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Role and Therapeutic Potential of Astrocytes in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is characterized by the progressive degeneration of motor neurons in the spinal cord, brain stem, and motor cortex. The molecular mechanism underlying the progressive degeneration of motor neuron remains uncertain but involves a non-cell autonomous process. In acute injury or degenerative diseases astrocytes adopt a reactive phenotype known as astrogliosis. Astrogliosis is a complex remodeling of astrocyte biology and most likely represents a continuum of potential phenotypes that affect neuronal function and survival in an injury-specific manner. In ALS patients, reactive astrocytes surround both upper and lower degenerating motor neurons and play a key role in the pathology. It has become clear that astrocytes play a major role in ALS pathology. Through loss of normal function or acquired new characteristics, astrocytes are able to influence motor neuron fate and the progression of the disease. The use of different cell culture models indicates that ALS-astrocytes are able to induce motor neuron death by secreting a soluble factor(s). Here, we discuss several pathogenic mechanisms that have been proposed to explain astrocyte-mediated motor neuron death in ALS. In addition, examples of strategies that revert astrocyte-mediated motor neuron toxicity are reviewed to illustrate the therapeutic potential of astrocytes in ALS. Due to the central role played by astrocytes in ALS pathology, therapies aimed at modulating astrocyte biology may contribute to the development of integral therapeutic approaches to halt ALS progression.

Keywords

astrocytes; gliosis; motor neuron; neurodegeneration; oxidative stress; amyotrophic lateral sclerosis

1. INTRODUCTION

In 1856 Rudolf Virchow coined the term neuroglia to describe the elements filling the space not occupied by neurons in the central nervous system (CNS) [1]. Neuroglia can be further classified in macroglia and microglia. The macroglia derives from the ectoderm and includes

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astrocytes (astroglia cells), oligodendrocytes and NG2-positive progenitors. Microglia, the resident macrophages of the CNS, have a mesodermal origin. The ratio of glia to neurons varies according to the region of the CNS and species studied. However, the traditional notion that glial cells far outnumber neuronal cells does not appear to be true, with the human brain containing about an equal number of glial and neuronal cells [2–4]. Regardless of the actual glia:neuron ratio in the human brain [5, 6], astrocytes constitute a large portion of the glial cell population in the CNS and perform a plethora of essential functions.

Astrocytes are a heterogeneous population that displays regional and functional diversity [7]. Early histological analysis classified astrocytes as protoplasmic (grey matter) and fibrous (white matter), but other special types of astrocytes can be identified in specific regions of the CNS or in certain species [8]. Protoplasmic astrocytes exhibit an intricate bushy or spongiform morphology due to many fine membrane processes that become evident when cells are filled with dye [9]. Protoplasmic astrocytes occupy extended spaces with a multitude of lamellar processes and establish exclusive territories. In the human cortex, a single astrocyte enwraps more than one million synapses and most, if not all, have at least one process with endfeet contacting a blood vessel. Consequently, astrocytes establish structural and functional compartments and are in a key position to provide structural, metabolic and trophic support to neurons [10, 11].

While this review will focus on the potential pathological role of astrocytes in ALS, it is worth stressing that astrocytes play a role in almost all physiological processes that ensure the wellbeing of neurons in the healthy CNS. For example, astrocytes regulate the volume and composition of the extracellular space (by controlling ion concentration, water movement and uptake of neurotransmitters), regulate the establishment and maintenance of the blood brain barrier, control cerebral blood flow in response to neuronal activity and shape synaptic connectivity. In addition, astrocytes regulate synaptic transmission in what has been called the “tripartite synapse” and play a key role in the integration and processing of synaptic information [12–15]. Lastly, the capacity of astrocytes to produce and deliver energy metabolites and antioxidants is essential for normal neuronal metabolic function [16–18]. Neuronal activity is a high-energy demanding process, and through the astrocyte-neuron lactate shuttle, astrocyte-derived lactate sustains the energy needs of neurons. In addition, glutathione metabolism in astrocytes boosts neuronal antioxidant defenses and protects neurons from increased oxidative stress resulting from neurotransmission and metabolic activity [16, 19]. Dysregulation of this astrocyte-neuron coupling, key for normal brain function, is likely to contribute to neuronal death during neurodegeneration.

Astrocytes respond to CNS damage, such as trauma, infection, ischemia and neurodegeneration through a process called astrogliosis [20]. Astrogliosis is generally characterized by upregulation of glial fibrillary acidic protein (GFAP), vimentin and re-expression of nestin. This is accompanied by hypertrophy of cellular processes, changes in astrocytic biochemistry and altered expression of proteases, cytokines and growth factors. Although traditionally thought as an all-or-none stereotypical response, astrogliosis is a complex remodeling of astrocyte biology. Some features of reactive astrocytes vary according to the nature and severity of the insult, as well as with their relative location to the site of damage. For example, severe astrogliosis leads to substantial presence of proliferating

astrocytes and the formation of a glial scar, while mild astrogliosis occurs without astrocyte proliferation or loss of exclusive territories [20–22]. In addition, astrogliosis occurs in the context of a multicellular response that involves the interaction of neuronal and non-neuronal cells (e.g. microglia, oligodendrocytes and endothelial cells) [23]. Due to the key role played by astrocytes in the support of neuronal and CNS function, through gain and loss of functions, reactive astrocytes most probably affect neuronal function and survival in an injury-specific way. In animal models of amyotrophic lateral sclerosis (ALS), astrocytes play a major role influencing motor neuron fate and disease progression.

