.[77] It has proved that in the mouse models BM-MSC-derived exosomes keep the intestines from necrotizing enterocolitis (NEC).[78]

MicroRNAs are a type of small non-coding RNAs (~18-24 nucleotides) which regulate proliferation, differentiation, development, and cell death.[79] Due to exosomes enriched with microRNAs,[53,80] probably play a crucial role in cellular functions such as tissue homeostasis and hematopoiesis. Several miRNAs in adult MSC-derived exosomes, including *miR-191*, *miR-222*, *miR-21*, and *let-7a* adjust cell proliferation, *miR-222*, *miR-21*, and *let-7f* induce angiogenesis and *miR-6087* causes promotion of endothelial cell differentiation.[81]

MSC-derived exosomes accelerate muscle regeneration via promoting myogenesis and angiogenesis, which mediated by miRNAs (e.g. *miR-494*) to be dependent on the effect of cytokines present in exosomes.[82] One study at 2013 reported that MSC-DEs due to enriched with *miR-16*, suppress tumor progression and angiogenesis via down-regulation of the expression of vascular endothelial growth factor (VEGF) in tumor cells[83] and another study at 2012 was reported that MSC-DEs promoted tumor growth in *in vivo* through the increasing VEGF expression by activating extracellular signal-regulated kinase1/2 (ERK1/2) pathway in tumor cells.[84]

Xin et al. showed that intravenous infusion of MSC-derived exosomes after stroke improves neurogenesis, neurite remodeling and angiogenesis.[85] Exosomes have multimodal neuroprotective effects because they can pass over the blood-brain barrier in spite of most drugs.[86] In addition, MSC-exosomes induce axonal development,[87] so this can make a new window in treatment of the neurodegenerative disorders.

Exosomes derived from huc-MSC have the immunomodulatory effects through an increase in the percentage of T-regulatory cells (CD4+ CD25+ FoxP3+) and dissuasion the proliferation of T CD4+ and T CD8+ cells.[88] MSC-DEs improve the survival of allogenic skin graft in mice and delay the occurrence of GVHD for two days by the shift of activated T CD4+ cells to T-regulatory cells.[24]

Conclusions and Future Directions

There have been some attracting therapeutic effects of MSC-derived exosomes in various animal models. Exosomes are ideal vehicles for drug or gene delivery because they enriched with trophic factors. Over the past decades, some studies have been conducted on MSC-DEs showed that exosomes have the capacity in repairing tissue damages, suppressing inflammatory responses, and modulating the immune system but their effects on tumor progression remain controversial and require further studies. Exosome secretions and also its compositions rely on types of sources and environmental conditions; therefore, optimization of the exosome collection procedures from various sources of MSCs gives promising confidence to establish cell-free therapy based exosomes in future. MSCs have immunosuppressive capacities through inhibition of proliferation and maturation of most immune cells, also increasing regulatory T-cells.[7,89] In addition, MSCs are good vectors for *Mycoplasma hyorhinis* infection that had anti-proliferative effects on lymphocytes and MSCs; also, it increases the risk of graft versus host disease (GVHD) in hematopoietic stem cell transplantation (HSCT).[90] MSC-DEs have low immunogenicity capacity rather than MSCs[66,91] and have no infection risks. In addition, there is the risk of ectopic differentiation of MSCs after systemic infusion;[92] hence, exosome-based therapy can be a good replacement for mesenchymal stem cell-based therapy soon. Understanding the exact cellular and molecular mechanisms involved in the effect of MSC-DEs on tissue regeneration requires further investigations.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Koc ON, Lazarus HM. Mesenchymal stem cells: Heading into the clinic. *Bone Marrow Transplant* 2001;27(3):235-9. doi: 10.1038/sj.bmt.1702791
