Annals of Clinical Case Reports

Review Article
Published: 28 Aug, 2024

Astrocytes, SOD1 and Amyotrophic Lateral Sclerosis: Mechanisms and Implications in Neurodegeneration

*Queiroz DD and Eleutherio ECA**

Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro (UFRJ), Brazil

Abstract

As life expectancy increases, brain aging also rises, leading to cognitive and motor function decline. The accumulation of senescent glial cells during brain aging contributes to chronic inflammation and diseases such as Amyotrophic Lateral Sclerosis (ALS). These cells, including astrocytes, can undergo molecular and functional changes, exacerbating inflammation and inducing neuronal toxicity through the secretion of toxic factors, contributing to a pathogenic autotoxic cycle that drives disease progression. ALS, a severe neurodegenerative disease with reduced life expectancy, presents symptoms such as muscle atrophy, stiffness, and weakness due to motor neuron loss. ALS can be sporadic or familial, both linked directly or indirectly to Cu/Zn Superoxide Dismutase (SOD1) enzyme dysfunction. In addition to its role in eliminating superoxide radicals largely generated by respiration, SOD1 has been described as a possible transcription factor responsible for activating antioxidant protection mechanisms against oxidative stress. It is also implicated in signaling that shifts respiratory metabolism to aerobic glycolysis (glucose fermentation even in the presence of oxygen), affecting cellular protection against oxidative stress and longevity. Studies in animal models and post-mortem analyses of ALS patient tissues have demonstrated specific alterations in astrocytes, including changes in gene expression and secretion of pro-inflammatory factors. In summary, astrocytes play a complex and significant role in ALS, directly influencing disease progression through inflammatory processes, mitochondrial dysfunction, and oxidative stress. Better understanding of the roles of SOD1 and astrocytes in ALS pathogenesis is crucial for developing targeted therapeutic strategies aimed at modulating their protective and inflammatory functions.

Keywords: Astrocyte; Superoxide dismutase 1; Amyotrophic Lateral Sclerosis and neurodegeneration

OPEN ACCESS

Correspondence:
Elis Cristina Araujo Eleutherio, Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro (UFRJ), Av. Athos da Silveira Ramos, 149, Rio de Janeiro, RJ, 21941-909, Brazil, Tel: +55-21-3938-7735;

Received Date: *24 Jul 2024*
Accepted Date: *23 Aug 2024*
Published Date: *28 Aug 2024*

Citation:
Queiroz DD and Eleutherio ECA. Astrocytes, SOD1 and Amyotrophic Lateral Sclerosis: Mechanisms and Implications in Neurodegeneration. Ann Clin Case Rep. 2024; 9: 2666.
ISSN: 2474-1655.

Introduction

Aging is an inevitable consequence for all individuals, leading to a decline in cognitive and motor functions. According to the World Health Organization (WHO), the global population over 60 years old could reach 2 billion by 2050, resulting in a higher incidence of age-related diseases as well as neurodegenerative diseases such as Parkinson's, Alzheimer's, Huntington's, and Amyotrophic Lateral Sclerosis (ALS). Although ALS has a low global incidence, it affects approximately 1 to 4 per 100,000 people per year [1] and is recognized as the most severe among neurodegenerative diseases, with a life expectancy of 2 to 5 years after disease onset [2]. It is characterized by the progressive loss of upper and lower Motor Neurons (MNs). This neuronal death leads to progressive weakness and muscle atrophy, substantially reducing the quality of life for patients, and the majority of patients die from respiratory failure due to the weakening of respiratory muscles.

Although the majority (~90%) of ALS cases arise sporadically, the remaining proportion suggests a genetic basis and may provide insights into the pathophysiology, encouraging research into the genetic causes of this disease. Familial ALS (fALS) accounts for 10% of total cases and is linked to mutations in more than forty genes. The most common include Superoxide Dismutase 1 (SOD1), TAR DNA-binding Protein 43 (TDP-43), Fused in Sarcoma (FUS), and C9orf72 [3]. Collectively, these four genes are responsible for 60% of fALS cases and 11% of sporadic ALS (sALS) cases, with SOD1 responsible for 12% and 2% of fALS and sALS cases, respectively [4]. However, mutations in the SOD1 are the second most common genetic cause since the discovery of its association with ALS over 30 years ago [5]. More than 180 mutations in SOD1 have been described, and these mutations in the antioxidant enzyme Cu/Zn superoxide dismutase lead to changes in protein folding and function. An important pathological feature of ALS is the presence of insoluble protein aggregates

of misfolded proteins that tend to accumulate in motor neurons and associated glial cells [6].

