Glial Metabolic Reprogramming in Amyotrophic Lateral Sclerosis

Patricia Cassina^{a, b} Ernesto Miquel^{a, b} Laura Martínez-Palma^{a, b}
Adriana Cassina^{b, c}

^aDepartamento de Histología y Embriología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; ^bCentro de Investigaciones Biomédicas (CEINBIO), Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; ^cDepartamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Keywords

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Abstract

ALS is a human neurodegenerative disorder that induces a progressive paralysis of voluntary muscles due to motor neuron loss. The causes are unknown, and there is no curative treatment available. Mitochondrial dysfunction is a hallmark of ALS pathology; however, it is currently unknown whether it is a cause or a consequence of disease progression. Recent evidence indicates that glial mitochondrial function changes to cope with energy demands and critically influences neuronal death and disease progression. Aberrant glial cells detected in the spinal cord of diseased animals are characterized by increased proliferation rate and reduced mitochondrial bioenergetics. These features can be compared with cancer cell behavior of adapting to nutrient microenvironment by altering energy metabolism, a concept known as metabolic reprogramming. We focus on data that suggest that aberrant glial cells in ALS undergo metabolic reprogramming and profound changes in glial mitochondrial activity, which are associated with motor neuron

death in ALS. This review article emphasizes on the association between metabolic reprogramming and glial reactivity, bringing new paradigms from the area of cancer research into neurodegenerative diseases. Targeting glial mitochondrial function and metabolic reprogramming may result in promising therapeutic strategies for ALS.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a human neurodegenerative disease characterized by the loss of upper and lower motor neurons in the central nervous system (CNS), causing progressive paralysis and death. Despite enormous efforts in research, the etiology of most ALS cases remains unknown. Around 10% of ALS cases have a family history and dominant inheritance (familial ALS, FALS), and the remaining 90% of cases are sporadic (SALS), but both forms exhibit similar pathological features [1]. A growing list of mutations linked to both FALS and SALS involve to date >50 potentially causative or disease-modifying genes, but pathogenic variants in Cu/Zn superoxide dismutase (SOD1), C9ORF72 repeat expan-

sion, Fused in sarcoma (FUS), and TAR DNA-binding protein-43 (TDP-43) are the most frequently occurring [2].

Transgenic rodents expressing FALS-linked SOD1 mutations develop progressive motor neuron degeneration resembling many aspects of the human disease [3, 4] and have been the main source of ALS pathogenic investigations and therapeutic preclinical trials. Studies in these models have demonstrated cell-specific effects of mutant SOD1 expression beyond their influences on motor neurons, highlighting the important role of glia in motor neuron support [5]. These different effects depending on the cell type considered lead to the concept of “noncell autonomous” toxicity of SOD1 mutations coined by Cleveland’s laboratory in studies performed in chimeric mice [6, 7]. Reducing mutant SOD1 expression in astrocytes [8] or microglia [9] extended disease duration in ALS mice, with no effect on disease onset, which seems to be dependent on the expression of mutant SOD1 in motor neurons [9, 10]. Noncell autonomous toxicity has also been shown in knock-in or transgenic models for FUS, TDP-43, and c9orf72 [11–13], although data are not as consistent as with SOD1 mutations, and there are conflicting reports [14, 15]. Taken together, these studies suggest a scenario of disrupted cell-cell communications with motor neurons being the main target of mutant SOD1 toxicity and glial cells modulating the rate of degeneration. When carrying the SOD1 mutation, glial cells nearby damage motor neurons as well as other key partner cells that likely adopt aberrant responses and accelerate disease progression.

Glial Cells in ALS

The term neuroglia was coined by Rudolf Virchow to describe the elements filling the space not occupied by neurons in the CNS [16]. The neuroglia include the neuroectoderm-derived macroglia that comprise astrocytes, oligodendrocytes, and NG2-positive progenitors and the microglia, the resident macrophages of the CNS that have a mesodermal origin.

Astrocytes are a large portion of the glial cell population in the CNS and accomplish a plethora of essential functions that have been extensively summarized [17]. In the human cortex, a single astrocyte enwraps >1 million synapses and most, if not all, have at least 1 process with endfeet contacting a blood vessel [18]. Therefore, astrocytes are polarized cells that are in a key position to provide structural, metabolic, and trophic support to neurons.

