Chapter 16
Extra-Cellular Vesicles: A Promising Approach for Translating Cell-Based Therapy

Benjamin Buller, Michael Chopp, and Zheng Gang Zhang

Abstract As cell-based therapies have demonstrated efficacy in the treatment of experimental and clinical stroke, their mechanisms of action warrant intense investigation and are being investigated in greater depth. It is becoming increasingly clear that one of the main ways that cell therapies based on mesenchymal stem cells (MSC) and other cells impart functional benefits to animals is through release of exosomes and other extracellular vesicles *in vivo*. Mounting evidence shows that MSCs release exosomes, and that these exosomes induce predictable and impactful changes in recipient cells. These exosome-induced cellular changes are likely mediated through the content of the exosomes, which comprise mRNA, miRNA, proteins, and other macromolecules. Many studies that have been published in the last several years have shown that treatment of animals with exosomes, harvested from MSCs and other cells, after stroke and traumatic brain injury (TBI) recapitulate the effect of the parent cells. Exosomes lack the safety and manufacturability issues that plague cell therapy, and they therefore may represent the next generation of cell-free therapies. Their biology and potential use as therapies for CNS injuries are discussed herein.

Keywords MSC • Stroke • TBI • Exosome • Microvesicle • Extracellular vesicle • Neuroresoration

Abbreviations

Ago2	Argonaute-2
Ang1	Angiopoietin-1
BBB	Blood-brain barrier

B. Buller • Z.G. Zhang (✉)
Department of Neurology, Henry Ford Health System, Detroit, MI, USA
e-mail: zhazh@neuro.hfh.edu

M. Chopp
Department of Neurology, Henry Ford Health System, Detroit, MI, USA

Department of Physics, Oakland University, Rochester, MI, USA

© Springer International Publishing AG 2018

P.A. Lapchak, J.H. Zhang (eds.), *Cellular and Molecular Approaches to Regeneration and Repair*, Springer Series in Translational Stroke Research,
https://doi.org/10.1007/978-3-319-66679-2_16

CSF	Cerebral-spinal fluid
dll4	Delta-like 4
EV	Extracellular vesicle
lncRNA	Long non-coding RNA
MCAo	Middle cerebral artery occlusion
miRNA	MicroRNA
MSC	Mesenchymal stem/stromal cell
MVB	Multivesicular body
RISC	RNA-induced silencing complex
TBI	Traumatic brain injury

1 Introduction

Cell-based therapy may be the most promising approach to achieving a clinically viable restorative therapy for stroke and other neurologic diseases. In a series of preclinical studies first published by our laboratory beginning in 2000, we showed that administration of bone marrow mesenchymal stromal cells (MSC), leads to enhanced neurological recovery in rats that are subjected to middle cerebral artery occlusion (MCAo) [1–4]. This work has been repeated and reproduced by many laboratories around the world [5–8], and other cell types have also been shown to be effective in aiding recovery from stroke [9–12]. Despite the acceptance of cell therapy as an effective treatment for animal models of stroke, the mechanisms by which it imparts functional recovery have remained elusive.

It was first hypothesized that MSC transdifferentiate into neural cells and thereby regrow brain tissue. This hypothesis was abandoned relatively quickly in favor of a paracrine hypothesis—that the cells secrete factors that stimulate growth [13]. This hypothesis too evoked skepticism, because treating animals with any number of secreted growth factors from cells has never reproduced the effect of treating with cells directly.

Extracellular vesicles (EV), including exosomes and microvesicles, are small, membrane bound spheroids of approximately 30–200 nm in diameter. They contain macromolecular cargo that includes receptors, ligands, and nucleic acids. The nucleic acid cargo of EVs comprises a mixture of mRNA, tRNA, vault RNA, microRNAs (miRNA), and long non-coding RNA (lncRNA). While the biologic functions of the majority of the contents of EVs remain unclear, their miRNA contents have been shown to be functional *in vivo*, which is particularly important given that many roles have been described for miRNAs in both the pathogenesis of, and recovery from, stroke (for review of the miRNA and stroke, see e.g. [14]). The role of exosomes and other EVs in mediating cell therapy repair, with particular attention paid to MSCs, and the putative mechanism by which they operate are discussed below.

2 EV Biogenesis Pathways

EVs comprise several subtypes, and they are secreted by virtually all cells [15]. They exist in all body fluids, including blood, cerebrospinal fluid (CSF), urine, ascites, and saliva [16–20]. The two most well studied types of EVs are exosomes and microvesicles. Each of these two types has unique attributes and a distinctive biogenesis pathway. Exosomes are spheroids that have an approximate size of 30–100 nm [21] that are generated by the endosomal pathway [22]. In this pathway, the cell membrane invaginates to form an endosome, and then successive invaginations of the endosome create a multivesicular body (MVB). Fusion of the MVB with the cell membrane releases exosomes to the extracellular space where they may dock locally or distally with other cells, or perhaps be taken up by endocytosis or macropinocytosis [23]. By contrast, microvesicles are thought to bud directly from the membrane; they have more amorphous shapes than exosomes, and they have a much larger average diameter, perhaps any size up to 2000 nm [24]. Figure 16.1 describes these separate biogenesis pathways.

