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Review article:

Study of mesenchymal stem cells derived-exosomes in neurorestorative ability after ischemic stroke

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Abstract
Stroke is one of the leading causes of mortality and disability among adults worldwide. Recovery from stroke often requires highly interactive processes of the neurovascular unit and paracrine factors. The utilization of multipotent mesenchymal stromal cells (MSC) has shown the may promise better stroke treatment outcomes through stem cell therapy. Accumulating evidence suggests that the therapeutic effects of mesenchymal stem cells result from exosomes release – i.e., a membrane vesicle containing various molecular constituents including proteins and RNAs from maternal cells. For Emerging data suggest that exosomes mediate cell-cell communication in the nervous system by transporting protein and RNA cargo from the source cells to the target cells, contributing to neuronal development and maturation. The therapeutic potential of exosomes in treating stroke may result from the active participation of miRNAs in neurovascular remodeling events. Pre-clinical studies on cell-based therapies have confirmed the improvement of functional neural recovery, but such improvements need further confirmation on a clinical level. In this Review, we discuss the exosome effects on neurorestorative ability after stroke.
Keywords: Exosomes, Blood-Brain Barrier, Stroke, Mesenchymal Stem Cell

Introduction

Stroke is a leading cause of mortality and physical disability worldwide among adults. A large number of stroke survivors have significant disabilities [1]. The ischemic insult may cause brain tissue damage by destroying different brain cell types and disrupting the neuronal connections and vascular system functions [2]. The safety and efficacy of cellular-based treatment of ischemic strokes utilizing several types of stem cells, including embryonic stem cells (ESCs), neural stem/precursor cells, mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and induced neurons, have been investigated by some recent studies [3]. This treatment method is safe and improves the recovery of neurological functions [4].It is suggested to exert

its therapeutic effects not only through replacing dead neurons after the differentiation of grafted MSCs but also through promoting endogenous neuronal rewiring and increasing the angiogenesis and neurogenesis in the ischaemic brain by secreting factors that trigger the signaling pathways and amplify brain remodeling, as suggested by pre-clinical studies [5].

The neurovascular unit comprises endothelial cells, vascular smooth muscle, [8].

Exosome are endosome-derived small membrane vesicles –about 30 to 100 nm in diameter, released into extracellular fluids and biofluids (such as blood and cerebrospinal fluid, CSF) by cells in all living systems [9, 10]. Exosomes transfer proteins, lipids, and genetic materials and mediate cellular communication by transferring their cargo between source and target cells under physiological and pathophysiological conditions[8, 11]. These small-membrane vesicles can also regulate the intercellular communication among components of the neurovascular unit following an ischemic stroke. In this Review, we highlight recent insights into the role of exosomes and exosomal microRNAs (miRNAs) in brain repair processes after stroke and discuss potential applications of exosomes for stroke therapy[12].

There is contraindication between the results of cell-therapy studies. Some studies showed that cell-based therapy positively impacts survival, proliferation, differentiation, and restoration of lost neuronal and vascular elements [5, 8, 12, 13]. In contrast, some other studies have shown only a limited neurorestorative ability on the part of transplanted cells [2, 6, 14]. In this Review, we discuss recent advances that demonstrate the importance of exosomes in intercellular signaling in the brain and consider the use of exosomes to treat acute stroke and amplify brain remodeling to improve recovery of neurological function after stroke and TBI. We discuss evidence that shows how unmodified and modified exosomes can affect outcomes of stroke and TBI when used as therapy, and we examine cellular and molecular mechanisms that might underlie the benefits of exosome therapy. We also outline opportunities for and challenges in the translation of exosome-based therapy to clinical applications.

Exosomes Derived from NVUs in Response to Stroke

Pericytes are key contributors to changes in the NVU components following strokes. They can adopt the phenotype of stem cells following a stroke after activation and manifest the markers of neuro-epithelial stem cells. They also have the potential to convert to neurovascular precursor cells [15].

Paracrine signaling and bilateral communicative properties of exosomes help establish the endothelial cell-pericyte interactions, which are essential for preserving the stability of the function of the microvascular system. For example, it has been shown that endothelium and exosomes derived from hypoxic pericytes cause induction of angiogenesis [16, 17].

It has recently been found that perivascular MSCs are indeed endothelial cells that act as precursors of pericytes and other stromal cells during tissue homeostasis. Previous studies have indicated that different subsets of pericytes, and not all, can also act as stem cells; some can even play the role of fibroblasts [18].