SOD1 and ALS

SOD1 encodes a ubiquitously expressed cytosolic enzyme with a molecular mass of 32 kDa, consisting of two subunits, each composed of 153 amino acids, one copper ion (Cu^{2+}), and one zinc ion (Zn^{2+}) per active site. Its normal cellular function is to detoxify superoxide produced in the mitochondrial intermembrane space, cytosol, and peroxisomes, converting it into oxygen and hydrogen peroxide, thereby serving as an important cellular antioxidant defense mechanism. Although this is its primary function, recent studies have shown that SOD1 has other functions that diverge from this original role, including the activation of protective and repair gene transcription following exposure to oxidative stress and the modulation of glucose sensing pathways to regulate metabolic type, whether fermentative or respiratory [7].

All these functions performed by SOD1 are crucial for the proper functioning of neurons. Neurons are highly susceptible to oxidative stress and therefore depend on the efficient performance of the antioxidant system, in which SOD1 acts as a catalyst and inducer of the antioxidant response. On the other hand, neurons rely on lactate produced by astrocytes to generate ATP, conserving glucose for NADPH production for their antioxidant protection. SOD1 has also been described as a regulator of aerobic glycolysis. However, mutations such as hSOD1A4V have been shown to inadequately regulate PDH activity through phosphorylation, impairing aerobic glycolysis a process crucial in astrocytes and vital for neuronal health [8]. Therefore, non-functional or misfolded SOD1 poses significant harm to neurons [7]. In some cases of ALS, altered SOD1 protein accumulates within associated neuronal and non-neuronal cells, believed by researchers to cause neuronal damage leading to their death. It is important to note that normal SOD1 protein, present in sALS, can also generate protein aggregates, suggesting this is a central issue in ALS. Recently, it has been shown that various forms of SOD1 proteopathy are a common feature in all forms of ALS (sporadic and familial, whether or not linked to SOD1 mutations) [9]. Most of these alterations are specific to regions of neurodegeneration, emphasizing the importance of SOD1 proteopathy as a target for developing new therapies against ALS. Rodent models expressing missense mutations in SOD1 develop abnormal cytoplasmic protein aggregates and motor neuron degeneration, identical to human SOD1-ALS pathology, while SOD1 knockout mice fail to develop aggregates and NM loss [10]. This has also been observed with TDP-43 inclusions in neural and glial cells inducing degenerative motor neuron death in ALS through the pathogenic effect of TDP-43 protein resulting from the formation of toxic aggregates, rather than loss of function. It is known that TDP-43 inclusions are found in 95% of motor neurons and glia in ALS patients, and aggregation occurs due to phosphorylation controlled by the phosphatase Calcineurin (Cn) [11]. Previous *in vivo* and *in vitro* studies have shown that SOD1 interacts with Cn increasing its activity, consequently hyperphosphorylating TDP-43 [12]. Jeon and collaborators demonstrated that the expression and redistribution of TDP-43 protein in neurons and glial cells of SOD1G93A mice lead to cellular damage and death, indicating that SOD1 mutations alter modifications as well as phosphorylation in TDP-43, thereby supporting the hypothesis of an interaction between mutant SOD1 and TDP-43 in ALS pathogenesis [13]. However, further studies are needed to clarify the mechanism of TDP-43 modification induced by SOD1 mutation.

Pathogenic Mechanisms in ALS

Although many pathophysiological mechanisms have been proposed, such as excitotoxicity, oxidative stress, mitochondrial dysfunction, protein aggregation, and neuroinflammation, it is increasingly evident that ALS is not a disease purely related to motor neurons; both astrocytes and SOD1 mutations play crucial roles in these processes. The central nervous system (CNS), traditionally divided into the brain, spinal cord, and retina, is composed of neuronal and glial cells. Glia consists of astrocytes, microglia, and oligodendrocytes in the central nervous system, and satellite glial cells and Schwann cells in the peripheral nervous system. However, glial cells also play a role in neuroinflammatory mechanisms and neuronal death throughout the progression of the disease [14-16].