ALS or Lou Gehrig's disease is characterized by the progressive degeneration of motor neurons in the spinal cord, brain stem, and motor cortex. Motor neuron death leads to muscle weakness and paralysis, usually causing death in one to five years from the time of symptoms onset. In the United States and the United Kingdom, ALS accounts for about 1 in 500 to 1 in 1,000 adult deaths [24] and current estimates indicate that the number of individuals with ALS will increase by more than two thirds worldwide between 2015 and 2040 [25]. Approximately 10% of the ALS cases present with a familial history of the disease (familial ALS, FALS) and are most frequently linked to a dominant mutation. The rest of the cases do not have a familial history (sporadic ALS, SALS) and may result from yet unidentified environmental exposure or genetic mutations [26]. The first ALS-linked gene identified was superoxide dismutase 1 (SOD1) [27]. Up to 20% of FALS and 1–2% of SALS cases are linked to SOD1 mutations. An intronic hexanucleotide repeat expansion in C9orf72 (orf 72 on chromosome 9) and mutations in TARDBP, FUS and OPTN (among other genes) have been identified in many FALS cases [26, 28–30]. Each mutated gene has its own genetic and molecular signature, but FALS and SALS are phenotypically indistinguishable and a significant share of our understanding of the disease comes from the study of rodents overexpressing ALS-linked mutant human SOD1 (hSOD1), which develop an ALS-like phenotype [31]. The molecular mechanism underlying the toxic gain-of-function of mutant hSOD1s remains uncertain. However, several lines of evidence suggest that toxicity to motor neurons requires dysfunction of non-neuronal cells [32–34].

2. ASTROGLIOSIS IN ALS

In FALS and SALS patients, reactive astrocytes surround both upper and lower degenerating motor neurons, as well as the region where the descending fibers of corticospinal tracts enter the grey matter [35–38]. Reactive astrocytes in ALS show increased immunoreactivity for GFAP and the calcium binding protein S100 β , and express inflammatory makers such as cyclooxygenase-2, inducible nitric oxide synthase (iNOS) and neuronal NOS. In addition, cytoplasmic hyaline inclusions and markers of oxidative and nitrate stress accompany astrocyte pathology [13, 39, 40]. Aggregated TDP-43 (43 kDa TAR DNA-binding protein, coded by the TARDBP gene) is a major component of the ubiquitinated inclusions found in neurons and glia and it is now recognized as a common feature of most ALS cases, with exception of FUS- and SOD1-linked ALS [41].

Reactive gliosis is observed in transgenic mice over-expressing wild-type or mutant TDP-43 under the control of a prion promoter [42–44], and loss of TDP-43 function in astrocytes may be an important driver of motor neuron degeneration in some of these models [45].

Astrocytes differentiated from human induced pluripotent stem cells (iPSCs) carrying an ALS-linked mutant TDP-43 show subcellular mislocalization of TDP-43 and decreased cell survival [46]. In addition, reduction of mutant TDP-43 selectively in motor neurons does not attenuate astrogliosis, suggesting that astrocyte pathology may be an important component in models of TDP-43 proteinopathy [47]. Motor neuron degeneration in FUS and c9orf72 models is also associated with marked astrogliosis [48–51].

Mutant hSOD1 transgenic rodent models recapitulate the pattern of reactive astrogliosis observed in ALS patients [52–57]. However, the appearance of astrogliosis, relative to motor neuron degeneration and symptoms onset, seems to vary in different models. For example, in hSOD1^{G37R} and hSOD1^{G85R} mice, astrogliosis becomes evident before disease onset. In the most widely used mouse model overexpressing hSOD1^{G93A}, reactive astrogliosis develops at symptoms onset, after motor neuron degeneration is evident [13]. Interestingly, a detailed time course analysis of the pathology in the hSOD1^{G93A} model revealed that aside from an increase in the number of glial cells, no ultrastructural pathological abnormalities or hallmarks of degeneration are observed in glial cells [58, 59]. Reactive astrocytes in mutant hSOD1 mouse models express iNOS and display markers of oxidative and nitritative stress [13]. In addition, translational profiling of astrocytes from hSOD1^{G37R} mice identified upregulation of inflammatory genes and down-regulation of metabolic genes belonging to the pathways controlled by the liver X receptor and the peroxisome proliferator activated receptor [60]. Meanwhile, a transcriptome analysis of astrocytes isolated from hSOD1^{G93A} mice revealed early gene expression changes linked to ion homeostasis [61]. Therefore, significant changes in the biology of astrocytes accompany motor neuron degeneration in ALS-models, supporting the idea that astrocytes play an active role in mutant hSOD1-mediated toxicity.

3. ASTROCYTE-MEDIATED MOTOR NEURON TOXICITY

Initial evidence pointing to a non-cell autonomous toxic mechanism of mutant hSOD1 came from models where the mutant enzyme was expressed under cell-type specific promoters. While ubiquitous expression of mutant hSOD1 leads to motor neuron degeneration in transgenic mice, expression of mutant hSOD1 only in astrocytes or neurons fails to induce overt motor neuron disease *in vivo* [62–64]. These data suggested that coordinated dysfunction in different cell types is critical for the initiation of the disease. However, it is worth noting that under some circumstances very high levels of hSOD1^{G93A} neuronal expression can lead to very late onset motor neuron degeneration [65]. Additional evidence for a non-cell autonomous toxic mechanism came from chimeric mice displaying a mixture of normal and hSOD1 mutant-expressing cells. In the CNS of these mice, wild-type motor neurons surrounded by non-neuronal cells expressing mutant hSOD1 developed degenerative changes [32]. Subsequently, gene excision experiments demonstrated that the expression of the mutant enzyme in motor neurons affects disease onset, while the expression within the glial compartment influences disease progression [66]. It is now clear that dysfunction in several types of glial cells (microglia, oligodendrocytes and astrocytes) contributes to motor neuron degeneration in ALS-linked mutant SOD1. Several mechanisms have been proposed to contribute to motor neuron death in ALS, including excitotoxicity, oxidative stress, mitochondrial dysfunction, neuroinflammation, altered axonal transport, ER

