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Mesenchymal Stem Cells in the Treatment of Amyotrophic Lateral Sclerosis

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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder which is characterized by motor neuron (MN) dysfunction, progressive paralysis, and death. Although several therapeutic approaches have been used for treatment of ALS, little success has been achieved. Natural vectors such as mesenchymal stem cells (MSCs) can be a promising tool for overcoming therapeutic problems. MSCs have multipotential characteristics such as the ability to differentiate into variety of cell types, easy access, immunomodulation, tissue repair, exertion of trophic factors, exosome secretion and efficient homing. In this review, we will discuss the characteristics of MSCs and their possible therapeutic mechanisms in ALS patients.

Keywords: Amyotrophic lateral sclerosis, mesenchymal stem cells, stem cell, treatment.

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rare fatal neurodegenerative disease which is characterized by the loss of upper and lower motor neurons resulting in muscle weakness, progressive paralysis and mortality 3 to 5 years after the clinical onset due to respiratory failure [1]. It is estimated that about 30,000 people in United States are struggling with ALS [2] with a higher incidence rate in men [3]. ALS can be divided into two forms, including sporadic ALS (sALS) with 90% prevalence and the familial ALS (fALS) affecting only 10% of patients with a possible link to some genetic mutations [4]. Up to now 13 genes have been introduced that can affect ALS inheritance. *SOD1* (encodes for copper/zinc ion-binding superoxide dismutase), *TARDBP* (also known as *TDP-43*; encodes for TAR DNA binding protein), *OPTN* (encodes optineurin), *ANG* (encodes angiogenin, ribonuclease, RNase A family, 5) and *FUS* (encodes fusion in sarcoma) were clinically important. Mutated *SOD1* is the leading cause for 20% of familial ALS, although 5-10% of familial ALS mutations are related to *TARDBP*, mutations in *FUS* stands for 5%, and mutations in *ANG* is found in around 1% of ALS patients [5].

The presence of blood brain barrier (BBB) is the main problem in the treatment of central nervous system (CNS)-related diseases such as ALS [6]. The newly designated methods especially drug delivery vectors may help to solve this problem. There are two types of drug delivery tools, including synthetic and natural vectors. In general, synthetic vectors are products of chemical reactions from lipophilic compounds. Liposomes are the main type of synthetic tools utilized by researchers, however, regarding their possible side effects such as immunogenicity, accumulation in tissues and unknown consequences of this method, applying natural vectors instead is being considered [7].

Bacteria, viruses and stem cells are the most frequent vectors which have been used as drug delivery tools, however, application of stem cells is more interesting. Stem cell therapy as a branch of regenerative medicine is a method of employing natural vectors as therapeutic drug delivery systems [8, 9]. Among the cellular vectors that have been used for treatment of medical conditions, embryonic stem cells (ESCs) and adult stem cells (ASCs) are more popular. Regarding the ethical issues of ESCs, their employment is usually associated with limitations [10-12]. Therefore, ASCs are more attractive for clinical research. Adult stem cells (ASCs) especially mesenchymal stem cells (MSCs) have several features which make them an ideal option for drug delivery purpose. MSCs are multipotential [13] tissue resident cells [14] which have high capacity of differentiation into specialized cells especially mesodermal-derived types, however differentiation to other lineages is also possible [15, 16]. MSCs exhibit their therapeutic functions through different mechanisms such as tissue repair [17], immunomodulation [18], exertion of trophic factors [19] and efficient homing [20].

As there is no definite cure for ALS, transplantation of naive or engineered MSCs (EMSCs) may be considered as novel promising approach for treatment of ALS. There is evidence which shows EMSCs can control the disease pro-

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gression by several mechanisms in ALS animal models [21]. In this review, we will discuss about the new advances in biology of MSCs and their role in the treatment of ALS.