Role of Astrocytes in ALS

ALS is a complex and multifactorial disease, indicating that the crosstalk among different cell types contributes to triggering neurodegeneration rather than a single cell type. Astrocytes are the largest population of non-neuronal cells in the CNS and perform a wide range of functions in a healthy brain. Astrocytes express receptors, transporters, and neurotransmitters, in addition to releasing neurotrophic factors, inflammatory mediators, and cytokines. Therefore, astrocytes are crucial for providing structural, metabolic, and trophic support to neurons [17].

In response to a wide variety of stimulus and the context in which they find themselves, as well as during neurodegeneration, astrocytes can significantly alter their gene expression, morphology, and function in a process known as "reactivity," which can present neurotoxic, pro-inflammatory (A1) or neuroprotective, anti-inflammatory (A2) phenotypes [18-20]. Reactive astrogliosis is driven by inflammatory signals, loss of neuronal contact, and disease-associated proteinopathy [19,21]. For example, during aging, reactive A1 astrocytes exhibit altered morphologies and produce pro-inflammatory cytokines, leading to neuroinflammation, a process that plays a significant role in the CNS and associated pathological conditions [22]. In degenerative disorders, such changes disrupt brain homeostasis, causing astrocytes to acquire toxic gain-of-function or loss of essential metabolic functions that exacerbate neurodegenerative processes [23]. In astrocytes-ALS, evidence of both neuroprotective and neurotoxic effects has been reported [24].

It is well known that astrocyte dysfunction and neuroinflammation have been shown to accompany and likely even drive the loss of motor neurons in ALS. However, the role of reactive astrocytes in the progression of ALS involves several mechanisms that can result in the loss of physiological and homeostatic functions or the acquisition of a neurotoxic and aberrant phenotype [6,25]. For example, reactive astrocytes in ALS show increased immunoreactivity for GFAP and the calcium-binding protein S100β, expressing inflammatory agents such as cyclooxygenase-2, inducible nitric oxide synthase (iNOS), NOS and reactive oxygen species (ROS). Furthermore, C3, a recognized marker of A1 astrocytes, has been identified in post-mortem tissue from the cortex and spinal cord of ALS patients, both in cases of fALS and sALS [19,26]. However, the mechanisms that lead to astrocytic failure and hyperactivation remain unclear. Thus, identifying specific mechanisms and mediators of astrocytic toxicity may provide important insights into the pathways of motor neuron degeneration in ALS.

As observed, this spontaneous neurodegenerative phenotype has

been reported in results widely described in the literature, as well as the toxic effect on motor neurons (MNs). The presence of specific soluble factors from mutated astrocytes is sufficient to induce damage and loss of MNs, as observed when they were cultured in the presence of primary astrocytes expressing SOD1G93A or when exposed to astrocyte-conditioned medium (ACM) [27-30], as well as in ALS patients [31].

More evidence indicates that astrocytes in ALS exhibit dysfunctional mechanisms and actively induce toxicity in MNs. Among these mechanisms, selective impairment of glial glutamate transport, leading to the accumulation of excitotoxic levels of extracellular glutamate, is one of the earliest hypotheses proposed to explain MN death in ALS. Astrocytes maintain homeostatic levels of extra-synaptic glutamate within the synaptic cleft to regulate synaptic transmission, primarily through specific glutamate transporters such as GLAST and GLT-1 [32]. The GLT-1 transporter is found exclusively in astroglia, both in the brain and spinal cord, and is responsible for the uptake of nearly 90% of glutamate. If glutamate is not removed, activation of glutamate receptors leads to sustained elevation of intracellular calcium levels in neurons, initiating a cascade of events that culminate in cell death. Astrocytes expressing mutant hSOD1 have shown impaired glutamate clearance, suggesting a pathological feature of the disease [33-35]. Therefore, significant changes in astrocyte biology accompany MN degeneration in ALS models, further supporting the idea that astrocytes play an active role in toxicity mediated by mutant hSOD1.

