



Adipose-Derived Mesenchymal Stem Cells Combined With Extracellular Vesicles May Improve Amyotrophic Lateral Sclerosis

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The complexity of central nervous system diseases together with their intricate pathogenesis complicate the establishment of effective treatment strategies. Presently, the superiority of adipose-derived mesenchymal stem cells (ADSCs) on neuronal injuries has attracted significant attention. Similarly, extracellular vesicles (EVs) are potential interventional agents that could identify and treat nerve injuries. Herein, we reviewed the potential effects of ADSCs and EVs on amyotrophic lateral sclerosis (ALS) injured nerves, and expound on their practical application in the clinic setting. This article predominantly focused on the therapeutic role of ADSCs concerning the pathogenesis of ALS, the protective and reparative effects of EVs on nerve injury, as well as the impact following the combined usage of ADSCs and EVs in ALS.

Keywords: adipose-derived mesenchymal stem cells, amyotrophic lateral sclerosis, neuronal injury, combinational therapy, extracellular vesicles

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease. It is characterized by dysfunction of the upper and lower motor neurons (Hardiman, 2021). Clinical manifestations of ALS include adult-onset focal muscle weakness and emaciation, usually beginning in the muscles of the extremities and spreading as the disease progression. Some patients have different degrees of non-motor performance, including cognitive and behavioral changes (Phukan et al., 2007; Masrori and Van Damme, 2020). ALS is difficult to diagnose due to the high variability of clinical manifestations. Presently, its diagnosis is based on clinical signs and electromyography. In addition, ultrasound and magnetic resonance imaging are auxiliary methods that assist with disease

Abbreviations: ADSCs, Adipose-derived mesenchymal stem cells; ALS, Amyotrophic lateral sclerosis; NFL, Neurofilament Light chain protein; TDP-43, TAR DNA binding protein 43; MSCs, Mesenchymal stem cells; EVs, Extracellular Vesicles; BMSCs, Bone marrow mesenchymal stem cells; GDNE, Glial cell-derived neurotrophic factor; BDNE, Brain-derived neurotrophic factor; IL-1 β , Interleukin-1 β ; TrkB, Tyrosine receptor kinase B; TNF, tumor necrosis factor; ROS, Reactive oxygen species; Nrf2, Nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; ARE, Antioxidant response element; GJA1, Gap junction alpha 1; BBB, Blood-brain barrier; CXCR4, C-X-C chemokine receptor type 4; SDF-1 α , Stromal cell-derived factor-1 α ; CXCL12, C-X-C motif chemokine ligand 12; CUEDC2, CUE domain-containing 2; VCAM-1, The vascular cellular adhesion molecule-1; RRAS, Ras-related protein R-Ras; RAB11A, Ras-related protein Rab-11A.

diagnosis (Goedee et al., 2020; Johnsen, 2020). ALS patients have poor prognosis, and most ALS patients eventually die due to respiratory dysfunction. Although no medical intervention for the complete alleviation of ALS presently exists, studies have shown that non-invasive ventilation and medication (riluzole and edaravone) improve patients' quality of life and delay disease progression (Niedermeyer et al., 2019; Chiò et al., 2020). As a heterogeneous syndrome, the pathogenesis of ALS is still unclear. However, genetic abnormalities often cause changes in their corresponding molecular mechanisms (Blokhuys et al., 2013). ALS pathogenesis includes cell function aberrancy and changes regarding the molecular content (van Es et al., 2017). SOD1^{G93A}, as a marker protein of the ALS, activates NLRP3 inflammasome in microglia via its promotion of IL-1 β secretion, which in turn leads to neuroinflammation and neurotoxicity (Deora et al., 2020). Studies have shown that ERK1/2 kinase can drive accumulated TAR DNA binding protein 43 (TDP-43) to the cytoplasm. Aggregated TDP-43 binds to specific microRNAs, resulting in interruption of protein synthesis. This causes the overall imbalance of mitochondria and aggravates oxidative stress. The decrease of glutathione content in cytoplasm is significant to the occurrence of oxidative stress response (Romano et al., 2020; Zuo et al., 2021). Furthermore, glutamate transporter deficiency increases the content of extracellular glutamate, which culminates in the degeneration of motor neurons (Bonifacino et al., 2019).

The diversity of ALS pathogenesis has provided new ideas for the treatment of the disease. In recent years, mesenchymal stem cells (MSCs) and extracellular vesicles (EVs) from different sources have played a significant role in neurodegenerative diseases. MSCs, as pluripotent and self-renewing stem cells, have a wide range of sources, can be obtained from bone marrow and adipose tissue, and be easily expanded *in vitro* (Gugliandolo et al., 2019). Transplanted MSCs mitigated the autophagy and apoptosis of motor neurons, produced neuroprotective effects and prolonged life span in the models of the ALS mice. More interestingly, the repeated injection did enhance these effects (Řehořová et al., 2019). Several routes can achieve MSCs in ALS treatment: One is the obstruction of the disease by replacing and protecting the damaged neurons (Gao et al., 2019). Through its paracrine effects, MSCs can release nutritional factors to maintain the microenvironment of neurons (Sykova et al., 2021). Obviously, the extraction of MSCs is more convenient from adipose tissue (Strioga et al., 2012). In addition, its acquisition does not involve ethical issues like that of embryonic mesenchymal stem cells (Gugliandolo et al., 2019). Therefore, adipose-derived mesenchymal stem cell transplantation is a potential cell therapy for ALS (Marote et al., 2016).

