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Invited Mini Review

Stem cell-derived extracellular vesicle therapy for acute brain insults and neurodegenerative diseases

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Stem cell-based therapy is a promising approach for treating a variety of disorders, including acute brain insults and neurodegenerative diseases. Stem cells such as mesenchymal stem cells (MSCs) secrete extracellular vesicles (EVs), circular membrane fragments (30 nm−1 μm) that are shed from the cell surface, carrying several therapeutic molecules such as proteins and microRNAs. Because EV-based therapy is superior to cell therapy in terms of scalable production, biodistribution, and safety profiles, it can be used to treat brain diseases as an alternative to stem cell therapy. This review presents evidences evaluating the role of stem cell-derived EVs in stroke, traumatic brain injury, and degenerative brain diseases, such as Alzheimer's disease and Parkinson' disease. In addition, stem cell-derived EVs have better profiles in biocompatibility, immunogenicity, and safety than those of small chemical and macromolecules. The advantages and disadvantages of EVs compared with other strategies are discussed. Even though EVs obtained from native stem cells have potential in the treatment of brain diseases, the successful clinical application is limited by the short half-life, limited targeting, rapid clearance after application, and insufficient payload. We discuss the strategies to enhance the efficacy of EV therapeutics. Finally, EV therapies have yet to be approved by the regulatory authorities. Major issues are discussed together with relevant advances in the clinical application of EV therapeutics. [BMB Reports 2022; 55(1): 20-29]

INTRODUCTION

Stem cell-based therapy is a promising approach for treating acute brain insults such as stroke and traumatic brain injury (TBI), and neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Stem cells such as mesenchymal stem cells (MSCs) secrete extracellular vesicles (EVs), which carry several molecules such as proteins and microRNAs (miRNAs). Recent preclinical studies suggest that stem cell-derived EVs can be used to treat brain illness as an alternative to stem cell application.

This review presents evidence regarding the role of stem cell-derived EVs in acute and chronic neurological diseases in addition to discussing the advantages and disadvantages of EVs therapy versus other strategies. Major issues in the clinical application of EV therapeutics are discussed together with relevant advances in EV production/enrichment, isolation/purification, and quantification/characterization.

ADVANTAGES OF STEM CELL-DERIVED EXTRACELLULAR VESICLES OVER OTHER THERAPEUTIC STRATEGIES

Several therapeutic strategies have been introduced for preventing or slowing the progression of brain damages and each has its own advantages and disadvantages (Table 1).

Small chemicals or macromolecules showed a limited efficacy due to single mechanism of action and complex pathophysiology of brain diseases. For example, over 1,000 neuroprotective agents for acute stroke have been investigated in preclinical studies with promising results, but failed when tested in human (1). Similarly, trophic factors tested in various neurological diseases such as PD failed to show beneficial effects (2). For neuroprotection, a single target of neuroprotection will not provide the expected therapeutic effects, and signals that mediate cell death during the acute stage of ischemic insult might promote repair during the recovery phase (3). As a result, pleiotropic multitarget agents that act via multiple mechanisms of action to interrupt multiple steps may be more fruitful (4). In addition, almost no macromolecules and 98% of all small molecules do not cross the blood-brain barrier (BBB). Therefore, non-active vesicles such as adeno-associated virus capsids and polymer- or lipid-base nanoparticles were used to overcome the limitation. However, the use of drug delivery system increases the risk of toxicity, immunogenicity, and infection (5).

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Table 1. Strategies for acute and degenerative brain diseases

	Small chemicals or macromolecules	Drug delivery system	Stem cells	Stem cell-derived EV therapy
Advantages	Manufacture on a large scale	Manufacture on a large scale Delivery of a therapeutic to its target site, minimizing off-target accumulation	Various paracrine effects More complex MoA (trans-differentiation and responsiveness to microenvironment)	Pleiotropic multiple target regulatory components Better biocompatibility, immunogenicity, and safety
Disadvantages	Limited efficacy due to single MoA Limitation in crossing the BBB	Lack of intrinsic biological cargo beside the load Possible adverse effects, such as complement activation-related pseudo-allergy (107)	Possible cell-mediated adverse effects Industrially unfeasible Difficult to maintain cell viability and functionality Donor variation	Lack of standardization for EV production, isolation and storage Complex characterization of EV product Donor variation
Results of clinical trial	None of RCTs showed successful results	No RCTs available for acute and degenerative brain diseases	Mixed long-term effects of MSCs in RCTs	No RCT available

EV, extracellular vesicle; MoA, mode of action; BBB, blood-brain barrier; MSCs, mesenchymal stem cells; FDA, Food and Drug Administration; RCT, randomized controlled trial.