2. Kariminekoo S, Movassaghpour A, Rahimzadeh A, Talebi M, Shamsasenjan K, Akbarzadeh A. Implications of mesenchymal stem cells in regenerative medicine. *Artif Cells Nanomed Biotechnol* 2016;44(3):749-57. doi: 10.3109/21691401.2015.1129620
3. Abdi R, Fiorina P, Adra CN, Atkinson M, Sayegh MH. Immunomodulation by mesenchymal stem cells: A potential therapeutic strategy for type 1 diabetes. *Diabetes* 2008;57(7):1759-67. doi: 10.2337/db08-0180

4. Gallina C, Turinetto V, Giachino C. A new paradigm in cardiac regeneration: The mesenchymal stem cell secretome. *Stem Cells Int* 2015;2015:765846. doi: 10.1155/2015/765846
5. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, et al. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. *Hepatology* 2007;46(1):219-28. doi: 10.1002/hep.21704
6. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci* 2006;119(11):2204-13. doi: 10.1242/jcs.02932
7. De Miguel MP, Fuentes-Julian S, Blazquez-Martinez A, Pascual CY, Aller MA, Arias J, et al. Immunosuppressive properties of mesenchymal stem cells: Advances and applications. *Curr Mol Med* 2012;12(5):574-91. doi: 10.2174/156652412800619950
8. Kellner J, Sivajothi S, McNiece I. Differential properties of human stromal cells from bone marrow, adipose, liver and cardiac tissues. *Cytotherapy* 2015;17(11):1514-23. doi: 10.1016/j.jcyt.2015.07.009
9. Lai RC, Arslan F, Tan SS, Tan B, Choo A, Lee MM, et al. Derivation and characterization of human fetal mscs: An alternative cell source for large-scale production of cardioprotective microparticles. *J Mol Cell Cardiol* 2010;48(6):1215-24. doi: 10.1016/j.yjmcc.2009.12.021
10. Lotfinegad P, Shamsasenjan K, Movassaghpour A, Majidi J, Baradaran B. Immunomodulatory nature and site specific affinity of mesenchymal stem cells: A hope in cell therapy. *Adv Pharm Bull* 2014;4(1):5-13. doi: 10.5681/apb.2014.002
11. Lotfy A, Salama M, Zahran F, Jones E, Badawy A, Sobh M. Characterization of mesenchymal stem cells derived from rat bone marrow and adipose tissue: A comparative study. *Int J Stem Cells* 2014;7(2):135-42. doi: 10.15283/ijsc.2014.7.2.135
12. Gebler A, Zabel O, Seliger B. The immunomodulatory capacity of mesenchymal stem cells. *Trends Mol Med* 2012;18(2):128-34. doi: 10.1016/j.molmed.2011.10.004
13. Wang M, Yang Y, Yang D, Luo F, Liang W, Guo S, et al. The immunomodulatory activity of human umbilical cord blood-derived mesenchymal stem cells in vitro. *Immunology* 2009;126(2):220-32. doi: 10.1111/j.1365-2567.2008.02891.x
14. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008;8(9):726-36. doi: 10.1038/nri2395
15. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy* 2006;8(4):315-7. doi: 10.1080/14653240600855905
16. Smirnov SV, Harbacheuski R, Lewis-Antes A, Zhu H, Rameshwar P, Kotenko SV. Bone-marrow-derived mesenchymal stem cells as a target for cytomegalovirus infection: Implications for hematopoiesis, self-renewal and differentiation potential. *Virology* 2007;360(1):6-16. doi: 10.1016/j.virol.2006.09.017
17. Curley GF, Ansari B, Hayes M, Devaney J, Masterson C, Ryan A, et al. Effects of intratracheal mesenchymal stromal cell therapy during recovery and resolution after ventilator-induced lung injury. *Anesthesiology* 2013;118(4):924-32. doi: 10.1097/ALN.0b013e318287ba08
18. Reinshagen H, Auw-Haedrich C, Sorg RV, Boehringer D, Eberwein P, Schwartzkopff J, et al. Corneal surface reconstruction using adult mesenchymal stem cells in experimental limbal stem cell deficiency in rabbits. *Acta Ophthalmol* 2011;89(8):741-8. doi: 10.1111/j.1755-3768.2009.01812.x
19. Djouad F, Bouffi C, Ghannam S, Noel D, Jorgensen C. Mesenchymal stem cells: Innovative therapeutic tools for rheumatic diseases. *Nat Rev Rheumatol* 2009;5(7):392-9. doi: 10.1038/nrrheum.2009.104
20. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005;105(4):1815-22. doi: 10.1182/blood-2004-04-1559