Extracellular vesicles is a tiny vesicle that can be secreted by various cells in the body, and is formed after induction of plasma membrane and formation of intracellular multivesicular bodies. These are secreted to the outside of the cell by exocytosis, and transfer nucleic acids, proteins and lipids between cells, acting on target cells to induce their function and effect (He et al., 2018; Kalluri and LeBleu, 2020). EVs come from a wide range of sources in the human body, and mediate communications and maintenance of cells (Doyle and Wang, 2019). Studies have

demonstrated that endothelial cells, glial cells, and MSCs can produce EVs (Frühbeis et al., 2013; Song H. et al., 2019; Gonçalves et al., 2020), and exists in plasma, urine, saliva and cerebrospinal fluid (Elsharkawi et al., 2019; Jain et al., 2019; Rahman et al., 2019; Mi et al., 2020). More importantly, they can protect and repair nerve injury. This article reviews the potential effects of ADSCs and ADSCs EVs in the treatment of ALS. In addition, the therapeutic effects focusing on ADSCs EVs and ADSCs delivery pathways and the benefits associated with repeated delivery will be described.

THE ADVANTAGES OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS TRANSPLANTATION

Adipose tissue is abundant in the human body, thus, obtaining ADSCs is convenient for autologous transplantation (Marconi et al., 2013). The safety of autologous ADSCs for the treatment of ALS has been reported in a clinical study (Staff et al., 2016). ADSCs protect and repair nerves through differentiation and paracrine. In comparison to bone marrow mesenchymal stem cells (BMSCs), ADSCs can secrete more glial cell-derived neurotrophic factor (GDNF) and fibroblast growth factor 2 (Otsuka et al., 2021). Noteworthy is that GDNF can promote axonal growth and the formation of functional synapses, while fibroblast growth factor 2 can enhance the survival, proliferation and differentiation of nerve cells (Zhu S. et al., 2020; Shamadykova et al., 2021). Regarding the extraction of ADSCs from different parts of the body, several differences exist. For instance, subcutaneous ADSCs express higher levels of fibronectin and vimentin, which contributes to their directional migration. The expression of stemness-associated genes, such as c-MYC, SOX2, KLF4, and NANOG in visceral ADSCs are significantly upregulated, indicating that visceral ADSCs have better differentiation ability than subcutaneous ADSCs (Ritter et al., 2019). The subsequent sections will explicate some of the biological functions of ADSCs transplantation.

Provision of Nutrition and Support

Previous studies have shown that ADSCs can protect neurons in neurological diseases, such as traumatic brain injury and cerebral ischemia, reduce secondary injury and improve neurological function (Ma et al., 2019; Paudyal et al., 2021). ADSCs can improve nutrition and support the survival of neurons in two ways. (1) ADSCs can be differentiated into neural cells to enhance the effects of nerve cell loss in the injured area. Notably, ADSCs obtained from dogs showed immense ability in differentiating into neurogenic lineage (Roszek et al., 2017). Also, a study showed that ADSCs can undergo neural differentiation induced by olfactory ensheathing cells or Schwann cell cultures (Lo Furno et al., 2018). ADSCs differentiated into neural lineage can highly express markers of neurons and glial cells, such as neurofilament H and glial fibrillary acid protein (Prpar Mihevc et al., 2020). The neural differentiation potential of ADSCs under different inducers also indicates that ADSCs could differentiate into different types of nerve cells

in complex living environment *in vivo*. ADSCs transplantation can hinder myelin degeneration to promote increased number of myelin fibers in the injured area of the peripheral nerve, and enhance the regeneration of neurons in spinal ganglion (Masgutov et al., 2019). Moreover, ADSCs can differentiate into Schwann-like cells and improve the regeneration of motor neurons in the repair of peripheral nerve injury. In addition, undifferentiated ADSCs can secrete growth factors and have a synergistic effect with Schwann cells (Kingham et al., 2014). (2) Transplanted ADSCs secrete nerve growth factors, BDNF, and GDNF. BDNF-tyrosine receptor kinase B (TrkB) is an important mechanism that maintains neuromuscular junction (NMJ) stability. Thus, decreased activation of TrkB may reduce the signal transduction in cells, resulting in the instability of NMJ, and subsequently leading to motor neuronal death (Just-Borràs et al., 2019). Besides, transplanted ADSCs can secrete BDNF (Kim et al., 2014). BDNF can augment the signal transduction of BDNF-TrkB in neuronal cells to maintain normal NMJ and delay ALS progression (Just-Borràs et al., 2019). Interestingly, BDNF-TrkB signal transduction promoted the proliferation of oligodendrocyte precursor cells, culminating in its increment and repair of myelin sheath (Geraghty et al., 2019). And the combination of ADSCs with GDNF enhances the survival of ADSCs and increases their ability to differentiate into neural-like cells. This implies that the sustained release of GDNF may be the reason for the pluripotent differentiation of ADSCs (Sun S. et al., 2020). CRISPR activation-engineered ADSCs can activate endogenous neurotrophic factor genes to induce the expressions of BDNF, GDNF, and nerve growth factors. These neurotrophic factors, in turn, can stimulate the proliferation of neurons and enhance the growth of neurites (Hsu et al., 2019). Furthermore, ADSCs can directly secrete neurotrophic factors or indirectly regulate local glial cells, and transform them into neuroprotective secretory phenotypes (Marconi et al., 2013). Following ADSCs transplantation, BDNF and GDNF expressions in the neural microenvironment are upregulated. BDNF and GDNF do significantly improve the survival environment of neurons. In addition, the stimulation of ADSCs with a combination of growth factors promoted the secretion of vascular endothelial growth factor-A and angiopoietin-1. These secretions led to enhanced axonal and blood vessel regeneration. More importantly, in the 4th week after the transplantation of ADSCs, TUNEL positive cells are significantly decreased, indicating that various factors produced by ADSCs could block apoptosis of motor neurons (Kim et al., 2014). Following the injection of green fluorescent protein labeled ADSCs, inflammation within the spinal cord was observed (Constantin et al., 2009; Marconi et al., 2013). This suggests that ADSCs could locate the site of injury. Therefore, ADSCs may improve the microenvironment of neuronal survival by directly or indirectly secreting regulatory factors, and stimulating their own neural differentiation. The differentiation and secretion of ADSCs have significant nutritional and supportive effects on nerve cells.