Cell-based therapy is a promising therapeutic approach against a range of neurological diseases. Unlike to small chemicals or macromolecules, MSCs harbor specific functions, such as regenerative, cytoprotective, and immunomodulatory properties. Application of MSC transplantation was safe in patients with neurological diseases. However, the beneficial effects were diverse among patients. For example, four randomized clinical trials (RCTs) of stem cells have been conducted in stroke patients, with mixed results (6-9). There are several possible reasons for this inconsistent result, including heterogeneity of patients, timing of therapy, and donor-to-donor or batch-to-batch variations. Our pre-specified biomarker sub-study showed that circulating EVs were markedly increased immediately after intravenous injection of MSCs (10). In this study, the number of the circulating EVs varied among patients after the application of the same dose of MSCs, and was associated with motor function improvement, as assessed by clinical assessment and multimodal magnetic resonance imaging (MRI) as shown in Fig. 1 (10). These data raised the possibility of the use of MSC-EVs, instead of MSCs *per se*, given that the number of EVs determines the effects of MSC-based therapy. By contrast, plasma levels of trophic factors remained unchanged after the intravenous injection of autologous MSCs although the level of trophic factors in brain-derived EVs were increased. These findings suggest that the paracrine effects of MSCs are modulated by trophic factors in the brain indirectly via MSC-EVs.

EVs are circular membrane fragments measuring 30 nm to 1 μm in diameter that are shed from the cell surface. EVs represent a heterogeneous group of vesicles released from multiple cell types in the brain (neuron, astrocyte, oligodendrocytes, microglia, and endothelial cells/pericyte). EVs mediate cell-cell interaction and the complex and versatile EV signaling was shown to regulate neurogenesis, angiogenesis, and inflammation (11). EVs are important in sustaining the cellular function in the CNS, but they also participate in the pathophysiology of underlying neurodegenerative diseases. EVs are implicated in disease spreading by misfolded and pathological proteins engaged in transferring pathogenic molecules to neighboring cells, such as Aβ42 in AD, Huntingtin protein in HD, α-synclein, leucine-rich receptor kinase 2, vacuolar-sorting protein 35 in PD, and prion proteins (PrPc and PrPsc) in Prion disease (12). For example, EVs are involved in complex mechanisms of secretion, diffusion and degradation of Aβ or tau proteins. A study analyzing the physical properties of individual EVs using electrostatic force microscopy showed that EVs carried higher levels of Aβ42 when treated with to neuroblastoma cells with higher concentrations of Aβ42 oligomers, implying that it acts as a transport vesicle (13).

Stem cell-derived EVs are considered as naturally therapeutic agents and innate drug delivery systems for therapy of brain diseases. Unlike a sole protein or small molecule, EVs contain molecules with heterogenous function. EVs contain cellular proteins, DNAs, and RNAs. Among them, most studies have focused on the regulatory roles of non-coding RNAs components, such as miRNAs, in the CNS. EVs can also capture and transfer whole mitochondria or mitochondrial fragments, and mitochondrial transfer of stem cells to injured cells may be beneficial in ischemic diseases and mitochondria-related diseases, such as age-related neurodegenerative diseases (14-16). EVs exhibit multiple benefits related to biocompatibility, immunogenicity, stability, pharmacokinetics, biodistribution, and cellular uptake mechanism (17). EVs can transfer intravesicular cargo and vesicular membrane-bound receptors to recipient cells. EVs can cross the BBB and actively target specific cell types (18). Unlike to cell-based therapy, EVs can avoid the first pass effect and cell-mediated adverse effects, such as as tumor forma-