2. MSCs CHARACTERISTICS

The friedestein's group described the unique characteristics of MSCs for the first time, which has been further completed by Owen and coworkers [22]. MSCs are fibroblast-like, multipotent progenitors which have self-renewal capacity and high differentiation rate [23]. They usually differentiate into mesodermal-derived cells, however, they can also generate other cell lineages including endoderm and ectoderm-derived ones [15, 24-26]. Bio-preservation of MSCs is possible and during preservation they revealed minimum loss of potency [27]. It has been reported that MSCs are present in every vascularized tissues [28]. They can be easily isolated from bone marrow, adipose tissue, umbilical cord, derm, pancreas, liver, muscles and lungs. Moreover, under specific conditions, expansion of MSCs will be possible [29-32].

3. MSC ISOLATION

Due to lack of consensus marker for purification of MSCs, the International Society for Cellular Therapy (ISCT) has introduced minimum criteria containing adhesion to plastic surfaces and positive and/or negative expression of some markers. It has been demonstrated that MSCs can express molecules such as CD73, CD90 and CD105. On the other hand, CD34, CD45, CD19 and HLA class II molecules do not express on MSCs [33, 34]. In addition, presence of different surface receptors will be helpful for their isolation. Recently, the application of monoclonal antibodies for isolation of MSCs has become popular. One of the most useful monoclonal antibodies for this purpose is against Stro-1 [35]. Several markers beside Stro-1 have been proposed for isolation of MSCs, including CD146 (MUC-18), CD271 (low affinity nerve growth factor) [35] and the embryonic stem cell marker (SSEA-4) [36]. Other properties related to MSCs such as phenotypic difference [37, 38], required growth factors [39], chemokine [40, 41] and cytokine [42] receptors, cell-matrix and cell-cell receptors [43] may also be helpful for purification of different MSCs from different organs [35]. As the *in vitro* expansion of MSCs is associated with lower genetic abnormalities compared to other stem cell types, it seems that the rate of malignancy induction by MSC therapy is slight [44].

4. MSCs ORIGIN

Due to lack of discriminating markers, isolation of MSC progenitors is very difficult. Little is known regarding the genetic mechanisms behind the lineage differentiation management in MSC progenitors. Identification of these mechanisms may lead to the production of MSCs from ESCs [45].

As bone marrow aspiration method is very invasive, investigation for identification of new sources for isolation of MSCs is going on [46]. As mentioned previously wide range of organs are suitable for MSCs separation, however, umbilical cord blood [47], placental tissue [48] and adipose tissue [49] have been considered as potent substitutions of BM-MSCs. Despite the existence of phenotypic similarities between MSCs derived from different organs, they differ in function which is in part due to organ specific niche [47-49].

5. THERAPEUTIC MECHANISMS OF MSCs

As mentioned previously, the therapeutic mechanisms of MSCs are in part through the excretion of trophic factors, immunomodulation, anti-inflammatory effects, efficient homing and exosome secretion. Cooperation of these therapeutic mechanisms will lead to tissue repair and disease attenuation. Here, we will discuss each mechanism.

5.1. Generation of Trophic Factors

MSCs can generate several trophic factors and natural proteins which are crucial for neuronal survival. Interaction of MSCs with TLR ligands, inflammatory cytokines and conditions such as hypoxia are the main stimulators of various trophic factors secretion [50]. The majority of trophic factors are mediators of angiogenesis and apoptosis inhibitors [51, 52]. Among the MSCs-derived growth factors, brain-derived neurotrophic factor (BDNF) [53], cilliary neurotriphic factor (CNTF) [54], glial derived neurotrophic factor (GDNF) [55], nerve growth factor (NGF) [56], insulin-like growth factor1 (IGF-1) [57], and vascular endothelial growth factor (VEGF) play an important role in neurons replenishment and protection [58]. Transferring of growth factors which are usually large peptides to the CNS faced with an BBB obstacle [59]. It has been shown that genetic-modified stem cells generate neurotrophic and growth factors which are essential for MN regeneration through different mechanisms such as clearance of toxic compounds and production of glial cells [60].

