Astrocytes in amyotrophic lateral sclerosis: direct effects on motor neuron survival

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Abstract Selective motor neuron death during amyotrophic lateral sclerosis (ALS) is a non-cell autonomous process in which non-neuronal cells induce and/or contribute to the disease process. The non-neuronal cells that are clearly involved in the pathogenesis of the disease are the surrounding astrocytes. Under normal conditions, astrocytes remove glutamate from the synaptic cleft and release trophic factors. In addition, these cells determine the functional characteristics of motor neurons. Recent evidence suggests that activation of astrocytes in a degenerative disease like ALS disturbs the crosstalk between astrocytes and motor neurons, which could contribute to and/or accelerate selective motor neuron death. These new insights may contribute to the development of therapeutic approaches to slow this fatal neurodegenerative disease.

Keywords Amyotrophic lateral sclerosis · Glutamate uptake · Neurotrophic factors · Excitotoxicity · AMPA receptor

This overview focuses on the mechanisms through which astrocytes have been implicated in motor neuron function and/or survival. The different effects of astrocytes on motor neurons are discussed in view of the crucial role played by these cells in the initiation and progression of amyotrophic lateral sclerosis (ALS). Finally, a short overview is provided of the therapeutic strategies that could originate from this knowledge.

ALS is a fatal, adult-onset, neurodegenerative disease characterized by the selective death of motor neurons in the motor cortex, brain stem, and spinal cord. In 90% of cases, ALS is considered a sporadic disease as no family history of ALS is known. The remaining 10% of patients suffer from familial ALS of which approximately 20% is caused

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by mutations in the gene encoding superoxide dismutase 1 (SOD1) located on chromosome 21q; the corresponding protein is known to detoxify potentially cell-damaging free radicals [1]. Intriguingly, SOD1 knockout mice do not develop an ALS-similar phenotype [2] which implies that disease pathology occurs due to a “gain of function” in mutant SOD1 and not due to a loss of functioning protein. Clinically, sporadic and familial ALS are indistinguishable including symptoms of muscle weakness, atrophy, and spasticity caused by the loss of both upper and lower motor neurons. Ultimately, patients become paralyzed and denervation of respiratory muscles leads to the death of the patient, on average 3 to 5 years after the onset of the first symptoms. Similarly, transgenic mice and rats overexpressing mutant human SOD1 develop an age-dependent degeneration of motor neurons leading to paralysis and death and thus form a valuable tool for ALS research [3].

Research on transgenic animals overexpressing mutant SOD1 has demonstrated that non-neuronal cells contribute to the disease process of ALS, and, as a consequence, motor neuron death in ALS is considered as a “non-cell autonomous” process. The first argument in favor of this concept is that restricted expression of mutant SOD1 in motor neurons [4, 5] or in astrocytes [6] does not lead to motor neuron degeneration. In contrast, a (possibly more prominent) motor-neuron-specific expression of mutant SOD1 induces motor deficits at a later age [7], clearly indicating that SOD1 expression in other cell types is necessary for the accelerated phenotype detected in transgenic mice ubiquitously expressing mutant SOD1.

Chimeric animals containing both transgenic (mutant SOD1 expressing) and non-transgenic neighboring cells demonstrate a delayed degeneration and extended survival of mutant-SOD1-expressing motor neurons [8]. Interestingly, motor neurons not expressing mutant SOD1 are also affected, presumably by the non-neuronal cells expressing mutant SOD1.

The identity of the non-neuronal cell type(s) contributing to mutant-SOD1-induced motor neuron death was investigated using a floxed mutant SOD1 gene that is excised by Cre recombinase, which expression is driven by a cell-type-specific promoter. The selective removal of mutant SOD1 from microglia and peripheral macrophages significantly delays the progression of the disease [9]. Moreover, the same holds true for astrocytes as the reduction of mutant SOD1 expression in these cells also affects survival [10].