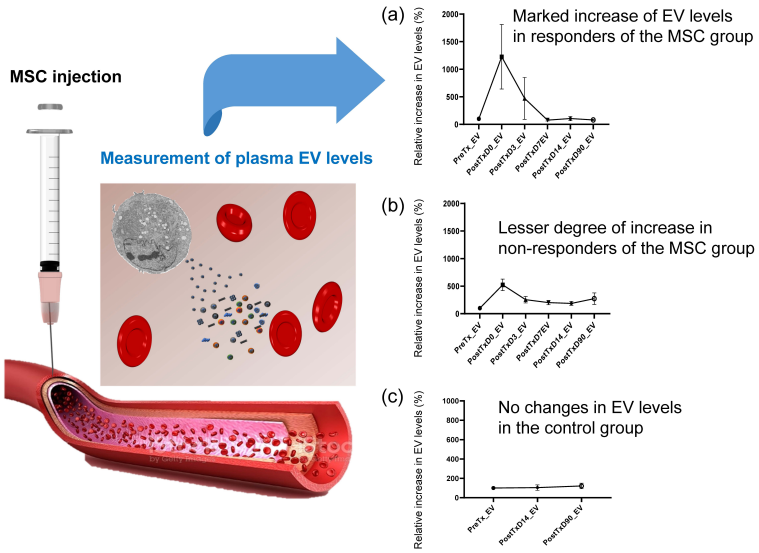

Fig. 1. Association between elevated levels of circular extracellular vesicles (EVs) and stroke outcome after mesenchymal stem cell (MSC) injection. Modified from Bang *et al*. (10) The levels of circulating EVs increased immediately after intravenous injection of autologous MSCs (A and B), but not after placebo treatment (C, right lanes). Although patients of the MSC group received the same number of MSCs (1×10^6 cells/kg), the levels of circulating EVs were varied among patients; (A) marked increase of EV levels in patients who showed clinically significant improvement, and (B) lesser degree of increase in those who showed no clinically significant improvement. The circulating EVs levels were correlated with improvement in MRI indices of neuroplasticity as well as in motor function.

tion, coagulopathy, and vascular occlusion, In addition, because lipids are essential components of EV membrane, nucleic acid components enriched in EVs are protected against RNase in the blood. A biodistribution study showed that systemically injected MSC-derived EVs can target the site of injury in proportion to the degree of tissue damage suggesting the possible role of signals from damaged tissue in homing EVs but not liposomes (19, 20). Further, EVs secreted by stem cells carry more complex cargo than those secreted by other cells (21), and EVs bearing MSC-specific membrane proteins on their surfaces potentially show disease-targeting ability as infused MSCs (22, 23).

Various cells have been used as a source of EVs. HEK293 cells are most commonly used as a source of EVs due to their high EV production capacity and easy transfection. However, HEK293 cells carry minimal intrinsic biological cargo, and the results of HEK293 cells cannot be translated to MSCs that are hard to transfect (24). MSCs represent a better source of EVs because the paracrine activity is responsible for at least 80% of their positive effects (25). As a result, most groups demonstrated therapeutic effects of MSC-EVs in preclinical models. However, EVs from different MSC sources exhibit different properties and carry cargo with distinct effects in the same diseases. The profile of miRNA within EVs varied greatly among the three common sources of MSCs, i.e., bone marrow, adipose, and umbilical cord (only 11 miRNAs were common), and the number of miRNAs was the highest in umbilical cord MSC-derived EVs (26). Compared with EVs derived from other MSC sources, EVs from umbilical cord showed superior therapeutic immunomodulation and protective effect (27). Further, fetal stem cells, such as umbilical cord/amniotic fluid stem exhibit a cellular phenotype intermediate between embryonic stem cells (ESCs)/induced pluripotent stem cells (iPSC) and adult MSCs (28).

It remains unknown whether MSCs are the best EV source for treatment of neurological disease. MSCs may not be an ideal cell source for EV manufacture on a clinical scale due to their limited lifespan, heterogeneity and batch-to-batch or donor-to-donor variations (29). ESCs or iPSCs, or ESC/iPSC-derived MSCs show better profiles in terms of cell numbers, senescence, and donor variation, while ethical concerns of ESCs are the main disadvantage in addition to the risk of immune response and teratoma involving iPSCs. Upadhya *et al*. showed that EVs isolated from iPSC-derived NSCs were enriched with miRNAs and proteins involved in neuroprotective, anti-apoptotic, anti-inflammatory, BBB repairing, neurogenic, and Aβ reducing activities, and are used in treating neurodegenerative disorders (30). Webb and colleagues found differences between EVs derived from different cell sources. Compared with MSC-EVs, NSC-derived EVs were superior in terms of modulation of post-stroke systemic immune response, neuroprotection, and functional recovery (31).