5.2. Immunomodulation

It has generally been accepted that MSCs have potent immunomodulatory functions [61]. It has been demonstrated that MSCs can shift immune response from TH1 (cell mediated) toward TH2 (humoral) [62]. It has also been suggested that MSCs may inhibit the production of $CD4^+$ T cell cytokines such as IL-2 and IFN-γ. The induced anergy in T cells following coculture with MSCs is in part due to the lack of costimulatory molecules on MSCs. This coculture may even lead to generation of suppressor T cells [63, 64].

The controversial results have been reported about the effect of MSCs on B cells. Suppression of B cell proliferation and stimulation of antibody secretion are some of MSCs effects on B cells [65]. It has been suggested that MSCs inhibit NK cells cytotoxic activity through down regulation NKp30 and NKG2 receptors [66].

The hMSCs exude several immunomodulatory factors such as IDO (Indoleamine 2,3-dioxygenase), Semaphorin-3A, B7-H4, HLA-G, LIF(Lukemia Inhibitory Factor), TSG6(Tumor necrosis factor-inducible gene 6 protein) and Galectins. These immunosuppressing factors can inhibit inflammatory process and induce T regulatory cells, reversibly [67].

5.3. Anti-Inflammatory Effects

The anti-inflammatory function of MSCs is in part due to up regulation of TGF-β. MSCs can also inhibit inflammatory

process through reduction of oxidative stress and peroxide dismutase secretion [68]. MSCs engraftment on the third day after spinal cord injury resulted in shifting of macrophage phenotype from M1 toward M2 [68]. M1 macrophage belongs to pro-inflammatory group whereas M2 phenotype defined as anti-inflammatory ones.

5.4. Efficient Homing

There are several problems regarding tracking of MSCs homing *in vivo*, including lack of discriminating markers and low concentration of MSCs in blood stream [69]. It has been shown that MSCs can infiltrate into different injured tissues such as ischemic brain injury [70], myocardial infarction [71] and acute renal failure [72]. Although expression of several chemokine receptors including CCR1, CCR4, CCR7, CCR9, CCR10, CXCR1, CXCR3, CXCR4, CXCR5 and CX3CR1 has been observed [40, 41], the predominant chemokines in MSCs homing are not recognized yet. Among the chemokines responsible for leukocytes homing, stromal derived factor -1 (SDF-1) has been studied more precisely [73]. It has been shown that SDF-1 is the main mediator of stromal progenitor cells migration into injured tissues in a rat model of myocardial infarction [74]. Abott and colleagues showed that SDF-1 was over expressed in ischemic muscles following myocardial infarction, which enhanced the mobilization of endothelial progenitor cells [75]. Subsequently, Ip *et al.* exhibited that β1 integrins (but not CXCR4) are the main molecules in MSCs homing [76].

The efficient homing of MSCs is under control of three molecular groups including chemokines (particularly CXCR4, CXCL12, CCL/R2), adhesion molecules (such as integrins), and matrix metalo proteases (especially MMP-2) [77, 78]. The transplantation of CXCR4 transfected-MSCs into rat model of myocardial infarction has been associated with ameliorative effects [79]. Furthermore, *in vitro* and *in vivo* analysis of MSCs revealed that P-selectin, vascular cell adhesion protein-1(VCAM-1) and very late antigen-4(VLA-4) are the most important factors for interaction of MSCs with endothelial cells [80]. Surprisingly, injection of VCAM-1 transfected MSCs in contrast with naive MSCs enhanced homing into inflammatory sites in a mouse model of inflammatory bowel disease [81]. This experiment implicates a manipulation possibility of homing molecules in order to improve MSCs homing ability which can lead to better therapeutic outcomes. Furthermore, efficient homing can be affected by the route of MSC administration and origin of cells, for example intravenously administered MSCs mostly entrapped in lungs and showed little migration to the CNS [82]. Eliopoulos *et al.* demonstrated that allogenic MSCs could be successfully administrated without considering of immune recognition and rejection by host immune system [83].

