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Far beyond the motor neuron: the role of glial cells in amyotrophic lateral sclerosis

Muito além do neurônio motor: o papel das células da glia na esclerose lateral amiotrófica

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ABSTRACT

Motor neuron disease is one of the major groups of neurodegenerative diseases, mainly represented by amyotrophic lateral sclerosis. Despite wide genetic and biochemical data regarding its pathophysiological mechanisms, motor neuron disease develops under a complex network of mechanisms not restricted to the unique functions of the alpha motor neurons but which actually involve diverse functions of glial cell interaction. This review aims to expose some of the leading roles of glial cells in the physiological mechanisms of neuron-glial cell interactions and the mechanisms related to motor neuron survival linked to glial cell functions.

Keywords: motor neurons; astrocyte; microglia; amyotrophic lateral sclerosis; motor neuron disease.

RESUMO

A doença do neurônio motor constitui um dos principais grupos de doenças neurodegenerativas, representadas principalmente pela esclerose lateral amiotrófica. Apesar dos amplos dados genéticos e bioquímicos em relação aos seus mecanismos fisiopatológicos, a doença do neurônio motor se desenvolve sob uma complexa rede de mecanismos não restritos às funções particulares dos neurônios motores alfa, mas, na verdade, envolvendo diversas funções interativas das células da glia. Esta revisão tem como objetivo expor alguns dos principais papéis das células da glia nos mecanismos fisiológicos de interações neurônio-glia e os mecanismos relacionados à sobrevivência do neurônio motor ligados a funções das células da glia.

Palavras-chave: neurônios motores; astrócitos; microglia; esclerose lateral amiotrófica; doença do neurônio motor.

Motor neuron disease represents an important group of adult-onset progressive neurodegenerative motor conditions, typified primarily by upper and lower motor neuron compromise in amyotrophic lateral sclerosis (ALS)[1,2]. Amyotrophic lateral sclerosis results from progressive neurodegenerative processes of the central nervous system (CNS) involving the motor cortex, brainstem motor nuclei and anterior horn of spinal cord[3]. Specific clinical, electrophysiological and neuroimaging findings guarantee a definitive diagnosis by using the revised El Escorial diagnostic criteria for ALS[1]. Although alpha motor neuron and cortical upper motor neuron diseases establish the motor clinical commitment, its pathophysiological mechanisms are wider and involve a complex network of cell interactions, non-neuronal cell roles and molecular mechanisms involving dysfunction of glial cells. Despite great improvement in its proper clinical, neurophysiological and radiological diagnosis, pharmacologic management is still based on symptom exclusion therapy measures[4].

Different mechanisms have been postulated as dysfunctions in ALS including motor neuron oxidative stress (including endoplasmatic reticulum stress), glutamate direct excitotoxicity, dysfunction in electrolytes and vesicular homeostasis, disruption of axonal transport (of proteins and mitochondria along microtubules), exosome and vesicular trafficking (including dysregulated endosomal trafficking), motor neuron apoptosis (including mitochondria-mediated mechanisms), dysfunction in the ubiquitin-proteasome system and in autophagy, mitochondrial dysfunction, aberrant RNA metabolism and processing, and glial cell pathology[5,6,7,8]. There is also growing evidence that other underlying hypothetical mechanisms affecting, primarily, the glial cells should include emergent latent virus infection and misfolded infectious protein (prion-like

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effect)[7]. This review aims to summarize the most important data established on the role of glial cell types in ALS.

PRIMARY GLIAL CELL TYPES AND FUNCTIONS RELATED TO MOTOR NEURODEGENERATION

Most information about physiopathological mechanisms involved in ALS has been through post-mortem histopathological evaluation and from mouse models related to different induced mutations, mainly the human transgenic mouse model with G93A mutation in the gene *SOD1* (Cu-Zn superoxide dismutase-1), involved in an autosomal inherited form of ALS, with overexpression of SOD1 protein. In motor neuron disease linked to TAR DNA-binding protein 43 (TDP43), fused in sarcoma protein (FUS), *ALS2* gene, *VAPB* gene, *OPTN* gene and *C9ORF72* gene (*chromosome 9 open reading frame 72*) mechanisms[7,9], few data regarding the involvement of glial cells are available, compared with cases related to *SOD1* gene mutations.