stress, protein aggregation (further aggravated by impaired autophagy and proteasome defects) and altered RNA metabolism. Whether these mechanisms preferentially contribute to the toxicity of mutant SOD1 in a cell-type specific manner has not been clearly established yet. The following sections focus on the potential pathogenic mechanisms in which astrocytes are likely to be involved (Figure 1).

3.1. Mutant hSOD1-expressing astrocytes

In transgenic mice, restricted overexpression of mutant SOD1 in astrocytes results in astrogliosis [62]. However, in cell culture, the expression of mutant hSOD1 *per se* does not cause the typical reactive phenotype found in ALS or ALS-models [67]. Yet, primary astrocytes isolated from rats and mice overexpressing different ALS-linked mutant hSOD1s induce motor neuron death in co-culture [68–71]. Accordingly, astrocytes differentiated from human postmortem ALS spinal cord-derived progenitor cells and astrocytes obtained from the trans-differentiation of fibroblasts from FALS and SALS patients are also toxic for motor neurons in co-culture [72, 73]. The identity of the astrocytic factor(s) that mediate this effect remains uncertain. However, it seems to involve a secreted factor(s) present in astrocyte-conditioned media [69, 74]. Unconventional secretory pathways may be involved in the secretion of the toxic mediators. Indeed, hSOD1^{G93A}-expressing astrocytes display increased exosome release, and exosomes derived from hSOD1^{G93A} astrocytes induce motor neuron death [75]. Increased nitric oxide production, mitochondrial dysfunction, altered connexin 43 expression, decreased lactate release, increased pro-nerve growth factor (proNGF) secretion, and astrocyte-mediated reduction in neuronal expression of major histocompatibility complex class I (MHC I) molecules are some examples of the mechanism suggested to participate in this toxicity [68, 74, 76–78]. *In vivo*, transplantation of hSOD1^{G93A} glial-restricted precursor cells (lineage-restricted glial progenitors) into the spinal cord of wild-type animals induces motor neuron death and motor deficits [79]. A similar result was obtained when astrocytes differentiated from ALS patient-derived iPSCs were used for the transplant [80]. Moreover, chronic infusion of conditioned media from hSOD1^{G93A}-astrocyte cultures induces motor neuron degeneration and neuromuscular dysfunction in wild-type rats [81].

3.2. Astrocytes expressing other ALS-linked mutations

It is not clear yet if mutant TDP-43 toxicity has a non-cell autonomous component. Astrocytes overexpressing mutant TDP-43, as well as astrocytes lacking TDP-43, do not cause the death of wild-type motor neurons in co-culture [46, 82]. However, in transgenic rats, restricted expression of an ALS-linked mutant TDP-43 in astrocytes causes progressive loss of motor neurons and denervation of skeletal muscles [83]. Lipocalin-2, a member of the secreted lipocalin protein family, is primarily released by glial cells and acts as an autocrine mediator of gliosis [84]. In transgenic rats with restricted neuronal expression of an ALS-linked mutant TDP-43 or FUS, lipocalin-2 is upregulated in reactive astrocytes and may have a direct neurotoxic effect [85]. Likely, glial and neuronal cells respond in a different manner to the toxic accumulation of TDP-43, but a growing body of evidence suggests an important role of TDP-43 dysfunction in astrocytes in the context of motor neuron degeneration in ALS [45, 86–88]. Interestingly, TDP-43 is present in the eosinophilic inclusions characteristically found within astrocytes in the brain of Alexander disease

patients [89], suggesting that primary alteration of TDP-43 biology within astrocytes can lead to neurodegeneration.

The non-cell autonomous component of other ALS-linked mutations remains under investigation and as other transgenic models become available, it will be interesting to establish if a common mechanism is responsible for astrocyte-mediated motor neuron toxicity in ALS. However, astrocytes differentiated from neuronal progenitors and iPSCs from sporadic ALS patients are neurotoxic towards co-cultured motor neurons [72, 73], indicating that astrocyte-mediated neurotoxicity is not restricted to familial ALS.

4. PATHWAYS INVOLVED IN ASTROCYTE-MEDIATED MOTOR NEURON TOXICITY

4.1. Excitotoxicity

Selective impairment in glial glutamate transport, leading to the accumulation of excitotoxic levels of extracellular glutamate, is one of the early hypotheses proposed to explain motor neuron death in ALS. Astrocytes uptake the glutamate released into the synaptic cleft through sodium-dependent excitatory amino acid transporters (EAAT1/GLAST) and EAAT2 (GLT1). If glutamate is not removed, activation of glutamate receptors leads to sustained elevation of neuronal intracellular calcium levels, initiating a cascade of events that leads to cell death. Loss of astrocytic EAAT2 (GLT1) has been observed in both ALS patients and animal models [53, 57, 83, 90]. However, in hSOD1^{G93A} mice, decreased GLT1 levels in spinal cord astrocytes is only detected after the onset of clinical symptoms, when motor neuron degeneration is already established [91]. On the other hand, in hSOD1^{G93A} overexpressing rats, the loss of GLT1 occurs before symptoms onset and further progresses with the course of the disease [57]. In hSOD1^{G93A} mice, astrocyte-specific overexpression of EAAT2 improves motor performance but does not modify disease onset or survival, while viral mediated focal restoration of cervical spinal cord GLT1 expression has no effect on forelimb disease onset or survival [92, 93]. However, it is worth noting that overexpression of ALS-linked mutant SOD1 leads to increased oxidative stress, and GLT1 is a selective target of post-translational oxidative modifications that inactivate the transporter [94].