Exosomes and microvesicles can be distinguished by their surface markers. Exosomes, uniform lipid bilayer spheroids, are generally marked by tetraspanin proteins including CD63, CD81, and CD9, as well as flotillin and Alix [25, 26]. Microvesicles, in contrast, generally lack tetraspanins and are of varying size, shape, and density [27]. Separating exosomes from microvesicles is difficult practically, as

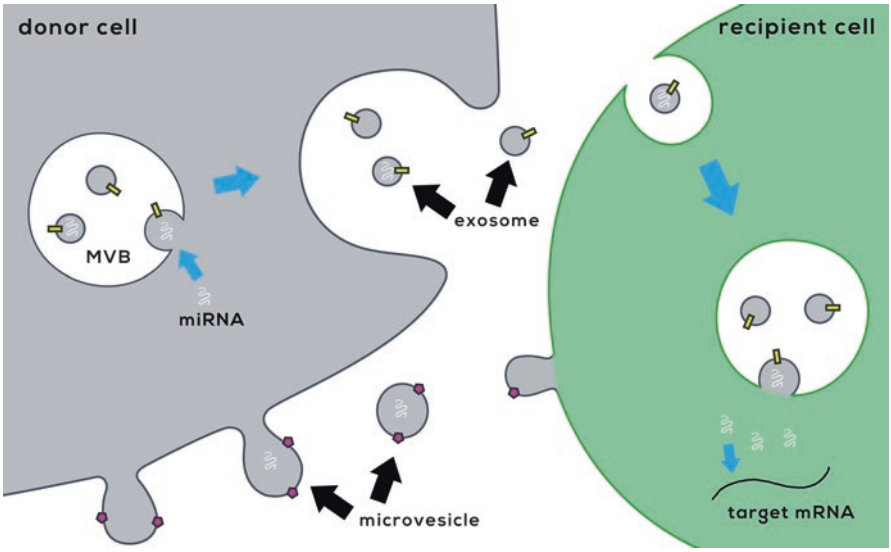

Fig. 16.1 Extracellular vesicles are shed by two primary mechanisms. In the endosomal pathway, exosomes are created by successive invaginations of the plasma membrane to create an MVB. The MVB then fuses with the plasma membrane to release the exosomes into the extracellular space. Microvesicles are created by direct budding of the membrane. EVs can be taken up by the recipient cell by direct membrane fusion or by endocytosis/macropinocytosis

their densities and sizes have a significant amount of overlap. However, separation of subpopulations of EVs may be more important from a technical scientific standpoint than a medical one. It is unclear which EV subtype, if any, contributes more to their therapeutic effects. Some studies have implicated exosomes [28], while others have implicated microvesicles [29] as the more important vesicle type in mediating the effects of parent cells. This debate may not be settled soon; however, exosomes are generally thought to be the more biologically relevant EV subtype, and the majority of the literature therefore focuses on exosomes, although it should be noted that much of the early work in EVs was clouded by a lack of consistent nomenclature to distinguish between the various subtypes of EVs.

3 Potential Therapeutic Applications

EVs have been shown to be therapeutic for many of the same neurologic diseases and injuries as have been demonstrated for their parent cells, including traumatic brain injuries and stroke [30, 31], and they are the only cell product that has been shown to recapitulate the therapeutic effect of the parent cells. EVs have only begun to be deployed clinically, so it is not entirely clear what their eventual impact may be; however, we can speculate that their adoption will be swift should they be shown to be as efficacious as parent cells in treatment of injury and disease. They have several inherent advantages over cells that make them ideal replacements for or adjuvants to cell-based therapy.

Foremost and most obvious, EVs do not divide. One of the biggest safety concerns with cell therapy is the risk of teratoma formation. Although rare, teratoma formation or other uncontrolled cell division is a real concern. Cultured cells often are observed to have genomic aberrations, and the risk of tumor transformation has tempered enthusiasm for their use, especially from regulatory agencies. Therefore, completely mitigating the risks posed by dividing cells can only be counted as a positive development. EVs are not cells, so their immediate effects are transient, and they cannot form tumors.

Second, exosomes appear to not cause microvascular embolization, nor do they induce formation of thrombi. MSC and other cells can lead to vascular occlusions in some circumstances, which can cause significant complications to the patient. By contrast, exosomes, perhaps owing to their small size, have never been reported to cause thrombosis or otherwise occlude vessels. This fact may make swifter clinical adoption more likely by further reducing risk.

