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Extracellular vesicle-based therapy for amyotrophic lateral sclerosis

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Abstract:

Amyotrophic lateral sclerosis (ALS) stands as a neurodegenerative disorder characterized by the rapid progression of motor neuron loss in the brain and spinal cord. Unfortunately, treatment options for ALS are limited, and therefore, novel therapies that prevent further motor neuron degeneration are of dire need. In ALS, the infiltration of pathological elements from the blood to the central nervous system (CNS) compartment that spur motor neuron damage may be prevented via restoration of the impaired blood-CNS-barrier. Transplantation of human bone marrow endothelial progenitor cells (hBM-EPCs) demonstrated therapeutic promise in a mouse model of ALS due to their capacity to mitigate the altered blood-CNS-barrier by restoring endothelial cell (EC) integrity. Remarkably, the hBM-EPCs can release angiogenic factors that endogenously ameliorate impaired ECs. In addition, these cells may produce extracellular vesicles (EVs) that carry a wide range of vesicular factors, which aid in alleviating EC damage. In an *in vitro* study, hBM-EPC-derived EVs were effectively uptaken by the mouse brain endothelial cells (mBECs) and cell damage was significantly attenuated. Interestingly, the incorporation of EVs into mBECs was inhibited via $\beta 1$ integrin hindrance. This review explores preclinical studies of the therapeutic potential of hBM-EPCs, specifically via hBM-EPC-derived EVs, for the repair of the damaged blood-CNS-barrier in ALS as a novel treatment approach.

Keywords:

Neurodegenerative disorder, ALS, blood-CNS-barrier, stem cell, extracellular vesicle, transplantation

Introduction

Previous research has demonstrated that extracellular vesicles (EVs) play a crucial role in regulating communication between cells in a variety of physiological and pathological circumstances and compose of microvesicles, apoptotic bodies, and exosomes.^[1-7] Microvesicles are 50–1,000 nm in size, exosomes are 40–120 nm, and apoptotic bodies are 500–2,000 nm.^[2,8] EVs are not only distinguished by constituent's sizes, but also by inner contents and biogenesis pathways.^[2,8,9] Importantly, EVs are largely involved in mediating cross-talk between cells by transporting a variety of biomolecules (e.g., proteins, lipids, peptides, mRNA, microRNA) among cells. As a result,

EVs contribute to stem cell plasticity,^[1,10] immune system responses,^[11-14] and angiogenesis.^[15,16] In addition, EVs may play a role in the transport of therapeutic factors from stem cells^[17] that bear regenerative capacity, promote angiogenesis, attenuate inflammation, and inhibit apoptosis.^[18]

Due to the capacity of nanovesicles to traverse the blood-brain barrier and their low tendency to evoke an immune response, nanoparticle-based therapies have arisen as potential deliverers of therapeutics for the treatment of neurodegenerative illnesses.^[2,4,19] Notably, human bone marrow (hBM) mesenchymal stromal cells (MSC)-derived EVs demonstrated rehabilitative capacity in animal models of lung injury induced by either LPS^[20] or ischemia-reperfusion,^[21] as well as of kidney damage^[22] and sepsis.^[23] In myocardial ischemia/reperfusion injury mice,

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exosomes derived from MSCs diminished infarct volume.^[24] Following treatment with MSC-isolated exosomes, healing of femur fracture in mice accelerated.^[25] In immunodeficient SCID mice, endothelial cell (EC) viability, proliferation, and angiogenesis were promoted by human endothelial progenitor cell (EPC)-derived microvesicle administration.^[16] Another study using SCID mice explored the efficacy of human pancreatic islet xenotransplantation in conjunction with microvesicles harvested from EPCs in ameliorating immune deficiency.^[15] Notably, this combined therapy enhanced angiogenesis and upregulated the production and release of insulin in treated mice.^[15] Thus, EVs may serve as effective transporters for the therapeutic delivery of various cargo proteins in the treatment of numerous diseases. Nonetheless, the mechanism underlying EV cargo delivery warrants further investigation.^[17] This review discusses the efficacy of EVs in treating amyotrophic lateral sclerosis (ALS), specifically EVs secreted by hBM-EPCs, and the mechanisms behind the therapeutic actions of these vesicles.