Finally, the ability of astrocytes to produce and provide energy metabolites and antioxidants is essential for normal neuronal metabolic function [36-38]. Neuronal activity is a process with high energy demands, and through the lactate shuttle from astrocytes to neurons, lactate derived from astrocytes supports the energy needs of neurons. Additionally, glutathione metabolism in astrocytes enhances neuronal antioxidant defenses and protects neurons from increased oxidative stress resulting from neurotransmission and metabolic activity [38]. Dysregulation of this astrocyte-neuron coupling, crucial for normal brain function, likely contributes to neuronal death during neurodegeneration.

Taken together, these studies suggest a scenario of disrupted cell-cell communication with NM being the primary target of the toxicity of the mutant hSOD1, and glial cells modulating the rate of degeneration. By carrying the hSOD1 mutation, nearby glial cells damage motor neurons, as well as other important partner cells, which likely adopt aberrant responses and accelerate disease progression. Together, these observations suggest that NM degeneration mediated by astrocytes is an important phenomenon in ALS, but we believe that further studies are necessary to better understand the role of astrocytes in this disease.

Intersection between Astrocytes, SOD1, and Neurodegeneration

The intersection between astrocytes, SOD1, and neurodegeneration in ALS reveals a pathogenic cycle where the dysfunction/gain of function of astrocytes and SOD1 enhances neuronal toxicity, perpetuating neurodegeneration. As mentioned, in neurodegenerative diseases such as ALS, astrocytes change their morphology and function and then become reactive in response to various stimulus.

As previously reported, the first gene identified related to ALS was *SOD1*, which led to the proposal of several relevant mechanisms in the pathogenesis of the disease. Although the cause is still unclear, we know that mechanisms such as excitotoxicity, oxidative stress, endoplasmic reticulum (RE) stress, mitochondrial dysfunction, disruption of axonal transport, prion-like propagation, and non-cell autonomous toxicity from glia are associated with SOD1 proteopathy. Post-mortem isolated astrocytes from ALS or fALS patients have been considered toxic to healthy motor neurons in culture, but this toxicity is alleviated by reducing SOD1 expression in astrocytes [39,40]. Reactive astrocytes induce neurofilaments and SOD1 aggregation, disrupting autophagy *via* TGF-β1 action, leading to motor neuron degeneration [41]. SOD1G93A astrocytes are larger than those from normal tissue, with more hypertrophied processes and express typical markers of astrogliosis, including GFAP [34,42]. Additionally, the expression of Connexin 43 (Cx43), an important astrocytic connexin that drives crucial homeostatic functions in the CNS, is elevated in SOD1G93A astrocytes. During the pre-symptomatic stages of ALS, Cx43 expression in astrocytes is slightly elevated above physiological levels, and this elevation becomes more significant as the disease progresses [43]. Therefore, significant changes in astrocyte biology accompany the degeneration of motor neurons in ALS models, reinforcing the idea that astrocytes play an active role in toxicity mediated by mutant hSOD1.

It is clear that many studies have implicated astrocytes in the pathogenesis of ALS. Recently, changes in the expression of genes associated with extracellular matrix dynamics, endoplasmic reticulum stress responses, and the immune system were reported in a meta-analysis of studies involving human iPSC-derived astrocytes with SOD1 mutations and astrocytes from mice expressing the SOD1G93A mutation [44]. Another recent study, published by Shen and collaborators assessed the function of Differentially Expressed Genes (DEGs) in non-neuronal cells from the primary motor cortex of ALS patients. The results showed that the functions of the DEGs in non-neuronal cells were primarily related to energy metabolism, especially oxidative phosphorylation, and protein synthesis. Additionally, SOD1 was positively regulated in glial cells, confirming that it could be a potential biomarker [45]. Abnormal oxidative phosphorylation is associated with the development of ALS. Analysis of transcriptomic datasets from humans and mice obtained from the GEO database revealed that oxidative phosphorylation was dysregulated in data from ALS patients and in models of mice transgenic for SOD1 [46]. Furthermore, it was observed that the genes involved in oxidative phosphorylation encoded by nuclear DNA showed heterogeneous expression, mostly decreased in the spinal cord tissue of ALS patients [47].

