

Complexity of Astrocyte-Motor Neuron Interactions in Amyotrophic Lateral Sclerosis

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Key Words

Amyotrophic lateral sclerosis · Astrocytes · Motor neurons · Fibroblast growth factor · Nerve growth factor

Abstract

Neurons and surrounding glial cells compose a highly specialized functional unit. In amyotrophic lateral sclerosis (ALS) astrocytes interact with motor neurons in a complex manner to modulate neuronal survival. Experiments using chimeric mice expressing ALS-linked mutations to Cu,Zn superoxide dismutase (SOD-1) suggest a critical modulation exerted by neighboring non-neuronal cell types on disease phenotype. When perturbed by primary neuronal damage, e.g. expression of SOD-1 mutations, neurons can signal astrocytes to proliferate and become reactive. Fibroblast growth factor-1 (FGF-1) can be released by motor neurons in response to damage to induce astrocyte activation by signaling through the receptor FGFR1. FGF-1 stimulates nerve growth factor (NGF) expression and secretion, as well as activity of the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor. Nrf2 leads to the expression of antioxi-

dant and cytoprotective enzymes such as heme oxygenase-1 and a group of enzymes involved in glutathione metabolism that prevent motor neuron degeneration. However, prolonged stimulation with FGF-1 or SOD-mediated oxidative stress in astrocytes may disrupt the normal neuron-glia interactions and lead to progressive neuronal degeneration. The re-expression of p75 neurotrophin receptor and neuronal NOS in motor neurons in parallel with increased NGF secretion by reactive astrocytes may be a mechanism to eliminate critically damaged neurons. Consequently, astrocyte activation in ALS may have a complex pathogenic role.

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Introduction

Amyotrophic lateral sclerosis (ALS) is characterized pathologically by the progressive and selective degeneration of cortical, bulbar and spinal motor neurons. The characteristic ALS symptoms of spasticity, paralysis and atrophy of skeletal muscles reflect the loss of both upper and lower motor neurons. Because the degeneration of motor neurons is so blatant, research on ALS has been

mainly focused on the mechanisms of motor neuron degeneration. The concomitant pathological changes in other cell types including astrocytes have been mostly neglected, often dismissed as a secondary and unspecific response to the ongoing neurodegeneration.

An important clue to the pathogenesis of ALS was provided by the development of transgenic animal models carrying mutations of the Cu,Zn superoxide dismutase (SOD-1) linked to familial cases of ALS. Toxicity of mutant SOD-1 involves a dominant gain-of-function instead of a loss of function [1, 2]. The selective vulnerability of spinal motor neurons was linked to the high levels of SOD-1 expression. However, current evidence indicates that the expression of SOD-1 mutations in neurons only may not be sufficient to induce the disease [3]. The enzyme must be expressed in both neuronal and non-neuronal cells to induce the disease. These findings suggest that interactions between motor neurons and surrounding cells in the spinal cord or skeletal muscle can greatly influence motor neuron degeneration in ALS. In agreement, using chimeric mice composed of mixtures of normal cells and cells expressing ALS mutant SOD-1, Clement et al. [4] showed that motor neuron pathology is not directly associated with the expression of SOD-1 mutations in the motor neuron but rather depends on the concomitant expression of the mutated enzyme in a critical number of neighboring non-neuronal cells.

In the adult CNS, glial cells and neurons compose a specialized functional unit. While neurons mainly integrate synaptic information and form networks, the adjacent interacting astrocytes provide highly specialized and localized structural, metabolic and trophic support to neurons. In addition, astrocytes modulate neuronal excitability and neurotransmission [5, 6]. In response to damage, astrocytes display highly plastic phenotypes and might proliferate when stimulated by diffusible and non-diffusible factors. Thus, astrocytes constitute a renewable and plastic cell type within the CNS.