21. Jia Z, Jiao C, Zhao S, Li X, Ren X, Zhang L, et al. Immunomodulatory effects of mesenchymal stem cells in a rat corneal allograft rejection model. *Exp Eye Res* 2012;102:44-9. doi: 10.1016/j.exer.2012.06.008
22. Johnson TV, Bull ND, Hunt DP, Marina N, Tomarev SI, Martin KR. Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma. *Invest Ophthalmol Vis Sci* 2010;51(4):2051-9. doi: 10.1167/iovs.09-4509
23. Kassis I, Grigoriadis N, Gowda-Kurkalli B, Mizrachi-Kol R, Ben-Hur T, Slavin S, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. *Arch Neurol* 2008;65(6):753-61. doi: 10.1001/archneur.65.6.753
24. Zhang X, Jiao C, Zhao S. Role of mesenchymal stem cells in immunological rejection of organ transplantation. *Stem Cell Rev* 2009;5(4):402-9. doi: 10.1007/s12015-009-9076-y
25. Dazzi F, Ramasamy R, Glennie S, Jones SP, Roberts I. The role of mesenchymal stem cells in haemopoiesis. *Blood Rev* 2006;20(3):161-71. doi: 10.1016/j.blre.2005.11.002
26. Horwitz EM, Maziarz RT, Kebriaei P. Mscs in hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2011;17(1 Suppl):S21-9. doi: 10.1016/j.bbmt.2010.11.026
27. Kfoury Y, Scadden DT. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell* 2015;16(3):239-53. doi: 10.1016/j.stem.2015.02.019
28. Levac K, Karanu F, Bhatia M. Identification of growth factor conditions that reduce ex vivo cord

blood progenitor expansion but do not alter human repopulating cell function in vivo. *Haematologica* 2005;90(2):166-72.
29. Mehrasa R, Vaziri H, Oodi A, Khorshidfar M, Nikogoftar M, Golpour M, et al. Mesenchymal stem cells as a feeder layer can prevent apoptosis of expanded hematopoietic stem cells derived from cord blood. *Int J Mol Cell Med* 2014;3(1):1-10.
30. Mohammadian M, Shamsasenjan K, Lotfi Nezhad P, Talebi M, Jahedi M, Nickkhah H, et al. Mesenchymal stem cells: New aspect in cell-based regenerative therapy. *Adv Pharm Bull* 2013;3(2):433-7. doi: 10.5681/apb.2013.070
31. Magnusson M, Sierra MI, Sasidharan R, Prashad SL, Romero M, Saarikoski P, et al. Expansion on stromal cells preserves the undifferentiated state of human hematopoietic stem cells despite compromised reconstitution ability. *PLoS One* 2013;8(1):e53912. doi: 10.1371/journal.pone.0053912
32. Bruno S, Collino F, Tetta C, Camussi G. Dissecting paracrine effectors for mesenchymal stem cells. *Adv Biochem Eng Biotechnol* 2013;129:137-52. doi: 10.1007/10_2012_149
33. Wang KX, Xu LL, Rui YF, Huang S, Lin SE, Xiong JH, et al. The effects of secretion factors from umbilical cord derived mesenchymal stem cells on osteogenic differentiation of mesenchymal stem cells. *PLoS One* 2015;10(3):e0120593. doi: 10.1371/journal.pone.0120593
34. Breakefield XO, Frederickson RM, Simpson RJ. Gesicles: Microvesicle "cookies" for transient information transfer between cells. *Mol Ther* 2011;19(9):1574-6. doi: 10.1038/mt.2011.169
35. Hannafon BN, Ding WQ. Intercellular communication by exosome-derived micrornas in cancer. *Int J Mol Sci* 2013;14(7):14240-69. doi: 10.3390/ijms140714240
36. Thery C, Zitvogel L, Amigorena S. Exosomes: Composition, biogenesis and function. *Nat Rev Immunol* 2002;2(8):569-79. doi: 10.1038/nri855
37. Admyre C, Johansson SM, Qazi KR, Filen JJ, Lahesmaa R, Norman M, et al. Exosomes with immune modulatory features are present in human breast milk. *J Immunol* 2007;179(3):1969-78. doi: 10.4049/jimmunol.179.3.1969
38. Bruschi M, Ravera S, Santucci L, Candiano G, Bartolucci M, Calzia D, et al. The human urinary exosome as a potential metabolic effector cargo. *Expert Rev Proteomics* 2015;12(4):425-32. doi: 10.1586/14789450.2015.1055324
39. Gallo A, Tandon M, Alevizos I, Illei GG. The majority of micrornas detectable in serum and saliva is concentrated in exosomes. *PLoS One* 2012;7(3):e30679. doi: 10.1371/journal.pone.0030679
40. Qiu S, Duan X, Geng X, Xie J, Gao H. Antigen-specific activities of cd8+ t cells in the nasal mucosa of patients with nasal allergy. *Asian Pac J Allergy Immunol* 2012;30(2):107-13.
41. Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early alzheimer disease. *J Biol Chem* 2012;287(6):3842-9. doi: 10.1074/jbc.M111.277061
42. Hyun KA, Kim J, Gwak H, Jung HI. Isolation and enrichment of circulating biomarkers for cancer screening, detection, and diagnostics. *Analyst* 2016;141(2):382-92. doi: 10.1039/c5an01762a
43. Soung YH, Nguyen T, Cao H, Lee J, Chung J. Emerging roles of exosomes in cancer invasion and metastasis. *BMB Rep* 2016;49(1):18-25. doi: 10.5483/BMBRep.2016.49.1.239
44. Tang H, Wu H, Yang Y, Zhao J, Chen J. Progress in study on the role of exosome-derived microrna in diagnosis and treatment of diseases. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2015;40(11):1270-5. doi: 10.11817/j.issn.1672-7347.2015.11.018
45. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, et al. Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. *Nanomedicine* 2011;7(6):780-8. doi: 10.1016/j.nano.2011.04.003
46. Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, et al. Vesiclepedia: A compendium for extracellular vesicles with continuous community annotation. *PLoS Biol* 2012;10(12):e1001450. doi: 10.1371/journal.pbio.1001450
47. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. Exocarta 2012: Database of exosomal proteins, rna and lipids. *Nucleic Acids Res* 2012;40(Database issue):D1241-4. doi: 10.1093/nar/gkr828
48. Wickman G, Julian L, Olson MF. How apoptotic cells aid in the removal of their own cold dead bodies. *Cell Death Differ* 2012;19(5):735-42. doi: 10.1038/cdd.2012.25
49. Mathivanan S, Ji H, Simpson RJ. Exosomes: Extracellular organelles important in intercellular communication. *J Proteomics* 2010;73(10):1907-20. doi: 10.1016/j.jprot.2010.06.006
50. Zeringer E, Barta T, Li M, Vlassov AV. Strategies for isolation of exosomes. *Cold Spring Harb Protoc* 2015;2015(4):319-23. doi: 10.1101/pdb.top074476
51. Wang R, Lin M, Li L, Li L, Qi G, Rong R, et al. Bone marrow mesenchymal stem cell-derived exosome protects kidney against ischemia reperfusion injury in rats. *Zhonghua Yi Xue Za Zhi* 2014;94(42):3298-303.