Neuronal exosomes are present at the site of synapses, such that they are inside the pre-synaptic and post-synaptic parts as well as neurotransmitter receptors. This localization pattern allows for preserving the neuronal flexibility both locally and systemically (AMPA and GPCRs receptors) [19].

The activity of glutamatergic synapses, which increases in post-stroke conditions, causes the secretion of exosomes from neurons, thereby

affecting the interneuronal relationship [20]. The released exosomes secreted secondary to depolarization of neurons are rich in miRNAs, providing synaptic flexibility through rapid translation of relevant proteins [21].

Regulation of the repair processes after stroke is regulated by reactions between neurons, glial cells, and microvascular cells and also intracellular chemical and physical communication and [22]. Developing a neuro-regenerative treatment is a real challenge [24], given the complexity of these systems, the functional and structural heterogeneity of the brains, and ischemic strokes –regarding their sizes, etiologies, and sites [23].

The Potentials of Using MSCs and Their Exosomes in Stroke Treatment

Ischemic stroke is the leading cause of mortality and long-term disability in industrial counties, where the only available treatment is the use of thrombolysis and vascular recanalization. Due to severe side effects and the short period of therapy, only a few patients receive these treatments. Thus, thinking about a new stroke treatment is essential [23].

Some strategies have been studied about strengthening the survival of neurons in the acute phase of stroke in experimental models, but they did not have successful results in clinical trials [23].

After confirming that the cells transferred to the nervous system have poor cooperation with neural networks, they induce brain restoration through paracrine secretion of nanovesicles. Thus, vesicles were known as crucial factors influencing the regeneration of stem cells and precursor cells in ischemic brain tissue. Neuroprotection, as observed after path of vesicles in experimental models of stroke, result in the participation of stem cells in stroke. Results from paracrine interactions of stem cells, in which vesicles derived from these cells have a significant conducting role [25].

Access to the CNS is denied or limited for therapeutic agents because of the blood-brain barrier (BBB). Thus, many CNS diseases lack effective treatment due to the powerful selective nature of BBB. Therefore, many current research projects focus on developing nano-sized transmitters which deliver the drug to the target tissue and help to exploit the capabilities of stem cells in secreting exosomes. The reason is that exosomes are natural nano-vesicles rich in active bio-molecules and can attach to lipids and cross the BBB and function through their active contents, including proteins, lipids, and genetic contents, or can be used as natural carriers of drugs [26].

The Exosomal Effects of MSCs

The database of exosomes known as ExoCata has reported over 900 protein samples associated with exosomes derived from MSCs. However, recent information has identified 2000 proteins in the exosome of MSCs, most of which have regenerative roles in the brain [27].

Anti-inflammatory and immune-modulating mediators (e.g., TGF-β and IL-10) enable glia for regeneration and NVU restoration [28]. Nevertheless, it has recently been found IL-10 is one of the neuroprotective factors through which the transferred MSCs exert their effects after the stroke. Thus, MSCs with over-secretion of IL-10 can expand the survival of neurons in the ischemic hemisphere [29]. Exosomes derived MSCs present post-stroke changes in their miRNA profile, where miRNAs participate by expressing these genes in the regeneration process, improving brain functioning [30].

It is believed that the effects of paracrine are conducted by soluble molecules, including growth factors, cytokines, chemokines, and hormones. Recent data has suggested that various pathophysiological and physiological processes are controlled by exosomes[31]. In experimental stroke models, some evidence has suggested that strengthening the neuronal

flexibility, protecting the neuronal tissue, and angiogenesis are performed by exosomes[32]. Thus, systemic injection of MSC-derived exosomes in a rat stroke model offered increased restoration of function [32]. Moreover, injection of exosomes derived from adipose tissue and adipose-derived MSCs (ADMSCs) diminished the range of the infarction in the brain and reinforced the neural regeneration in rats after the acute phase of stroke [33]. On a molecular level, beneficial effects of MSC-derived exosomes can be primarily created by miR-17-92. Thus, rats treated by exosomes rich in miR-17-92 functioned considerably better than the control group, which underwent treatment with MSC exosomes[34].

Similarly, injection of exosomes rich in miR-133b results in enhanced neuro-flexibility and improved functional regeneration in rats following stroke [35]. Based on these observations, we compared the therapeutic effects of MSCs and their exosomes in Chinese models in which mainly the striatum and most parts of the underlying cortex had been affected by stroke. It was found that injection of MSC and exosomes derived from it similarly cause improved motor movement under the influence of stroke and restoration of its associated part in the brain. Also, Xin et al. evidenced a considerable decline in defects during four weeks after systemic injection of MSCs-derived vesicles [32] in both neurogenesis and angiogenesis treatment and modulation of immune function as endogen following ischemia [36].