Preclinical Studies Supporting Human Bone Marrow-Endothelial Progenitor Cells Therapy in Amyotrophic Lateral Sclerosis

In contemplating the role EVs in hBM-EPC therapy, a brief introduction on the status of hBM-EPC therapy for ALS will provide a better appreciation of the scientific merit of EV contribution to regenerative medicine. In ALS studies of regenerative stem cell therapy targeting the impaired blood-central nervous system (CNS)-barrier, one of the pathogenic disease mechanisms, may serve as a potent therapeutic strategy for this aggressive neurodegenerative malady.^[17,26-28] When hBM-EPCs were delivered intravenously to symptomatic G93A SOD1 mutant mice (an ALS model), the blood-CNS-barrier was significantly restored potentially due to the replacement of damaged ECs with “healthy” transplanted cells.^[29] Barrier functionality in gray matter horns and white matter columns in the spinal cord, as well as in gray and white matter in the cerebral motor cortex/brainstem was substantially ameliorated following extensive hBM-EPC engraftment.^[29] This improvement of barrier structure and function in the SOD1 mutant mice was observed through a significant reduction in capillary permeability and bolstering of perivascular astrocyte end-feet function.^[29] Therefore, the manifestations of disease in these mice were greatly mitigated via blood-CNS-barrier restoration, resulting in augmented motor neuron viability in the spinal cord.^[29]

A subsequent *in vitro* study examined the mechanism underlying hBM-EPC-mediated blood-CNS-barrier repair.^[30] Cultured hBM-EPCs were observed in a normogenic environment at various time points.^[30]

Notably, the cells demonstrated a steady secretion of VEGF-A and angiogenin-1, as well as structural changes in cytoskeletal F-actin filaments, and immunoexpression of zonula occludens 1 and occludin.^[30] Furthermore, these results indicate that hBM-EPCs may provide endogenous endothelium repair behind the rehabilitative capacity of these cells along with cell replacement of damaged ECs.^[30] In a similar investigation, human peripheral blood-derived EPCs promoted angiogenesis and renewal of impaired brain tissue injury via secretion of biomolecules.^[31] On account of hBM-EPCs’ capacity to replace damaged ECs and also bolster EC functionality, the efficacy of both of these reparative mechanisms in alleviating ALS-induced blood-CNS-barrier injury should be examined.

The Therapeutic Potential of Human Bone Marrow-Endothelial Progenitor Cells-Derived Extracellular Vesicles in Amyotrophic Lateral Sclerosis

Recently, the preclinical study has explored hBM-EPC-derived EVs as cell-free therapeutics for blood-CNS-barrier restoration in conditions mirroring ALS.^[17] An *in vitro* investigation found that hBM-EPC-derived EVs served as nanosized vesicles and after adding to culture media at concentration of 1 µg/ml, were effectively integrated into mouse brain endothelial cells (mBECs) and ameliorated ALS-induced injury of mBECs following exposure to plasma obtained from symptomatic ALS mice.^[17] The total protein content and size of the EVs were noted at specific culture time points during normogenic conditioning.^[17] Indeed, EVs are heterogeneous in size and content; however, these vesicles primarily consist of exosomes and microvesicles. Interestingly, the apoptotic bodies in EVs were noted upon vesicle isolation from the hBM-EPC cultures at 5 DIV.^[17] The appearance of these EV constituents may be explained by the natural variations in cellular nuclei and cytosol shapes that ensue with apoptosis.^[17] Of note, another *in vitro* study found morphological variations in hBM-EPCs, as the cells transformed from more rounded at 24 h to more elongated at 72 h.^[30]

The hBM-EPCs were subject to ALS plasma derived from symptomatic G93A SOD1 mice in culture. It has been shown that G93A SOD1 mutant mice during disease progression contain high levels of the unfavorable humoral factor, as well as heightened concentrations of pro-inflammatory type 1 cytokines and comparatively lower levels of anti-inflammatory type II cytokines.^[32] As exposure to ALS mouse plasma grew, cell death accompanied by morphological changes in hBM-EPCs was also increased.^[17] Notably, 97 cytokines in the blood plasma of G93A SOD1 mutant mice over the course of disease were delineated, and an upregulated

concentration of multiple cytokines correlated with increased mortality rate for mice.^[33] However, these biomolecules may not be effective as a prognostic tool due to disparities in levels of expression.^[33]