Microglia invade the brain early in development, convert into a highly ramified cell type, and constantly screen their environment. Microglia are activated by any type of pathologic event or change in brain homeostasis and can strongly influence the outcome or response to a stressor due to the release of a variety of substances, including cytokines, chemokines, and growth factors. They are the professional phagocytes of the brain and help orchestrate the immunological response by interacting with infiltrating immune cells [19].

Astrocytes and microglia are major players in neuroinflammatory response. Both cell types can be polarized to a neurotoxic/proinflammatory or neurotrophic/resolutive phenotype in response to different signals, and close molecular conversations and reciprocal modulation are established between them during the course of activation [20].

Astrocytes react to CNS damage with a phenotypic shift characterized by morphological, molecular, and functional changes including increased proliferation rate, higher levels of astrocytic glial fibrillary acidic protein expression, and extension of thicker processes [21]. Since many terms have been used to describe these astroglial responses leading to confusing terminologies, it was recently proposed to reserve “reactive astrocytes” as an umbrella term encompassing multiple potential states of astrocytes undergoing disease-associated remodeling [22]. During ALS progression, increased number of reactive astrocytes and hypertrophic microglia are identified in the spinal cord, even before motor symptoms are detected [23, 24].

Astrocytes overexpressing the FALS-linked SOD1 mutation (G93A) reduce motor neuron survival in coculture conditions, whether they are isolated from neonatal rats [25], mice [26], or derived from human cells [27]. The mechanism of neurotoxicity has not been fully identified, but NGF-related species (proNGF or nitrated NGF), nitric oxide (NO), and a reduced lactate release have been suggested as candidates [28, 29]. In addition, oligodendrocytes have also been shown to contribute to neurodegeneration in ALS by reduced lactate support to axons [30] and motor neurons in culture [31]. Elimination of the SOD1G93A astrocyte toxicity can be achieved in culture by depleting NGF levels or blocking the p75 receptor [28, 32] and also by providing lactate to the culture media [29]. Increased NO production and mitochondrial dysfunction in SOD1G93A astrocytes may also contribute to the observed toxicity [25, 33]. For example, pretreatment of mutant SOD1G93A astrocyte monolayers with NOS inhibitors, mitochondrial-targeted antioxidants (ubiquinone and carboxy-proxyl nitroxide) [33], or NADPH ox-

idase inhibitors (NOX2) [34, 35] prevented motor neuron loss, suggesting that multiple pathways could be targeted to improve the antioxidant status of the astrocytes reducing motor neuron death in ALS.

In addition to the pathogenic role of astrocytes and microglia, we have previously described a novel type of aberrant glial cells (AbGC) isolated from the degenerating spinal cord of SOD1G93A rats. AbGC appear after paralysis onset, increase their number toward the final stages of the disease, and are highly toxic to motor neurons in culture [36]. AbGC were initially described as aberrant astrocytes and then AbGC, as they simultaneously express astrocytic glial fibrillary acidic protein, S100 β , and connexin-43 (Cx-43) [36] and microglial Iba-1, CD11b, and CD163 markers [37] and are possibly originated from aberrant microglia [24]. Furthermore, they exhibit features of cellular stress and inflammatory activation [38], are highly proliferative [36], and induce extensive gliosis after focal transplantation into the spinal cord of nontransgenic (non-tg) rats [39]. Such AbGC, typically localized adjacent to motoneurons, are abundant in the symptomatic phase of disease, suggesting a link between their emergence and the rapidly progressing neurodegeneration characteristic of the SOD1G93A rat model. Similar features have been defined in the spinal cord of SOD1G93A mice during paralysis progression, involving a subset of astrocytes strictly confined to the microenvironment of motor neuron cell bodies described as abnormal spheroid-shaped astrocytes [40]. In addition, as the selective isolation of CD11b+ cells rendered AbGC-like cells in culture [37], the possibility exists that AbGC are linked to “disease-associated microglia” that have been identified by different groups using single-cell RNAseq and express damage-associated molecules such as ApoE, Cst7, or LPL [41, 42].