There is no doubt about the importance of cellular and non-neuronal extracellular microenvironments in the regulation of mechanisms involved in neuron survival[10] and in early stage activation of astroglial and microglial cells in ALS pathogenesis[11]. Changes in the adequate formation and balance of the neuron-glia network and abnormal assembly of its components represent one of the main mechanisms related to different, slowly progressive, focal and global neuropsychiatric dysfunctions, such as ALS[10].

Although nearly all glial cells are involved in ALS pathogenesis, astrocytes and microglia have major roles[12] (Figure 1). Protoplasmic and fibrous astrocytes, microglial cells and oligodendrocytes represent the major types of glial cells in the CNS and most non-neuronal pathological processes related to neurodegeneration involve these cell types. The role of ependymal cells and choroid plexus epithelial cells are not yet understood, specifically in ALS pathogenesis, and have not been adequately studied[10]. Mechanisms involving other glial cell types including Schwann cells, oligodendrocytes and NG2 (nerve-glia factor 2 proteglycan antibody) positive cells are also not well established[12].

Neuropathological studies have shown that reactive astrocytes, microglia activation, and macrophage and T-lymphocyte infiltration of neural tissues overcoming classical motor neuron dysfunction in ALS[3] (Figure 2). There is a fine immune cross-talk in ALS pathogenesis in a suggestive immunopattern shown by reactive microglia (with a mixture of minor expression of CX3CL1 and its receptor and CD200 and its receptor and major expression of IL-6, IL-12, TNF-α and reactive oxygen species); activated macrophages (with higher expression levels of IL-1β, COX2 and glycoprotein CD68), reactive astrocytes (raised SDF-1α); and reactive T-lymphocytes (starting with a preponderance of regulatory T lymphocytes, and, during