As a diagnostic marker for ALS, peripheral blood levels of particular cytokines may be useful.^[34] Plasma extracted from ALS patients exhibited upregulated concentrations of pro-inflammatory cytokines tumor necrosis factor- α (TNF- α), TNF receptor 1, interleukin-6 (IL-6), and IL-1 β .^[35-37] Through the trans-signaling pathway, humoral IL-6 may contribute to inflammation of ECs.^[38] Furthermore, the heightened levels of inflammatory cytokines associated with ALS, inducing neuroinflammation, may exacerbate transplanted cell viability in the bloodstream.^[17] In addition, ALS engenders deleterious humoral conditions with greater levels of injurious factors in the blood circulation, which may also negatively affect the functionality of endogenous ECs.^[17] Nevertheless, further investigation into the correlation between cytokine levels and their impact on ECs in ALS at various stages of the disease are warranted. An *in vitro* study utilizing mBECs examines this point. Upon 3% ALS mouse plasma treatment, cell survival of mBECs substantially decreased *in vitro*.^[17] However, adding 1 $\mu\text{g}/\text{ml}$ of hBM-EPC-derived EVs to culture media greatly mitigated cell deterioration and warped morphology.^[17] Conversely, at an EV dose of 5 $\mu\text{g}/\text{ml}$, cell viability plummeted, indicating an EV level toxic to ECs. Furthermore, before clinical trials, extensive preclinical investigation is necessary to elucidate optimal dosage and timing of EV treatment.^[17]

MSCs have also risen as a potential source of EVs for cell-free therapeutics.^[21-25,39-42] Human BM-EPC-derived microvesicles have demonstrated therapeutic potency in animal models, specifically via the augmentation of angiogenesis.^[15,16] The microvesicles were incorporated in ECs and showed the ability to carry angiogenic factors to regions of brain injury.^[15] Importantly, the microvesicles mirrored the activity of the hBM-EPCs from which they were harvested and were critical for intercellular communication.^[6,43] Therefore, these EVs have been coined “nanosized extracellular organelles.”^[3]

Potential Mechanisms Underlying Human Bone Marrow-Endothelial Progenitor Cells-Derived Extracellular Vesicles-Induced Neuroprotection in Amyotrophic Lateral Sclerosis

Preclinical studies have also explored the mechanisms behind EV uptake by cells, as well as mitigation of EC impairment spurred by ALS. Remarkably, hBM-EPCs secrete protein and lipid-based therapeutic factors that upregulate neuronal progenitor cell survival and

differentiation *in vitro*.^[44] Moreover, EVs harvested from hBM-EPCs may serve as potent carriers for therapeutic biomolecules in neurodegenerative disorders. The cellular and molecular links between neuroprotection to EC restoration remain an outstanding issue that requires further investigations. In mBEC culture, following the addition of hBM-EPC-derived EVs, the vesicles were found ubiquitously throughout the cytosol and cellular projections.^[17] Therefore, the therapeutic effects of these EVs may be due to their capacity to carry a wide range of biomolecules to various cell compartments, thereby aiding the preservation of cellular function.^[17] Nonetheless, a multi-omic investigation is warranted to delineate the types of biomolecules involved with the rehabilitative capacity of these EVs.^[17] In general, EVs vary significantly in structure, content, and biological mechanisms.^[2,45,46]