APPLICATIONS OF EXTRACELLULAR VESICLES IN BRAIN DISEASES

Cerebrovascular disease

Many preclinical studies have recently shown that stem cell-derived EVs can be used in stroke therapy (32). In 2013, Xin *et al*. reported that intravenous injection of MSC-EVs in a rat model of stroke improved the neurological outcomes and increased angiogenesis and neurogenesis (33). Other investigators have also demonstrated the beneficial effects of stem cell-derived EVs in various animal models of stroke. Several advances in EV-based strategy have been reported: (a) the use of EVs derived from stem cells other than MSCs, such as ESCs, neural stem cells (NSCs), and iPSC-derived MSCs/NSCs (31,

34, 35), (b) application of EVs intranasally (34), (c) production of EVs via 3D dynamic culture to increase the synthesis and regulate the payload of EVs (36) and stimulation with ischemic brain extracts (37, 38), and (d) evaluation of therapeutic effects of EVs on stroke in large animal models such as pigs and monkeys (35, 39). Stem cell-derived EVs contain many molecules that may have therapeutic effects in stroke, and miRNA-mediated effects was most widely studied. EV cargo miRNAs and other components related to the action of stem cell-derived EV in animal models of stroke were summarized elsewhere (32).

Traumatic brain injury

TBI represents acute damage of brain tissue caused by trauma. Together with stroke, TBI is one of the most common causes of disability and death in adults. Gao *et al*. showed that EVs derived from human umbilical cord blood-derived endothelial colony-forming cells have beneficial effects on BBB integrity in mice with TBI (40). Astrocyte-derived EVs carrying gap junction alpha 1 (GJA1) transmitted to neurons reduced apoptosis, increased mitochondrial function, and alleviated neuronal damage (41). Zhang *et al*. showed that MSC-derived EVs significantly improved functional recovery in rats after TBI, by promoting angiogenesis and neurogenesis and reducing neuroinflammation (42). In a porcine model of TBI, administration of EVs excreted from human MSCs reduced brain edema and lesion size, and improved BBB integrity (43). The therapeutic effects of EVs in TBI are attributed to their miRNA content. Yin *et al*. showed that miR-21-5p contained within EVs secreted from neurons promotes microglial M2 polarization to alleviate neuroinflammation after TBI (44). In contrast, Long and colleagues showed that miR-873a-5p carried by astrocyte-derived EVs suppresses neuroinflammation by inhibiting the NF-κB signaling pathway in neurons after TBI (45).

Neurodegenerative diseases

Similar to acute brain insults, such as stroke and TBI, EVs play an important role in neurodegenerative diseases, as both disease biomarkers and therapeutic targets. Dysregulation of circulating levels of specific EV-miRNAs has been reported in patients with neurodegenerative diseases. Recent evidences implicate EVs in the etiology and spread of neurogenerative disease. In preclinical models of AD, EVs reduced oxidative stress and neuroinflammation, inhibited the progression of neurodegeneration, and induced clearance of amyloid and neurofibrillary tangles (46, 47). Intranasal administration of MSC-derived EVs and iPSC-derived NSCs inhibited microglial activation, increased synaptogenesis, and rescued memory loss in animal models of AD (30, 48, 49). The beneficial effects of EVs obtained from MSCs preconditioned by cytokines or hypoxia and 3D culture methods were also reported recently in animal models of AD (48, 50, 51). Narbute and colleagues showed that intranasal administration of stem cell-derived EVs from teeth improved motor symptoms and normalized tyrosine hydroxylase expression in a rat model of PD (52). Several EV-miRNAs have been reported to show potential effects in AD models, such as miR-124a, miR-146a, miR-21, and miR-29b (53). Katsuda *et al*. showed that adipose tissue-derived MSCs secrete EVs carrying enzymatically active neprilysin, the most important Aβ-degrading enzyme in the brain (54).

Relatively few studies carried out to data have revealed the effects of stem cell-derived EV therapy in PD. Engineered EVs have been used to regulate specific proteins related to PD pathogenesis, such as antioxidant catalase, α-synuclein, and dopamine. The α-synuclein protein accumulates in brains of individuals with PD. Investigators have designed shRNA minicircles delivered by RVG EVs to treat dopaminergic neurons and reduce α-synuclein aggregation in PD (55, 56). Chen *et al*. showed that EVs from umbilical cord MSCs inhibited apoptosis by inducing autophagy in an in vitro model of PD (57). Upregulation of autophagy may clear accumulated α-synuclein, and miRNAs play major regulators of autophagy pathway (58). In HD, EVs transport mutant huntingtin between cells and trigger HD-related behavior and pathology (59). Didiot *et al*. reported the efficacy of small-interfering RNAs-loaded EVs delivered to the brain of a HD model in silencing HD mRNA suggesting the role of EVs as a gene-modifying strategy (60).