52. Yang Y, Bucan V, Baehre H, von der Ohe J, Otte A, Hass R. Acquisition of new tumor cell properties by msc-derived exosomes. *Int J Oncol* 2015;47(1):244-52. doi: 10.3892/ijo.2015.3001
53. Chen TS, Lai RC, Lee MM, Choo AB, Lee CN, Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-micrornas. *Nucleic Acids Res* 2010;38(1):215-24. doi: 10.1093/nar/gkp857
54. Subra C, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, et al. Exosomes account for vesicle-

mediated transcellular transport of activatable phospholipases and prostaglandins. *J Lipid Res* 2010;51(8):2105-20. doi: 10.1194/jlr.M003657
55. Yoon YJ, Kim OY, Gho YS. Extracellular vesicles as emerging intercellular communicasomes. *BMB Rep* 2014;47(10):531-9. doi: 10.5483/BMBRep.2014.47.10.164
56. Savina A, Furlan M, Vidal M, Colombo MI. Exosome release is regulated by a calcium-dependent mechanism in k562 cells. *J Biol Chem* 2003;278(22):20083-90. doi: 10.1074/jbc.M301642200
57. Blanchard N, Lankar D, Faure F, Regnault A, Dumont C, Raposo G, et al. TCR activation of human t cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J Immunol* 2002;168(7):3235-41. doi: 10.4049/jimmunol.168.7.3235
58. Salomon C, Ryan J, Sobrevia L, Kobayashi M, Ashman K, Mitchell M, et al. Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis. *PLoS One* 2013;8(7):e68451. doi: 10.1371/journal.pone.0068451
59. Faure J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, et al. Exosomes are released by cultured cortical neurones. *Mol Cell Neurosci* 2006;31(4):642-8. doi: 10.1016/j.mcn.2005.12.003
60. Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol* 2006;Chapter 3:Unit 3 22. doi: 10.1002/0471143030.cb0322s30
61. Nordin JZ, Lee Y, Vader P, Mager I, Johansson HJ, Heusermann W, et al. Ultrafiltration with size-exclusion liquid chromatography for high yield isolation of extracellular vesicles preserving intact biophysical and functional properties. *Nanomedicine* 2015;11(4):879-83. doi: 10.1016/j.nano.2015.01.003
62. Ban JJ, Lee M, Im W, Kim M. Low pH increases the yield of exosome isolation. *Biochem Biophys Res Commun* 2015;461(1):76-9. doi: 10.1016/j.bbrc.2015.03.172
63. Ge M, Ke R, Cai T, Yang J, Mu X. Identification and proteomic analysis of osteoblast-derived exosomes. *Biochem Biophys Res Commun* 2015;467(1):27-32. doi: 10.1016/j.bbrc.2015.09.135
64. Lopez-Verrilli MA, Caviedes A, Cabrera A, Sandoval S, Wyneken U, Khoury M. Mesenchymal stem cell-derived exosomes from different sources selectively promote neuritic outgrowth. *Neuroscience* 2016;320:129-39. doi: 10.1016/j.neuroscience.2016.01.061
65. Sokolova V, Ludwig AK, Hornung S, Rotan O, Horn PA, Epple M, et al. Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy. *Colloids Surf B Biointerfaces* 2011;87(1):146-50. doi: 10.1016/j.colsurfb.2011.05.013
66. Konala VB, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: A new paradigm for cell-free regeneration. *Cytotherapy* 2016;18(1):13-24. doi: 10.1016/j.jcyt.2015.10.008
67. Fierabracci A, Del Fattore A, Luciano R, Muraca M, Teti A, Muraca M. Recent advances in mesenchymal stem cell immunomodulation: The role of microvesicles. *Cell Transplant* 2015;24(2):133-49. doi: 10.3727/096368913x675728
68. Maumus M, Jorgensen C, Noel D. Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: Role of secretome and exosomes. *Biochimie* 2013;95(12):2229-34. doi: 10.1016/j.biochi.2013.04.017
69. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int* 2010;78(9):838-48. doi: 10.1038/ki.2010.278
70. Sun L, Xu R, Sun X, Duan Y, Han Y, Zhao Y, et al. Safety evaluation of exosomes derived from human umbilical cord mesenchymal stromal cell. *Cytotherapy* 2016;18(3):413-22. doi: 10.1016/j.jcyt.2015.11.018
71. Lai RC, Yeo RW, Lim SK. Mesenchymal stem cell exosomes. *Semin Cell Dev Biol* 2015;40:82-8. doi: 10.1016/j.semcdb.2015.03.001