Notably, the following biomarkers have been found on the surface of exosomes: CD9, CD63, CD81, tetraspanins, and flotillin.^[47-50] With respect to microvesicles, integrins, selectins, and CD40 have been identified.^[16,51] Finally, annexin V, C2b, and thrombospondin have been delineated in apoptotic bodies.^[52-54] To more effectively distinguish EV subtypes, a ratio comparing the number of a particular biomarker with the others in the vesicles should be determined.^[17] Additionally, proteomic studies and analysis of RNA in EVs are key factors to delineate the molecular cargo of EVs and may aid in elucidating the EV mechanisms in ALS.^[17,54-57] Since EVs are enclosed in a heterogeneous phospholipid bilayer, investigation into how components of the membrane factor into EV structure, function, and stability is warranted.^[7] Regarding hBM-EPC-derived EVs specifically, delineating the composition of the lipid membrane may provide a molecular understanding of cellular fusion and fission for these vesicles.^[58] Moreover, in-depth investigations that aim to identify the protein and lipid composition of EVs may serve as a robust area of future research.^[59]

In addition to further elucidating the molecular heterogeneity of EVs isolated from hBM-EPCs, the mechanisms behind the incorporation of these vesicles into cells must be more extensively evaluated. Indeed, EVs may be uptaken by cells via membrane fusion, endocytosis, phagocytosis, and macropinocytosis.^[5] The particular pathways that drive EV incorporation are influenced by the membrane composition of the target cell and the vesicle, indicating the importance of identifying the proteins and/or glycoproteins involved in EV internalization.^[5] While EVs can be internalized into target cells via endolysosomal mechanisms or budding, endocytosis seems to be the most prominent pathway.^[2,5,8] Nevertheless, it is also important to assess

the fusion mechanism where the EV lipid membrane fuses directly with the membrane of target cells.^[17,60] Future studies should investigate the mechanisms of EV uptake into target cells despite hindering or blocking by ligands or receptor-mediated incorporation.^[2,5,12] Notably, microvesicle uptake by human EPCs was attenuated via the inhibition of $\alpha 4$ integrin and $\beta 1$ integrin (CD29).^[16] *In vitro*, preconditioning hBM-EPC-derived EVs with anti-CD29 inhibiting antibodies resulted in reduced uptake of EVs by mBECs.^[17] Consequently, cell death was significantly exacerbated following exposure to 3% ALS mouse plasma compared to the effect of EV treatment.^[17] Moreover, cell adhesion molecules may play a critical role in the uptake of EVs by receiving cells.^[17]

Conclusion

In summary, EVs isolated from hBM-EPCs demonstrate potential therapeutic promise in ALS. Animal models of ALS showed substantial EC damage, leading to impairment of blood-CNS-barrier integrity. The effects of hBM-EPC-derived EVs culminated in ameliorated EC deterioration *in vitro* [Figure 1], most likely due to EV uptake into ECs and the release of therapeutic factors from the vesicles. This effect likely results from hBM-EPC transplantation. Nonetheless, the specific biomolecules secreted by these EVs have not been fully illuminated. Additionally, the safety and efficacy of these vesicles need to be investigated *in vivo* to further elucidate the therapeutic potency of hBM-EPC-derived EVs in ALS. This review paper is limited to the potential contribution of EVs derived from hBM-EPCs in the observed functional recovery in ALS following hBM-EPC transplantation. Other mechanisms of action, including but not limited to hBM-EPC differentiation and integration into the host brain tissue, equally warrant consideration in advancing hBM-EPC transplantation for ALS.

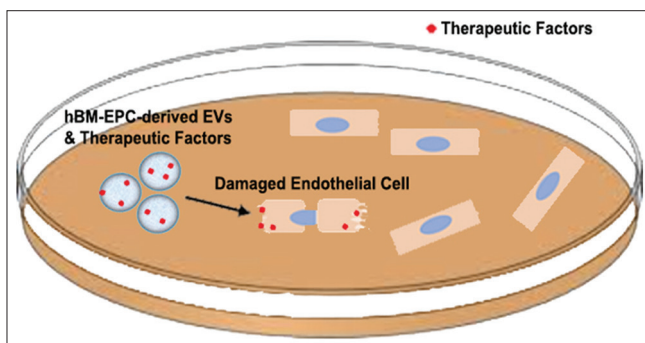


Figure 1: Extracellular vesicle-mediated repair of damaged endothelial cells. Schematic diagram shows how extracellular vesicles derived from human bone marrow endothelial progenitor cells are secreted and uptaken by damaged endothelial cells in an *in vitro* setting

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Conflicts of interest

There are no conflicts of interest.

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