Third, manufacture and delivery of EVs may prove to be simpler and produce a more reliable supply chain than cells. Cell therapy requires that cells be grown for each patient, and that they then be delivered intact and sterilely at the point of care. This requires the infrastructure to thaw and formulate the cells on site, or else a manufacturing facility in very close proximity. By contrast, EVs are stable at 4 °C for relatively long periods of time, with little detectable difference in the cargo of

exosomes that were collected fresh or stored for several weeks [32]. This remarkable property makes central manufacturing and formulation much nearer to reality than could ever be possible with parent cells. For example, EVs could potentially be loaded into premade IV bags that could be stored on site at hospitals that serve stroke and TBI patients. Because of their relative stability, the product could be on site for immediate use, with a time buffer of potentially many weeks. Pre-formulation of a hypothetical exosome product obviates the need for experienced technicians to prepare treatments on site on a patient-by-patient basis, and allows for central quality control in a way that cell therapy does not.

The most pressing barrier to quick clinical adoption of EVs for treatment of stroke is their relatively short history compared to cell therapy. Some of the earliest investigations of EVs for treatment of any neurologic disease were published by our lab in 2013 [30]. These first reports demonstrated that MSC derived EVs could impart therapeutic benefits to rats after stroke when delivered at 24 h after MCAo, and that the functional recovery of these animals is caused by enhanced white matter remodeling, including new axon growth and myelination, as well as angiogenesis. MSCs have long been known to cause remodeling of neurites and angiogenesis [33–36], further evidence that MSCs enhance functional outcomes via release of exosomes and other EVs. This finding that MSC exosomes promote recovery after stroke has been reproduced and verified by several independent labs in rats and in mice [37, 38]. Furthermore, using human cells to generate exosomes does not impact their ability to enhance neurologic recovery in rats subjected to TBI [39]. The above renders it likely that exosome-induced functional recovery after neurologic injury is generalizable across multiple species, and thus also likely applicable to human disease.

To date, the therapeutic potential of EVs derived from MSCs has been investigated most extensively preclinically. MSCs are a robust source of exosomes and other EVs, producing an abundance of them compared to other cell types [40]. However, the majority of cell types produce exosomes and microvesicles, and several of these cell types have been explored as potential sources of therapeutic EVs. For example, exosomal miR-126 is pro-angiogenic [41], and may underlie human cord blood cell mediated recovery from stroke in diabetic animals [42]; endothelial cell derived exosomes have been used to treat hindlimb ischemia [43]; and dendritic and other immune cell derived exosomes are being explored extensively as a therapy for cancer [44–48].

Most of the clinical work focused on exosomes has been dedicated to their potential as biomarkers (for review see e.g., [49, 50]). Despite their short history as a therapeutic agent, exosomes have begun to appear in clinical trials. Table 16.1 is a list of all current registered trials on clinicaltrials.gov for which ‘exosome’ is a keyword and that are targeting therapy and not biomarkers. The range of diseases is diverse, and only one so far uses MSC as a source. However, this is likely to change rapidly in the coming years.

Table 16.1 List of trials using exosomes as a therapeutic

Identifier	Institution	Disease	Source	Phase
NCT02565264	Kumamoto University	Cutaneous wound healing/ulcers	Plasma	I
NCT02138331	General Committee of Teaching Hospitals and Institutes, Egypt	Type I diabetes mellitus	MSC	II/III
NCT01159288	Gustave Roussy, Cancer Campus, Grand Paris	Non-small cell lung cancer	Dendritic cells	II
NCT01668849	James Graham Brown Cancer Center, University of Louisville	Chemoradiation-induced oral mucositis	Grape	I
NCT01294072	James Graham Brown Cancer Center, University of Louisville	Colon cancer	Curcumin-loaded plant	I

3.1 Mechanism

It is apparent that among the most important cargo that exosomes carry are miRNAs. miRNAs are often highly conserved across disparate organisms, and although they are frequently gained during evolution, they are rarely lost [51]. The number of miRNA that a species possesses correlates well with morphologic complexity [52], and any given miRNA may target many genes in a single gene network, thereby possessing the ability to efficiently shut down redundant systems [53]. More than 700 miRNAs can be detected in exosomes and other EVs [54], and they are mostly bound to Argonaute 2 (Ago2) [55, 56], a major constituent of the RNA-induced silencing complex (RISC). Silencing of targets by miRNA is RISC-dependent, so the fact that miRNA in exosomes are bound to Ago2 suggests that they are destined to bind to mRNAs in recipient cells (i.e. be functional). All this points to exosomes being a potent system to pass "information" from cell to cell in a manner that other macromolecules cannot.