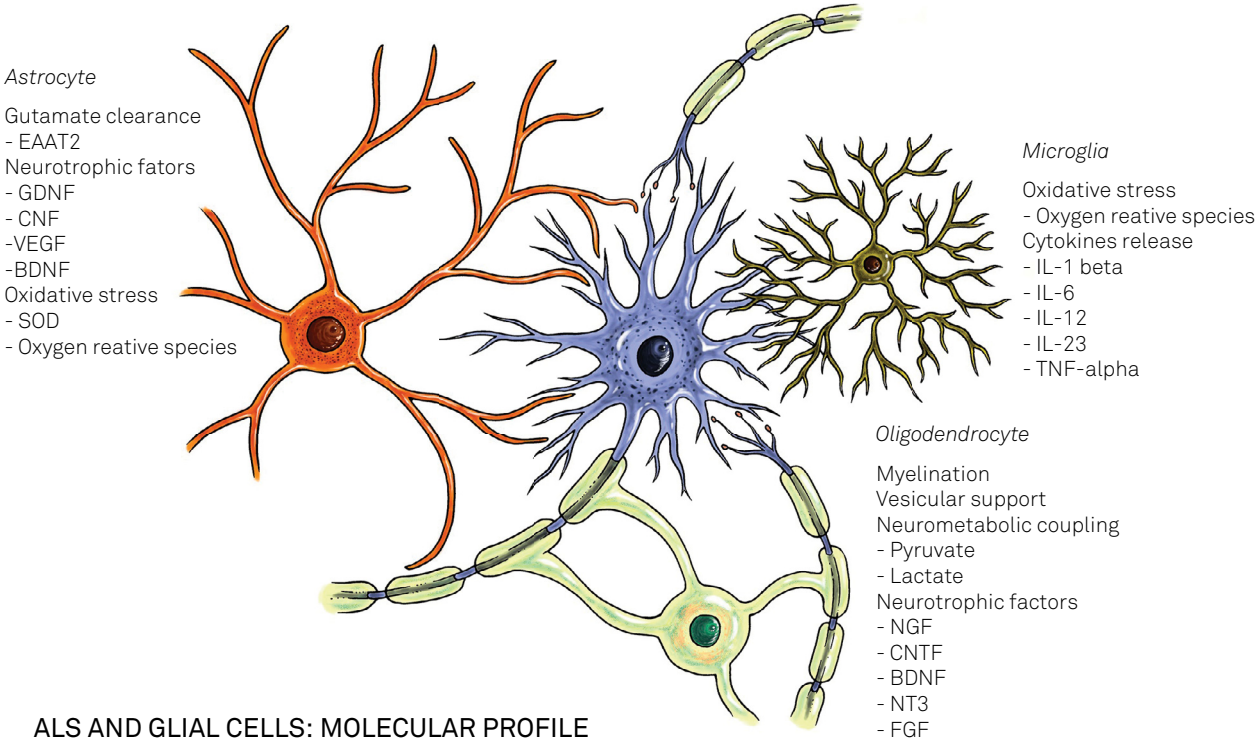

Figure 1. The ALS molecular profile. Glial cells interact with neurons in many different ways to maintain neural tissue health, for which a peculiar cytoarchitecture is required, as shown. Molecular mechanisms and molecules recently implicated in the pathophysiology of motor neuron disease are listed, according to cell types.

progression, with a predominance of homolog effector T lymphocytes with reduction of their neuroprotector effect with raised reactive oxygen species, inducible nitric oxide synthase and NOX2/phagocyte oxidase gp91phox)[3,13].

Environmental factors and extraneuronal disturbances are also essential in its immunopathogenesis. For example, it has also been proven that the direct modulatory effect of vitamin D in glial cell function in patients with ALS affects different cell and extracellular matrix mechanisms: cell-signaling mechanisms (glutamate, matrix metalloproteinases, mitogen-activated protein kinase pathways, Wnt/β-catenin signaling pathway, prostaglandins and reactive oxygen species release, and nitric oxide synthase), major histocompatibility complex class II molecules, toll-like receptors, poly (ADP-ribose) polymerase-1, heme oxygenase-1, calcium-binding proteins, and a reduced form of nicotinamide adenine dinucleotide phosphate[14].