The cell confluence and number of passages are two most important factors in culture of MSCs. It has been shown that increased conflunecy in cultured MSCs leads to secretion of inhibitory compounds like TIMP-3 (a natural MMP-inhibitor) and dysregulated trans-endothelial migration following injection. However, *in vitro* expanded MSCs in contrast with freshly isolated MSCs, do not express some critical receptors for homing, particularly CXCR4. Moreover, this loss can be compensated by cytokine cocktail in MSCs culture medium [69]. Choi *et al.* showed that approximately after ten passages, the ability of MSCs for neuroprotection, immunomodulation and differentiation has been decreased significantly, so which it seems that the use of freshly isolated MSCs must be a priority [84].

5.5. Exosome Secretion

One of the newly discovered aspects of MSCs is their high capacity of exosome secretion. Exososomes can be defined as secreted membrane vesicles with the ability to carry cargos such as proteins and RNAs for intercellular communications [85]. Tian sheng chen *et al.* have been shown that MSCs derived exosomes were enriched in pre-miRNAs. Their team detected 106 miRNA in MSCs [86]. MSCs exosomes has gained widespread attention not only for their specific composition but also for their successfully therapeutic outcomes in animal models and immunosuppressive activity. Yu B *et al.* reported that transferring exosomes derived from MSC overexpressing GATA-4 (MSC(GATA-4)) upon transplantation in injured cardiomyocytes of rat heart contributed to tissue protection by secreting different miRs, particularly miR-19a, working as inducers of cell survival signaling pathway [86]. In another study Amarnath S *et al.* showed that in a human-into-mouse xenogeneic graft-versus-host disease (x-GVHD) model, mediated by human CD4+ Th1 cells, BMSCs inverted experimental x-GVHD through marked inhibition of Th1 cell effecter functions. Serum CD73 expressing exosomes were detected in BMMSC recipients which promoted adenosine accumulation immunosupression [87]. In a recent study, transplanted MSCs in rats after traumatic brain injury (TBI) revealed functional recovery and neurovascular remodeling through endogenous angiogenesis and neurogenesis by reduction of inflammation process [88]. Nakamura Y *et al.* exhibited that MSC-derived exosomes were able to enhance myogenesis and angiogenesis and induce muscle regeneration, by miRNAs such as miR-494. Therefore MSC derived exosomes could be a useful cell-free therapy tool for different types of neurological disorders [89].

6. ROUTES OF MSCs DELIVERY

There are two major drug delivery methods including remote delivery and direct delivery, which have their own advantages and disadvantages. The intramuscular, intraneural and intravascular delivery routes are examples of remote delivery. Although privileged options as noninvasiveness seems quite enough for efficiency of this method, high dosage of therapeutic agents is required. However direct injection and intrathecal delivery provide the chance to distribute greater therapeutic agents, they are highly invasive [90].

7. ALS AND CURRENT TREATMENTS

Obvious genetic linkage to point mutations in cytosolic Cu^{2+}/Zn^{2+} superoxide dismutase1 (SOD1) has been reported in minority of fALS. Several transgenic animal models which have mutations in different genes such as SOD1, fused in sarcoma (FUS), TAR DNA binding protein (TARDBP) and angiogenine have been generated for fALS [91, 92]. Unfortunately the exact etiology of sALS remains

elusive [5]. Destruction of MNs is complex and it is probably under control of diverse pathways. These pathways may lead to protein aggregation, axonal transport deficiency, changes in calcium concentration, impairment of mitochondrial functions and consequently cellular death [93, 94]. Current treatments for ALS are usually based on maintaining quality of life. Rilozule, baclofen, tizanadine, non-steroidal anti inflammatory drugs (NSAIDS), tramadol, physical/occupational and speech therapy, and anti-depressant medications are current treatments involved in ALS therapy [95]. All mentioned drugs have some side effects such as dizziness, elevated liver enzymes, granulocytopenia and weakness [96]. Unfortunately, none of the treatments applied for ALS patients are effective and rilozule (the only specific approved drug), is just useful for relieving symptoms and increase patients survival up to two or three months [97]. It seems that the occurrence of hundreds mutations in dozens of genes is involved in etiology of ALS, so which investigators should focus on more genes rather than SOD1. Thus, necessity of approaching to new methods is clear. Development of stem cell therapy as a branch of regenerative medicine, particularly MSCs application is growing rapidly in order to achieve appropriate treatment for ALS [97].