Glial cells are considered one of the largest producers of ROS and reactive nitrogen species (RNS) under pathological conditions in the CNS, including motor neuron diseases [48]. An increase in cellular respiration contributes to elevated ROS levels in astrocytes, activating an inflammatory response that results in non-cell autonomous degeneration of motor neurons. We know that the regulation of the redox balance between astrocytes and neurons is determined by the equilibrium between GSSG/GSH, NAD/NADH, and NADP/NADPH, which directly affects the balance of metabolites such as lactate and pyruvate, and β-hydroxybutyrate and acetoacetate, whose interconversion depends on these ratios. Studies have shown that GSH levels are lower in the motor cortex of ALS patients compared to healthy volunteers [49]. In hSOD1G93A and hSOD1WT mice, it has been demonstrated that GSH depletion promotes neurological

deficits, mitochondrial pathology, and motor neuron degeneration [50,51]. In ALS, significant efforts have provided deep insights into the dysregulated secretome of astrocytes. Recently, an analysis of the proteome and metabolite secretome of astrocytes expressing hSOD1G93A showed alterations in GSH metabolism and signaling that were negatively regulated, while proteolytic processes were positively regulated [52]. In summary, astrocytes express and release antioxidants as part of their function in regulating redox balance by removing ROS to prevent oxidative damage to neurons [53].

Finally, we know that misfolded proteins are transmitted between cells in neurodegenerative diseases, particularly with SOD1 in ALS. Several studies have described "prion-like" characteristics of misfolded SOD1, including its ability to transfer between cells, causing the misfolding of wild-type SOD1 within those cells. Astrocyte-neuron communication can also be modulated by the secretion of extracellular vesicles (EVs), such as microvesicles and exosomes. Misfolded SOD1, whether wild-type or associated with mutations, is secreted *via* EVs in NSC-34 and HEK cells [54]. EVs containing SOD1 aggregates have also been found in the plasma of fALS patients [55], as well as in the brains and spinal cords of SOD1G93A mice, where they were efficiently transferred to spinal neurons, inducing selective motor neuron death [56,57], further reinforcing their involvement in the pathogenesis of the disease. As observed, alterations in the production and composition of exosomes in astrocytes have been previously reported in ALS [58]. Recently, a study demonstrated that exosomes derived from SOD1G93A astrocytes are sufficient to reduce the survival of MN as well as the length and branching of neurites [59]. However, the transmission of toxic aggregates *via* EVs is still not well understood.

Taken together, all these data demonstrate that the dysfunction of the astrocytic secretome in ALS impairs many key functions of motor neurons involving autophagy, growth, and neurite length, resulting in accelerated protein aggregation, excitotoxicity, cellular stress and degeneration [60].

Concluding Remarks

Based on the factors leading to the gain of function/dysfunction of SOD1, the role of astrocytes in neurodegeneration, and their correlation in the pathogenesis of ALS, this review aims to understand the altered molecular pathways that may underlie the dysfunctions of astrocytes in ALS and the altered astrocyte-motor neuron crosstalk in the pathology. Collectively, astrocytes play a significant role in the pathology of ALS in humans and in mouse models, affecting various cell types, particularly MNs, whose survival is severely compromised by astrocytic influence. Therefore, there is growing evidence that both astrocytes and SOD1 are important contributors to neurodegeneration. Understanding the processes occurring in the damaged central nervous system is crucial for developing therapies that may improve the prognosis and quality of life for patients affected by this devastating neurodegenerative disease.

Funding

This work was supported by grants from FAPERJ (CNE 201.174/2022), CAPES-DAAD (PROBRAL 88881.371325/2019-01) and CNPq Universal (401780/2023-6).