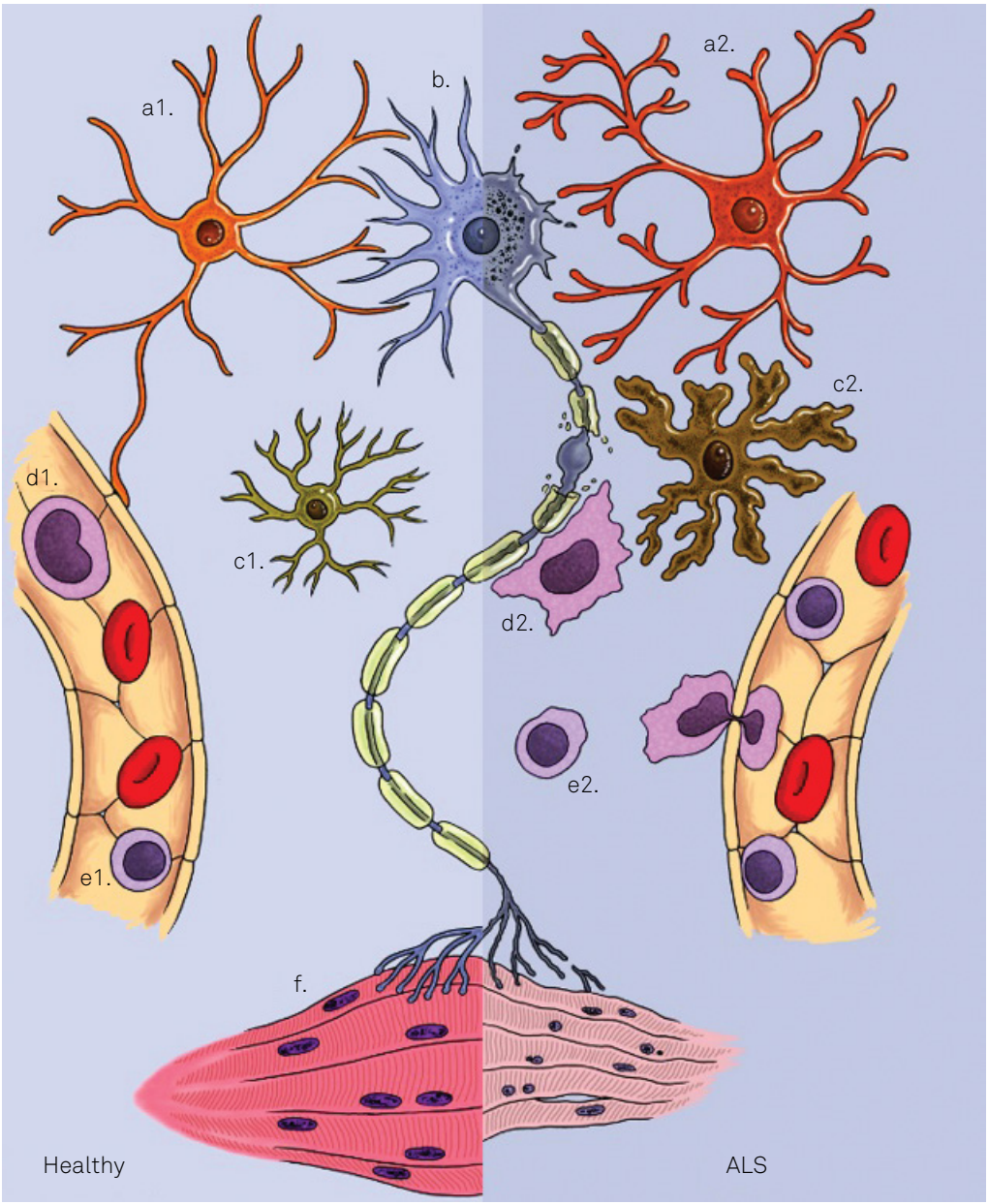

Figure 2. Glial cells functioning in healthy subjects and in ALS patients. a1. Astrocyte: nourishes motor neurons through the synthesis of amino acids, neurotransmitters and neurotrophic molecules; clears extracellular glutamate via specific transporters (EAAT2); offers antioxidant defenses. a2. Reactive astrocyte: reduced production of neurotrophic factors; inefficient glutamate clearance; induces metabolic pathways that favor oxidative stress. b. Motor neuron. c1. Microglia: expresses ligands and receptors with neuroprotective roles (CX3CL1/ CX3CL1R, CD200/CD200R); minimal production of oxygen and nitrogen reactive species. c2. Reactive microglia: increased synthesis and release of oxidant agents (H_2O_2, NO_2) and cytokines (IL-6, IL-12, IL-23, TNF-α). d1. Monocyte: no chemotactic stimulus. d2. Macrophage: invades neural tissue, secretes molecules that perpetuate inflammation and neuron loss (IL-1 beta), increased expression of COX-2. e1. T-lymphocyte: neuroprotective production of IGF-1, brain-derived neurotrophic factor and glial-derived neutrotrophic factor. e2. Reactive T-lymphocyte: secretes IL-4, IL-10, TGF-β; increased expression of INOS and NOX-2. f. Skeletal muscle fiber.

At different stages of evolution, there are remarkable changes in neuroinflammation patterns and cell activation in ALS. Briefly, there is no doubt that the balance of astrocyte neuroprotective or microglia pro-inflammatory functions generate a progression, or decrease, in the rate of primary intrinsic motor neuron degeneration[15,16]. During the late stages of ALS, there is a wide increase in cytotoxic T cell infiltration of the spinal cord leading to a pro-neurotoxic profile of cytokines and chemokines and decreases in local levels of neuroprotective neurotrophic factors, such as GLT1 (SLC1A2) and GLAST (SLC1A3)[16]. Next, we will sumarize the most relevant physiopathological functions described in each glial cell type.

ASTROCYTE ROLE IN ALS PATHOGENESIS

Although classically related to neuronal regulation functions, astrocytes are involved in regulation of the extracellular microenvironment in neurotransmitters at synapses and during their developmental stages; control of signaling of CNS vascular development (including modulation of blood-brain-barrier development); neurotrophic support and stimulation for diverse neurons, maintenance of intercellular signaling (including modulation of excitatory synaptic transmission via release and propagation of glutamatergic stimuli); and neurometabolites and ionic regulation and homeostasis (acid and fluid equilibrium)[3,8,13,17,18,19].

