Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, which up to date remains incurable. Multiple experimental approaches toward finding an effective way of reducing ALS progression and improving patients’ condition have been proposed but none of them brought significant desired effects. In recent years, studies focused on stem cells (SCs) have proven that not only cells themselves but also trophic factors, which they secrete, may cause positive effects on neural tissue environment. Crucial issues that have to be considered in any study implementing SC’s secreted trophic factors are administration route and type of administered cells. Furthermore, the understanding of trophic factor function, secretion manner, and their potential influence on damaged cells may be immensely beneficial. This chapter focuses on recent studies exploiting trophic factors to improve ALS patients and animal ALS models’ condition.

Keywords: amyotrophic lateral sclerosis, trophic factors, neurotrophins, BDNF, GDNF, VEGF, IGF-1, GLP-1

1. Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease, is a fatal neurodegenerative disease, characterized by progressive loss of motor neuron functions in the spinal cord, cortex, and brainstem [1]. ALS was first described in the 1870s by the French neurologist Jean-Marie Charcot. The incidence of ALS ranges from 1 to 4 new cases per year. Typical first symptoms, which occur because of gradual loss of neuron functions, are muscle weakness, impaired reflexes, and speech difficulties. Patients in the later stages of the disease
may also suffer from cognitive impairments, among which aphasia and semantic dementia are the most frequently reported [2]. Eventually, in most cases, weakening of respiratory muscles results in death caused by asphyxiation within 3–5 years after diagnosis. From a pathophysiologic point of view, two forms of ALS are widely described—familial ALS (fALS) and sporadic ALS (sALS). fALS affects approximately 10% of patients, whereas sALS is responsible for the other 90%. Mutations in SOD1 gene, which encodes Cu/Zn superoxide dismutase 1, are common among patients with fALS, and more than 160 different mutations in SOD1 have been described to date [2]. However, recent studies have reported potential relationships between other genes and fALS, including those encoding transactive response DNA-binding protein (TARDBP), fused in sarcoma (FUS) and c90RF72 [3]. The precise cause of sALS has not been identified, but environmental factors, lifestyle, and genetic predispositions are considered as key factors in the disease development [4]. Evidence has demonstrated that especially exposure to tobacco smoke and the quantity of cigarettes smoked are significantly correlated with ALS [5]. Pathophysiological mechanisms that result in neurodegeneration involve protein aggregation, mitochondrial dysfunction, generation of free radicals, excitotoxicity, disrupted axonal transport, and dysregulation of neurotrophic factor levels [6]. Notably, no effective cure for ALS has been discovered, leaving newly diagnosed patients with no chance for complete recovery. The only remedy approved by the Food and Drug Administration (FDA) is riluzole, an antiglutamatergic agent, which may cause excitotoxicity reduction and, therefore, extend survival by up to few months [7]. Because of the lack of an effective treatment and the relatively late manifestation of first symptoms, often when neuron loss is already in an advanced stage, the search for new ways to manage ALS turns to stem cell (SC)-based therapy. SCs are considered to be a promising tool of modern medicine because of their ability to transform into almost any other cell type and their capability of an infinite divisions number. Two main directions in SC-based therapies for ALS are considered—“structural” cell replacement and humoral neuroprotection via secretion of trophic factors. The main issues that must be considered in all forms of SC-based therapies are the proper administration method, the optimal type of administered cells, and the identification of factors that are crucial for the survival and differentiation of transplanted cells [8]. Recently, the greatest effect evidenced from the studies implementing various types of SCs is their ability to improve neural tissue microenvironment and provide soluble neurotrophic factors rather than structural replacement of lost cells [9]. Accordingly, this review focuses on the role of trophic factors in ALS progression and the chance of implementing therapies that take advantage of their properties.