References

1. Longinetti E, Fang F. Epidemiology of amyotrophic lateral sclerosis: An update of recent literature. Curr Opin Neurol. 2019;32(5):771-6.
2. Petrov D, Mansfield C, Moussy A, Hermine O. ALS clinical trials review: 20 years of failure. Are we any closer to registering a new treatment? Front Aging Neurosci. 2017;9:68.
3. Ghasemi M, Brown RH. Genetics of amyotrophic lateral sclerosis. Cold Spring Harb Perspect Med. 2018;8(5).
4. Akçimen F, Lopez ER, Landers JE, Nath A, Chiò A, Chia R, et al. Amyotrophic lateral sclerosis: translating genetic discoveries into therapies. Nat Rev Genet. 2023;24:642-58.
5. Rosen 1993.
6. Peggion C, Scalcon V, Massimino ML, Nies K, Lopreiato R, Rigobello MP, et al. SOD1 in ALS: Taking stock in pathogenic mechanisms and the role of glial and muscle cells. Antioxidants. 2022;11(4):614.
7. Eleutherio ECA, Silva Magalhães RS, de Araújo Brasil A, Monteiro Neto JR, de Holanda Paranhos L. SOD1, more than just an antioxidant. Arch Biochem Biophys. 2021;697.
8. de Holanda Paranhos L, Magalhães RSS, de Araújo Brasil A, Neto JRM, Ribeiro GD, Queiroz DD, et al. The familial amyotrophic lateral sclerosis-associated A4V SOD1 mutant is not able to regulate aerobic glycolysis. Biochim Biophys Acta Gen Subj. 2024;1868(8).
9. Trist BG, Genoud S, Roudeau S, Rookyard A, Abdeen A, Cottam V, et al. Altered SOD1 maturation and post-translational modification in amyotrophic lateral sclerosis spinal cord. Brain. 2022;145(9):3108-30.
10. Abati E, Bresolin N, Comi G, Corti S. Silence Superoxide Dismutase 1 (SOD1): A promising therapeutic target for Amyotrophic Lateral Sclerosis (ALS). Expert Opin Ther Targets. 2020;24(4):295-310.
11. Liachko NF, Saxton AD, McMillan PJ, Strovas TJ, Currey HN, Taylor LM, et al. The phosphatase calcineurin regulates pathological TDP-43 phosphorylation. Acta Neuropathol. 2016;132(4):545-61.
12. Agbas A, Hui D, Wang X, Tek V, Zaidi A, Michaelis EK. Activation of brain calcineurin (Cn) by Cu-Zn superoxide dismutase (SOD1) depends on direct SOD1-Cn protein interactions occurring *in vitro* and *in vivo*. Biochem J. 2007;405(1):51-9.
13. Jeon GS, Shim YM, Lee DY, Kim JS, Kang MJ, Ahn SH, et al. Pathological modification of TDP-43 in amyotrophic lateral sclerosis with SOD1 mutations. Mol Neurobiol. 2019;56(3):2007-21.
14. Ilieva H, Polymenidou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J Cell Biol. 2009;187(6):761-72.
15. Robberecht W, Philips T. The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci. 2013;14:248-64.
16. Yamanaka K, Komine O. The multi-dimensional roles of astrocytes in ALS. Neurosci Res. 2018;126:31-8.
17. Verkhratsky A, Ho MS, Zorec R, Parpura V. Neuroglia in neurodegenerative diseases. Adv Exp Med Biol. 1175.
18. Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, et al. Astrocyte scar formation AIDS central nervous system axon regeneration. Nature. 2016;532(7598):195-200.
19. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481-7.
20. Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, Serrano-Pozo A, et al. Reactive astrocyte nomenclature, definitions, and future directions. Nature Neurosci. 2021;24(3):312-25.
21. Burda JE, Sofroniew MV. Reactive gliosis and the multicellular response to CNS damage and disease. Neuron. 2014;81(2):229-48.
22. Verkerke M, Hol EM, Middeldorp J. Physiological and pathological ageing of astrocytes in the human brain. Neurochem Res. 2021;46(10):2662-75.

23. Jiwaji Z, Tiwari SS, Avilés-Reyes RX, Hooley M, Hampton D, Torvell M, et al. Reactive astrocytes acquire neuroprotective as well as deleterious signatures in response to Tau and Aß pathology. Nat Commun. 2022;13(1):135.

24. Pehar M, Harlan BA, Killoy KM, Vargas MR. Role and therapeutic potential of astrocytes in amyotrophic lateral sclerosis. Curr Pharm Des. 2018;23(33):5010-21.