Inhibition of Inflammatory Response

Adipose-derived mesenchymal stem cells can secrete a variety of anti-inflammatory factors, inhibit the activation of inflammatory

cells, and repress the secretion of pro-inflammatory factors. Also, ADSCs promote the transformation of microglia and other cells to neuroprotective phenotype that in turn protects motor neurons from the influence of inflammatory environment and prevents motor neuronal degeneration and death.

Inflammatory markers, interleukin-1 β (IL-1 β), IL-6, IL-8, tumor necrosis factor- α (TNF- α), and TNF receptor-1, are significantly increased in ALS patients (Hu et al., 2017). Furthermore, the number of activated astrocytes and activated microglia increases in the damaged area of ALS. Also, there are inflammatory cells within the periphery, such as lymphocytes, mast cells, dendritic cells, and neutrophils, but the correlation between these inflammatory cells is not clear (Trias et al., 2018; McCauley and Baloh, 2019; McCauley et al., 2020). Astrocytes and microglia are key players in neuroinflammation. In particular, microglia release dysfunctional or broken mitochondria into the environment, which causes activated astrocytes to spread inflammation. The spread of inflammation leads to the degeneration and death of neurons (Joshi et al., 2019). Therefore, the inhibition and alleviation of the activation of glial cells and their signaling pathway can effectively reduce the occurrence of neuroinflammation.

Adipose-derived mesenchymal stem cells treatment downregulates the expression of inflammatory cytokines and the number of Iba1⁺ microglia, implying that ADSCs suppress microglia activation (Huang X. et al., 2020). Moreover, ADSCs induce microglia phenotype transformation from M1 to M2, and minimizes the release of pro-inflammatory factors (Angeloni et al., 2020). M2 microglia can secrete interleukin (IL)-4, IL-10, and IL-13. These pro-inflammatory factors play an anti-inflammatory role and can alleviate immune responses (Lyon et al., 2019). Moreover, the expressions of BDNF and TrkB are downregulated due to inflammation. However, ADSCs transplantation reverses these effects (Huang X. et al., 2020). BDNF-TrkB signal has been related to neuronal cell injury. The inhibition of this signal transduction may decrease the activated number of astrocytes and microglia to alleviate neuroinflammation (Ding et al., 2020). Monocytes/macrophages and CD3-positive lymphocytes at the site of neuroinflammation in ADSCs treated mice are markedly decreased (Marconi et al., 2012), implying that ADSCs can suppress neuroinflammation. The silencing of the nuclear factor erythroid 2-related factor 2 (Nrf2) expression downregulates HO-1 downstream expression, and increases the level of NLRP3 (Chen Z. et al., 2019). NLRP3 enhances IL-1 β secretion, which leads to neuroinflammation (Deora et al., 2020). Also, the expression of M2 microglia markers, Ym1 and Arg1, were increased after treatment with overexpressed-Nrf2 ADSCs (Huang X. et al., 2020). The above studies demonstrate that ADSCs could reverse nerve cell injuries and release inflammatory factors via the Nrf2/HO-1 signaling pathway.

Curtailment of Oxidative Stress Response

Oxidative stress is an important mechanism in ALS. ROS or reactive nitrogen species accumulated in cells can induce