Astrocytes can also regulate the vulnerability of motor neurons to excitotoxic insults by regulating the influx of calcium through alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors in these cells. The calcium permeability of AMPA receptors is largely determined by the presence of the GluA2 (GluR2) subunit. Its presence renders the channel impermeable to calcium, and astrocytes are able to modulate GluA2 expression in motor neurons. The expression of ALS-linked mutant hSOD1 in astrocytes abolishes their GluA2-regulating capacity, leading to decreased GluA2 expression in co-cultured motor neurons and increased vulnerability to AMPA receptor-mediated glutamate excitotoxicity [95]. The pre-mRNA of the GluA2 subunit is normally edited at the glutamine/arginine (Q/R) site, changing the glutamine residue into a positively charged arginine residue that is responsible for the impermeability to calcium of GluA2-containing AMPA receptors. The editing efficiency of the GluA2 subunit is significantly decreased in ventral spinal cord motor neurons of SALS patients, and in a FUS^{P525L}-linked FALS patient,

but not in SOD1-linked FALS patients [96, 97]. Moreover, the presence of the unedited GluA2Q subunit has been associated to the development of TDP-43 pathology [96]. Accordingly, the AMPA receptor antagonist perampanel rescues motor neuron degeneration in a transgenic model with altered GluA2 editing, which develops a progressive ALS-like phenotype with TDP-43 pathology [98]. Thus, altered clearance of glutamate by astrocytes, together with abnormal expression of GluA2 receptor subunits in motor neurons may facilitate neuronal excitotoxic death in ALS. Additionally, the reduced expression of the astrocytic inwardly rectifying potassium channel 4.1 (Kir4.1) observed in hSOD1^{G93A} models may contribute to motor neuron degeneration and hyperexcitability through the impairment of potassium homeostasis [99, 100]. Lastly, abnormal voltage-gated calcium channel function has been reported in hSOD1^{G93A} cultured spinal motor neurons [101] and could be yet another mechanism influencing the sensitivity of motor neurons to excitotoxicity.

4.2. Dysregulation of neuronal metabolic support

Astrocyte-neuron metabolic coupling plays a fundamental role in the regulation of extracellular glutamate levels, CNS energy storage, disposal of catabolic products and oxidative stress defenses, but perhaps it is best exemplified by the “outsourcing” of glycolysis by neurons to astrocytes [102]. Since neuronal glucose utilization favors reducing equivalents production (pentose phosphate pathway) over energy production, astrocyte-derived lactate plays a fundamental role as an energy substrate for neurons [17, 18, 102]. Decreased expression of the astrocytic lactate transporter (monocarboxylate transporter 4, MCT4/Slc16a4) and decreased lactate spinal cord levels have been described in hSOD1^{G93A} mice [74]. Mutant hSOD1-expressing astrocytes display significant alterations of their metabolic fingerprint and altered cellular lactate shuttling function when in co-culture with motor neurons [103, 104]. Accordingly, lactate supplementation rescues motor neurons in co-culture with hSOD1^{G93A} astrocytes [74]. On the other hand, an increase in lactate levels has been described in the brain of a TDP-43 mouse model [105]. Regardless, a better understanding of the changes in astrocyte-neuron metabolic coupling in the disease may lead to therapeutic interventions aimed at enhancing astrocytic neuronal metabolic support.

4.3. Oxidative stress and mitochondrial dysfunction

In ALS and ALS-animal models reactive astrocytes upregulate iNOS expression and display makers of oxidative and nitrative stress [13, 106, 107]. In co-culture, an increase in astrocytic production of nitric oxide can inhibit neuronal mitochondrial respiration, induce death and exacerbate NMDA-induced excitotoxicity [108–112]. Interestingly, conditioned media from reactive astrocytes as well as spinal cord lysates from symptomatic hSOD1^{G93A} mice can induce the death of cultured motor neurons when incubated with an exogenous source of nitric oxide [113]. In addition, cultured motor neurons isolated from different ALS mouse models overexpressing ALS-linked hSOD1 mutants are more sensitive to the death triggered by an exogenous flux of nitric oxide or other toxic stimuli such as FasL/Fas or NGF/p75^{NTR} signaling [114, 115]. The increased susceptibility of hSOD1^{G93A} overexpressing-motor neurons to NGF/p75^{NTR}-mediated death was associated to decreased nuclear factor erythroid 2-related factor 2 (Nrf2) expression and downregulation of the enzymes involved in glutathione biosynthesis [115].

Moreover, mitochondrial dysfunction in ALS-astrocytes can lead to increased superoxide production and the subsequent formation of peroxynitrite, by a diffusion-limited reaction with nitric oxide [76, 116]. Peroxynitrite can move across cell membranes through either anion channels (anionic form) or passive diffusion (protonated forms) and can induce toxicity approximately 10 μm from its site of formation [116]. Hence, in addition to the direct effect on astrocyte biology [112], the production of peroxynitrite by astrocytes can induce oxidative and nitrative stress in motor neurons, affecting mitochondrial function and inducing cell death [113, 115, 117–120]. Thus, oxidative stress and mitochondrial dysfunction in ALS astrocytes have the potential to induce motor neuron death and/or contribute to propagate the disease.