8. MSCs IN THE TREATMENT OF ALS

Recently rodents with G93A-SOD mutations showed conscription of endogenous neural progenitors in deteriorating lumbar spinal cord which was correlated with progression of ALS. This finding provides hopes for creating new therapeutic approach based on stem cells in order to decrease the disease progression. Consistently, it has recently been shown that intravenous, intrathecal, intracerebral and intraspinal administration of MSCs to G93A-SOD1 mice led to advanced motor function, decreased loss of MNs and long time survival [98, 99]. Moreover, intraspinal administration of MSCs to ALS mouse model was decreased the microglial activation [98]. Interestingly, the intravenous injected MSCs to ALS mice could effectively differentiate to neural lineage (as was neurogenin 1 positive), and improve motor function and delay disease onset [100]. Forostyak *et al.* demonstrated that administration of hMSCs in SOD1 rats was associated with prolonged survival, remodeled gene expression paradigm, neurotrophic effects and immunonomodulation [101].

The intracerebroventricular administration of glucagon-like peptide1(peptide with antioxidant functions)-transfected MSCs to G93A-SOD1 mice led to delayed disease onset, generation of astrocyte, microglial activation, decreased neuroinflammation and prolonged survival [102]. Moreover, intramuscular injection of GDNF-transfected MSCs into G93A-SOD1 rats showed improved survival and healthy motor functions. The neuroprotective features of MSCs have been shown by evaluating their ability in secretion of glial-derived neurotrophic factor (GDNF), the vital factor which conserve MNs after brain injury [103]. Although differentiation of MSCs leads to responsiveness of cells to rilozule and upregulation of GDNF, however BM-MSCs in hSOD ^{G93A} with the same interventions showed moderate aspartate uptake and unresponsiveness to rilozule therapy [104]. Moreover, Krakora and colleagues showed that application of h-GDNF(human-GDNF) and h-VEGF(human-VEGF) synergistically increased life span [105].

The intraspinal administration of autologous MSCs into 11 Spanish ALS patients was associated with high rate of MN generation [106]. Moreover, it has been demonstrated that intraspinal administration of autologous MSCs was safe and no sign of toxicity or deformity has been showed by two years [107, 108]. Furthermore, there was no report regarding the toxicity or possible tumor generation in the similar studies performed in Israel, India, South korea and Mexico [109-112] (Fig. **1**).

A number of studies have been emphasized on systemic behavior of ALS with effects both on MSC function and motor neurons [84, 113, 114]. Consistently, it has recently been reported that BM-MSCs of ALS patients and healthy individuals could be different in several features despite of their identical cell surface markers and morphology. So which, analysis of Nanog, Oct-4 and Nestin-1 as accepted markers of pluripotency, revealed low expression of Nanog and Oct-4 in ALS MSCs (A-MSCs) [115]. In another study, comparison of ALS hMSCs with hMSCs showed the decreased expression of cytoplasmic FMR interacting protein 2 (CyFIP2) and retinoblastoma (Rb) binding protein 9 (RbBP9) in ALS hMSCs led to dysregulation of their translation [116]. Koh *et al.* have suggested that β-PIX, an intracellular factor involved in migration, is responsible for impaired migration of ALS-hMSCs compared to hMSCs [117]. These findings put some discrepancies in application of autologus stem cell therapy in ALS patients which suggests further investigations.