Exosome and functional recovery after stroke

In preclinical studies, significant improvement in neurological function, attenuation in brain swelling, and reduced brain shrinkage have been observed by receiving xenogenic adipose MSC and ADMSC-derived exosome therapy after acute ischemic stroke [33, 10]. Also, MCAO-induced infarct volume decreased due to exosome injection [46, 47].

Similarly, Nalamolu et al. confirmed that infarct size is attenuated after exosome implantation, and neurological function may be preserved. [48]. In another research, BMSCs-derived exosomes containing overexpressed miR-138-5p decreased apoptosis rate in neurons and cerebral infarction [25].

Conclusion

Nano-sized exosomes are capable of establishing intracellular communication and are thus beneficial to the regeneration of NVUs. Concerning the side effects of the current treatments, especially malignant conversion of transferred cells, MSCs-derived exosomes are attractive candidates for treating stroke [26]. Systemic injection of MSCs-derived exosomes can effectively improve motor areas affected by the stroke and maintain the regeneration of their connected areas in the experimental models of stroke; clinical studies on these therapeutic potentialities will be appreciated. Therefore, to begin clinical studies, we need further studies focusing on 1) the mechanisms of reactions between exosomes and target cells, 2) kinetic and bio-distribution in the blood circulation system, 3) biogenesis mechanisms and 4) potential side effects. For example, the exosomes secreted by tumors may be known as metastasis mediators in cancer through preserving and improving the tumor in the environment [37-39].

Further, various studies have reported concerns over high levels of Cholesteryl ester(CE), Triacylglycerol (TAG), and cardiolipin in the products of exosomes, increasing the risk of stroke [40-42]. Eventually, as stroke usually occurs in the elderly, the experimental models in old mice before initiation of clinical studies in patients with stroke are highly required and are recommended to be examined [43-45].

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References

1. Chen J, Chopp M. Exosome therapy for stroke. Stroke. 2018;49(5):1083-90.
2. Chen Y, Song Y, Huang J, Qu M, Zhang Y, Geng J, et al. Increased circulating exosomal miRNA-223 is associated with acute ischemic stroke. Frontiers in neurology. 2017;8:57.
3. Doeppner TR, Herz J, Görgens A, Schlechter J, Ludwig A-K, Radtke S, et al. Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. Stem cells translational medicine. 2015;4(10):1131-43.
4. Hong S-B, Yang H, Manaenko A, Lu J, Mei Q, Hu Q. Potential of Exosomes for the Treatment of Stroke. Cell transplantation. 2018:0963689718816990.
5. Otero-Ortega L, Laso-García F, Gómez-de Frutos M, Fuentes B, Diekhorst L, Díez-Tejedor E, et al. Role of exosomes as a treatment and potential biomarker for stroke. Translational stroke research. 2018:1-9.
6. Shen Y, Venkat P, Chopp M, Chen J. Mesenchymal Stromal Cell Therapy of Stroke. Cellular and Molecular Approaches to Regeneration and Repair: Springer; 2018. p. 217-37.
7. Venkat P, Chen J, Chopp M. Exosome-mediated amplification of endogenous brain repair mechanisms and brain and systemic organ interaction in modulating neurological outcome after stroke. Journal of Cerebral Blood Flow & Metabolism. 2018;38(12):2165-78.
8. Venkat P, Chopp M, Chen J. Cell-Based and Exosome Therapy in Diabetic Stroke. Stem cells translational medicine. 2018;7(6):451-5.
9. Wang J, Wang M, Xu J, Wang Y-g, Liu M, Ashraf M, et al. Exosomal Treatment of Stroke Injury. Circulation. 2018;138(Suppl_1):A15905-A.
10. Xin H, Katakowski M, Wang F, Qian J-Y, Liu XS, Ali MM, et al. MicroRNA-17–92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. Stroke. 2017;48(3):747-53.
11. Xin H, Wang F, Li Y, Lu Q-e, Cheung WL, Zhang Y, et al. Secondary release of exosomes from astrocytes contributes to the increase in neural plasticity and improvement of functional recovery after stroke in rats treated with exosomes harvested from microRNA 133b-overexpressing multipotent mesenchymal stromal cells. Cell transplantation. 2017;26(2):243-57.
12. Xiong Y, Mahmood A, Chopp M. Emerging potential of exosomes for treatment of traumatic brain injury. Neural regeneration research. 2017;12(1):19.
13. Zhang ZG, Buller B, Chopp M. Exosomes—beyond stem cells for restorative therapy in stroke and neurological injury. Nature Reviews Neurology. 2019:1.
14. Zhang ZG, Chopp M. Exosomes in stroke pathogenesis and therapy. The Journal of clinical investigation. 2016;126(4):1190-7.
15. Nakagomi T, Kubo S, Nakano-Doi A, Sakuma R, Lu S, Narita A, et al. Brain vascular pericytes following ischemia have multipotential stem cell activity to differentiate into neural and vascular lineage

cells. Stem cells (Dayton, Ohio). 2015;33(6):1962-74.