Astrocytes, microglia, pericytes, endothelial and immune cells become activated to a different extent during the progression of motor neuron degeneration in ALS. Morphologically, reactive astrocytes display hypertrophic nuclei and cell bodies and increased elaboration of processes with increased content of glial fibrillary acidic protein (GFAP). In addition, activated astrocytes express a wide variety of markers such as cytoskeleton proteins, cell surface and matrix molecules, proteases, protease inhibitors, and several growth factors and cytokines [7, 8]. Thus, astrocytes interact in a complex manner with im-

mune cells and microglia. In turn, microglia are a source of proinflammatory mediators, nitric oxide and excitotoxins, which can potently modulate astrocyte activation and neuronal survival [9].

Recent evidence indicates the existence of complex molecular mechanisms by which activated astrocytes may contribute to promote either neuronal survival or death in response to damage. Some reviews have been recently published about the pathogenesis of ALS [1, 10, 11] and also about the role of glial and inflammatory cells in ALS [12–14]. This review will consider the emerging evidence supporting the complex influence of astrocytes on motor neuron survival. It will also discuss the significance of astrogliosis in ALS and the potential pathogenic role played by astrocytes displaying abnormal phenotypes.

Motor Neuron Damage and the Triggering of Astrocytosis

ALS pathology is characterized by overt glial reaction that surrounds both the affected upper and lower motor neurons [15–19]. Reactive astrocytes show increased immunoreactivity for GFAP and the calcium binding protein S100 β [20] and express inflammatory makers such as COX-2 [21], iNOS and neuronal NOS [22, 23]. Astrocyte activation in ALS can be comparable with the ‘isomorphic’ gliosis described in other neuropathologies, with the appearance of proliferative, hypertrophic and globular astrocytes [8]. In addition, astrocytes might be subject to oxidative and nitrative stress as evidenced by the expression of specific markers such as nitrotyrosine [23–25]. Astrocyte activation can be observed in both ALS patients and in mice and rats carrying different SOD-1 mutations. In transgenic mice, the extent of astrocyte activation correlates with the degree of neuronal degeneration. In G93A SOD-1 transgenic rats, astrocyte activation coincides with early vacuolization of mitochondria and with a striking focal loss of the GLT-1 glutamate transporter in the ventral horn [26].

Little is known about the neuron-glia signaling that leads to astrocyte activation. Damaged motor neurons can trigger the activation of neighboring astrocytes, as shown by studies in axotomy and spinal cord injury. Damaged motor neurons upregulate diffusible inflammatory molecules [27, 28] capable of eliciting secondary glial responses resembling those observed in ALS animal models. Motor neurons can also produce vascular endothelial growth factor, which can promote proliferation

and migration of astrocytes [29]. In addition, we have showed that oxidative stress caused by increased production of nitric oxide and peroxynitrite by damaged motor neurons can induce astrocyte activation in ALS models [30]. Peroxynitrite production by motor neurons has been demonstrated in response to trophic factor deprivation [31], Fas pathway activation [32] or loading with zinc-deficient SOD-1 [33].

Fibroblast Growth Factor-1 Induces Astrocyte Activation

Fibroblast growth factor-1 (FGF-1), as well as other members of the FGFs family is involved in the regulation of several biological processes including cell growth and differentiation, inflammation and angiogenesis [34]. Compared to other neuronal types, motor neurons contain high levels of FGF-1 [35–37], which represent approximately 0.1% of soluble protein in the cytoplasmic compartment [35]. FGF-1 levels in motor neurons are altered following spinal cord injury or nerve lesions [38, 39], which might be due to an increased rate of release. FGF-1 is not released by a classical secretion pathway but rather by an alternative mechanism triggered by cell damage. For example, FGF-1 is secreted from cells in a thiol-dependent manner in response to heat-shock and oxidative stress [40, 41]. It has been recently shown that peroxynitrite promotes the formation of thiol-crosslinked FGF-1 dimers [42], a releasable form of FGF-1 containing latent biological activity. Since FGF-1 is highly concentrated in motor neurons and mobilized during sublethal cell injury and oxidative stress, we have proposed that FGF-1 might play a role in ALS pathogenesis. In agreement, we have recently showed dramatic changes in the pattern of FGF-1 immunoreactivity in the spinal cord of symptomatic G93A SOD mice. Damaged motor neurons show low levels of FGF-1 immunoreactivity with a concurrent increase of FGF immunoreactivity in the neuropil, suggesting increased mobilization from the motor neurons [43].