25. Guttenplan KA, Weigel MK, Adler DI, Couthouis J, Liddelow SA, Gitler AD, et al. Knockout of reactive astrocyte activating factors slows disease progression in an ALS mouse model. Nat Commun. 2020;11(1):3753.

26. Reischauer C, Gutzeit A, Neuwirth C, Fuchs A, Sartoretti-Schefer S, Weber M, et al. *In-vivo* evaluation of neuronal and glial changes in amyotrophic lateral sclerosis with diffusion tensor spectroscopy. Neuroimage Clin. 2018;20:993-1000.

27. Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. Nat Neurosci. 2007;10(5):608-14.

28. Marchetto MCN, Muotri AR, Mu Y, Smith AM, Cezar GG, Gage FH. Non-cell-autonomous effect of human SOD1G37R astrocytes on motor neurons derived from human embryonic stem cells. Cell Stem Cell. 2008;3(6):649-57.

29. Re DB, Le Verche V, Yu C, Amoroso MW, Politi KA, Phani S, et al. Necroptosis drives motor neuron death in models of both sporadic and familial ALS. Neuron. 2014;81(5):1001-8.

30. Ikiz B, Alvarez MJ, Ré DB, Le Verche V, Politi K, Lotti F, et al. The regulatory machinery of neurodegeneration in *in vitro* models of amyotrophic lateral sclerosis. Cell Rep. 2015;12(2):335-45.

31. Filipi T, Hermanova Z, Tureckova J, Vanatko O, Anderova M. Glial cells—The strategic targets in amyotrophic lateral sclerosis treatment. J Clin Med. 2020;9(1):261.

32. Gonçalves-Ribeiro J, Pina CC, Sebastião AM, Vaz SH. Glutamate transporters in hippocampal LTD/LTP: Not just prevention of excitotoxicity. Front Cell Neurosci. 2019;13:357.

33. Van Den Bosch L, Van Damme P, Bogaert E, Robberecht W. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. Biochim Biophys Acta. 2006;1762:1068-82.

34. Wallis N, Lau CL, Farg MA, Atkin JD, Beart PM, O'Shea RD. SOD1 mutations causing familial amyotrophic lateral sclerosis induce toxicity in astrocytes: Evidence for bystander effects in a continuum of astrogliosis. Neurochem Res. 2018;43(1):166-79.

35. Dafinca R, Barbagallo P, Farrimond L, Candalija A, Scaber J, Ababneh NA, et al. Impairment of mitochondrial calcium buffering links mutations in C9ORF72 and TARDBP in iPS-derived motor neurons from patients with ALS/FTD. Stem Cell Reports. 2020;14(5):892-908.

36. Bélanger M, Allaman I, Magistretti PJ. Brain energy metabolism: Focus on Astrocyte-neuron metabolic cooperation. Cell Metab. 2011;14(6):724-38.

37. Fernandez-Fernandez S, Almeida A, Bolaños JP. Antioxidant and bioenergetic coupling between neurons and astrocytes. Biochem J. 2012;443(1):3-11.

38. Bolaños JP. Bioenergetics and redox adaptations of astrocytes to neuronal activity. J Neurochem. 2016;139:115-25.

39. Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, et al. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nat Biotechnol. 2011;29(9):824-8.

40. Meyer K, Ferraiuolo L, Miranda CJ, Likhite S, McElroy S, Renusch S, et al. Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS. Proc Natl Acad Sci U S A. 2014;111(2):829-32.

41. Tripathi P, Rodriguez-Muela N, Klim JR, de Boer AS, Agrawal S, Sandoe J, et al. Reactive astrocytes promote ALS-like degeneration and intracellular protein aggregation in human motor neurons by disrupting autophagy through TGF-β1. Stem Cell Reports. 2017;9(2):667-80.

42. Qian K, Huang H, Peterson A, Hu B, Maragakis NJ, Ming G li, et al. Sporadic ALS astrocytes induce neuronal degeneration *in vivo*. Stem Cell Reports. 2017;8(4):843-55.

43. Almad AA, Doreswamy A, Gross SK, Richard JP, Huo Y, Haughey N, et al. Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis. Glia. 2016;64(7):1154-69.