4.4. Death receptor signaling

Several death receptors from the tumor necrosis factor receptor superfamily have been implicated in ALS pathology, including tumor necrosis factor- α receptor (TNFR), Fas/CD95, lymphotoxin- β receptor (LT- β R), and p75 neurotrophin receptor (p75^{NTR}). Depending on the cellular context, death receptors can signal cell death or induce survival and tissue regeneration [121]. For example, in the MPTP model of Parkinson's disease Fas signaling protects dopaminergic neurons [122]. However, in the context of ALS death receptor signaling has been linked to autonomous and non-cell autonomous toxic mechanisms [121, 123].

4.4.1. TNF α signaling—The levels of TNF α and its receptors (TNFR1/TNFR2) are elevated at presymptomatic stage in the lumbar spinal cord of hSOD1^{G93A} mice and continue to increase during the progression of the disease [124–126]. Evidence of a possible interplay between TDP-43 pathology and TNF α signaling has also been reported [127–129]. In addition, both TNF α and soluble TNF receptor are elevated in serum of ALS patients [130]. Glial cells are a major source of TNF α in the CNS and treatment of primary embryonic motor neurons with TNF α induces cell death [131]. Thalidomide lowers TNF α expression in the spinal cord and reduces motor neuron loss in hSOD1^{G93A} mice [132]. However, genetic ablation of TNF α had no effect on motor neuron degeneration in ALS-linked mutant hSOD1 mouse models [133]. In addition, deletion of TNFR2 in hSOD1^{G93A} mice partially protects motor neurons but does not improve motor deficits or survival [134]. Moreover, TNFR1 deletion accelerates motor neuron degeneration and disease progression in the same model, an effect attributed to the disruption of the astrocytic TNFR1-GDNF axis [135]. Thus, the role of TNF α in ALS pathogenesis remains controversial and likely reflects the yet incompletely understood effects of this factor in the biology of the different cell types involved in the disease.

4.4.2. Fas/FasL signaling—Neurons and glial cells broadly express both Fas and Fas ligand (FasL) during embryonic development and adult life. A strong immunoreactivity for FasL is evident in neurons and astrocytes in the lumbar spinal cord of asymptomatic hSOD1^{G93A} mice, as well as in the spinal cord of familial and sporadic ALS cases [132]. FasL can induce the death of cultured motor neurons through a motor neuron-specific signaling pathway downstream of Fas that involves nitric oxide production [114]. Motor neurons from transgenic mice overexpressing ALS-linked hSOD1 mutants displayed

increased susceptibility to activation of this pathway [114]. Moreover, a mechanism linking the activation of the ligand-gated ion channel P2X7 to the mobilization of FasL to the plasma membrane has been recently proposed to mediate motor neuron cell death [119, 136]. A partial loss-of-function mutation for FasL confers a modest extension of survival in hSOD1^{G93A} mice [137]. *In vivo*, reactive astrocytes could be the source of both nitric oxide and FasL, although an inhibitor of soluble Fas ligand failed to rescue motor neuron death induced by co-cultured ALS-astrocytes [69].

4.4.3. LIGHT/LT- β R signaling—Another motor neuron-selective death pathway triggered by activation of LT- β R by LIGHT (tumor necrosis factor superfamily member 14, TNFSF14), has been proposed to mediate motor neuron toxicity induced by ALS-astrocytes. In the spinal cord of sporadic ALS patients there is increased interferon- γ (IFN γ) immunoreactivity in large ventral horn neurons as well as in glial cells, which coincides with increased LIGHT levels [138]. In co-culture, ALS-astrocytes can induce selective motor neuron death through the secretion of IFN γ , which in turn activates a LIGHT-LT- β R death pathway in motor neurons [139]. Interestingly, FasL and LIGHT can have an additive effect mediating motor neuron death in culture [139].

4.4.4. NGF/p75^{NTR} signaling—Mature nerve growth factor (NGF) binds to and activates TrkA and p75^{NTR} receptors. p75^{NTR} increases the selectivity and affinity of TrkA for NGF, and in cells that express both receptors NGF signaling promotes cell survival, differentiation, and/or plasticity [140]. However, in cells that express p75^{NTR} in the absence of TrkA, NGF promotes cell death [141, 142]. Neither TrkA nor p75^{NTR} are expressed by adult motor neurons, but p75^{NTR} is re-expressed by motor neurons following axotomy and in ALS [143–148]. Moreover, in hSOD1^{G93A} mice, reactive astrocytes surrounding degenerating motor neurons express NGF [113], while large p75^{NTR}-expressing alpha motor neurons are immunoreactive for the apoptotic marker cleaved caspase 3 [149]. Evidence supporting a role for astrocytes in p75^{NTR}-mediated motor neuron death comes from co-culture studies in which treatment of astrocytes with fibroblast growth factor-1, LPS or peroxynitrite leads to increased production of NGF [113, 150, 151]. In turn, secreted NGF induces the death of motor neurons in a nitric oxide and p75^{NTR}-dependent manner. Blocking antibodies against NGF or p75^{NTR}, and a small non-peptidyl blocking ligand of p75^{NTR}, prevent motor neuron death induced by hSOD1^{G93A} spinal cord extracts [113, 152]. In the absence of nitric oxide, NGF does not alter the survival of wild-type motor neurons but it is selectively toxic to hSOD1^{G93A}-expressing motor neurons [113, 115]. p75^{NTR}-mediated apoptosis in motor neurons involved neutral sphingomyelinase activation, increased mitochondrial superoxide production, peroxynitrite formation, cytochrome c release to the cytosol and caspase activation [113, 115]. p75^{NTR}-mediated apoptosis triggered by the precursor form of NGF (proNGF) has also been implicated in the neurotoxicity induced by ALS-astrocytes [74]. Further stressing the potential role of p75^{NTR}-death signaling in ALS pathology, it has been shown that ALS patients display increased levels of NGF in the cerebrospinal fluid [74] while motor neurons re-express p75^{NTR} [74, 144, 147]. Moreover, increased levels of p75^{NTR} extracellular domain in the urine of ALS patients have been identified as a potential biomarker of disease activity and progression [153, 154]. Nevertheless, earlier attempts to modulate p75^{NTR}-mediated neuronal death in ALS-models only modestly delayed the