Astrocytes represent a potential target for extracellular FGF-1 released by damaged motor neurons. FGFs signal through four closely related high-affinity tyrosine kinase-linked FGF receptors (FGFR1–4) [44]. Typically, astrocytes activated by FGF accumulate the FGFR-1 in the nucleus, which is considered a hallmark of astrocyte activation and proliferation [45–47]. We have recently reported that about 80% of the astrocytes display nuclear FGFR-1 immunoreactivity in the ventral horn of symp-

tomatic G93A SOD-1 mice, as compared with less than 25% in non-transgenic spinal cord [43]. These results suggest that FGF-1 signaling through FGFR-1 can activate astrocytes in ALS.

Activation of the Transcription Factor Nuclear Factor Erythroid 2-Related Factor 2 in Astrocytes

It is well established that FGF-1 exerts potent neurotrophic effects and promotes motor neuron survival and axonal growth following injury [48–50]. Remarkably, we have showed that FGF-1, through activation of the FGFR-1, induces the expression of the antioxidant enzyme heme oxygenase-1 (HO-1) in cultured spinal cord astrocytes [51]. FGF-1 regulates HO-1 expression primarily at a transcriptional level and requires new protein synthesis, which is consistent with the involvement of the nuclear factor erythroid 2-related factor 2 (Nrf2) in the transcriptional regulation of the HO-1 gene. The activation of Nrf2 results in increased expression of ARE-containing genes including antioxidant and phase II detoxification enzymes, which can potentially confer protection to neighboring neurons [52, 53]. In agreement, increased Nrf2 activity in astrocytes improved the survival of co-cultured motor neurons [51], suggesting the potential role of astrocytic Nrf2 in regulating the crosstalk between glia and neurons in neurodegeneration. Among the Nrf2-driven genes expressed in astrocytes are those involved in the synthesis of glutathione, the most abundant thiol present in mammalian cells. Extracellular glutathione exerts potent neuroprotective activity and antagonize the deleterious actions of NO in the nervous system. Consequently, astrocytes can export glutathione to the extracellular media which can exert a direct antioxidant effect in the extracellular compartment and also boost glutathione content in neurons. Taken together, these data emphasize the role of astrocytes in supporting motor neuron survival and predict that upregulation of ARE-driven genes in astrocytes may serve as a therapeutic approach in ALS and other neurodegenerative diseases.

HO-1 is a microsomal enzyme that catalyzes the degradation of the pro-oxidant heme group yielding equimolar quantities of biliverdin, iron, and carbon monoxide [54]. Biliverdin is subsequently converted to bilirubin, which exerts potent antioxidant activity and neuroprotective effects [55–57]. Two isoforms of heme oxygenase have been characterized: a constitutive isoform, HO-2, and an inducible enzyme, HO-1 [58, 59]. HO-1 is poorly

expressed in normal conditions and mainly restricted to a subset of neurons in the brain and motor neurons in the ventral horn of the spinal cord [60–62]. However, HO-1 is quickly and highly induced in glial cells by a variety of pro-oxidant and inflammatory stimuli [63–69]. A common feature of HO-1 inducers is their ability to cause oxidative stress, which is in agreement with its antioxidant activity. In fact, HO-1-deficient mouse and human show an increased susceptibility to oxidative stress [70, 71] and overexpression of HO-1 confers resistance against oxidative stress [72]. In neurodegenerative diseases, HO-1 is augmented in neurons and astrocytes, the percentage of HO-1-immunoreactive astrocytes being notably increased compared to normal controls [73]. Although little is known about HO-1 expression in ALS patients, we have recently reported that both Nrf2 and HO-1 expression is induced in astrocytes at the onset of the disease in G93A SOD1 rats [51].