44. Ziff OJ, Clarke BE, Taha DM, Crerar H, Luscombe NM, Patani R. Meta-analysis of human and mouse ALS astrocytes reveals multi-omic signatures of inflammatory reactive states. Genome Res. 2022;32(1):71-84.

45. Shen D, Ji Y, Qiu C, Wang K, Gao Z, Liu B, et al. Single-cell RNA sequencing analysis of microglia dissected the energy metabolism and revealed potential biomarkers in amyotrophic lateral sclerosis. Mol Neurobiol. 2023;61(7):4473-87.

46. Swaroop S, Akhil PS, Pradhan SS, Prasad B, Rao R, Manjunath M, et al. Integrated multi-omic data analysis and validation with yeast model show oxidative phosphorylation modulate protein aggregation in amyotrophic lateral sclerosis. Sri Sathya Sai Institute of Higher Learning. 2021.

47. Xue YC, Ng CS, Xiang P, Liu H, Zhang K, Mohamud Y, et al. Dysregulation of RNA-binding proteins in amyotrophic lateral sclerosis. Front Mol Neurosci. 2020;13:78.

48. D'Ambrosi N, Cozzolino M, Carrì MT. Neuroinflammation in amyotrophic lateral sclerosis: Role of redox (dys)regulation. Antioxid Redox Signal. 2018;29:15-36.

49. Weiduschat N, Mao X, Hupf J, Armstrong N, Kang G, Lange DJ, et al. Motor cortex glutathione deficit in ALS measured in vivo with the J-editing technique. Neurosci Lett. 2014;570:102-7.

50. Vargas MR, Johnson DA, Johnson JA. Decreased glutathione accelerates neurologiCal deficit and mitochondrial pathology in familial ALS-linked hSOD1G93A mice model. Neurobiol Dis. 2011;43(3):543-51.

51. Killoy KM, Harlan BA, Pehar M, Helke KL, Johnson JA, Vargas MR. Decreased glutathione levels cause overt motor neuron degeneration in $hSOD1^{WT}$ over-expressing mice. Exp Neurol. 2018;302:129-35.

52. Stella R, Bonadio RS, Cagnin S, Massimino ML, Bertoli A, Peggion C. Perturbations of the proteome and of secreted metabolites in primary astrocytes from the hsod1 (G93a) als mouse model. Int J Mol Sci. 2021;22(13):7028.

53. Jiwaji Z, Hardingham GE. The consequences of neurodegenerative disease on neuron-astrocyte metabolic and redox interactions. Neurobiol Dis. 2023;185.

54. Gomes C, Keller S, Altevogt P, Costa J. Evidence for secretion of Cu, Zn superoxide dismutase *via* exosomes from a cell model of amyotrophic lateral sclerosis. Neurosci Lett. 2007;428(1):43-6.

55. Sproviero D, La Salvia S, Giannini M, Crippa V, Gagliardi S, Bernuzzi S, et al. Pathological proteins are transported by extracellular vesicles of sporadic amyotrophic lateral sclerosis patients. Front Neurosci. 2018;12:487.

56. Basso M, Pozzi S, Tortarolo M, Fiordaliso F, Bisighini C, Pasetto L, et al. Mutant copper-zinc Superoxide Dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes: Implications for disease spreading and motor neuron pathology in amyotrophic lateral sclerosis. J Biol Chem. 2013;288(22):15699-711.

57. Silverman JM, Christy D, Shyu CC, Moon KM, Fernando S, Gidden Z, et al. CNS-derived extracellular vesicles from superoxide dismutase 1 (SOD1)G93A ALS mice originate from astrocytes and neurons and carry misfolded SOD1. J Biol Chem. 2019;294(10):3744-59.

58. Gharbi T, Zhang Z, Yang GY. The function of astrocyte mediated extracellular vesicles in central nervous system diseases. Front Cell Dev Biol. 2020;8.

59. Marton S, Miquel E, Acosta-Rodríguez J, Fontenla S, Libisch G, Cassina P. SOD1G93A astrocyte-derived extracellular vesicles induce motor neuron death by a miRNA-155-5p-mediated mechanism. ASN Neuro. 2023;15.

60. Jiang S, Xu R. The current potential pathogenesis of amyotrophic lateral sclerosis. Mol Neurobiol. Springer; 2024.