In addition, a prominent histopathological characteristic of ALS is astrogliosis, both in animal models and in patients [11, 12]. Originally, this astrogliosis was considered secondary to the loss of motor neurons. However, an increasing body of evidence strongly indicates that glial cells may be crucially involved in the pathogenesis of ALS.

1 Astrocytes and extracellular glutamate

The first important function of astrocytes is to maintain a low extraneuronal concentration of glutamate by the clearance of this neurotransmitter from the synaptic cleft (Fig. 1). By far, the most important glutamate transporter expressed in astrocytes is EAAT2/GLT-1. This transporter has the highest affinity for glutamate and is widely expressed in astrocytes throughout the central nervous system. Insufficient clearance of glutamate could lead to overstimulation of glutamate receptors and neuronal death, a process called excitotoxicity (for a review, see [13]).

Many results indicate that scavenging of glutamate from the synaptic cleft is compromised in ALS. Diminished glutamate transport is found in synaptosomes isolated from affected brain areas and spinal cord of sporadic ALS patients [14]. Moreover, a reduction of the density of glutamate transporters was observed in spinal cords from sporadic ALS

Fig. 1 Schematic overview of the different interactions between astrocytes and motor neurons. The EAAT2/GLT1 transporter present on the astrocytes removes glutamate from the synaptic cleft. In addition, astrocytes secrete neurotrophic factors and influence the characteristics of the surrounding motor neurons (e.g., the GluR2 expression level). Under pathological conditions, these different functions could be compromised. In addition, astrocytes could secrete toxic factors that induce and/or enhance motor neuron death

patients [15]. The underlying mechanism is a selective loss of EAAT2/GLT1 in the motor cortex and spinal cord [16]. The loss of EAAT2/GLT1 is not limited to sporadic ALS patients [16–18] but is also observed in familial cases [16]. In addition, a clear loss of EAAT2/GLT1 immunoreactivity is also found in the ventral horn of mutant SOD1 mice and rats [19–21].

Both in vitro and in vivo, loss of EAAT2/GLT1 function causes selective motor neuron degeneration. Treatment of organotypic spinal cord cultures with antisense oligonucleotides to EAAT2/GLT1 results in diminished transporter protein expression and induction of progressive motor neuron loss. This process is sensitive to an α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor antagonist [22]. Furthermore, chronic intraventricular administration of these antisense oligonucleotides in rats results in a rise of extracellular glutamate levels and a progressive motor syndrome [22].

A direct link between ALS-causing SOD1 mutations and a decreased EAAT2/GLT1 function was found in vitro [23]. In the presence of mutant SOD1, H_2O_2 oxidatively damages the intracellular carboxyl-terminal part of EAAT2/GLT1, decreasing glutamate transport (Fig. 1). Similarly, exposure of cultured motor neurons to glutamate receptor agonists results in the generation of reactive oxygen species [24], damaging neighboring

astrocytes and interfering with glutamate uptake in these cells [25]. The source of the reactive oxygen species is most likely the mitochondria and Ca^{2+} overload of these organelles may be involved. Indeed, in the absence of Ca^{2+} -binding proteins in motor neurons [26], the mitochondria seem to play a crucial role in buffering Ca^{2+} [24, 27].

2 Astrocytes and neurotrophic or toxic factors

Insufficient release of neurotrophic factors by astrocytes could contribute to the motor neuron loss in ALS (Fig. 1). Indeed, astrocytes are an important source of neurotrophic factors affecting motor neuron survival. Brain-derived neurotrophic factor, glial-derived neurotrophic factor, and ciliary neurotrophic factor can all rescue motor neurons, both in vitro and in vivo (for a review, see [28]). Recently, vascular endothelial growth factor (VEGF) was added to this list as it was released by astrocytes and was neurotrophic for motor neurons [29]. The importance of VEGF was discovered after the generation of transgenic mice with lower VEGF levels than normal by deletion of the hypoxia-response element in the VEGF promoter. These mice show progressive motor neuron loss and motor deficits [30]. One mechanism involved in the neuroprotective action of growth factors could be the protection against apoptosis through activation of the Akt pathway, as we and others have shown [31, 32].