Secretion of Nerve Growth Factor by Activated Astrocytes and Motor Neuron Apoptosis

It has been previously shown that FGF-1 induces astrocyte activation and nerve growth factor (NGF) secretion [74, 75]. NGF plays a key role modulating neuronal survival and differentiation through activation of the tyrosine kinase receptor TrkA, but it can also stimulate neuronal death by activation of the p75 neurotrophin receptor (p75) [76, 77]. NGF is a mediator in tissue inflammation and accumulates in several neuropathologies [78]. In agreement, we found a 2-fold increase in NGF levels in the spinal cord of symptomatic G93A SOD-1 mice [79]. In these mice, NGF is localized in a subset of reactive astrocytes expressing GFAP that surround the degenerating spinal motor neurons.

Signaling through p75 receptor, in the absence of the corresponding Trk receptor, has been shown to promote apoptosis in specific neuronal types during normal CNS development. Such a mechanism is probably used to eliminate damaged neurons and oligodendrocytes in the mature nervous system [80, 81]. Although motor neurons do not express TrkA, they express the p75 receptor during the embryonic period of naturally occurring cell death when over half of the motor neurons die, but its expression gradually ends after birth [82–84]. Although p75 is not present in mature motor neurons, the receptor can be re-expressed following nerve injury [82, 85–88] and has been shown to mediate motor neuron loss after facial

nerve lesion in newborn [89] and adult mice [85]. Moreover, p75 is found in motor neurons of ALS patients [90, 91], suggesting that re-expression of the receptor might modulate the elimination of neurons in damaged areas. We have showed evidence that in cultures of spinal cord astrocytes, FGF-1 increased by 3- to 4-fold NGF expression and secretion, which in turn induced apoptosis of co-cultured motor neurons expressing the p75 receptor [79].

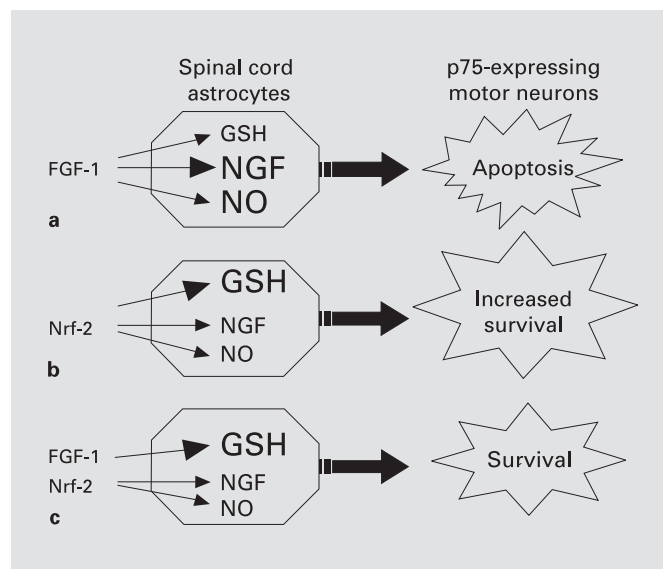
Thus, we hypothesized that FGF/FGFR-1 signaling in degenerating spinal cords of ALS mice contribute to astrocyte activation and may have a dual role. Through Nrf2 induction it promotes neuroprotection of healthy neurons. On the other hand, by NGF secretion it may eliminate damaged motor neurons expressing the p75 receptor. Interestingly, the vulnerability of motor neurons to NGF is potently modulated by the availability of nitric oxide, suggesting that the NGF/p75 pathway requires a concurrent oxidative stress or mitochondrial dysfunction.