It has been shown that miRNA expressed in one cell can suppress protein expression in another cell through innate mechanisms [57]. Although more than one pathway for targeted inhibition of translation from one cell to another may exist, exosomes represent a major mechanism by which this information transfer occurs. It has been shown in many studies across multiple independent labs that specific proteins can be suppressed in cells in a predictable way when the cells are incubated with exosomes containing targeting miRNA [57–60]. Therefore, the likeliest way that exosomes function is to release their miRNA contents into target cells upon being internalized, thereby affecting gene networks in the recipient cells. This hypothesis is supported by a number of studies in which the miRNA cargo of exosomes was altered to target specific genes. Xin et al. showed that over-expressing miR-133b in MSC exosomes enhances functional recovery after MCAo to an even greater extent that naïve exosomes [61, 62]. Additionally, miR-17-92 cluster

expression can target neurons and promote axonal growth via suppression of PTEN [63], and exosomes enriched in miR-17~92 constituents promote functional recovery and axonal growth more efficiently than naïve exosomes [64, 65]. It is likely that in the future, better methods of expression and more predictive targeting algorithms will allow for even more refined tuning of the therapeutic properties of exosomes.

3.2 Neurovascular Niche

Recovery from stroke is dependent on remodeling of the neurovascular niche [66, 67]. Exosomes have been shown to affect multiple aspects of the neurovascular unit during recovery from stroke and brain injury, and in *in vitro* injury models. For example, when exposed to MSC exosomes, astrocytes are stimulated to release exosomes of their own, which in turn induce downstream remodeling of axons [68]. Indeed, exosome treatment after stroke is associated with improved axonal growth and myelination [30, 64, 68]. In an apparent feedback loop, neuronal exosomes also contain biomolecules that target astrocytes, including PTEN and miR-124, which limit astrocyte proliferation and increase expression of the amino acid transporter GLT-1, respectively [69]. Furthermore, oligodendrocytes secrete exosomes that impact neuronal behavior, helping to coordinate myelination [70, 71] and supplying protective molecules in stress conditions [72].

As the name suggests, the other half of the neurovascular unit comprises cerebral blood vessels, whose function after stroke is coupled to recovery of brain parenchyma, and exosomes from MSCs promote angiogenesis [30, 37, 73]. Endothelial cells communicate with each other via exosome secretion. For example, endothelial cell exosomes contain miR-214 and miR-126, both of which are pro-angiogenic miRNAs, and they also contain angiopoietin-1 (Ang1), the primary ligand of Tie2 receptor and a potent inducer of angiogenesis [43, 74]. Endothelial exosomes also contain delta-like 4 (dll4), a notch ligand that maintains endothelial stasis [75]. The exosome system may represent a way for a large and distributed tissue such as the endothelium to maintain homeostasis over a large surface area and long distances. Treatment of hindlimb ischemia with endothelial derived exosomes significantly improves recovery of function and angiogenic sprouting [76], suggesting that supporting the natural cell communication system in the endothelium could be a strategy for treating cardiovascular disease. Dysfunction of the natural endothelial cell exosome axis may lead to pathologic conditions that are prevalent in stroke and other cardiovascular disease, such as atherosclerosis. Endothelial cells from sclerotic vessels secrete exosomes with cargo that is distinct from healthy cells [77], which may trigger damage and recruitment of inflammatory cells.

The other relevant question in cell therapy with respect to the cerebral endothelium is whether MSC exosomes can cross the blood-brain barrier (BBB). Several lines of evidence suggest that they can. First, brain tumor exosomes can readily be detected in blood, which suggests crossage of the BBB [78]. Second, *in vitro* evidence shows that endothelial cells can actively transport exosomes across the BBB [79]. Although circumstantial, these reports provide clear evidence of instances in which exosomes can in

fact cross the BBB intact. Due to their heterogeneous nature as aggregates of biomolecules that can be disaggregated *in vivo*, directly observing exosomes *in vivo* after injection is difficult, but it seems likely that therapeutic exosomes can enter the brain [80].

3.3 Inflammatory System

The inflammatory system, both in the brain and in the periphery, may mediate cell therapy after stroke [81]. When introduced IV, exosomes encounter macrophages and other immune cells of the periphery almost immediately, and macrophage depleted animals clear exosomes much slower than wild-type animals [82]. The half-life of injected exosomes in wild type rats may be as little as 2 min, with total clearance happening by 4 h [83]. The exact role of the peripheral immune system in mediating cell therapy has not been fully described, but some evidence suggests that its presence is necessary for enhancing recovery [84, 85]. Additionally, the majority of injected exosomes lodge in the peripheral organs, including the lungs, liver, and spleen [82, 83, 86], although these studies do not agree as to which organ is the primary point of exosome uptake, which may be cell source dependent. An open question for scientists who are developing exosome therapies is whether the interaction of exosomes with peripheral organs contributes to or inhibits their effectiveness as therapeutic agents. It may be that one of the ways in which exosomes from MSCs and other cells impart functional benefits is by "reprogramming" the immune system to behave in a way that supports recovery. Secreted vesicles from MSCs suppress secretion of pro-inflammatory cytokines from stimulated microglia *in vitro* [87]. In turn, secreted inflammatory factors from microglia, such as IL-1α and TNFα, stimulate astrocytes to suppress synapse formation [88], which may have serious deleterious effects on recovery. Indeed, microglia help coordinate tissue remodeling after injury, and can encourage oligodendrocyte differentiation and myelination during recovery [89–91]. Conversely, neuronal secreted exosomes can recruit microglia to prune synapses [92], which may be an innate mechanism for normal function, but also could potentially contribute to dysfunction in degenerative disease states, as aberrant synapse pruning is a hallmark of early Alzheimer's disease and other forms of dementia, for example [93, 94]. Therefore, the potential of MSC exosomes to reprogram microglia to adopt a pro-recovery phenotype is perhaps one of their greatest assets.