Energy Metabolism and Metabolic Reprogramming in ALS

The brain is widely recognized as a highly oxidative organ, and emphasis is frequently placed on the disproportionately high fraction of oxygen consumed by adult brain per unit mass [43]. Within the CNS, neurons are the highest energy-demanding cells, and accordingly they are also the most sensitive to energy stress. Consistent with their higher energy requirements, neurons sustain a high rate of oxidative phosphorylation (OXPHOS) at the mitochondria compared to astrocytes that are mainly glycolytic [44, 45]. In addition to glucose, neurons can efficient-

ly use lactate as an energy substrate [46] and even show a preference for lactate over glucose when both substrates are present [45]. The full oxidation of glucose, or its metabolites pyruvate or lactate, in the mitochondria, requires the crucial activity of the pyruvate dehydrogenase (PDH), the enzymatic complex that mediates pyruvate conversion to acetyl-CoA, which enters the TCA cycle and the electron transport chain (reviewed in [47, 48]). Hence, PDH activity controls oxidative versus anaerobic catabolism. Accordingly, high PDH activity has been demonstrated in neurons, compared to that found in astrocytes [49], supporting the different metabolic patterns of both cell types. Furthermore, permanent downregulation of the enzyme 6-phosphofructose-2-kinase/fructose-2,6-bisphosphatase-3 (Pfkfb3), which is a key positive modulator of glycolysis, by constant proteasomal degradation in neurons, accounts for their slow glycolytic rate and diverts part of glucose usage to the pentose-phosphate pathway, which is linked to the regeneration of reduced glutathione, an antioxidant critical for neuroprotection, at the expense of NADPH(H+) [50]. As a result, neurons undergo oxidative stress and apoptosis after glycolysis activation via Pfkfb3 overexpression [50] or stabilization [51].

In contrast, high Pfkfb3 levels detected in astrocytes [50], along with a low PDH activity [49], would underlie their high glycolytic rate and lower oxidative metabolism compared to neurons. Furthermore, according to the astrocyte-neuronal lactate shuttle hypothesis, lactate would be transferred from astrocytes to neurons to match the neuronal energetic needs and to provide signals that modulate neuronal physiology, affecting several homeostatic functions (for review see [52]). However, this hypothesis has been controversial [53].

Despite their glycolytic nature, astrocytes exhibit a profuse mitochondrial network. When both neurons and their neighboring astrocytes are subjected to an identical degree of mitochondrial respiratory chain damage by excess of reactive oxygen species, the neurons rapidly undergo cell death, whereas the astrocytes resist by reducing their mitochondrial respiratory activity [54]. SOD1G93A-expressing astrocytes exhibit reduced mitochondrial respiratory activity with no compromise in their viability. Moreover, they show a higher proliferation rate compared to non-tg astrocytes and undergo phenotypic changes that modify cell-cell communications in a way that no longer sustains neuronal survival [33, 55]. As mentioned in the previous section, SOD1G93A astrocyte toxicity has been related to reduced lactate support [29]. However, another study showed higher lactate concentrations in culture media from SOD1G93A astrocytes

compared to those from non-tg ones cocultured with spinal neurons [56]. The beneficial impact of lactate was restricted to a concentration range from 1 to 5 mM, being ineffective at 0.5 or 10 mM [29]. The discrepant data from these 2 studies may be in part derived from differences in time or other culture conditions needed to accumulate the metabolite in the culture media.

Additionally, the mitochondrial fatty acid oxidation capacity of astrocytes was recently demonstrated, highlighting their versatility and the contribution of lipid metabolism to brain bioenergetics [57], signaling in astrocyte-neuron cross-talk, and neurodegeneration [58, 59]. Furthermore, as occurs in immune cells [60], lipid droplets accumulate in astrocytes in response to stress [61], including that originated by mitochondrial defects [62, 63] and mutated SOD1 expression in astrocytes from ALS models [64].