LIMITATIONS OF CURRENT MSC-EV THERAPEUTICS

Although the use of stem cell-derived EV therapy has several benefits in preclinical studies, there exist some limitations of the use of EVs obtained from naïve stem cells for patients with brain diseases.

Donor heterogeneity

One of the main obstacles hindering the clinical application of MSCs and EV therapeutics is the large variability in cell quality, due to the usage of different donors and their tissues, known as donor heterogeneity. Interestingly, Wang *et al*. showed that individual MSC-EV preparations from healthy human donors may differ in their therapeutic potency, suggesting donor-to-donor variation (61). The therapeutic potential of independent MSC-EV preparation may differ due to donor age, comorbidity (obesity and disease condition), artificial niche of MSCs (preconditioning or external stimuli), and culture methods used (62, 63). Along with the development of a production method that minimizes donor-to-donor and batch-to-batch variations, a robust quality control for each EV production lot is required.

Inherited undesirable features of MSC-EVs

Nalamolu *et al*. tested the efficacy of EVs secreted by MSCs under standard culture conditions against post-stroke brain damage and neurological outcomes in a rat model of stroke. The treatment attenuated ischemic brain damage without improving the post-stroke neurological outcome, suggesting the need for modification of MSC culture conditions (64).

Even though native EVs have potential in the treatment of brain disease, the successful clinical application is limited by

the short half-life, limited targeting, rapid clearance after application, and insufficient payload (65). Although native EVs cross the BBB under stroke-like, inflamed conditions, whether they can cross the intact BBB has yet to be firmly established (66, 67). In both preclinical and human studies, blood levels of EVs decreased rapidly after systemic application of EVs, and EVs accumulated in the lung, liver and spleen until day 10 after administration (68, 69). In addition, the circulation time of EVs is shortened by macrophage/microglial clearance.

ENHANCING THE EFFICACY OF THERAPEUTIC EXTRACELLULAR VESICLES

Two strategies to enhance the efficacy of EV therapeutics.

Production or selection of optimal EVs

Brain pharmacokinetics of EVs may differ among EVs of different origin (68) and may depend on the characteristics of EV membrane proteins or receptors. For example, CD46, integrins, and intercellular adhesion molecule-1 on the surface of EVs were associated with the rate and mechanisms of BBB crossing of EVs (70, 71). In addition to surface molecules, intravesicular miRNAs and VEGF-A may also influence the BBB integrity (72, 73). Caveolin-mediated endocytosis and integrins and phosphatidylserine ligand-receptor interaction are involved in EV uptake by recipient cells in the brain (65). Membranous lipid-draft protein caveolin-1 and phosphatidylserine were highly expressed in microvesicles than in exosomes (74). CD47, a transmembrane protein that enables cancer cells to evade clearance by macrophages ('don't eat me signal'), prolongs the circulation time of EV after systemic administration (75). EV surface features, such as tetraspanin (CD63) and integrin profiles, may influence the targeting capacity (76). Selection of EVs with optimal characteristics in terms of surface and cargo molecules represents a safer alternative than bioengineered EVs. Ideal EVs for brain therapeutics exhibit the aforementioned characteristics of surface molecules and intravesicular cargo, as shown in Fig. 2.

Modification of EV culture conditions or exposure to external stimuli may enhance the therapeutic potential of MSC-EVs (77, 78). Preconditioning (hypoxia, ischemia or inflammation), supplementation of culture medium with bioactive factors, and modification of cell-cell interaction (spheroid culture) or cell-substrate interaction (collagen microgel) were used to enhance their therapeutic properties and possibly minimize donor variation. Interestingly, our and other studies showed that the cellular yield of EVs was increased compared with standard 2D culture and EV cargo undetected by standard 2D conditioning of cells were enriched in EVs obtained from 3D culture (36, 79).