Two different types of adult SCs are under extensive investigation in the perspective of ALS therapy: mesenchymal stem cells (MSCs) and SCs connected with the organization of nerve tissue. MSCs are an attractive source for potential therapeutic approaches for several reasons. Firstly, MSCs are characterized by great plasticity [10] and can be easily obtained from different sources, including bone marrow [bone marrow stem cells (BMSCs)], adipose tissue [adipocyte stem cells (ASCs)], or umbilical cord blood [11]. Moreover, MSCs can differentiate into cells of all three germ layers (ectoderm, endoderm, and mesoderm) when cultured under specific conditions [12], and their expansion in vitro does not involve any changes in function or chromosome structure, as observed in cells obtained from ALS patients [13]. It has been also
found that MSCs can support regeneration of damaged parenchymal cells by scavenging toxic inflammatory cytokines and secreting trophic cytokines that are involved in neuroprotection [14]. Cells connected with nerve tissue organization are also being used in ALS research, including neural progenitor cells (NPCs), astrocyte precursor cells [15], and olfactory ensheathing stem cells (OESCs), which have been recently used in treatment of spinal cord injury [16]. Embryonic SCs have also been extensively investigated, but their implementation raises major clinical and ethical concerns. Although the physiological relevance of pluripotency is of significant importance, induced pluripotent stem cells (iPSCs) have been demonstrated to differentiate into a variety of cell types, including muscle, cardiac muscle, and hepatocyte cells. All of the abovementioned cell types might present different advantages when used in ALS management. Because ALS affects motor neurons at different levels and in various ways, attention is focusing more on restoring neuronal tissue homeostasis than on cell replacement. In addition, conditions in the adult spine do not favor the differentiation of transplanted cells into neural ones. However, it has been observed that the environment in spinal cord fluid from ALS patients stimulates transplanted MSCs to secrete factors that relieve ALS symptoms [17].

Figure 1. Classification of neurotrophic factors according to their structure and function. NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; GDNF, glial-derived neurotrophic factor; NTN, neurturin; ARTN, artemin; PSPN, persephin; EGF, epidermal growth factor; IGF-1, insulin-like growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; EPO, erythropoietin; VEGF, vascular endothelial growth factor; CNTF, ciliary neurotrophic factor; TNF, tumor necrosis factor.
These factors are mainly neurotrophins, proteins synthesized and secreted by the brain, spinal cord, and other cells that are dependent on peripheral sensory neurons. Neurotrophins are responsible for nerve growth and survival, synapse formation, and axonal growth [18]. In general, neurotrophins provide neuroprotection, thus slowing down neurodegeneration. Neurotrophins also prevent oxidative stress and inhibit apoptosis [19]. Neurotrophic factors can be categorized in different ways based on their activities in preventing neuronal cell death [19] or by their structural and functional affiliations (Figure 1). Accordingly, “classic” neurotrophins, ligands of glial-derived neurotrophic factor (GDNF), and neuroprotective cytokines can be distinguished. The group of classic neurotrophins includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) [20]. Furthermore, factors that are connected with more than just neural cells, such as IGF-1, also demonstrate potential as ALS therapies. Growth factors involved in vasculogenesis, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and angiopoietin, might also be correlated with ALS progression.

2. Trophic factors correlated with ALS

Previous studies have investigated the expression patterns of trophic factors in both in vitro cultures and animal models of ALS. Data accumulated in the last decade suggest that trophic factors are notable not only for monitoring ALS therapy and disease progression but also for potentially helping in ALS diagnosis. SCs, particularly MSCs, exhibit higher levels of NGF, BDNF, and VEGF expression after stimulation with protein extracts from the brains and spinal cords of transgenic SOD1 (G93A) rats (an animal ALS model) [21]. The fact of changing levels of these proteins at various disease stages demonstrates that they might be essential during ALS development and progression. Therefore, exploiting their potential in treatment is highly desirable. Unfortunately, their pharmacokinetic constraints, including their restricted ability to cross the blood-brain barrier (BBB) [22] and penetrate gray matter, limited bioavailability, and relatively short half-life [23], hinder their efficient use. Hence, other approaches of neurotrophin delivery, e.g., with viral vectors or by SCs grafts, are worth investigation. Recently, it has been reported that SCs derived from umbilical cord blood, an ethically sound source of SCs, express higher levels of neurotrophic factors when cultured under stress conditions [24]. Furthermore, in patients with ALS who received autologous transplant of bone marrow-derived stem/progenitor cells, slower disease progression was positively correlated with higher NTs expression levels [25].