Microglial cells exhibit the ability to undergo a metabolic switch from oxidative phosphorylation toward glycolysis upon activation by proinflammatory stimuli. This shift enables them to meet cellular demands required by high production and secretion of signaling factors and allows for cell survival, similar to the Warburg effect demonstrated in cancer cells. In contrast, surveillance microglial fate or stimulation to a resolutive phenotype relies primarily on mitochondrial oxidative phosphorylation and fatty acid oxidation (reviewed in [65]). Metabolic changes involving lipid droplet accumulation in microglia have also been demonstrated after proinflammatory stimulation [66] and associated with aging and neuroinflammation, as shown in aging mouse and human brains [67].

In addition, loss of C9orf72 led to metabolic modifications and lysosomal accumulation in microglia, with age-related neuroinflammation similar to C9orf72 ALS but not SALS patient tissue [68, 69], further indicating that metabolic alterations in microglia contribute to neuroinflammation. Remarkably, AbGC are also characterized by an increased proliferation rate and reduced mitochondrial bioenergetics [70], associated with abundance of lipid droplets, as well as ER cistern dilatation when analyzed by electron microscopy [38]. Altogether, features of cellular stress and inflammation linked to metabolic changes indicate that glial proliferation is associated with a metabolic reprogramming.

Mitochondrial Dysfunction in Neurotoxic Astrocytes

For many years, defects in mitochondrial bioenergetics and oxidative stress have been included in the mechanisms put forward to explain pathogenesis of various neurodegenerative disorders, including ALS. Morpho-

logical, ultrastructural, and functional abnormalities of mitochondria have been detected in patients and animal models of the disease, either in motor neurons, skeletal muscle [71–75], or glial cells [33, 70, 76].

A mitochondrial role in ALS pathogenesis was strengthened by the fact that mutated forms of SOD1 have been repeatedly shown to be present in the mitochondria [77, 78], either in the intermembrane space, bound to the cytoplasmic side, or even in the matrix compartment [79, 80]. TDP-43 mutated forms can also be found in mitochondria [81], either in its full-length or truncated form, where it may disrupt mitochondrial fission and fusion dynamics, trafficking, and respiratory function [81]. FUS mutations have also been associated with mitochondrial alterations, such as fragmentation [82], disruption of ATP synthase activity, increased mitochondrial ROS production, and decreased mitochondria-ER associations [83].

Overexpression of SOD1G93A in the NSC-34 cell line reproduces the mitochondrial abnormal morphology and decreased respiration rate [84] and makes the cells highly dependent on glycolysis, suggesting that SOD1G93A expression may induce a switch to anaerobic metabolism to maintain ATP levels.

Isolated mitochondria from SOD1G93A astrocytes demonstrate defects in state 3 (ADP-dependent) respiration resulting in reduced mitochondrial respiratory activity and loss of membrane potential [33]. Further analysis of astrocyte mitochondrial function by high-resolution respirometry in intact cultured cells also revealed reduced respiratory capacity in neonatal SOD1G93A astrocytes [76]. Importantly, defective astrocytic mitochondria respiratory activity was directly associated with the ability of SOD1G93A astrocytes to induce motor neuron death. It is interesting to note that mitochondria from transgenic mice expressing SOD1G93A and wild-type hSOD1 contain 100-fold more hSOD1 than the content of mSOD1 in control mice. This overloading could alter the structure of affected mitochondria and lead to mitochondrial dysfunction [80]. However, no changes in mitochondrial bioenergetics were detected on wild-type hSOD-expressing astrocytes [33].

One potential functional consequence of mitochondrial dysfunction in astrocytes is the impairment of energy metabolism and increased generation of oxidants. Overproduction of NO [25] and mitochondrial O₂ formation [33] have been reported in SOD1G93A astrocytes. Since mutant SOD1 has been shown to mediate aberrant oxidative chemistry in neuronal cells [85], increased NO and O₂ would facilitate the formation of peroxynitrite (ONOO⁻)

that can damage key mitochondrial enzymes [86]. Indeed, we found increased nitrotyrosine labeling of mitochondrial proteins obtained from SOD1G93A astrocytes indicating nitrooxidative damage inside mitochondria. Accordingly, administration of DMPO, L-NAME, or MnTE-2-PyP restored mitochondrial respiration. Furthermore and particularly interesting, the same antioxidant treatment also prevented astrocyte-derived neurotoxicity in SOD1G93A astrocytes [33]. In addition, decreasing mitochondrial oxidative damage in astrocytes with the mitochondria-targeted antioxidant MitoQ also reestablished their respiratory capacity as well as the ability to support motor neuron survival [33]. These studies suggest a link between astrocyte mitochondrial OXPHOS activity and neuronal survival support capacity.