4 Summary and Conclusion

The use of cell therapies for recovery from stroke has gained prominence and traction in recent years due to their effectiveness in treating animal models of brain injury. Their use in clinical trials, of which hundreds now are registered, is therefore warranted, as no other regenerative or restorative treatment is available to patients.

However, exosomes are only now beginning to be investigated as a potential next generation replacement for or adjuvant to cell therapy, but awareness of them is rising quickly. Several clinical trials have been registered to investigate the use of exosomes for diseases such as cancer, wound healing, and diabetes. Should they prove to be safe and effective, exosomes will become one of regenerative medicine's best hopes for treating patients with stroke and other debilitating CNS diseases. In animal models of stroke, TBI, cognitive decline, and other CNS diseases, they have been shown to have a great impact at lessening the disease severity and quickening and deepening recovery.

The therapeutic impact of exosomes is multifactorial, but is certainly dependent on three identifiable factors: (a) the surface proteins that determine the targeted cell type; (b) the miRNA cargo that determines their function in target cells; and (c) secondary release of exosomes and paracrine factors from target cells. Deeper understanding of each of these factors will doubtlessly affect our ability to design custom treatments for stroke and other CNS diseases that are currently untreatable. Exosomes represent a unique opportunity to advance cell therapy to a place of safety, efficacy, and manufacturability that currently does not exist.

References

1. Li Y, Chopp M, Chen J, Wang L, Gautam SC, Xu YX, et al. Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. J Cereb Blood Flow Metab. 2000;20(9):1311–9.
2. Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M. Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J Neurol Sci. 2001;189(1-2):49–57.
3. Li Y, Chen J, Wang L, Lu M, Chopp M. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology. 2001;56(12):1666–72.
4. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, et al. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res. 2003;73(6):778–86.
5. Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Exp Neurol. 2002;174(1):11–20.
6. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, et al. BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. Mol Ther. 2004;9(2):189–97.
7. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, et al. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. Mol Ther. 2005;11(1):96–104.
8. Hanabusa K, Nagaya N, Iwase T, Itoh T, Murakami S, Shimizu Y, et al. Adrenomedullin enhances therapeutic potency of mesenchymal stem cells after experimental stroke in rats. Stroke. 2005;36(4):853–8.
9. Liu YP, Seckin H, Izci Y, Du ZW, Yan YP, Baskaya MK. Neuroprotective effects of mesenchymal stem cells derived from human embryonic stem cells in transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab. 2009;29(4):780–91.
10. Lin YC, Ko TL, Shih YH, Lin MY, Fu TW, Hsiao HS, et al. Human umbilical mesenchymal stem cells promote recovery after ischemic stroke. Stroke. 2011;42(7):2045–53.