The main problem regarding the application of MSCs in ALS patients is their low differentiation rate to MNs. One solution for this problem is genetic engineering of MSCs. Consistently, it has been shown that the genetically engineered hMSCs for expression of MNs specific transcription factors such as Olig2 and Hb9, expressed higher levels of MN markers (30% of total cells) and was able to create connections with muscular fibers [118]. In another study, hypoxia-cultured human adipose-derived MSCs (hAMSC-H) applied in GBM showed no sign of tumor forming cooperated with improved viability, tropism and motility toward tumorigenic cells [119].

Kwon *et al.* reported that intrathecal injected MSCs in ALS patients exerted their action by recruitment of immune cells into CNS which was associated with shifting infiltrated cells to T regulatory and TH2 subpopulation. Secretion of IL-4, IL-10 and TGF-β by these cells is one of the indirect immunomodulatory effects of MSC therapy in ALS patients [120].

Regarding the decreased levels of TIMP in ALS patients, pathological upregulation of MMPs may be predictable. As mentioned previously, MMPs are one of the important molecules for MSCs homing into injured MNs. The administration of autologous MSC to patients may associate with three main outcomes including constitution of lost MNs, activation of endogenous neurogenesis and neuroprotective or neurotrophic effects [121].

It has recently been reported that MSCs-derived CX3CL1 can shift activated glial cells toward neuroprotective phenotype [122].

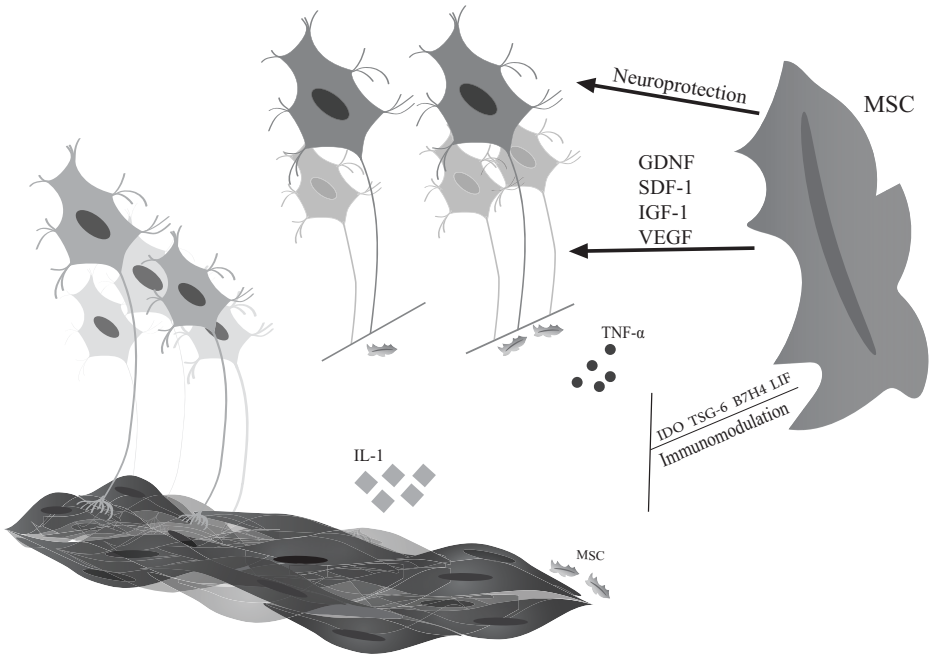

Fig. (1). During onset of ALS and progressive loss of neuromuscular connections, releasing of inflammatory cytokines such as TNF-α and IL-1 leads to recall of MSCs. Neuroprotective effects of migrated MSCs on degenereated neurons is managed by trophic factor secretion where as Immunomodulation aspects are presented by inflammation supressors.

Table 1. Studies related to application of MSC therapy in mouse model of ALS.