72. Huang L, Ma W, Ma Y, Feng D, Chen H, Cai B. Exosomes in mesenchymal stem cells, a new therapeutic strategy for cardiovascular diseases? *Int J Biol Sci* 2015;11(2):238-45. doi: 10.7150/ijbs.10725
73. Bian S, Zhang L, Duan L, Wang X, Min Y, Yu H. Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. *J Mol Med (Berl)* 2014;92(4):387-97. doi: 10.1007/s00109-013-1110-5
74. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, et al. Exosome secreted by msc reduces myocardial ischemia/reperfusion injury. *Stem Cell Res* 2010;4(3):214-22. doi: 10.1016/j.scr.2009.12.003
75. Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. *Stem Cells Dev* 2013;22(6):845-54. doi: 10.1089/scd.2012.0395
76. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, et al. 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(3):301-12. doi: 10.1016/j.scr.2013.01.002
77. Dorronsoro A, Robbins PD. Regenerating the injured kidney with human umbilical cord mesenchymal stem

cell-derived exosomes. *Stem Cell Res Ther* 2013;4(2):39. doi: 10.1186/scrt187
78. Rager TM, Olson JK, Zhou Y, Wang Y, Besner GE. Exosomes secreted from bone marrow-derived mesenchymal stem cells protect the intestines from experimental necrotizing enterocolitis. *J Pediatr Surg* 2016;51(6):942-7. doi: 10.1016/j.jpedsurg.2016.02.061
79. Hwang HW, Mendell JT. Micrornas in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2006;94(6):776-80. doi: 10.1038/sj.bjc.6603023
80. Baglio SR, Rooijers K, Koppers-Lalic D, Verweij FJ, Perez Lanzon M, Zini N, et al. Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive mirna and trna species. *Stem Cell Res Ther* 2015;6:127. doi: 10.1186/s13287-015-0116-z
81. Merino-Gonzalez C, Zuniga FA, Escudero C, Ormazabal V, Reyes C, Nova-Lamperti E, et al. Mesenchymal stem cell-derived extracellular vesicles promote angiogenesis: Potencial clinical application. *Front Physiol* 2016;7:24. doi: 10.3389/fphys.2016.00024
82. Nakamura Y, Miyaki S, Ishitobi H, Matsuyama S, Nakasa T, Kamei N, et al. Mesenchymal-stem-cell-derived exosomes accelerate skeletal muscle regeneration. *FEBS Lett* 2015;589(11):1257-65. doi: 10.1016/j.febslet.2015.03.031
83. Lee JK, Park SR, Jung BK, Jeon YK, Lee YS, Kim MK, et al. Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating vegf expression in breast cancer cells. *PLoS One* 2013;8(12):e84256. doi: 10.1371/journal.pone.0084256
84. Zhu W, Huang L, Li Y, Zhang X, Gu J, Yan Y, et al. Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth in vivo. *Cancer Lett* 2012;315(1):28-37. doi: 10.1016/j.canlet.2011.10.002
85. 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(11):1711-5. doi: 10.1038/jcbfm.2013.152
86. Jarmalaviciute A, Pivoriunas A. Exosomes as a potential novel therapeutic tools against neurodegenerative diseases. *Pharmacol Res* 2016. doi: 10.1016/j.phrs.2016.02.002
87. Zhang Y, Chopp M, Liu XS, Katakowski M, Wang X, Tian X, et al. Exosomes derived from mesenchymal stromal cells promote axonal growth of cortical neurons. *Mol Neurobiol* 2016. doi: 10.1007/s12035-016-9851-0
88. Liu M, Wang J, Liu M, Hu X, Xu J. Study of immunomodulatory function of exosomes derived from human umbilical cord mesenchymal stem cells. *Zhonghua Yi Xue Za Zhi* 2015;95(32):2630-3.
89. Keating A. Mesenchymal stromal cells: New directions. *Cell Stem Cell* 2012;10(6):709-16. doi: 10.1016/j.stem.2012.05.015
90. Abumaree M, Al Jumah M, Pace RA, Kalionis B. Immunosuppressive properties of mesenchymal stem cells. *Stem Cell Rev* 2012;8(2):375-92. doi: 10.1007/s12015-011-9312-0
91. Han C, Sun X, Liu L, Jiang H, Shen Y, Xu X, et al. Exosomes and their therapeutic potentials of stem cells. *Stem Cells Int* 2016;2016:7653489. doi: 10.1155/2016/7653489
92. Breitbach M, Bostani T, Roell W, Xia Y, Dewald O, Nygren JM, et al. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood* 2007;110(4):1362-9. doi: 10.1182/blood-2006-12-063412