oxidative stress. ROS mainly comes from damaged mitochondria in the motor neurons of ALS patients (Wang et al., 2019). During ALS, the microglia in the central nervous system highly express NOX2, which is a source of ROS (Seredenina et al., 2016). In oxidative stress, increased reactive oxygen species (ROS) triggers the loss of motor neurons as well as the proliferation of glial cells. In addition, the increase of intracellular ROS content induces the aggregation of TDP-43 and damages the mitochondria (Ohta et al., 2019; Zuo et al., 2021). Because oxidative stress considerably impairs nerve cells in ALS, it is important to eliminate ROS/RNS. Interestingly, the activation of the Nrf2 triggers the expression of antioxidant enzymes (Munguía-Martínez et al., 2019). In the cytoplasm, the Nrf2 binds to Kelch-like ECH-associated protein 1 (Keap1) to form a complex that inhibits the Nrf2 activity. When the Keap1-Nrf2 complex is stimulated by external IL-4 and IL-13, the binding of the Keap1 and the Nrf2, causing the release of the Nrf2 (Furue, 2020). The Nrf2 released into the cytoplasm gradually translocate to the nucleus. In the nucleus, the Nrf2 binds to the antioxidant response element (ARE) to activate downstream pathways (Zhang et al., 2019, 2020). In addition, phosphorylated SQSTM1 interacts with Keap1 to promote the release of Nrf2, which in turn upregulates the expression of Nrf2 targeted genes (Deng et al., 2020). ADSCs can express IL-6, and IL-6 combined with IL-6 receptor augments p62 expression in cells (Noman et al., 2020; Zhu et al., 2021). The increased p62 binds to Keap1, causing the release of Nrf2 (Sun Y. et al., 2020). The downregulation of Keap1 gene expression using the CRISPR/Cas9 system caused the release of Nrf2 from the outcome of ubiquitination and protein degradation. This provides a premise for Nrf2 to enter the nucleus. Immunofluorescence analysis did show the location of the Nrf2 to be the nucleus (Hu et al., 2020). Besides, increased nuclear Nrf2 can enhance the expression of antioxidant enzymes in ADSCs (Garrido-Pascual et al., 2020). MDA (an oxidative stress marker) level was decreased after Nrf2 expression in ADSCs (Hu et al., 2020), indicating the antioxidant capacity of Nrf2 and its downstream pathway. Interestingly, HO-1 expression was downregulated after the knockdown of Nrf2 expression, implying that HO-1 is a downstream product of Keap1/Nrf2. Moreover, the expression of endoplasmic reticulum stress-related protein is considerably increased after HO-1 knockout. This demonstrates that Nrf2/HO-1 pathway can inhibit endoplasmic reticulum stress and improve cell damage, while also curtailing ROS that is generated during oxidative stress (Xu B. et al., 2020). The schematic diagram of intracellular ROS elevation stimulating Keap1/Nrf2/HO-1 signaling pathway activation against oxidative stress is depicted in **Figure 1**. In the oxidative stimulation environment, ADSCs pretreated with 0.25 mM H₂O₂ expresses higher Nrf2 and related antioxidant enzymes than untreated ADSCs, resulting in better antioxidant effect. This shows that the complete expression of Nrf2 in ADSCs improves ADSCs own oxidative tolerance, while also promoting survival and proliferation. Moreover, it can counteract the secretion of oxidative stress factors (Garrido-Pascual et al., 2020). ADSCs can resist oxidative stress through Nrf2 and its downstream antioxidant proteins.

THE PROTECTIVE AND REPARATIVE EFFECTS OF EXTRACELLULAR VESICLES ON NEURONAL INJURY

Extracellular vesicles from different sources have disparate degrees of protection against nerve injury. For instance, astrocyte-derived EVs delivered GJA1-20k to reduce the damage to mitochondrial structure caused by neuronal injury and mitigate the degree of neuronal apoptosis (Chen W. et al., 2020). EVs of oligodendrocytes can be internalized by neurons, which may enhance neuronal metabolism and survival under the regulation of cell stress. In addition, EVs derived from M2 microglia and Schwann cells can also maintain intracellular homeostasis and promote neuronal survival by transmitting miRNA (Song Y. et al., 2019; Gonçalves et al., 2020). Several studies have shown that ADSCs-derived EVs have more significant neuroprotective effects, and immunostaining analysis demonstrated that neurons preferentially ingest MSCs-derived EVs (Guo et al., 2019). Therefore, ADSCs-derived EVs may be used as a potential therapeutic mechanism for nerve injury. Also, EVs can be used as biomarkers to detect and treat diseases (Jain et al., 2019), especially in ALS (**Table 1**). IL-6 level in astrocytic-derived EVs in the central nervous system of ALS patients gradually increases with the development of the disease, indicating its potentiality in being a biological marker for the disease (Chen Y. et al., 2019). Furthermore, miR-27a-3p in serum EVs in ALS patients is downregulated when compared with normal serum EVs, suggesting that EVs miR-27a-3p could be a detection marker for ALS (Xu et al., 2018).

The biological functions of MSCs EVs have been reported in several studies. Proteomic analysis of MSCs EVs did show most of them to contain proteins related to cell adhesion and apoptotic regulation, as well as proteins related to inflammatory response and myelination (Bonafede et al., 2019; Li et al., 2019). Noteworthy is that MSCs EVs could enhance cell viability by promoting the phosphorylation of AKT and ERK in periodontal ligament cells, and induce cell migration and proliferation (Chew et al., 2019). EVs have dose-dependent characteristics (Arslan et al., 2013; Chew et al., 2019), and can locate the damaged site. Notably, MSCs EVs labeled with gold nanoparticles evidenced their capabilities of crossing the blood-brain barrier (BBB) and migrating to the injured area under the influence of chemokines (Guo et al., 2019).

Extracellular vesicles secreted by ADSCs can promote the differentiation of M1 macrophages into M2 phenotype through miR-21 and CSF-1R, and improve vascular regeneration. Furthermore, ADSCs can secrete more EVs in hypoxic environment, which can strengthen its neuroprotective effect (Zhu D. et al., 2020). Also, ADSCs EVs can transfer miR-25-3p into target cells, and interfere with p53/BNIP3 activation, which can inhibit autophagy and reduce neuronal death (Kuang et al., 2020). This can significantly improve the nerve injury induced by excessive autophagy and promote the recovery of nerve function. Neurite growth is closely related with the formation of synapses. In Alzheimer's disease, the atrophy of neurites can cause neural dysfunction. A study showed that ADSCs EVs can promote the growth of neurites and participate in synaptic formation