pathology in hSOD1^{G93A} female mice [155, 156]. However, the p75^{NTR} knockout mice used in one of these studies still expressed a full p75^{NTR} intracellular domain with signaling capabilities [157]. In addition, the modest effect of p75^{NTR} deletion in ALS-mice could also be explained by the fact that p75^{NTR} can also promote motor neuron survival by interacting with TrkB and TrkC to form a high affinity receptor complex for other neurotrophins [158]. Moreover, p75^{NTR} also regulates Schwann cell migration and myelination [142]. Thus, the targeting of p75^{NTR} signaling in ALS demands a better understanding of the regulatory mechanisms defining its differential effects in specific cell types.

4.5. Interaction with other glial cells

Reactive astrocytes can secrete proinflammatory mediators and affect microglia activation in the CNS. In turn, the role of microglia activation during CNS injury can be deleterious or beneficial [159, 160]. In mutant hSOD1 mouse models microglial cells display a neurotoxic phenotype and appear to significantly contribute to disease progression [33, 161]. In these models, removal of mutant hSOD1 from astrocytes delays microglial activation and extends survival [34, 162], evidencing a role for a crosstalk between astrocytes and microglia in the toxicity of mutant hSOD1. The purinergic receptor P2X7 is a key player in microglial response and ATP released from astrocytes may increase pro-inflammatory actions of ALS microglia through activation of this receptor [163, 164]. However, ablation of P2X7 receptor exacerbates gliosis and motor neuron death in hSOD1^{G93A} mice [165]. These results may be explained by the observation that astrocyte-mediated deactivation of microglia may be detrimental in ALS models. Astrocytes from ALS-patients and ALS-mice display elevated levels of transforming growth factor- β 1 (TGF- β 1). Transgenic astrocyte-specific overexpression of TGF- β 1 in mutant hSOD1 mice causes microglia deactivation, interferes with the neuroprotective functions of microglia and accelerates disease progression in ALS mice [166]. *Vice versa*, the status of the microglia can affect the response of astrocytes during CNS injury. Classically activated neuroinflammatory microglia can induce a subtype of reactive astrocytes with neurotoxic properties [167]. This subtype of astrocytes is present in various human neurodegenerative diseases, including ALS, suggesting that activated microglia could be responsible for the neurotoxic phenotype displayed by ALS-astrocytes. Since, microglial cells co-express potentially neurotoxic and neuroprotective factors during the course of the disease [168], the role of astrocyte-microglia interaction in ALS remains to yet be fully characterized.

Changes in reactive astrocytes also have the potential to affect another nearby glial cell type. In addition to participating in myelin formation, oligodendrocytes provide crucial metabolic support to neurons [169]. Myelinating oligodendrocytes release lactate through the monocarboxylate transporter (MCT1/Slc16a1), which in turn is used by neurons as an energy-producing substrate. MCT1 is downregulated in hSOD1^{G93A} mice and ALS patients [170], an observation likely due to oligodendrocyte degeneration and impaired maturation of oligodendrocyte progenitor cells (OPCs) [171]. The extent to which astrocytes regulate oligodendrocyte proliferation and OPCs differentiation is not fully characterized yet. However, astrocytes appear to create a permissive environment that promotes OPC recruitment, migration and differentiation [172]. Accordingly, astrocytes promote oligodendrogenesis through the secretion of tissue inhibitor of metalloproteinase-1 [173,

174], and inhibition of astroglial NF- κ B signaling enhances oligodendrogenesis following spinal cord injury [175]. Moreover, despite successful recruitment of OPC within a demyelinating injury in the adult rat spinal cord, these precursors fail to remyelinate the axons in the absence of astrocytes [176]. Thus, functional alterations in ALS-astrocytes may contribute to the impaired regeneration of oligodendrocytes observed in ALS. Interestingly, the connexins that participate in the formation of astrocyte-oligodendrocyte networks appear deregulated in ALS models [78, 177, 178].

5. THERAPEUTIC POTENTIAL OF ASTROCYTES

Based on the severity and the distance to the injury site, reactive astrocytes can undergo a variety of changes that range from reversible changes in gene expression and cellular hypertrophy to pronounced cell proliferation with compact scar formation. These observations suggest that astrogliosis is a continuum of potential phenotypes [20]. Although this review focuses on the potential pathogenic role of astrocytes in ALS, mild astrogliosis can be neuroprotective. Even the traditional notion that glial scar formation is detrimental for axonal regeneration has been challenged by recent findings demonstrating that scar formation, rather than preventing, aids axonal regeneration in the CNS [179]. Although it is difficult to predict the exact consequences of reactive astrogliosis, the loss of normal function or acquired new characteristics by astrocytes can directly impact neuronal fate. The therapeutic potential of astrocytes in ALS can be exemplified by the beneficial effect of cell replacement therapies aimed to restore normal astrocyte function or strategies that alter astrocyte biology which subsequently modify astrocyte-motor neuron interaction.