11. Mohamad O, Drury-Stewart D, Song M, Faulkner B, Chen D, Yu SP, et al. Vector-free and transgene-free human iPS cells differentiate into functional neurons and enhance functional recovery after ischemic stroke in mice. PLoS One. 2013;8(5):e64160.
12. Yuan T, Liao W, Feng NH, Lou YL, Niu X, Zhang AJ, et al. Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion. Stem Cell Res Ther. 2013;4(3):73.
13. Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. Lancet Neurol. 2002;1(2):92–100.
14. Bhalala OG. The emerging impact of microRNAs in neurotrauma pathophysiology and therapy. In: Kobeissy FH, editor. Brain neurotrauma: molecular, neuropsychological, and rehabilitation aspects. Boca Raton, FL: Frontiers in Neuroengineering; 2015.
15. Maas SL, Breakefield XO, Weaver AM. Extracellular vesicles: unique intercellular delivery vehicles. Trends Cell Biol. 2017;27(3):172–88.
16. Zocco D, Ferruzzi P, Cappello F, Kuo WP, Fais S. Extracellular vesicles as shuttles of tumor biomarkers and anti-tumor drugs. Front Oncol. 2014;4:267.
17. Cheow ES, Cheng WC, Lee CN, de Kleijn D, Sorokin V, Sze SK. Plasma-derived extracellular vesicles contain predictive biomarkers and potential therapeutic targets for myocardial ischemic (MI) injury. Mol Cell Proteomics. 2016;15(8):2628–40.
18. Foster BP, Balassa T, Benen TD, Dominovic M, Elmadjian GK, Florova V, et al. Extracellular vesicles in blood, milk and body fluids of the female and male urogenital tract and with special regard to reproduction. Crit Rev Clin Lab Sci. 2016;53(6):379–95.
19. Keller S, Ridinger J, Rupp AK, Janssen JW, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. J Transl Med. 2011;9:86.
20. Choi DS, Park JO, Jang SC, Yoon YJ, Jung JW, Choi DY, et al. Proteomic analysis of microvesicles derived from human colorectal cancer ascites. Proteomics. 2011;11(13):2745–51.
21. Rak J. Extracellular vesicles - biomarkers and effectors of the cellular interactome in cancer. Front Pharmacol. 2013;4:21.
22. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008;319(5867):1244–7.
23. Tian T, Zhu YL, Zhou YY, Liang GF, Wang YY, Hu FH, et al. Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21 delivery. J Biol Chem. 2014;289(32):22258–67.
24. Vader P, Breakefield XO, Wood MJ. Extracellular vesicles: emerging targets for cancer therapy. Trends Mol Med. 2014;20(7):385–93.
25. Park JE, Tan HS, Datta A, Lai RC, Zhang H, Meng W, et al. Hypoxic tumor cell modulates its microenvironment to enhance angiogenic and metastatic potential by secretion of proteins and exosomes. Mol Cell Proteomics. 2010;9(6):1085–99.
26. Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci U S A. 2016;113(8):E968–77.
27. Mrvar-Brecko A, Sustar V, Jansa V, Stukelj R, Jansa R, Mujagic E, et al. Isolated microvesicles from peripheral blood and body fluids as observed by scanning electron microscope. Blood Cells Mol Dis. 2010;44(4):307–12.
28. Bruno S, Tapparo M, Collino F, Chiabotto G, Deregibus MC, Soares Lindoso R, et al. Renal regenerative potential of different extra-cellular vesicle populations derived from bone marrow mesenchymal stromal cells. Tissue Eng Part A. 2017;PMID:28471327.
29. Akyurekli C, Le Y, Richardson RB, Fergusson D, Tay J, Allan DS. A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles. Stem Cell Rev. 2015;11(1):150–60.
30. 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.

31. Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A, et al. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. J Neurosurg. 2015;122(4):856–67.
32. Ge Q, Zhou Y, Lu J, Bai Y, Xie X, Lu Z. miRNA in plasma exosome is stable under different storage conditions. Molecules. 2014;19(2):1568–75.
33. Zacharek A, Chen J, Cui X, Li A, Li Y, Roberts C, et al. Angiopoietin1/Tie2 and VEGF/Flk1 induced by MSC treatment amplifies angiogenesis and vascular stabilization after stroke. J Cereb Blood Flow Metab. 2007;27(10):1684–91.
34. Xin H, Li Y, Shen LH, Liu X, Wang X, Zhang J, et al. Increasing tPA activity in astrocytes induced by multipotent mesenchymal stromal cells facilitate neurite outgrowth after stroke in the mouse. PLoS One. 2010;5(2):e9027.
35. van Velthoven CT, Kavelaars A, Heijnen CJ. Mesenchymal stem cells as a treatment for neonatal ischemic brain damage. Pediatr Res. 2012;71(4 Pt 2):474–81.
36. Guo F, Lv S, Lou Y, Tu W, Liao W, Wang Y, et al. Bone marrow stromal cells enhance the angiogenesis in ischaemic cortex after stroke: involvement of notch signalling. Cell Biol Int. 2012;36(11):997–1004.
37. 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 Transl Med. 2015;4(10):1131–43.
38. 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. J Cereb Blood Flow Metab. 2017;1:271678X17708917.
39. Zhang Y, Chopp M, Zhang ZG, Katakowski M, Xin H, Qu C, et al. Systemic administration of cell-free exosomes generated by human bone marrow derived mesenchymal stem cells cultured under 2D and 3D conditions improves functional recovery in rats after traumatic brain injury. Neurochem Int. 2016;pii:S0197-0186(16)30251-0.
40. Yeo RW, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, et al. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. Adv Drug Deliv Rev. 2013;65(3):336–41.
41. Du W, Zhang K, Zhang S, Wang R, Nie Y, Tao H, et al. Enhanced proangiogenic potential of mesenchymal stem cell-derived exosomes stimulated by a nitric oxide releasing polymer. Biomaterials. 2017;133:70–81.
42. Chen J, Ning R, Zacharek A, Cui C, Cui X, Yan T, et al. MiR-126 contributes to human umbilical cord blood cell-induced neurorestorative effects after stroke in type-2 diabetic mice. Stem Cells. 2016;34(1):102–13.
43. Mathiyalagan P, Liang Y, Kim D, Misener S, Thorne T, Kamide CE, et al. Angiogenic mechanisms of human CD34+ stem cell exosomes in the repair of ischemic hindlimb. Circ Res. 2017;120(9):1466–76.
44. Besse B, Charrier M, Lapierre V, Dansin E, Lantz O, Planchard D, et al. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. Oncoimmunology. 2016;5(4):e1071008.
45. Que RS, Lin C, Ding GP, Wu ZR, Cao LP. Increasing the immune activity of exosomes: the effect of miRNA-depleted exosome proteins on activating dendritic cell/cytokine-induced killer cells against pancreatic cancer. J Zhejiang Univ Sci B. 2016;17(5):352–60.
46. Lu Z, Zuo B, Jing R, Gao X, Rao Q, Liu Z, et al. Dendritic cell-derived exosomes elicit tumor regression in autochthonous hepatocellular carcinoma mouse models. J Hepatol. 2017;pii:S0168-8278(17)32055-X.
47. Bu N, Wu H, Zhang G, Zhan S, Zhang R, Sun H, et al. Exosomes from dendritic cells loaded with chaperone-rich cell lysates elicit a potent T cell immune response against intracranial glioma in mice. J Mol Neurosci. 2015;56(3):631–43.
48. Romagnoli GG, Zelante BB, Toniolo PA, Migliori IK, Barbuto JA. Dendritic cell-derived exosomes may be a tool for cancer immunotherapy by converting tumor cells into immunogenic targets. Front Immunol. 2014;5:692.