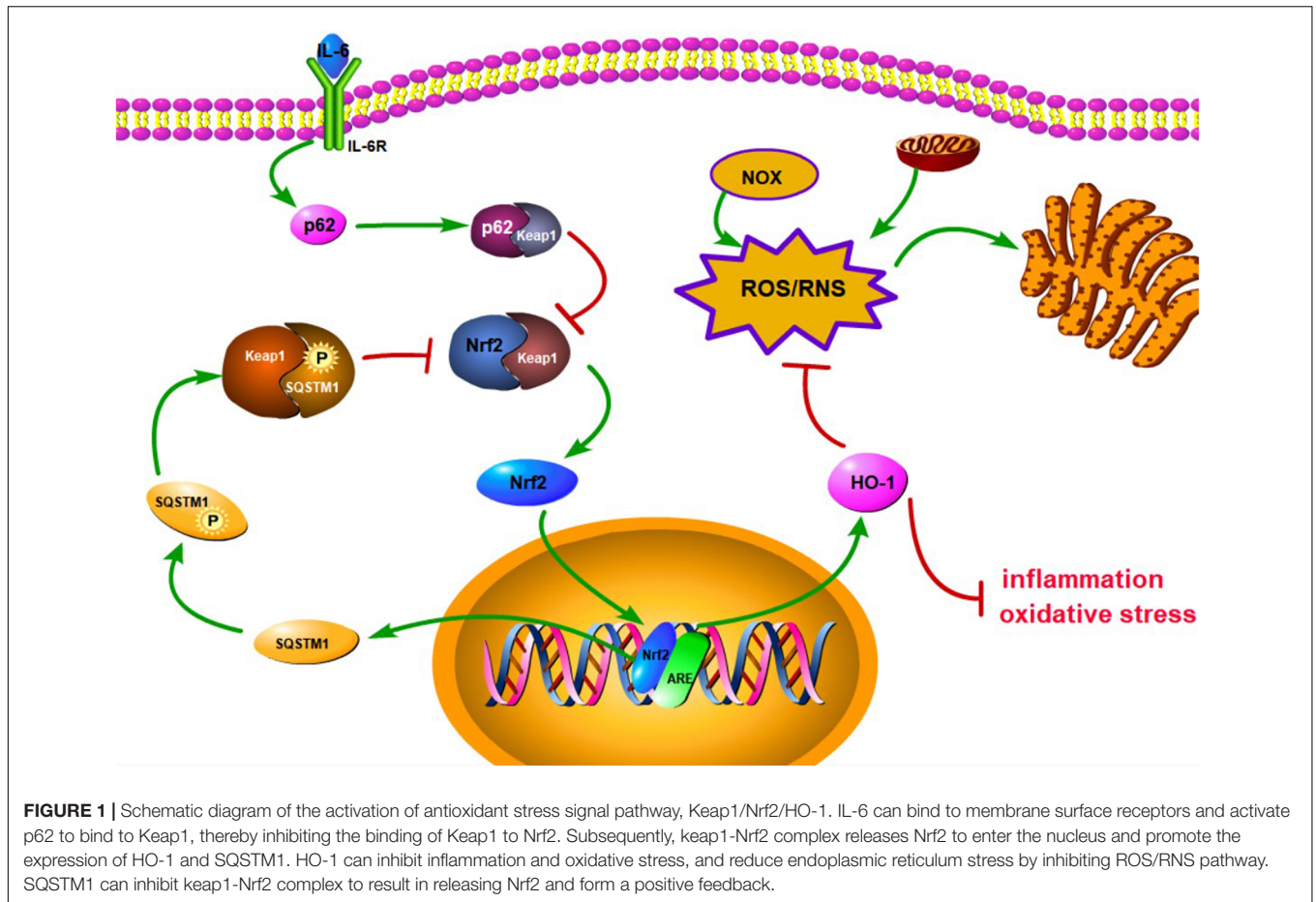


TABLE 1 | The potential biomarkers of ALS.

Exosomal source	Species	Alteration markers in ALS patients	ALS biological markers	References
Cerebrospinal fluid	Human	CUE domain-containing 2 (CUEDC2) and Ras-related protein Rab-11A (RAB11A) are highly expressed in cerebrospinal fluid exosomes of ALS patients	CUEDC2 RAB11A	Otake et al., 2019
Blood plasma	Human	miR-146a-5p in exosomes may lead to the loss of motor neurons, and the upregulation of miR-199a-3p affects neuronal regeneration	miR-146a-5p miR-199a-3p	Banack et al., 2020
Blood plasma	Human	The TDP-43 level in plasma exosomes of ALS patients is altered, and the NfL is related to the progression of ALS disease	TDP-43 NfL	Chen P. C. et al., 2020
Serum	Human	Compared with the healthy group, miR-27a-3p level in serum exosomes of ALS patients is decreased	miR-27a-3p	Xu et al., 2018
Central nervous system	Mouse	The presence of misfolded SOD1 in CNS derived exosomes in SOD1 ^{G93A} ALS mouse model can instigate ALS	SOD1	Silverman et al., 2019
Astrocyte	Human	IL-6 expression in astrocytic exosomes is increased in ALS patients	IL-6	Chen Y. et al., 2019
Spinal cord neuron	Mouse	miR-124-3p secreted by spinal cord neurons is increased in patients with advanced ALS	miR-124-3p	Yelick et al., 2020
Motor cortex	Human	Proteomics showed the contents of the vascular cellular adhesion molecule-1 (VCAM-1), Endoglin and Ras-related protein R-Ras (RRAS) to be downregulated in ALS	VCAM-1 Endoglin RRAS	Vassileff et al., 2020
Cerebrospinal fluid	Human	The proteasome in the cerebrospinal fluid of ALS patients is decreased, while bleomycin hydrolase expression is downregulated	Proteasome bleomycin hydrolase	Thompson et al., 2020