The most promising studies aimed at assessing the involvement of neurotrophins and other growth factors in ALS and their potentials in ALS treatment are summarized below.

3. Brain-derived neurotrophic factor

BDNF was discovered in 1982 by purification from pig brain as a protein responsible for promoting survival of sensory neurons [26]. BDNF is considered to be the best characterized
neurotrophic factor in the central nervous system (CNS), mainly because of its crucial role in the maintenance of normal brain function. Similar to other neurotrophins, BDNF may interact via a common p75NTR receptor or a specific tropomyosin-related kinase receptor, TrkB [27]. After binding of BDNF to TrkB and autophosphorylation of the receptor, three signaling pathways are activated: the phosphatidylinositol 3-kinase pathway (PI3K), mitogen-activated protein kinase/extracellular signal-regulated protein kinase pathway (MAPK/ERK), and phospholipase Cγ pathway (PLCγ) [28]. Expression of BDNF is increased in the hippocampus, cerebral cortex, cerebellum, and amygdala of the brain [29]. BDNF has many functions during both the development and normal function of the mature human brain, such as promoting the elongation and growth of neurites and initiating axon formation [30]. Notably, effects exerted by BDNF on neural cells differ depending on the method of stimulation. Dendritic cells exposed to increasing levels of neurotrophin for a certain time exhibit enlarged branching, whereas acute, transient elevation of BDNF concentrations results in neurite elongation and spine head enlargement [31]. BDNF is also involved in the formation, stabilization, and modulation of synaptic connections [32, 33]. Consequently, BDNF is deeply associated with normal CNS functions and exerts a plethora of actions that are crucial for neural tissue survival. Therefore, impaired expression of BDNF appears to be implicated in many diseases connected with the nervous system, including ALS.

Preclinical studies performed on a mice model of ALS, SOD1 (G93A), demonstrated that BDNF promotes the survival of neuronal cells [18]. A study conducted in wobbler mutant mice (another ALS model) injected with recombinant human BDNF resulted in a slower rate of grip strength decrease and greater muscle tensions compared with control animals [34]. However, clinical trials based on BDNF injections in humans, both subcutaneous and intrathecal, have not demonstrated noticeable improvements in ALS patients’ conditions [35, 36]. Clinical trials with intrathecal delivery of BDNF were conducted in 2003 and 2005 but failed to demonstrate any meaningful effect of BDNF on motor function because of small study sizes [37, 38]. Recently, it has been reported that muscle progenitor cells (MPCs) expressing BDNF, GDNF, and insulin-like growth factor (IGF-1) injected into the legs of SOD1 mice delayed disease onset and even prolonged the lifespans of treated animals by 13 days. Decreases in neuromuscular junction degeneration and increased axonal survival were also observed [39]. However, another study observed no prolonged lifespan in ALS mutant rats after intramuscular transplantsations of human mesenchymal stem cells (hMSCs) modified by lentiviral infection to express BDNF. The same study reported that infection of the same hMSCs expressing GDNF and VEGF into the muscle tissue of SOD1 (G93A) rats caused improvement in survival and motor function of the subjects [40], suggesting that the positive effects observed in different studies implementing neurotrophins against ALS were exerted by trophic factors other than BDNF.