Species	MSC type	Interventions	Results	Reference
G93A-SOD1 murine	hBM-MSCs	Intravenous Intrathecal Intracerebral Intraspinal	Advanced motor function, alleviated loss of MNs, long time survival	[98, 99]
mdf/ocd murine	BM-MSCs	BM-MSCs intraspinal injection	GDNF secretion Neuroprotective effects	[103]
G93A-SOD1 murine	BM-MSCs	Autologous grafts	Moderate aspartate uptake Unresponsiveness to Rilozule	[104]
G93A-SOD1 murine	hBM-MSCs	Intraspinal	Moderating neuroinflammation and activation of microglial cells	[98]
G93A-SOD1 murine	hBM-MSCs expressing neurogenin-1	Intravenous	excellent homing to CNS, improvement in motor function and delayed onset of disease	[100]
SOD1rats	hBM-MSCs	Intrathecal	Rearrangement of gene expression, immuno-modulation, neurotrophic effects prolong survival	[101]
G93A-SOD1 murine	Engineered hBM-MSCs expressing GLP-1	Intra cerebro ventricular	generation of astrocyte, microglial activation, decreased rate of neuro inflammation and pro-longed survival	[102]
C57BL/6J mice	BM-MSCs	Analyzing MSCs and micro-glial interactions	Interaction of MSCs CX3CLand microglial CX3CR phenotypic and functional changes of microglia to neuro protective type	[122]
Sprague-Dawley rats	hBM-MSCs	Engineering hMSCs for high expression of olig-2 and Hb-9	Significant neuron features Ability to create connection with muscular fibers	[118]

SOD1: super oxide dismutase1, hBM-MSC: human Bone Marrow- Mesenchymal Stem Cell, MN: Motor Neuron, BM-MSC: Bone Marrow MSC, mdf/ocd: muscle-deficient osteo-chondrodystrophy, GDNF: Glial Derived Neurotrophic Factor, CNS: Central Nervous System GLP1: Glucagon-Like Peptide 1, olig-2: oligodendrocyte transcription factor gene, Hb-9: home box gene.

Table 2. Studies related to application of MSC therapy in ALS patients.

Species	MSC type	Interventions	Results	Reference
Human	BM-MSCs	Analysis of cells surface markers in ALS patients and healthy individuals	Identical surface markers and morphology Low expression of NANOG, Oct-4, Nestin-1 in ALS MSCs	[115]
Human	BM-MSCs	intrathecally intravenously	Extraordinary rise in CD4 CD25 T regulatory in peripheral blood	[120]
Human	hBM-MSCs	Intraspinal Intrathecal Intraventricular Intravenous	No sign of toxicity and possible tumor forming Prolong survival	[107, 108]
Human	Curcumin nanoparticle loaded hAMSCs	Intravenous	Low cytotoxicity	[123]

MSC: Mesenchymal Stem Cell, ALS: Amyotrophic Lateral Sclerosis, BM-MSC: Bone Marrow MSC, NANOG: transcription factor encoded by NANOG gene, Oct-4: Octamer-biding transcription factor4, hBM-MSC: human BM-MSC, hAMSC: human Adipose MSC.

Table 3. The underway clinical trials related to MSC-based ALS therapy (clinicaltrial.org).

Trial code	Route of injection	Intervention	Status	Country	phase
NCT01609283	Intraspinal	Autologous MSC	Recruiting	USA	I
NCT01142856	Intraspinal	AMSC	completed	USA	I
NCT01494480	Intrathecal	UCMSC	Enrolling by invitation	China	II
NCT01759784	Intraventricular	BMMSC	Not yet recruiting	Iran	I
NCT01759797	Intravenous	BMMSC	completed	Iran	I
NCT02017912	Intramuscular/ Intrathecal	BM MSC-NTF	Recruiting	USA	II
NCT02290886	Intravenous	AMSC	Recruiting	Spain	I/II
NCT01777646	Intramuscular	BMMSC-NTF	Active / not recruiting	Israel	II
NCT01051882	Intramuscular	BM MSC-NTF	completed	Israel	I/II

MSC: Mesenchymal Stem Cell, ALS: Amyotrophic Lateral Sclerosis, AMSC: Adipose MSC, UCMSC: Umbilical Cord MSC, BMMSC: Bone Marrow MSC, BMMSC-NTF: Bone Marrow MSC secreting Neurotrophic Factors.