Although a general impairment in the production of neurotrophic factors has been proposed as direct or indirect cause for motor neuron loss during ALS [28], the opposite may also hold true. ALS astrocytes could be responsible for the release of hazardous rather than trophic factors (Fig. 1), as is suggested by a set of in vitro and in vivo studies. For instance, culture media from activated astrocytes from symptomatic mutant SOD1 mice induce apoptosis of purified motor neuron cultures. This effect is prevented by either neurotrophic growth factor (NGF) or p75 neurotrophin receptor blocking antibodies, suggesting that it is mediated by NGF [33].

In a recent study, embryonic stem-cell-derived motor neurons containing mutant SOD1 show a lower survival in comparison to non-transgenic or normal SOD1-overexpressing cells [34]. As only a subset of embryonic stem cells differentiate into motor neurons and as a considerable number of glial-fibrillary-acidic-protein-positive astrocytes are present, a non-cell-autonomous interaction between both cell types seems to be responsible for this negative effect. Culturing motor neurons in the presence of astrocytes overexpressing mutant SOD1 does not only enhance the motor neuron death of mutant SOD1-containing motor neurons but also induces cell death in normal motor neurons. Wild-type SOD1-overexpressing astrocytes do not exert this negative effect, strongly indicating that the genotype of the astrocytes influences the survival of motor neurons.

A similar conclusion is reached in another study using embryonic stem-cell-derived or primary motor neurons cultured in combination with primary astrocytes from spinal cord [35]. The effect is seen with different mutations that result in either catalytically active or inactive SOD1. Moreover, it was found that the release of a soluble factor by the astrocytes is responsible for this toxic effect on motor neurons. The identity of the astrocytic factor(s) is unknown, although several potential candidates (glutamate, several interleukins, NGF, soluble Fas ligand) have already been excluded.

Astrocytes may also be the source of extracellular mutant SOD1. Chromogranins can act as chaperone-like proteins promoting the secretion of mutant SOD1 [36]. This extracellular SOD1 is furthermore suggested to trigger microglial activation which can induce motor neuron death. The exact role of this microglial activation in ALS pathology is not yet clear,

although it has been suggested that inhibition of microglial activation could be beneficial [37, 38].

3 Astrocytes and Ca^{2+} metabolism

Astrocytes also induce neuronal differentiation and potentiate the sensitivity of motor neurons to AMPA-receptor-mediated glutamate stimulation [39]. This effect has a downside: a portion of the AMPA type of glutamate receptors lacks the GluR2 subunit and is therefore permeable to Ca^{2+} . This induces vulnerability of motor neurons to glutamate-induced excitotoxicity, a phenomenon that could contribute to the selectivity of the motor neuron loss in ALS [40, 41]. Increasing GluR2 expression in motor neurons attenuates, while decreasing GluR2 expression in these cells aggravates, motor neuron degeneration in mutant SOD1 mice [42, 43]. Astrocytes also influence the sensitivity of motor neurons to excitotoxicity by changing the expression of the GluR2 subunit in motor neurons and thus the relative amount of Ca^{2+} -permeable AMPA receptors [44] (Fig. 1). This is a very specific effect, as only astrocytes from spinal cord secrete factor(s) which affect GluR2 expression, and only motor neurons are responsive. Astrocytes thus appear to determine the vulnerability of motor neurons to excitotoxicity by affecting the receptor profile of these cells. One of the secreted factors increasing the GluR2 expression is VEGF [45]. We recently discovered that VEGF increases the GluR2 messenger RNA expression level through interaction with the VEGFR2. On the molecular level, the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway seems to be involved. This is indicated by the fact that the protective effect of VEGF on chronic excitotoxicity in organotypic spinal cord sections is linked to PI3-K activation [46] and by the observation that VEGF clearly induces Akt activation *in vivo* [31].