4. Glial-derived neurotrophic factor

GDNF was first isolated as a factor promoting the survival of dopaminergic neurons in embryonic ventral midbrain cell cultures [41]. GDNF also improves motor neuron functions
and decreases the rate of apoptotic dopamine neurons in vitro [43] and in vivo after an ischemic stroke [44]. In ALS patients, the levels of GDNF in the cerebrospinal fluid and muscle biopsies are increased [45, 46]. Compared with other NTFs (including BDNF, NT-3, and NT-4), GDNF exhibits almost 100 times higher efficacy in supporting the survival of spinal motor neurons [47]. GDNF also has beneficial effects on neuronal cells in SOD-1 (G93A) mice. NPCs expressing GDNF decreased the loss of motor neurons and stimulated changes in trophic factor expression in motor neurons of the spinal cord [23]. However, the same study did not observe any correlation between the presence of GDNF and the integration of neuromuscular junctions or disease progression [23]. In another study, hMSCs were genetically engineered to express GDNF and subsequently injected into three muscle groups. The injected cells were able to survive, graft, and further secrete GDNF. Moreover, an increase in the number of neuromuscular connections and motor neuron cell bodies in the spinal cord was also observed. As a result, hMSC-GDNF increased the lifespan of SOD-1 rats by up to 28 days [48]. Other studies using intramuscular injections of cells expressing GDNF from adenoviral transduction have also observed beneficial effects of GDNF on neurons in ALS animal models [49, 50]. These effects may be exerted by preventing apoptosis by preserving the Akt signaling pathway [51]. Taken together, GDNF seems to be a promising candidate for ALS treatment. However, there remain no clinical studies with this trophic factor in ALS patients. Moreover, GDNF may not have the ability to cross BBB [52], which compels the need to consider other routes of administration to the CNS, such as intracerebroventricularly.

5. Vascular endothelial growth factor

The first member of VEGF protein family was described from the discovery of vascular permeability factor (VPF), which is responsible for increasing tumor-induced vascular permeability [53]. VPF has also been observed in other cell types and named VEGF (now it is known as VEGF-A). VEGF is produced by many cell types, including macrophages, platelets, keratinocytes, and renal mesangial cells. Expression of VEGF initiates during gastrulation and is crucial for vasculogenesis and angiogenesis [54, 55]. In addition to the established roles of the VEGF family in angiogenesis and lymphangiogenesis, it has also been suggested that these proteins have prominent neurotrophic effects. VEGF family members are also involved in nerve migration [56] and axonal guidance [57] and protect cells from the effects of damaging agents, such as hypoxia, excitotoxicity, mechanical trauma, and serum deprivation [58–59]. In 2001, it was found that mice with deletion of the HIF-response (HIF - Hypoxia-Inducible Factor) element in the VEGF promoter present adult-onset progressive degeneration of motor neurons, similar to that encountered in ALS [60]. There have been several gene therapy approaches using VEGF in animal models of ALS. In one of the first studies, delivery of VEGF into the diaphragm, intercostal, facial, and tongue muscles before initial symptoms resulted in delayed ALS onset. Furthermore, improved motor function and prolonged survival were observed [61]. In another study, expression of VEGF was increased by the use of a zinc-finger protein (transcription factor) delivered with an adenoviral vector to primary motor neurons. It has been shown that increased VEGF expression in those cells increased stimulated axon
outgrowth and enhanced nerve regeneration overall [62]. Despite the fact that the abovementioned study was conducted in rats that underwent nerve-crush injuries and not in the SOD-1 (G93A) ALS model, their method of delivery has potential as an approach to regenerate neural cells in ALS therapy. To date, the only study which implemented zinc-finger proteins as transcription modulating factors in SOD-1 rats was based on the delivery of plasmid DNA to a single muscle and did not result in any difference in lifespan between treated and control animals. However, after six injections (one per week), better performance in behavioral tests was observed in the treated SOD-1 (G93A) group [63]. Of note, SCs have also been implemented as a method of VEGF delivery. Intrathecal transplantation of human neural stem cells (NSCs), which were modified to overexpress VEGF, significantly delayed disease onset and prolonged lifespan in ALS model mice [64]. Transplanted cells have also exhibited the ability to migrate to gray matter, integrate into the spinal cord anterior horn, and transform into motor neurons [64]. Further examination of the expression levels of proteins associated with apoptosis revealed that neuroprotective mechanisms might have been caused by apoptotic regulation. In control animals injected with PBS, expression levels of proapoptotic Bax and caspase 3 were significantly higher than antiapoptotic Bcl-2 and Bcl-xL, whereas the opposite effect was observed in subjects injected with NSCs expressing VEGF [64]. Intracerebroventricular delivery of recombinant VEGF in an ALS rat model improved motor performance and prolonged the survival of treated animals by up to 22 days [65]. VEGF also has the ability to protect the innervation of neuromuscular junctions (NMJs) in ALS [66]. Currently, there is one ongoing clinical trial that is based on intracerebroventricular administration of sNN0029 (rhVEGF165). To date, no results from this trial have been published.