The main mechanism involving astrocytes in ALS pathophysiology is dysfunction of glutamate transporters[20] with loss of the astroglial glutamate transporter EAAT2 (by aberrant RNA splicing, exon skipping and intron retention) in the motor cortex and in the anterior horn of the spinal cord, disclosing its important function in excitotoxic damage in sporadic ALS. As previously mentioned, astrocytes participate in glutamate clearance from the synaptic clefts providing more balanced levels of extracellular excitatory neurotransmission, the defective reuptake of which being the key mechanism in mouse models linked to EAAT2 transporter dysfunctions. It has also been proved in mutant *SOD1* mice that astrocytes directly regulate the expression of glutamate receptor subunit GluR2 in AMPA receptors of motor neurons[12]. Other proven mechanisms included impaired release of multiple neurotrophic factors, including glial-derived neutrotrophic factor, ciliary neurotrophic factor, vascular endothelial growth factor and brain-derived neurotrophic factor[12]. Astrocytes with *SOD1* gene mutation produce reactive oxygen species and soluble molecules with a selective toxicity pattern to spinal cord motor neurons. Another proven mechanism in the *SOD1* mouse model includes activation of the pro-nerve growth factor (NGF)-p75 receptor-signaling pathway involved in direct astrocyte toxicity to motor neurons[8].

It has also been established that high levels of cyclooxygenase 2 is involved in prostaglandin E2 synthesis and hyperstimulation of NMDA glutamate receptor activation of COX2 and subsequent production of reactive oxygen species and prostaglandin E2, and this enhances glutamate release from astrocytes[20].

THE ROLE OF MICROGLIA IN ALS PATHOGENESIS

Microglia represent the major primary resident phagocytic immune cells of the CNS associated with some astrocyte immune functions, secreting pro-inflammatory immune response molecules including cytokines and chemokines and anti-inflammatory molecules during resolution of neural damage, and stimulating the release of neurotrophic growth factors. Microglia result from differentiation of precursors of the monocyte/mesodermal lineage of hematopoietic stem cells that normally protect against microbial infection, abnormal aggregated protein, immunoglobulin-antigen complexes and microhemorrhagic content[3,21].

Microglial activation is a common hallmark of many neurodegenerative diseases, including ALS, despite the fact that it results mainly from proliferation of myeloid precursor cells[22]. Activated microglia releases proinflammatory cytokines (tumor necrosis factor-α, interleukin-1β, interleukin-12, interferon-γ), mitogenic factors (monocyte chemoattractant protein 1, macrophage colony stimulating factor), neurotrophic factors (insulin-like growth factor-1), and anti-inflammatory cytokines (tumor growth factor-β)[12]. There is a direct neuropathological correlation of microglial activation with severity of upper motor neuron damage. Diminishing the toxicity of mutant SOD1 transgene within microglia of mice has been shown to significantly slow disease progression of ALS[23]. It has also been shown that the CCAAT/enhancer binding protein-β is enhanced in activated microglial cells of the spinal cord of ALS mouse models with *SOD1* gene mutation, promoting higher expression of nitric oxide synthase-2, cyclooxygenase-2 and upregulation of other proinflammatory gene expression[24].

Reactive oxygen species and cytokines increase motor neuron susceptibility to glutamate excitotoxicity and inhibit expression of astrocytic glutamate transporters diminishing glutamate uptake and perpetuating this neurotransmitter's neurotoxicity mechanism[25].

Nuclear factor-kappa B (NF-κB) is upregulated in the spinal cord of mouse models and patients with ALS, although inhibitory effects of its pathway in astrocytes did not prevent neurodegeneration or rescue motor neuron death induced by microglia. However, the modulatory effect in NF-κB pathway impairs proinflammatory activation of microglia[26].

Neuropathological studies have also established the activation of microglia and T-cell infiltration in different stages

of ALS evolution[25]. It is widely known that the peripheral immune system represents a crucial stage in the main pathophysiological mechanism: T-lymphocytes directly cross the bood-brain barrier, and interact with resident primary microglia triggering two different immunophenotypes depending on the clinical and pathological stage of the ALS: an M2 protective anti-inflammatory profile in early processes involving regulatory T-cells and IGF-1, or, an M1 cytotoxic profile in late processes induced by fractalkine (CX3CL1) and CD200 involving Th1 cells and interleukin, and other substances such as IL-1β, IL-6, IL-12, IL-23, reactive oxygen species (mainly H_2O_2) and TNF-α[15,27]. Microglia production of IL-10, induced by cytokines from leptomeningeal cells, has also been shown.