Application of MSCs in combination with nanoparticles is another promising approach in the treatment of ALS. Tripodo *et al.* reported that targeted drug delivery of incorporated hydrophobic therapeutic agents in a systemic manner via MSCs is a new drug delivery approach which is named carrier in carrier method. The combination therapy with Inulin-d-alfa-tocopherol succinate micelles (INVITE M) and curcumin-loaded MSCs revealed low cytotoxicity compared to delivery of uncoated curcumin by MSCs [123] (Tables **1**, **2**).

9. MSC BASED THERAPY: TRANSLATION TO CLINIC

The several clinical trials regarding the use of MSCs transplantation in the treatment of different diseases have been registered at www.clinicaltrial.org. Myocardial infarction, graft versus host disease and diabetes are the main diseases that MSC therapy has been used for them.

There are about 30 trials related the CNS diseases such as Alzheimer, multiple sclerosis, Parkinson, glioblastoma, stroke and ALS. Recently 11 studies registered for ALS treatment by different types of MSCs, however most of them hired BM-MSC, UC-MSC and A-MSC [124] (Table **3**).

In all of the studies which have been done to evaluate MSCs possible therapeutic mechanisms, there are a number of questions which still remains unanswered and need further investigations. Among the struggling questions we can name the right origin of MSCs, exact route of delivery, best cell population and possible risk of oncogenesis, which have to be considered for each type of diseases.

The best cell numbers injection for optimal responsiveness in human disorders has not been calculated, yet. Optimal cell dosage must be considered as critical issue because of possible toxicity or tolerance mechanisms [125].

Allogenicity of MSCs didn't show any adverse effects after transplantation for tissue repair, however use of fresh MSCS is preferred because *in vitro* expansion of MSCs increases the expression of five major histocampatibilty complex class II genes [126, 127].

It has been shown that *in vitro* expansion of MSCs in order to reach adequate number of cells for therapeutic application can be led to malignant transformation of these cells. Consistently, Rubio *et al.* and Wang *et al.* achieved to deliberating data. Former group demonstrated that upregulation of c-myc and downregulation of p16 in human AMSCs will lead to transformation of cultured cells after 4-5 months [128]. However, in later experiment, cultured hBM-MSCs revealed the capability of tumor forming in NOD/SCID mice due to increased activation of telomerase, translocation and chromosome aneuploidism [129]. Regarding these controversial results, the possibility of oncogenesis by MSC based stem cell therapy remains as a conflicting field of study which requires further investigations. As mentioned previously, one of the essential terms of clinical application of MSC therapy is administration of adequate number of cell contents. Based on adverse consequences of increased passage rates, researchers generate MSCs with human telomerase reverse transcriptase gene (hTERT) which provides maintenance of their stemness aspects. Unfortunately genetic abnormalities is the main expecting side effect in this method which probably can become a leading way to oncogenesis [130]. Finally, making definite decision for this challenging item of MSC stem cell therapy needs further investigations.

CONCLUSION

In general the efficacy of MSC therapy in ALS patients is a matter of controversy. The application of MSCs in ALS patients has no ethical issues along with having benefits such as easy access, low risk of immunogenicity and multiple cell sources. Little is known regarding the therapeutic mechanisms exerted by MSCs in treatment of ALS. Improvement of MSC therapeutic functions through MSC engineering is novel promising approach in preclinical studies involved in ALS therapy. It seems that efforts have to be focused on creating the second generation MSCs with optimized activity in comparison with naive MSCs. Application of MSCs in clinic still needs further authorizations from governmental agencies about standard protocols for cell expansion, quality controls for safety issues and introduction of clinical monitoring parameters. We look forward to use MSCs products instead of whole cell injection in order to achieve an easy method of MSCs administration with high safety.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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