16. Fan GC. Hypoxic exosomes promote angiogenesis. Blood. 2014;124(25):3669-70.
17. Mayo JN, Bearden SE. Driving the Hypoxia-Inducible Pathway in Human Pericytes Promotes Vascular Density in an Exosome-Dependent Manner. Microcirculation (New York, NY : 1994). 2015;22(8):711-23.
18. de Souza LE, Malta TM, Kashima Haddad S, Covas DT. Mesenchymal Stem Cells and Pericytes: To What Extent Are They Related? Stem cells and development. 2016;25(24):1843-52.
19. Koniusz S, Andrzejewska A, Muraca M, Srivastava AK, Janowski M, Lukomska B. Extracellular Vesicles in Physiology, Pathology, and Therapy of the Immune and Central Nervous System, with Focus on Extracellular Vesicles Derived from Mesenchymal Stem Cells as Therapeutic Tools. Frontiers in cellular neuroscience. 2016;10:109.
20. Chivet M, Javalet C, Laulagnier K, Blot B, Hemming FJ, Sadoul R. Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. Journal of extracellular vesicles. 2014;3:24722.
21. Goldie BJ, Dun MD, Lin M, Smith ND, Verrills NM, Dayas CV, et al. Activity-associated miRNA are packaged in Map1b-enriched exosomes released from depolarized neurons. Nucleic acids research. 2014;42(14):9195-208.
22. Rosell A, Lo EH. Multiphasic roles for matrix metalloproteinases after stroke. Current opinion in pharmacology. 2008;8(1):82-9.
23. Hermann DM, ElAli A. The abluminal endothelial membrane in neurovascular remodeling in health and disease. Science signaling. 2012;5(236):re4.
24. Hermann DM, Buga AM, Popa-Wagner A. Neurovascular remodeling in the aged ischemic brain. Journal of neural transmission (Vienna, Austria : 1996). 2015;122 Suppl 1:S25-33.
25. Doeppner TR, Bahr M, Giebel B, Hermann DM. Immunological and non-immunological effects of stem cell-derived extracellular vesicles on the ischaemic brain. Therapeutic advances in neurological disorders. 2018;11:1756286418789326.
26. Lener T, Gimona M, Aigner L, Borger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. Journal of extracellular vesicles. 2015;4:30087.
27. Otero-Ortega L, Gomez de Frutos MC, Laso-Garcia F, Rodriguez-Frutos B, Medina-Gutierrez E, Lopez JA, et al. Exosomes promote restoration after an experimental animal model of intracerebral hemorrhage. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2018;38(5):767-79.
28. Burrello J, Monticone S, Gai C, Gomez Y, Kholia S, Camussi G. Stem Cell-Derived Extracellular Vesicles and Immune-Modulation. Frontiers in cell and developmental biology. 2016;4:83.
29. Nakajima M, Nito C, Sowa K, Suda S, Nishiyama Y, Nakamura-Takahashi A, et al. Mesenchymal Stem Cells Overexpressing Interleukin-10 Promote Neuroprotection in Experimental Acute Ischemic Stroke. Molecular therapy Methods & clinical development. 2017;6:102-11.
30. Liu FJ, Lim KY, Kaur P, Sepramaniam S, Armugam A, Wong PT, et al. microRNAs

Involved in Regulating Spontaneous Recovery in Embolic Stroke Model. PloS one. 2013;8(6):e66393.