OTHER GLIAL CELLS INVOLVED IN ALS PATHOGENESIS

NG2 cells, synantocytes or pericytes, participate in CNS immune mechanisms of defense, producing new astrocytes, oligodendrocytes and neurons, in some situations and specific areas of the CNS. NG2 cells become astrocytes by proinflammatory cytokine signaling in most ALS stages[12]. Oligodendrocytes also regulate local neuronal microenvironments. They represent the main myelinating cells of the CNS[28]. Oligodendrocytes are involved in mechanisms of central myelination and provide metabolic sustenance to motor neurons. Amyotrophic lateral sclerosis does not represent a primary oligodendrocyte disease.

The effective role of $NG2^+$ cells in the astrogliosis process in ALS is still uncertain. $NG2^+$ cells produce new oligodendrocytes, neurons and perhaps astrocytes after an injury, changing the cellular microenvironment. In ALS, cell proliferation rates are enhanced in regions of motor neuron degeneration, but myelination and remyelination defects are also found[3]. Few studies have explored the role of myelinated oligodendrocytes in ALS pathogenesis, which influence motor neurons through neurotrophic factors release and regeneration after neuronal injury. It has also been shown how oligodendrocytes and their $NG2^+$ progenitors mediate neuron loss in ALS, the abnormal rate of proliferation in mouse models secondary to oligodendrocyte degeneration, and to the progressive neuroinflammatory and neurodegenerative processes. The role of Schwann cells in ALS pathogenesis remains undefined[12].

FINAL REMARKS

Motor neuron disease represents an important group of neurodegenerative disorders, mainly represented by ALS. Primary alpha motor neuron involvement and degeneration and secondary mechanisms including glial cell pathological processes represent the most important features in the pathophysiology of ALS. Astrocyte and microglial dysfunctions have been widely demonstrated in patients and animal models of ALS. More studies are needed to establish specific molecular features linked to glial dysfunction at the microcellular level and its extracellular involvement, to allow new pharmacological perspectives and provide data about the natural history of the different genetic forms and variants of ALS.

References

1. Oliveira AS, Pereira RD. Amyotrophic lateral sclerosis (ALS): three letters that change the people's life. For ever. Arq Neuropsiquiatr. 2009;67(3A):750-82. doi:10.1590/S0004-282X2009000400040
2. Radunović A, Mitsumoto H, Leigh PN. Clinical care of patients with amyotrophic lateral sclerosis. Lancet Neurol. 2007;6(10):913-25. doi:10.1016/S1474-4422(07)70244-2
3. Rizzo F, Riboldi G, Salani S, Nizzardo M, Simone C, Corti S et al. Cellular therapy to target neuroinflammation in amyotrophic lateral sclerosis. Cell Mol Life Sci. 2014;71(6):999-1015. doi:10.1007/s00018-013-1480-4
4. Andrews J. Amyotrophic lateral sclerosis: clinical management and research update. Curr Neurol Neurosci Rep. 2009;9(1):59-68. doi:10.1007/s11910-009-0010-0
5. Shi P, Wei Y, Zhang J, Gal J, Zhu H. Mitochondrial dysfunction is a converging point of multiple pathological pathways in amyotrophic lateral sclerosis. J Alzheimers Dis. 2010;20:S311-24. doi:10.3233/JAD-2010-100366
6. Brites D, Vaz AR. Microglia centered pathogenesis in ALS: insights in cell interconnectivity. Front Cell Neurosci. 2014;8:117. doi:10.3389/fncel.2014.00117
7. Sica RE, Nicola AF, Deniselle MC, Rodriguez G, Monachelli GM, Peralta LM et al. Sporadic amyotrophic lateral sclerosis: new hypothesis regarding its etiology and pathogenesis suggests that astrocytes might be the primary target hosting a still unknown external agent. Arq Neuropsiquiatr. 2011;69(4):699-706. doi:10.1590/S0004-282X2011000500023
8. Ferraiuolo L, Kirby J, Grierson AJ, Sendtner M, Shaw PJ. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. Nat Rev Neurol. 2011;7(11):616-30. doi:10.1038/nrneurol.2011.152
9. Mackenzie IR, Rademakers R, Neumann M. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol. 2010;9(10):995-1007. doi:10.1016/S1474-4422(10)70195-2
10. Araque A. Astrocyte-neuron signaling in the brain: implications for disease. Curr Opin Investig Drugs. 2006;7(7):619-24.
11. Neusch C, Bähr M, Schneider-Gold C. Glia cells in amyotrophic lateral sclerosis: new clues to understanding an old disease? Muscle Nerve. 2007;35(6):712-24. doi:10.1002/mus.20768
12. Lasiene J, Yamanaka K. Glial cells in amyotrophic lateral sclerosis. Neurol Res Int. 2011;2011:718987. doi:10.1155/2011/718987
13. Blackburn D, Sargsyan S, Monk PN, Shaw PJ. Astrocyte function and role in motor neuron disease: a future therapeutic target? Glia. 2009;57(12):1251-64. doi:10.1002/glia.20848
14. Long K, Nguyễn LT. Roles of vitamin D in amyotrophic lateral sclerosis: possible genetic and cellular signaling mechanisms. Mol Brain. 2013;6(1):16. doi:10.1186/1756-6606-6-16
15. Evans MC, Couch Y, Sibson N, Turner MR. Inflammation and neurovascular changes in amyotrophic lateral sclerosis. Mol Cell Neurosci. 2013;53:34-41. doi:10.1016/j.mcn.2012.10.008