5.1. Candidates to revert astrocyte-mediated motor neuron toxicity

Through the use of astrocyte-motor neuron co-cultures, several treatments that modify the biology of astrocytes and revert the neurotoxic phenotype of ALS-astrocytes have been described. Activation of the transcription factor Nrf2 was one of the first approaches identified [68]. Nrf2 controls the expression of antioxidant and phase II detoxifying enzymes, and its activity is upregulated in ALS models [180, 181]. Glutathione (GSH) plays a key role in neuronal antioxidant defenses and increased astrocytic GSH secretion induced by Nrf2 activation could account for the motor neuron protection observed in co-culture with ALS-astrocytes [68, 182]. GSH is synthesized by the consecutive action of two enzymes, glutamate-cysteine ligase and glutathione synthetase. The transcription factor Nrf2 regulates both enzymes and hence increase GSH production and release to the extracellular medium upon activation. Astrocytes can efficiently release a large amount of GSH in the extracellular space, where it is cleaved by the ectoenzyme γ -glutamyl transpeptidase. The resulting dipeptide CysGly, can be hydrolyzed by the neuronal aminopeptidase N, to release cysteine and glycine. Astrocytes also release glutamine, and hence make available all the necessary precursors for enhanced neuronal GSH synthesis [16, 183]. Accordingly, Nrf2 overexpression restricted to astrocytes delays disease onset, extends survival and lowers glial reactivity in ALS-mice [184]. Several Nrf2 activators have been shown to be protective in co-culture or animal models of ALS [185–187]. Interestingly, Nrf2 overexpression in neuronal and skeletal muscle cells does not affect survival in ALS-linked mutant hSOD1

mouse models [188]. These results indicate that the cell type targeted is relevant when considering an Nrf2-mediated therapeutic approach for ALS.

In general, enhancing antioxidant defenses in ALS-astrocytes reverts their toxicity towards co-cultured motor neurons. Nicotinamide adenine dinucleotide (NAD⁺) is an essential redox molecule in metabolism and a co-substrate for a series of enzymes that regulate fundamental biological processes [189–191]. Sirtuins (Sir2-like enzymes) are NAD⁺-dependent enzymes with deacetylase or mono-ADP-ribosyltransferase activity that have a major effect in the capacity of the cell to modulate mitochondrial function and counteract the deleterious effects of oxidative stress [190]. By increasing NAD⁺ levels, either with precursor supplementation or overexpression of the rate limiting enzyme in the NAD⁺ salvage pathway (nicotinamide phosphoribosyltransferase), the toxic effects from ALS-astrocytes on motor neurons can be alleviated [192]. Elevated NAD⁺ levels in hSOD1^{G93A}-astrocytes leads to a reduction of reactive oxygen species production, possibly by a sirtuin-dependent mechanism, that is protective to motor neurons. Nitro-fatty acids are electrophilic signaling mediators formed in tissues during inflammation and can induce pleiotropic cytoprotective and antioxidant pathways, including upregulation of Nrf2. Nitro-fatty acids induce a potent Nrf2-dependent antioxidant response in astrocytes and revert the neurotoxic phenotype of hSOD1^{G93A} astrocytes [187]. Whether these strategies translate to improved motor neuron survival *in vivo* remains to be established. In co-culture, astrocyte-mediated toxicity can be prevented by strategies that specifically improve mitochondrial antioxidant defenses [76, 193], indicating a critical role of mitochondrial dysfunction in the neurotoxic phenotype. However, *in vivo*, these strategies have yielded mixed results regarding the overall effect on disease onset and survival in mouse models [193–195].

Dehydroepiandrosterone derivatives mimic the pro-survival effects of neurotrophins and revert the motor neuron loss observed in co-cultures with ALS-astrocytes. However, this treatment did not improve functional outcomes or survival in ALS-mice [196]. hSOD1^{G93A} astrocytes also display increased expression of α -adducin. A complex formed between α -adducin and the ion pump α 2-Na/K ATPase seems to participate in the development of the neurotoxic phenotype of ALS-astrocytes. Knockdown of α 2-Na/K ATPase or α -adducin in mutant hSOD1-expressing astrocytes protected in co-cultured motor neurons and increased the lifespan of ALS-mice [197]. An astrocyte-induced reduction in the expression of MHC I molecules on co-cultured motor neurons has also been proposed to explain astrocyte-mediated motor neuron toxicity. This effect is apparently mediated by soluble factors secreted from astrocytes that cause endoplasmic reticulum stress in motor neurons. Although the mechanism is not completely clear, overexpression of HLA-F, an MHC I molecule, protects motor neurons from the toxicity mediated by ALS-astrocytes, increases motor performance and extends survival in a mouse model of ALS [77].

Astrocyte-motor neuron co-cultures have allowed the identification of several therapeutic candidates (as discussed above) to reduce astrocyte-mediated motor neuron toxicity. However, no general statement can be made about the capacity of these approaches to translate into increased survival *in vivo* and ultimately these therapeutic strategies need to be verified in animal models in order to provide valid pre-clinical evidence.