ALS, Neuroinflammation and Gliogenesis

Neuroinflammation is a common feature of most neurodegenerative diseases. In ALS, neuroinflammation has been suggested to play a role in the progression of the disease [12]. Inflammatory mediators and newly expressed extracellular matrix proteins can stimulate endogenous stem cells proliferation [92]. Moreover, astrocytes might undergo a process of de-differentiation during neuroinflammation acquiring the potential of neural stem cells [93]. Preliminary evidence suggests increased cell proliferation in the spinal cord of ALS mice [94], as expected from the increased number of glial cells previously described. However, little is known about the functional impact of this process. Proliferating astrocytes could modulate the ongoing neuroinflammation, providing further trophic and metabolic support to damaged neurons. However, the possibility exists that some astrocytes differentiate to atypical or ‘aberrant’ phenotypes that allow them to avoid the anti-inflammatory signals. Such cells might contribute to neuronal damage and/or to the spreading of the disease to neighboring regions.

Further complexity is given by the possibility that blood stem cells could migrate into the parenchyma of degenerating CNS and contribute with new populations of cells. A mechanism of trans-differentiation into glial cells has been proposed. In a model of spinal cord injury, transplantation of stem cells has been reported to amelio-

Fig. 1. Modulation of motor neuron survival by astrocytic mediators in different experimental conditions. **a** Treatment of astrocytes with FGF-1 strongly upregulates NGF expression and secretion, in concert with increased NO production, leading to p75-dependent apoptosis of motor neurons. FGF-1 treatment would increase glutathione levels by Nrf2 activation, which cannot counteract apoptosis [43, 79]. **b** Increasing Nrf2 activity by its overexpression leads to increased glutathione content and secretion in astrocytes, which is sufficient to support motor neuron survival [51]. **c** Nrf2 overexpression prevents motor neuron apoptosis induced by astrocytes treated with FGF-1, possibly by a mechanism involving increased glutathione synthesis and blockade of nitrate stress in motor neurons [51].



rate tissue damage, induce axonal regeneration, and improve locomotion [95]. Similar mechanisms might explain the apparent beneficial effects of intravenous administration of human umbilical cord blood cells and wild-type bone marrow stem cells in SOD-1 transgenic mice [96–98]. The authors showed significant incorporation of transplanted cells in spinal cord and brain, displaying a predominant phenotype of microglia. Further studies are needed to determine how neurogenesis, gliogenesis and cell renewal influence the neuron-glia functional unit in ALS. The increased number of activated microglia may lead to astrocyte dysfunction and increased cytotoxicity. Microglia from SOD-1 mice showed an increased production of TNF- α and decreased IL-6 release when stimulated by LPS [99]. Astrocytes in turn, can downregulate microglial reaction [100], but this ability can be disrupted by the expression of SOD-1 mutations.

Conclusions

The toxic gain of function of SOD-1 mutations is likely dependent on the triggering of specific interactions between neurons and glial cells. The concept of the ‘functional unit’ formed by a neuron together with the adjacent glial cells may help to understand the complexity of these interactions in pathological conditions such as ALS. When the functional unit is perturbed by primary neuronal damage (e.g. expression of mutated SOD), glial cells can proliferate, be replaced or adopt reactive phenotypes

that can largely counteract the defect and prevent neuronal degeneration. Such an influence might be exerted by the expression of cytoprotective genes and the production of trophic factors by astrocytes as summarized in figure 1. However, the re-expression of p75 in motor neurons in parallel with increased NGF secretion by reactive astrocytes, may be a mechanism to eliminate critically damaged neurons. Finally, the neuron-glia functional unit must be considered as potentially dynamic. Not only the phenotypes may greatly vary but also new astrocytes and cells coming from resident or peripheral precursor cells can migrate and integrate to the unit. Future studies will have to elucidate how important the neuron-glia interactions are in the pathogenesis of ALS and other neurodegenerative disorders.

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