16. Philips T, Robberecht W. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. Lancet Neurol. 2011;10(3):253-63. doi:10.1016/S1474-4422(11)70015-1

17. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology". Acta Neuropathol. 2010;119(1):7-35. doi:10.1007/s00401-009-0619-8

18. Boillée S, Vande Velde C, Cleveland DW. ALS: a disease of motor neurons and their nonneuronal neighbors. Neuron. 2006;52(1):39-59. doi:10.1016/j.neuron.2006.09.018

19. Maragakis NJ, Rothstein JD. Mechanisms of Disease: astrocytes in neurodegenerative disease. Nat Clin Pract Neurol. 2006;2(12):679-89. doi:10.1038/ncpneuro0355

20. Seifert G, Schilling K, Steinhäuser C. Astrocyte dysfunction in neurological disorders: a molecular perspective. Nat Rev Neurosci. 2006;7(3):194-206. doi:10.1038/nrn1870

21. Graeber MB, Streit WJ. Microglia: biology and pathology. Acta Neuropathol. 2010;119(1):89-105. doi:10.1007/s00401-009-0622-0

22. Gowing G, Philips T, Van Wijmeersch B, Audet JN, Dewil M, Van Den Bosch L et al. Ablation of proliferating microglia does not affect motor neuron degeneration in amyotrophic lateral sclerosis caused by mutant superoxide dismutase. J Neurosci. 2008;28(41):10234-44. doi:10.1523/JNEUROSCI.3494-08.2008

23. Yamanaka K, Yamashita H. [ALS and microglia: a player for non-cell-autonomous neuron death]. Brain Nerve. 2007;59(10):1163-70. Japanese.

24. Valente T, Mancera P, Tusell JM, Serratosa J, Saura J. C/EBPβ expression in activated microglia in amyotrophic lateral sclerosis. Neurobiol Aging. 2012;33(9):2186-99. doi:10.1016/j.neurobiolaging.2011.09.019

25. Henkel JS, Beers DR, Zhao W, Appel SH. Microglia in ALS: the good, the bad, and the resting. J Neuroimmune Pharmacol. 2009;4(4):389-98. doi:10.1007/s11481-009-9171-5

26. Frakes AE, Ferraiuolo L, Haidet-Phillips AM, Schmelzer L, Braun L, Miranda CJ et al. Microglia induce motor neuron death via the classical NF-κB pathway in amyotrophic lateral sclerosis. Neuron. 2014;81(5):1009-23. doi:10.1016/j.neuron.2014.01.013

27. Zhao W, Beers DR, Appel SH. Immune-mediated mechanisms in the pathoprogression of amyotrophic lateral sclerosis. J Neuroimmune Pharmacol. 2013;8(4):888-99. doi:10.1007/s11481-013-9489-x

28. Bradl M, Lassmann H. Oligodendrocytes: biology and pathology. Acta Neuropathol. 2010;119(1):37-53. doi:10.1007/s00401-009-0601-5