An interesting and intriguing observation is that the expression of mutant SOD1 in the astrocytes affects the secretion of factor(s) which lowers the expression of GluR2, resulting in more Ca^{2+} -permeable AMPA receptors and a higher sensitivity of the motor neurons to excitotoxicity [44]. In contrast, the presence of mutant SOD1 in motor neurons does not influence their vulnerability to excitotoxicity.

Using a motor neuron–astrocyte coculture model, it was recently shown that mutant SOD1 expression in astrocytes induces changes in the mitochondrial homeostasis in motor neurons [47]. In the presence of mutant SOD1 astrocytes, the mitochondrial redox state is more reduced and mitochondrial membrane potential is decreased in motor neurons [47]. These early deficits in mitochondrial function induced by surrounding astrocytes may increase the vulnerability of motor neurons to other neurotoxic mechanisms involved in ALS pathogenesis. Naturally, a word of caution must be placed concerning altered expression patterns between cells *in vitro* and *in vivo* [48].

4 Therapeutic strategies targeting astrocytes

As disturbances of the normal astrocytic function by mutant SOD1 or by other factors could have a direct or indirect effect on the survival of motor neurons, astrocytes may be an interesting therapeutic target. Interfering with the hazardous factors released by ALS astrocytes or enhancing insufficient release of growth factors by ALS astrocytes may be possible in the near future. Cell therapy approaches may particularly benefit from the knowledge of the interaction between neurons and astrocytes as it is more feasible and efficient to transplant healthy astrocytes rather than motor neurons into a hostile environment.

The proof of principle is provided by Lepore et al. using lineage-restricted astrocyte precursors, called glial-restricted precursors (GRPs). These GRPs were transplanted in the vicinity of cervical spinal cord respiratory motor neuron pools and survive in diseased tissue, differentiate efficiently into astrocytes, and reduce microgliosis in the cervical spinal cords of mutant SOD1 rats. Moreover, GRPs extend survival and disease duration, attenuate motor neuron loss, and slow decline in forelimb motor and respiratory physiological functions [49].

In order to counteract the decrease in glutamate uptake by the astrocytes, several drugs and nutritional have been screened. In one of these screenings, β -lactam antibiotics were discovered as potent stimulators of EAAT2/GLT1 expression [50]. Both in vitro and in vivo administrations of ceftriaxone, a β -lactam antibiotic, provide a threefold increase in protein levels and a comparable increase in EAAT2/GLT-1 glutamate transport. Ceftriaxone treatment of mutant SOD1 mice starting at the onset of the disease significantly increases the life span of the mutant SOD1 mice. Moreover, ceftriaxone prevents motor neuron loss and astrogliosis [50]. These data indicate that an induction of the EAAT2/GLT1 expression and a higher clearance of glutamate from the synaptic cleft protect motor neurons during ALS, at least in the mutant SOD1 mouse model.

A lot of research has focused on the potential use of trophic factors to treat ALS. A number of clinical trials using these factors have already been performed, but, to this moment, none of them have been successful (for a review, see [51]). One problem could be that delivery of the trophic factors to their target cells is inadequate. As intrathecal or intracerebroventricular administration of insulin-like growth factor 1 or VEGF extends survival in mutant SOD1 mouse and rats [32, 52], these factors may be still be candidates for ALS patient trials.

In view of the important role played by disturbances of the Ca^{2+} metabolism in motor neuron death, drugs interfering with excitotoxicity remain potential therapeutics. Riluzole, to this day the only drug which proves effective against disease progression in ALS patients [53–55], is thought to exert its effect through inhibition of glutamate release. Treatments that interfere with excitotoxicity delay selective motor neuron death and prolong survival of the mutant SOD1 mice [56–59]. Moreover, the AMPA receptor antagonist LY300164/talampanel was tested in a very small double-blind placebo trial and is currently being tested on a larger scale [60, 61].

In conclusion, the effects of astrocytes on motor neuron survival in ALS are increasingly recognized and investigated. This will hopefully facilitate the development of new therapeutic strategies for ALS. With respect to this, the newest and most promising approach seems to be the replacement of the bad environment of the motor neurons by transplanting healthy astrocytes.

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