(Lee et al., 2018). Additionally, ADSCs EVs can increase the expression of Bcl-2 and reverse neuronal apoptosis. In intranasal administration, EVs combined with gold nanoparticle entered the brain region through the olfactory bulb, and inflammation triggered EVs to migrate to neurons in the injured area (Perets et al., 2019). Because EVs can pass through the BBB and migrate to the damaged site, they have significant protective effects on nerves. The overexpression of SOD1 mutant in motor neuron-like cell line NSC-34 caused oxidative damage to neurons in the H₂O₂ environment, though ADSCs EVs treatment reversed the cell damage in an anti-dose-dependent manner (Bonafede et al., 2016). This was in contrast to the study by Chew et al. (2019). Moreover, ADSCs EVs can increase the cell viability of motoneuron like NSC-34 cells transfected with ALS mutant SOD1 and reduce the excessive apoptosis caused by oxidative damage. Also, the ribonuclease RNase 4 contained in ADSCs EVs can play a neuroprotective role (Bonafede et al., 2019). EVs can transfer microRNA from BMSCs to target cells. EVs released by BMSCs, which are perfused with IFN- γ may significantly inhibit immune response (Giunti et al., 2021). Moreover, EVs can reduce the aggregations of TDP-43, SOD1, and FUS. In particular, ADSCs EVs treatment markedly improved the accumulation of SOD1 protein in G93A neurons. In addition, ADSCs EVs prevented the decrements of p-CREB and PGC-1 α contents in cells, leading to enhanced conduction of the p-CREB-PGC-1 α signaling pathway and mitochondrial function (Lee et al., 2016). Another study showed ADSCs EVs to improve the activity of mitochondrial complex I and membrane potential. Normal SOD1 in ADSCs EVs could neutralize the effect of mutant SOD1, reduce neuronal damage caused by oxidative stress, and exert neuroprotective effects (Calabria et al., 2019).

THE COMBINATION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS AND EXTRACELLULAR VESICLES IN IMPROVING AMYOTROPHIC LATERAL SCLEROSIS

The global prevalence of ALS is 4.42 per 100,000 people, and the incidence rate appears to be increasing annually (Xu L. et al., 2020). Owing to the increasing burden of ALS, the search for a more effective treatment to delay and improve its development has become imperative in recent times. Both ADSCs and EVs have been suggested as potential interventions for nervous system diseases. However, the bioavailability of EVs seems limited. Chew et al. (2019) showed that injected EVs are considerably reduced after 48 h, which might be due to the degradation of EVs or phagocytosis by cells, or the destruction of the structural integrity of EVs. Transplanted ADSCs may be influenced by the microenvironment, such as local ischemia and hypoxia, oxidative stress and nutritional deficiency that could lead to loss of their therapeutic effects (Shende and Gandhewar, 2020). Also, most of the transplanted cells might die within 1 week after injection, implying that the therapeutic effect of stem cell transplantation alone may be limited (Zhao et al., 2019). The above studies show

that the survival status of EVs or ADSCs after injection could be affected by the immune and microenvironment in the body, and hence the therapeutic efficacy of the sole use of either EVs or ADSCs may be limited. Therefore, the combination of ADSCs and EVs could present an effective way to improve the local microenvironment and minimize their loss after injection.

Intravenous and intranasal administration are common methods of delivering EVs. A minimal dose of EVs intravenously administered crosses the BBB to the brain, although the efficiency is not as great as expected (Tian et al., 2018). However, a study showed that EVs enter the brain through the olfactory bulb in the nose (Perets et al., 2019). Some of the EVs via the intranasal route pass through the neural channel formed by olfactory ensheathing cells, bypass the BBB to reach the cerebrospinal fluid, and resulting in EVs distribution in the brain. Also, some EVs pass through the systemic circulation, through the BBB to reach the target area (Agrawal et al., 2018; Bahadur et al., 2020; Hayes et al., 2021; **Figure 2**).

Different transplantation methods have different effects on neurodegenerative diseases (**Table 2**). Intrathecal injection of ADSCs expresses higher levels of C-X-C chemokine receptor type 4 (CXCR4). Interestingly, CXCR4 can bind to stromal cell-derived factor-1 α (SDF-1 α) that are expressed at the injured site, and promote the migration of ADSCs to the damaged site (Ji et al., 2020). Furthermore, ADSCs can relieve neuropathic pain (Jwa et al., 2020). Noteworthy is that intravenous injection does not enhance the passage of ADSCs through the BBB. Although intrathecal injection of ADSCs can pass through the BBB, factors such as gravity and normal circulation of the cerebrospinal fluid can prevent the ADSCs from entering the deep part of brain parenchyma, which will then intricate the required therapeutic concentration of the stem cells (Duma et al., 2019). With most of the ADSCs ending up in the blood vessels of the lungs (Harting et al., 2009), the proposal of a new strategy (i.e., the intracerebroventricular injection) has been suggested (Duma et al., 2019). Although intracerebroventricular injection could impair the blood-brain barrier, it may enhance the conveyance of ADSCs into the brain parenchyma, and thus, increase its level for the patients' benefit. Also, by protecting motor neurons and secreting neurotrophic factors, the repeated injection of MSCs effectively prolonged the life span of ALS mice and reduced the deterioration of motor function when compared with single injection (Magota et al., 2021). Therefore, in ADSCs treatment, multiple administration may be more effective in improving the pathological degree of ALS.