Bioengineering of source cells or EVs

Engineered EVs may be used to enhance the efficacy of cell-derived EVs for treating neurological diseases. In this review, artificial engineering techniques for surface modification and cargo loading to enhance therapeutic efficacy of EVs are not discussed in detail because MSCs are hard to transfect and this topic was covered by other reviews (24, 80, 81). EV surface engineering can be achieved indirectly by genetic modification of the EV-secreting cells or via direct modification of EV surface to improve stability, targeting ability and EV tracking (82). However, such EV surface engineering techniques may be associated with toxicity and alteration of the characteristics of stem cell-derived EVs.

Nucleic acids, proteins, and small molecules can be loaded into EVs. To improve the loading efficacy, EV can be physically or chemically manipulated. However, most studies focused on specific EV-miRNAs or EV proteins to evaluate the mechanisms of EVs, and used engineered EVs from non-stem cells (e.g., HEK293) that contain selected EV cargos. It is likely that multi-

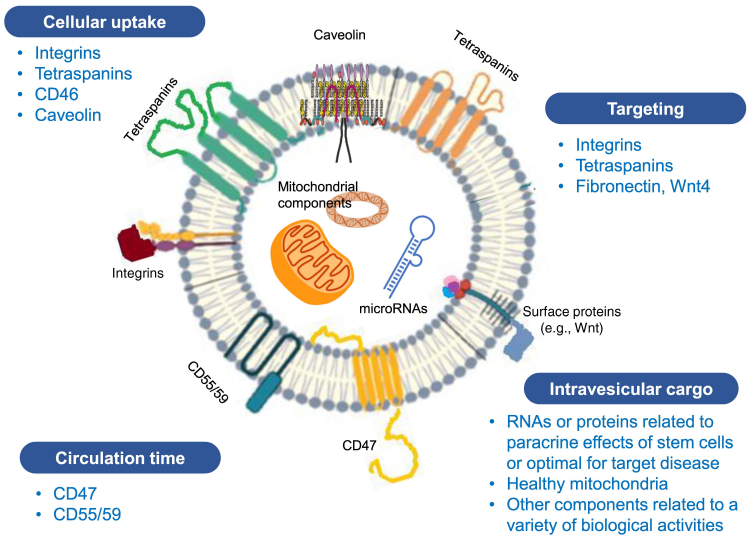

Fig. 2. Surface molecules and cargo related to efficacy and biodistribution of stem cell-derived extracellular vesicles (EVs).

ple EV-associated cargos rather than a single candidate molecule elicit therapeutic effects of MSC-EVs, in a synergistic manner. Further, active bioengineering techniques recently introduced may influence the characteristics of EVs and induce toxicity or be clinically undesirable. All the techniques can increase loading efficiency; however, they are often associated with negative effects such as loss of membrane integrity, aggregate formation and cargo impurity (83). Although bioengineering of EV-producing cells is attractive, most investigators prefer post-EV modification for rapid results and yield as well as viability for clinical feasibility (65).

EVs harbor bioactive molecules. Among them, miRNAs regulate gene expression and protein synthesis, while proteins have biochemical effects. The majority of RNAs found in EVs are less than 200 nucleotides in length. Unlike the abundance of ribosomal RNAs in the parent cells, EVs are mainly enriched in small RNAs, such as miRNAs, long non-coding RNAs, and circular RNAs (84). The miRNA is of prime importance in mediating therapeutic effects (32, 85). Differential miRNA expression in angiogenesis, neuroprotection, and immunomodulation may be associated with stroke and neurodegenerative diseases (32). For example, loading MSCs with miR-124, a neuroprotectant and inflammatory modulator induced cortical neurogenesis via EVs (86). Application of EVs from MSCs overexpressing miRNA-133b showed neurite remodeling and neuroprotection (87). In addition, the miRNA-17-92 cluster is associated with neuroplasticity, and treatment of EVs from MSCs loaded with these miRNAs increased neural plasticity and functional recovery in a rat model of stroke compared with EVs derived from naïve MSCs (88).