5.2. Drug discovery

The use of patient-derived iPSCs has become a widely used tool for many areas of research, including ALS. Since astrocytes differentiated from fibroblast-derived iPSCs from SALS and FALS patients exhibit a neurotoxic phenotype similar to that of human NPC-derived astrocytes [72, 73], they represent a reasonable platform for high-throughput drug screening. Screenings can be aimed to identify compounds that modify a particular aspect of astrocyte biology (e.g. oxidative stress) [198] or to identify candidates that revert astrocyte-mediated motor neuron toxicity in ALS [199]. However, implementation of effective drug treatments in ALS may be challenging in view of the changes in the ATP-binding cassette (ABC) transporters observed in the disease. In ALS, expression and function of ABC transporters is increased at the blood-brain barrier, potentially limiting entry of drugs to the CNS [200]. ABC transporter expression has also been detected in neurons and glia, which may further reduce the therapeutic action in target cells [200]. ABCB1, one of the members of the ABC transporter family involved in multidrug resistance, is upregulated in endothelial cells of the brain capillaries in ALS. In blood-brain barrier models, the expression of mutant hSOD1 and mutant FUS in astrocytes drives ABCB1 upregulation in endothelial cells [201]. Thus, the potential role of astrocytes affecting drug bioavailability may need to be addressed in order to implement effective drug therapies in ALS.

5.3. Cell replacement therapies

Since the hallmark of ALS pathology is motor neuron degeneration, replacing dead or dying motor neurons is a desirable therapeutic objective. However, the intrinsic complexity of replacing a highly specialized cell makes astrocytes a much more reasonable target [202]. There are two main types of stem cells that are showing promise in cell-replacement therapies: neural progenitor stem cells and glial-restricted precursors. Transplanted rat wild-type glial-restricted precursors survived in the diseased tissue, differentiated efficiently into astrocytes and reduced microgliosis in the cervical spinal cord of hSOD1^{G93A} rats. The transplants extended survival and disease duration, attenuated motor neuron loss and slowed the decline in forelimb motor function and respiratory physiological function [203]. On the other hand, although human glial-restricted precursors transplanted in hSOD1^{G93A} mice survived and differentiated into astrocytes, the transplant did not confer any therapeutic benefit [204, 205]. During chronic neurodegeneration the newly transplanted/differentiated astrocytes will face the same environment that adversely affects the capacity of endogenous astrocytes to support neuronal survival or that promotes a neurotoxic phenotype. Thus, transplantation of modified precursors, capable of fending off toxic stimuli (e.g. oxidative stress, inflammatory mediators, etc) might represent an alternative strategy that could confer increased protection.

5.4. Enhanced trophic support

Riluzole, one of the U. S. Food and Drug Administration approved drugs for the treatment of ALS is believed to confer moderate protection in ALS due to its anti-glutamatergic properties [206]. Interestingly, riluzole may also confer protection by increasing the trophic support provided by astrocytes [207]. However, chronic riluzole treatment has an inhibitory effect on trophic factor production by astrocytes, suggesting that adjustments in the

administration regimen could be beneficial [208]. Different growth factors such as glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor I (IGF-I), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) have been evaluated in experimental models of ALS [209–212]. Although positive results were obtained in animal models, there has been a constant lack of success when translated into human clinical trials [213]. Transplantation of pluripotent precursors genetically engineered to produce growth factors is being explored with mixed outcomes [214–218]. Since astrocytes can be an alternative source of trophic support for motor neurons during neurodegeneration, transplantation of glial-restricted precursors genetically engineered to produce growth factors could represent a more efficient delivery method.

CONCLUSIONS

Over the last decades our understanding of ALS pathology has significantly evolved. However, the identification of therapeutic approaches that ultimately improve symptoms and lifespan for ALS patients has been elusive. Understanding the role of astrocytes in the neurodegenerative process, and their interaction with neuronal and non-neuronal cells, provides a conceptual framework that highlights the potential of this cell type as the focus of therapeutic efforts. The central role that astrocytes play in ALS suggests that therapies aimed at modulating astrocyte biology may contribute to the development of integral therapeutic approaches to halt the progression of the disease.

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Figure 1.

Schematic representation of potential pathways involved in astrocyte-mediated motor neuron death in ALS. Astrocytes in ALS display reduced ability to provide metabolic support to motor neurons and to effectively regulate the extracellular levels of ions and neurotransmitters. Impaired glutamate transport in astrocytes leads to accumulation of excitotoxic levels of extracellular glutamate. Excitotoxicity is further facilitated by increased permeability of AMPA receptors to calcium as a consequence of decreased expression and pre-mRNA editing of GluA2 AMPA receptor subunits in motor neurons. In addition, the potassium buffering capacity of astrocytes is negatively affected by the reduced expression of the astrocytic inwardly rectifying potassium channel 4.1 (Kir4.1). The concomitant rise in extracellular potassium levels contributes to neuronal hyperexcitability and further decreases the electrogenic uptake of glutamate. Reactive astrocytes display increased production of nitric oxide (NO) due to iNOS upregulation. Moreover, mitochondrial dysfunction leads to increased production of superoxide ($O_2^{\bullet -}$) and the subsequent formation of peroxynitrite ($ONOO^-$), leading to oxidative and nitrative stress. Nitric oxide and peroxynitrite can diffuse across cellular membranes to directly affect mitochondrial function and induce oxidative and nitrative stress in motor neurons. Reduced Nrf2 expression by motor neurons could further aggravate this process. Increased levels of nitric oxide also sensitize motor neurons to the apoptotic signaling mediated by FasL/Fas and NGF/p75^{NTR}. The release of other neurotoxic factors by ALS-astrocytes has also been described (see text for further details). The cross-

talk between astrocytes and microglia is affected by the release of different proinflammatory mediators that promote a neurotoxic phenotype in both cell types (see text for further details).

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