MiR-21-encompassed ADSCs EVs could upregulate SDF-1 α expression in surrounding cells (An et al., 2019), and EVs derived from ADSCs overexpressing Sirtuin 1 may promote SDF-1 α expression in endothelial progenitor cells (Huang H. et al., 2020). Besides, SDF-1 α has a significant inducing effect, which can enhance the migration of ADSCs to the damaged area. The involvement of ADSCs in tissue remodeling has been showed recently (Zheng et al., 2020). Motor neurons within the spinal cord expresses higher SDF-1 α protein, also known as C-X-C motif chemokine ligand 12 (CXCL12). Intriguingly, the level of CXCL12 increases during the progression of ALS, though the increased content is small. Hence, it cannot play a strong

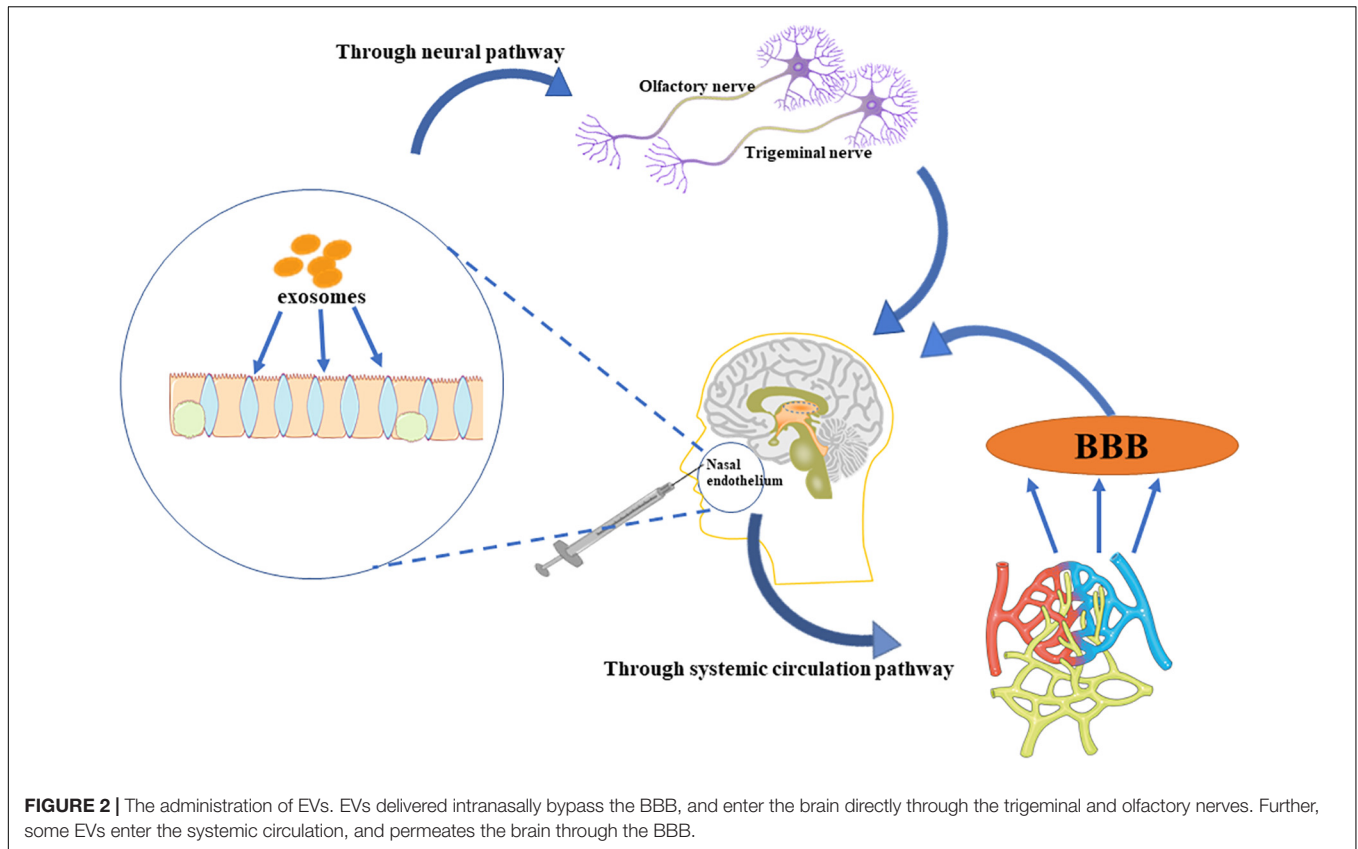


TABLE 2 | The different injection methods of ADSCs to ameliorating neurodegenerative diseases.

Diseases	Route of administration	Species	Results	References
Traumatic brain injury	Intravenous injection	Rat	ADSCs regulate inflammation and improve surrounding environment in early stages of trauma	Ruppert et al., 2020
Spinal cord injury	Intrathecal injection	Human	ADSCs injection improve the neurological function of patients, along with continuous recovery in 2 months	Hur et al., 2016
Spinal cord injury	Intravenous injection	Mouse	Most ADSCs remain in the spleen and thymus, with a small amount entering the spinal cord. Also, high dose injection does not cause tumor	Ra et al., 2011
Alzheimer's disease	Intravenous injection	Rat	ADSCs can migrate to brain tissue and improve learning and memory functions, and melatonin can significantly increase ADSCs effect	Nasiri et al., 2019
Alzheimer's disease	Intracerebral injection	Mouse	Intravenous injection reduces amyloid deposition and promotes synaptic stability	Chang et al., 2014
Parkinson's disease	Intracerebral injection	Mouse	Transplanted ADSCs can secrete GDNF to promote their survival and differentiation. However, ADSCs activity decreases with time	Sun S. et al., 2020
Parkinson's disease	Intravenous injection	Mouse	ADSCs transplantation upregulates the expressions of GDNF and BDNF, leading to the enhanced survival of dopaminergic neurons. More importantly, repeated injection can enhance these effects	Park and Chang, 2020
ALS	Intrathecal injection	Human	High-dose injections are tolerable and safe, but may cause pain	Staff et al., 2016
ALS	Intravenous injection	Human	Intravenous injection of ADSCs prolongs the life of patients and improves ALS symptoms	Shigematsu et al., 2021