Brain-derived neurotrophic factor (BDNF) is a clinically relevant candidate for drug delivery, potentially for neuroprotective effects both at and across the BBB (89). BDNF plays important roles in a variety of brain diseases. BDNF rescues neurons from apoptotic cell death, promotes neuronal development and regeneration of synaptic connections, and improves the overall neuroplasticity of recovery from brain injury and cognitive processes (90). Further, BDNF is expressed in many different brain regions and is decreased during the aging process. The levels of BDNF and netrin-1 (a laminin-related hormones) are strongly reduced in Parkinson's disease brains and gut tissues (91). D'Souza and colleagues susscessfuly transferred the plasmid DNA expressing BDNF and mitochondria/mitochondrial DNA to brain endothelial cells (89). Yang *et al*. evaluated the potential therapeutic effects of EV-mediated targeted delivery of NGF in ischemic cortex (92). In this study, HEK293 cells were transfected with RVG-Lamp2b and NGF vectors. Systemic administration of EVs resulted in a burst of encapsulated NGF protein released in the brain. Similarly, Zha *et al*. encapsulated the VEGF gene into chondrogenic ATDC5-derived EVs, which induced vascularized bone regeneration (93).

Therapeutic agents such as curcumin and catalase can be loaded in EVs to enhance the therapeutic potential of naïve EVs. For example, catalase, a potent antioxidant, was loaded into EVs ex vivo using different methods, and treatment via EVs provided neuroprotective effects in both in vitro and in vivo models of PD (94). Conversely, curcumin which exhibits anti-inflammatory and anti-oxidant properties, was loaded by passive incubation into MSCs and HEK293 cells resulting in enhanced protective effects in models of osteoarthritis and myocardial infarction, respectively (95, 96).

CLINICAL SCALE PRODUCTION OF EXTRACELLULAR VESICLES

The clinical use of EVs requires mass production of EVs. Strategies to increase the yield of EV production include large-scale methods for EV generation, such as artificial EV generation (e.g., extrusion via porous membrane) and large-scale natural EV generation (e.g., bioreactor use) as well as aforementioned methods for modification of culture conditions or external stimuli (97, 98). Various methods of EV production, including different bioreactors and isolation methods, are being used for clinical trials of EV therapeutics (98). In addition to culture of EV source cells, isolation methods also affect the EV cargos. Therefore, selection and validation of isolation methods of choice are needed to avoid confounding results regarding EV-specific content and function.

Haraszti *et al*. showed that microcarrier-based 3D culture and tangential flow filtration (TFF) facilitate scalable production of biologically active EVs from umbilical cord-derived MSCs. The yield of EVs using this combination system was robust compared with 2D-cultures (99). We have recently introduced a novel method for clinical scale MSC-EV production using a micro-patterned well-based 3D-spheroid system. Using this culture method, we were able to upregulate miRNAs related to neurogenesis/axonal outgrowth and reduce the donor variation.

CONCLUSIONS

Stem cell-derived EV therapy represents a promising approach for patients with acute and degenerative brain disease, as MSC therapies have already been tested in clinical trials. EV-mediated therapy is superior to cell therapy in terms of scalable production, biodistribution, and safety profiles. However, continuous efforts are needed to control the heterogeneity of cargo, optimization of EV surface molecules, and increasing EV production yield.

Currently, MSC-EV therapy is still in the process of development. The results of clinical trials of the application of MSC-EVs have been reported in graft versus host disease, chronic kidney disease, and COVID-19, which showed no adverse effects related to the administration of MSC-EVs (100-102). In addition, clinical safety and possible beneficial effects of MSC-EVs or secretome were reported in patients with alveolar bone regeneration, alopecia, Meniere's disease undergoing intracochlear application, and refractory macular degeneration (103-106). As of June 2021, only two clinical trials are evaluating the role of EV therapeutics in brain diseases including a small clinical trial

of safety involving intravenous application of MSC-EVs engineered to express miR-124 in stroke patients (clinicalTrial.gov identifier NCT3384433) and a phase I/II clinical trial of safety and efficacy of EVs derived from allogeneic adipose MSCs administered for nasal drip in patients with AD (NCT0438982).

EV therapies have yet to be approved by the regulatory authorities. Therefore, further studies evaluating the efficacy of MSC-EVs in RCTs are required. In addition, the conventional 2D culture method has been used in the aforementioned clinical studies. EV characterization and isolation methods show substantial heterogeneity. Therefore, further clinical studies are needed to address the limitations of clinical progression of EV therapeutics, such as scalability and GMP of source cells and EV preparation, in addition to extensive quality control. Finally, the biodistribution of EVs and the route and dose of application should be defined as they differ depending on the characterization of target diseases, in terms of acute insults vs. chronic neurodegeneration and intact vs. inflamed BBB.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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