Metabolic Reprogramming as a Target to Reduce Neuronal Death

The main question raised from our observations was whether compounds that increase mitochondrial bioenergetics might have beneficial effects on ALS progression, considering that astrocytes are in part responsible for the disease spreading along the CNS. We have employed the organohalide dichloroacetate (DCA), a well-characterized stimulator of mitochondrial bioenergetics, in cultured SOD1G93A astrocytes and in SOD1G93A mice and found that stimulating mitochondria resulted in improvement of motor function and survival [76]. DCA effects on bioenergetics come from the inhibition of the protein kinase of the PDH (PDK) [87] by blocking its binding to active or allosteric domains [88–90], keeping the PDH in its active unphosphorylated state, therefore switching glucose metabolism toward oxidation to CO₂ in the mitochondria. DCA completely prevented the toxicity of SOD1G93A astrocytes to motor neurons in coculture conditions [76]. Furthermore, chronic administration of DCA in the drinking water to SOD1G93A mice significantly increased survival, reestablished the respiratory control ratio value measured in lumbar spinal cord tissue, and improved grip strength performance [76]. Pathological features in the spinal cord were also improved by DCA treatment, as astrocyte reactivity and motor neuron loss were highly decreased. DCA enhances glucose and lactate oxidation to CO₂ and reduces lactate release mainly in astrocytes compared to having almost no effects on neurons [45], which supports that modulation of mitochondria respiratory activity in astrocytes may affect astrocyte-motor neuron interaction.

A complementary approach employed was to selectively mitigate nitrooxidative damage in mitochondria. Compounds that can improve bioenergetics of mitochondria either by increasing mitochondrial antioxidant activity, such as Mn porphyrins, or by decreasing oxygen free radical propagation such as DMPO have been shown to increase the lifespan in SOD1G93A transgenic mice [91, 92]. The mitochondria-targeted antioxidant MitoQ, developed by conjugating the lipophilic triphenylphosphonium cation to the antioxidant moiety ubiquinone, exerts protective effects by a catalytic detoxification cycle that involves its reduction to the active ubiquinol state by complex II followed by its oxidation by 1- and 2-electron oxidants [93]. MitoQ prevented mitochondrial dysfunction, reduced superoxide production in SOD1G93A astrocytes, and reversed their neurotoxic phenotype [33]. Importantly, MitoQ administration to SOD1G93A mice at symptoms onset restored mitochondrial function and reduced astrogliosis and motor neuron loss in the spinal cord, along with an improvement in motor performance and survival [94].

Further evidence linking mitochondria dysfunction and astrocyte-mediated toxicity comes from studies in non-tg astrocytes. Treatment with electron transport chain inhibitors causes astrocytes to induce motor neuron death, resembling the effect of the SOD1 mutation [33]. These findings clearly demonstrate that decreasing OXPHOS activity in astrocytes is sufficient to reduce their survival support to motor neurons.

Astrocyte mitochondria dysfunction linked to oxidative stress has been previously reported in other CNS injuries such as ischemia [95], Alzheimer's disease [96], glutaric acidemia [97], Parkinson's disease [98], and chronic pain [99]. Furthermore, inhibition of astrocyte mitochondria with fluorocitrate (an aconitase inhibitor) increases the vulnerability of cocultured cortical neurons to glutamate toxicity [100]. Taken together, available evidence indicates that nitrooxidative stress and related mitochondrial defects in astrocytes are, at least in part, triggering or maintaining a neurotoxic phenotype.