inducing role, and can only be used as a supplementary diagnostic biomarker of ALS (Andrés-Benito et al., 2020). BMSCs expresses higher CXCR4 levels after hypoxia treatment. Therefore, the upregulation of CXCL12 expression in injured neurons may induce MSCs to reach the injured site of motor neurons.

Besides, activating the CXCL12/CXCR4 pathway could promote neuronal repair and regeneration (Hu et al., 2019). Hence, by injecting ADSCs EVs, cells can express more SDF-1 α (CXCL12). Upregulated expression of SDF-1 α can enhance ADSCs homing effect, which can cause its migration to the damaged area.

The combined treatment of ADSCs and EVs has been applied in several diseases. A study showed that the combined treatment of ADSCs and EVs significantly improved the neurological damage after ischemic stroke (Chen et al., 2016). In addition, both anti-inflammatory and antioxidant stress effects formed following the combined treatment was better than the single treatment (Chen et al., 2016). In a mouse skin defect model, the combined treatment of ADSCs and EVs considerably enhanced angiogenesis, inhibited scar, and promoted wound healing. More importantly, the combined treatment of intravenous injection of ADSCs and EVs was better than the single therapy (Zhou et al., 2021). In other studies, the combination of ADSCs and EVs mitigated kidney injury after ischemia-reperfusion and safeguarded the functional and structural integrity of the kidney (Lin et al., 2016), while also playing a protective role by decreasing myocardial infarction insult (Wang et al., 2020). On the basis of the outcomes from the above investigations, we believe that ADSCs combined with EVs could be a potential intervention for the treatment of ALS. However, the advantages and disadvantages of the different injection methods need further investigations. Also, strategies related to enhancing the target effects following the combined use of EVs and ADSCs need to be explored.

Clinically, intrathecal injection of ADSCs is relatively non-invasive, and can alleviate the degree of spinal cord injury and improve nerves (Bydon et al., 2020). Far more, the safety of intrathecal injection of ADSCs has been documented, with the absence of tumorigenicity being reported (Staff et al., 2016). ADSCs come from a wide range of sources and are less difficult to extract, thus, the cost involved in acquiring ADSCs is low (Harasymiak-Krzyżanowska et al., 2013). Intrathecal injection of ADSCs may improve the living environment of ALS motor neurons. This mitigates degeneration and motor neuronal death (Ciervo et al., 2021). Also, intracerebroventricular injection can improve the amount of ADSCs that enters the injured brain injury site of ALS patients, with its safety having been reported in a clinical study (Duma et al., 2019). Interestingly, multiple infusion can significantly minimize dyskinesia in ALS. A study showed CD36 could be used as a specific marker of ADSCs, and both CD271 and CD273 may be used to distinguish BMSCs, which is conducive to the standardization of clinical ADSCs production and ensure patients' safety (Camilleri et al., 2016). However, long-term culture may reduce the differentiation and nutritional activity of ADSCs, thus, necessitating the need for

future investigations to address this problem. Also, a study showed that ADSCs can be separated from the culture medium and survive in normal saline for at least 3 days (Ra et al., 2011), further illustrating the feasibility of ADSCs in clinical treatment. In early clinical trials, the application of MSCs EVs inhibited inflammation and promoted the integrity of lung barrier. However, more cells were needed to produce EVs, making the treatment cost of EVs being relatively high (Liu et al., 2020). Yang et al. (2020) reviewed the available methods for EVs separation. This is paramount to the development of efficient EVs separation technology, which may reduce the cost and contribute to the clinical application of EVs on a global scale (Yang et al., 2020).

In summary, the combined usage of ADSCs EVs and ADSCs may mitigate the degree of motor neuronal degeneration and delay ALS progression.

CONCLUSION

A number of studies have evidenced that ADSCs and ADSCs EVs can protect and repair nerve injury in ALS. Moreover, clinical trials have shown that different delivery methods may promote ADSCs and EVs to enter the brain and spinal cord injured regions. ADSCs EVs can enhance the survival and proliferation of ADSCs. Furthermore, ADSCs EVs can form chemokines to induce the migration of ADSCs. Although some amounts of ADSCs and EVs are lost after delivery, the usage of combined therapy via multiple injection methods as well as repeated injections could counteract this problem. ADSCs are easy to obtain and can be used in the treatment of autologous and allogeneic transplantation. Besides, in hypoxia conditions, cells may secrete more EVs. Through *in vitro* isolation and modification, EVs have stronger effects. Therefore, the combinational employment of ADSCs and ADSCs EVs may be a potential treatment for ALS.

AUTHOR CONTRIBUTIONS

XL designed the study. XW, YZ, TJ, BB, RF, LW, and XL prepared the first draft of the manuscript and revised the manuscript. All authors approved the final manuscript.

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