49. Kawikova I, Askenase PW. Diagnostic and therapeutic potentials of exosomes in CNS diseases. Brain Res. 2015;1617:63–71.
50. Rao P, Benito E, Fischer A. MicroRNAs as biomarkers for CNS disease. Front Mol Neurosci. 2013;6:39.
51. Hertel J, Lindemeyer M, Missal K, Fried C, Tanzer A, Flamm C, et al. The expansion of the metazoan microRNA repertoire. BMC Genomics. 2006;7:25.
52. Berezikov E. Evolution of microRNA diversity and regulation in animals. Nat Rev Genet. 2011;12(12):846–60.
53. Gurtan AM, Sharp PA. The role of miRNAs in regulating gene expression networks. J Mol Biol. 2013;425(19):3582–600.
54. Zhang X, Yuan X, Shi H, Wu L, Qian H, Xu W. Exosomes in cancer: small particle, big player. J Hematol Oncol. 2015;8:83.
55. Melo SA, Sugimoto H, O'Connell JT, Kato N, Villanueva A, Vidal A, et al. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. Cancer Cell. 2014;26(5):707–21.
56. McKenzie AJ, Hoshino D, Hong NH, Cha DJ, Franklin JL, Coffey RJ, et al. KRAS-MEK signaling controls Ago2 sorting into exosomes. Cell Rep. 2016;15(5):978–87.
57. Katakowski M, Buller B, Wang X, Rogers T, Chopp M. Functional microRNA is transferred between glioma cells. Cancer Res. 2010;70(21):8259–63.
58. Katakowski M, Buller B, Zheng X, Lu Y, Rogers T, Osobamiro O, et al. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett. 2013;335(1):201–4.
59. Bovy N, Blomme B, Freres P, Dederen S, Nivelles O, Lion M, et al. Endothelial exosomes contribute to the antitumor response during breast cancer neoadjuvant chemotherapy via microRNA transfer. Oncotarget. 2015;6(12):10253–66.
60. Wei Y, Lai X, Yu S, Chen S, Ma Y, Zhang Y, et al. Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. Breast Cancer Res Treat. 2014;147(2):423–31.
61. Xin H, Li Y, Buller B, Katakowski M, Zhang Y, Wang X, et al. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. Stem Cells. 2012;30(7):1556–64.
62. Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, et al. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. Stem Cells. 2013;31(12):2737–46.
63. Zhang Y, Ueno Y, Liu XS, Buller B, Wang X, Chopp M, et al. The MicroRNA-17-92 cluster enhances axonal outgrowth in embryonic cortical neurons. J Neurosci. 2013;33(16):6885–94.
64. 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.
65. 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. 2017;54(4):2659–73.
66. Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci. 2003;4(5):399–415.
67. Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. Lancet Neurol. 2009;8(5):491–500.
68. 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 Transplant. 2017;26(2):243–57.
69. Morel L, Regan M, Higashimori H, Ng SK, Esau C, Vidensky S, et al. Neuronal exosomal miRNA-dependent translational regulation of astroglial glutamate transporter GLT1. J Biol Chem. 2013;288(10):7105–16.