31. Cramer SC, Wolf SL, Adams HP, Jr., Chen D, Dromerick AW, Dunning K, et al. Stroke Recovery and Rehabilitation Research: Issues, Opportunities, and the National Institutes of Health StrokeNet. Stroke. 2017;48(3):813-9.
32. 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. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2013;33(11):1711-5.
33. Chen KH, Chen CH, Wallace CG, Yuen CM, Kao GS, Chen YL, et al. Intravenous administration of xenogenic adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes markedly reduced brain infarct volume and preserved neurological function in rat after acute ischemic stroke. Oncotarget. 2016;7(46):74537-56.
34. Xin H, Katakowski M, Wang F, Qian JY, Liu XS, Ali MM, et al. MicroRNA cluster miR-17-92 Cluster in Exosomes Enhance Neuroplasticity and Functional Recovery After Stroke in Rats. Stroke. 2017;48(3):747-53.
35. Xin H, Wang F, Li Y, Lu QE, Cheung WL, Zhang Y, et al. Secondary Release of Exosomes From Astrocytes Contributes to the Increase in Neural Plasticity and Improvement of Functional Recovery After Stroke in Rats Treated With Exosomes Harvested From MicroRNA 133b-Overexpressing Multipotent Mesenchymal Stromal Cells. Cell transplantation. 2017;26(2):243-57. Doeppner TR, Herz J, Gorgens A, Schlechter J, Ludwig AK, Radtke S, et al. Extracellular Vesicles Improve Post-Stroke Neuroregeneration and Prevent Postischemic Immunosuppression. Stem cells translational medicine. 2015;4(10):1131-43.
37. Chang M, Lee CW, Ahn JM, Cavalcante R, Sotomi Y, Onuma Y, et al. Comparison of Outcome of Coronary Artery Bypass Grafting Versus Drug-Eluting Stent Implantation for Non-ST-Elevation Acute Coronary Syndrome. The American journal of cardiology. 2017;120(3):380-6.
38. Li Y, Cheng Q, Hu G, Deng T, Wang Q, Zhou J, et al. Extracellular vesicles in mesenchymal stromal cells: A novel therapeutic strategy for stroke. Experimental and therapeutic medicine. 2018;15(5):4067-79.
39. Salido-Guadarrama I, Romero-Cordoba S, Peralta-Zaragoza O, Hidalgo-Miranda A, Rodriguez-Dorantes M. MicroRNAs transported by exosomes in body fluids as mediators of intercellular communication in cancer. OncoTargets and therapy. 2014;7:1327-38.
40. Strauss K, Goebel C, Runz H, Mobius W, Weiss S, Feussner I, et al. Exosome secretion ameliorates lysosomal storage of cholesterol in Niemann-Pick type C disease. The Journal of biological chemistry. 2010;285(34):26279-88.
41. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nature reviews Molecular cell biology. 2008;9(2):112-24.
42. Llorente A, van Deurs B, Sandvig K. Cholesterol regulates prostasome release from secretory lysosomes in PC-3 human prostate cancer cells. European journal of cell biology. 2007;86(7):405-15.

43. Popa-Wagner A, Glavan DG, Olaru A, Olaru DG, Margaritescu O, Tica O, et al. Present Status and Future Challenges of New Therapeutic Targets in Preclinical Models of Stroke in Aged Animals with/without Comorbidities. International journal of molecular sciences. 2018;19(2).
44. Balseanu AT, Buga AM, Catalin B, Wagner DC, Boltze J, Zagrean AM, et al. Multimodal Approaches for Regenerative Stroke Therapies: Combination of Granulocyte Colony-Stimulating Factor with Bone Marrow Mesenchymal Stem Cells is Not Superior to G-CSF Alone. Frontiers in aging neuroscience. 2014;6:130.
45. Popa-Wagner A, Dinca I, Yalikun S, Walker L, Kroemer H, Kessler C. Accelerated delimitation of the infarct zone by capillary-derived nestin-positive cells in aged rats. Current neurovascular research. 2006;3(1):3-13.
46. Gutierrez-Fernandez M, Laso-Garcia F, Gomez-de Frutos MC, Otero-Ortega L, Lopez EA, Arroyo AM, Diekhorst L, Gonzalo MA, Fuentes B, Tejedor ED (2018) Exosomes Role in Brain Repair and Recovery in Stroke. A Dose Response Study. Stroke 49
47. Jiang M, Wang H, Jin M, Yang X, Ji H, Jiang Y, Zhang H, Wu F, Wu G, Lai X, Cai L, Hu R, Xu L, Li L (2018) Exosomes from MiR-30d-5p-ADSCs Reverse Acute Ischemic Stroke-Induced, Autophagy-Mediated Brain Injury by Promoting M2 Microglial/Macrophage Polarization. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 47 (2):864-878.
48. Nalamolu KR, Venkatesh I, Mohandass A, Klopfenstein JD, Pinson DM, Wang DZ, Veeravalli KK (2019) Exosomes Treatment Mitigates Ischemic Brain Damage but Does Not Improve Post-Stroke Neurological Outcome. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 52 (6):1280-1291. doi:10.33594/000000090

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