6. Insulin-like growth factor 1

IGF-1 was discovered in 1957 as a factor stimulating the incorporation of sulfate into rat cartilage [67]. IGF-1’s insulin-like activity was described later [68], and the growth factor mediator of anabolic and mitogenic function was named insulin-like growth factor. IGF-1 is secreted mostly by the liver and acts as an endocrine hormone after transportation to other tissues [69]. The effects of IGF-1 on neural cells were first reported in 1987 when the expression of IGF-1 receptors was observed in CNS neural cells. Afterward, other studies have reported that IGF-1 is involved in signaling between developing muscle and motor neurons in spinal cord and induces proliferation and nerve sprouting in adult muscle [70]. Moreover, IGF-1 promotes GDNF actions and stimulates neuroprotection [71]. Preclinical studies testing IGF-1 in animal models have validated these abilities. SOD-1 (G93A) mice injected with an adeno-associated virus 2 (AAV2)-based vector encoding IGF-1 into their spinal cords presented delayed disease onset, slower weight loss, and increased survival in treated objects [72]. A similar study conducted in a rat ALS model employing AAV2 to deliver IGF-1 into the spinal cord reported a reduced loss of motor neurons [73]. In 2008, a clinical trial on IGF-1 with ALS patients was performed. In this study, 330 patients were subjected to subcutaneous injections of recombinant human IGF-1 daily for 2 years [74]. After 2 years, no differences in muscle function or survival rate were observed between treated patients and the placebo group. The
authors concluded that IGF-1 does not provide benefits for ALS patients. No preclinical studies have demonstrated any beneficial effects of subcutaneously administered IGF-1 either. Positive outcomes were only observed after intrathecal injections or when the trophic factor was delivered with a viral vector, indicating that the method of administration might be crucial for successful treatment. Another study conducted on nine ALS patients intrathecally injected with IGF-1 every 2 weeks for 40 weeks reported that their method of administration caused modest but significant beneficial effects [75].

7. Glucagon-like peptide 1

GLP-1 is an endogenous peptide responsible for controlling plasma glucose levels by stimulating insulin synthesis and its secretion from pancreatic β cells [76]. So far, little is known regarding the role of GLP-1 in the physiology of neural tissue. However, GLP-1 receptors have been observed on the neurons of various brain regions, including the hippocampus, cerebellum, and cerebral cortex [77], indicating that it is involved in proper neuronal function. GLP-1 protects neural cells from excitotoxicity [78] and participates in learning and memory processes [79]. All of these features make GLP-1 a potentially useful factor to investigate for ALS therapy. An in vitro study using motor neurons derived from Sod-1 (G93A) transgenic mice that were exposed to kainite (excitotoxic stimulus) reported that a synthetic analogue of GLP-1 exhibited neuroprotective effects [80]. The neuroprotective potential of GLP-1 has also been tested in a mouse model of ALS. Human MSCs were transfected with a plasmid vector encoding GLP-1, encapsulated and subsequently injected into the cerebral ventricle [81]. This method of treatment significantly prolonged the lifespans of treated animals (by 13 days), delayed symptom onsets, decreased weight loss, and caused improvements in motor performance tests. These data suggest that GLP-1 may become a new target for ALS therapeutic research. However, further in vivo studies optimizing administration route and delivery methods should be conducted in animal models before effective use in any clinical trial.

8. Conclusions

As long as the exact cause of ALS remains elusive, it will be hard to propose any fully effective and accurately targeted form of therapy. Therefore, clinical trials should consider the broadest spectrum of neuroprotection possible. However, the methods employed have not always been standardized based on the results of preclinical research in animal models, resulting in ambiguous results in different studies performed in ALS patients. Since trophic factors have the ability to support survival and strengthen the functions of neural cells, further, more extensive studies are required to precisely investigate the real role of humoral activity in the natural history of ALS. To date, the most promising effects appear to be those obtained in studies employing VEGF and GDNF; however, clinical trials conducted on large numbers of patients remain lacking.
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