70. Bakhti M, Winter C, Simons M. Inhibition of myelin membrane sheath formation by oligodendrocyte-derived exosome-like vesicles. J Biol Chem. 2011;286(1):787–96.
71. Fruhbeis C, Frohlich D, Kuo WP, Kramer-Albers EM. Extracellular vesicles as mediators of neuron-glia communication. Front Cell Neurosci. 2013;7:182.
72. Fruhbeis C, Frohlich D, Kuo WP, Amphornrat J, Thilemann S, Saab AS, et al. Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. PLoS Biol. 2013;11(7):e1001604.
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.
74. van Balkom BW, de Jong OG, Smits M, Brummelman J, den Ouden K, de Bree PM, et al. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. Blood. 2013;121(19):3997–4006. S1–15
75. Schulz GB, Wieland E, Wustehube-Lausch J, Boulday G, Moll I, Tournier-Lasserve E, et al. Cerebral cavernous malformation-1 protein controls DLL4-notch3 signaling between the endothelium and pericytes. Stroke. 2015;46(5):1337–43.
76. Hu GW, Li Q, Niu X, Hu B, Liu J, Zhou SM, et al. Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells attenuate limb ischemia by promoting angiogenesis in mice. Stem Cell Res Ther. 2015;6:10.
77. Goetzl EJ, Schwartz JB, Mustapic M, Lobach IV, Daneman R, Abner EL, et al. Altered cargo proteins of human plasma endothelial cell-derived exosomes in atherosclerotic cerebrovascular disease. FASEB J. 2017;31(8):3689–94.
78. Graner MW, Alzate O, Dechkovskaia AM, Keene JD, Sampson JH, Mitchell DA, et al. Proteomic and immunologic analyses of brain tumor exosomes. FASEB J. 2009;23(5):1541–57.
79. Chen CC, Liu L, Ma F, Wong CW, Guo XE, Chacko JV, et al. Elucidation of exosome migration across the blood-brain barrier model in vitro. Cell Mol Bioeng. 2016;9(4):509–29.
80. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Mol Ther. 2011;19(10):1769–79.
81. Satani N, Savitz SI. Is immunomodulation a principal mechanism underlying how cell-based therapies enhance stroke recovery? Neurotherapeutics. 2016;13(4):775–82.
82. Imai T, Takahashi Y, Nishikawa M, Kato K, Morishita M, Yamashita T, et al. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. J Extracellular Vesicles. 2015;4:26238.
83. Takahashi Y, Nishikawa M, Shinotsuka H, Matsui Y, Ohara S, Imai T, et al. Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. J Biotechnol. 2013;165(2):77–84.
84. Vendrame M, Gemma C, Pennypacker KR, Bickford PC, Davis Sanberg C, Sanberg PR, et al. Cord blood rescues stroke-induced changes in splenocyte phenotype and function. Exp Neurol. 2006;199(1):191–200.
85. Walker PA, Shah SK, Jimenez F, Aroom KR, Harting MT, Cox CS Jr. Bone marrow-derived stromal cell therapy for traumatic brain injury is neuroprotective via stimulation of non-neurologic organ systems. Surgery. 2012;152(5):790–3.
86. Sakamoto W, Masuno T, Yokota H, Takizawa T. Expression profiles and circulation dynamics of rat mesenteric lymph microRNAs. Mol Med Rep. 2017;15(4):1989–96.
87. Jaimes Y, Naaldijk Y, Wenk K, Leovsky C, Emmrich F. Mesenchymal stem cell-derived microvesicles modulate lipopolysaccharides-induced inflammatory responses to microglia cells. Stem Cells. 2017;35(3):812–23.
88. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481–7.
89. Doring A, Sloka S, Lau L, Mishra M, van Minnen J, Zhang X, et al. Stimulation of monocytes, macrophages, and microglia by amphotericin B and macrophage colony-stimulating factor promotes remyelination. J Neurosci. 2015;35(3):1136–48.

90. Fu PC, Tang RH, Wan Y, Xie MJ, Wang W, Luo X, et al. ROCK inhibition with fasudil promotes early functional recovery of spinal cord injury in rats by enhancing microglia phagocytosis. J Huazhong Univ Sci Technolog Med Sci. 2016;36(1):31–6.
91. Lin X, Zhao T, Walker M, Ding A, Lin S, Cao Y, et al. Transplantation of pro-oligodendroblasts, preconditioned by LPS-stimulated microglia, promotes recovery after acute contusive spinal cord injury. Cell Transplant. 2016;25(12):2111–28.
92. Bahrini I, Song JH, Diez D, Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Sci Rep. 2015;5:7989.
93. Lui H, Zhang J, Makinson SR, Cahill MK, Kelley KW, Huang HY, et al. Progranulin deficiency promotes circuit-specific synaptic pruning by microglia via complement activation. Cell. 2016;165(4):921–35.
94. Southam KA, Vincent AJ, Small DH. Do microglia default on network maintenance in alzheimer's disease? J Alzheimers Dis. 2016;51(3):657–69.