Finally, another possible link between mutant SOD1-induced mitochondria dysfunction and the associated neurotoxic phenotype in astrocytes is strengthened by our recent identification that AbGC in the spinal cords of diseased SOD1G93A rats exhibit a very low mitochondrial respiratory activity [70]. These cells show increased proliferation rates, even higher than neonatal SOD1G93A astrocytes [36]. Recent studies have indicated that mitochondria can regulate signal transduction pathways such as MAP kinases and AKT phosphorylation that in turn

Fig. 1. Metabolic reprogramming in glial cells determines a phenotypic transition to AbGC in ALS. **a** The complex interactions between motor neurons (gray) and glial cells (astrocytes: red; microglia: blue) in healthy conditions (1) involve contact connections and released factors including trophic factors, cytokines, and metabolic mediators. ALS pathogenic factors provoke changes in the different cell types that lead to an altered, neuroinflammatory microenvironment. Inflammatory stimuli induce metabolic changes, mainly a decreased OXPHOS and increased mitochondrial fragmentation and glycolysis, in astrocytes and/or microglia (mitochondria are represented as green structures) (2), which in turn

determines the emergence of AbGC (magenta) (3) that induce motor neuron death. **b** Drawings representing the changing cell microenvironment in the ventral horn of the spinal cord during the course of disease from presymptomatic to end stages, showing the atrophic motor neurons and higher number of transitional glial phenotypes toward the end stage. In this scenario, targeting mitochondrial metabolism (e.g., with DCA, MitoQ, or antioxidants) can modulate (reprogram) the glial phenotype toward a neuroprotective one and prevent the metabolic shift in neighboring regions. AbGC, aberrant glial cells; ALS, amyotrophic lateral sclerosis; OXPHOS, oxidative phosphorylation; DCA, dichloroacetate.

control cell proliferation and differentiation [101]. This mechanism may explain the origin of astrocytosis and inflammation. Accordingly, AbGC exhibit mitochondrial fragmentation and a reduced mitochondrial respiratory control ratio [70]. Notably and further supporting a link between mitochondrial activity and AbGC toxic phenotype, DCA administration enhanced respiratory parameters and also strikingly reduced their toxicity, even when lactate levels were decreased [70]. Indeed, lactate measurements in CNS tissue from different regions of SOD1G93A mice have yielded different results. In some studies, the metabolite was found diminished, like in lumbar segments from SOD1G93A mice at presymptomatic stages, coincident with results from culture media [29] and with recent findings in other CNS regions (brain stem, striatum, and motor cortex) obtained by *in vivo* ^1H magnetic resonance spectroscopy at different stages [102]. Conversely, in a recent study performed in 2 SOD1G93A models (fast and slow progressing), Valbuena et al. [103] found no differences in lactate levels at the lumbar spinal cord in any stages (presymptomatic, onset, and late stage)

of disease, but an increase in the thoracic segments at the end stage of disease, in agreement with previous findings [104]. Furthermore, the AbGC proliferation rate response was also reduced by DCA treatment. The fact that mitochondrial respiration can be restored indicates that mitochondria are not irreversibly damaged, and that instead the organelle reprograms its metabolism to cope to different energy demands. The reviewed data demonstrate that this glial metabolic reprogramming that resembles cancer cells physiology critically influences neuronal death and disease progression, suggesting that targeting metabolic reprogramming may induce a disease-modifying effect.

Concluding Remarks

AbGC are identified surrounding motor neurons as motor symptoms occur in ALS models. Astrocytes and microglia may contribute to the emergence of these cells and may play a role in spreading disease by adopting phenotypic changes that promote neuronal death. Questions remain

about whether these AbGC may have a role in the pathology which seems to start focally and then spread to neighboring structures. Mitochondria are key players in this phenotypic transition, and evidence indicates that increased astrocyte toxicity is associated with reduced mitochondrial bioenergetics and increased proliferation resembling cancer cells as depicted in Figure 1. The metabolic reprogramming can be reverted to reduce toxicity and delay disease progression, a strategy brought from the cancer area into neurodegenerative diseases. This new paradigm may offer alternative strategies for possible future therapies in ALS.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

P.C. contributed to concept development and wrote most sections of the manuscript, E.M. wrote part of the manuscript, L.M.P. wrote part of the manuscript and designed and drew the figure, and A.C. wrote part of the manuscript. All authors revised the manuscript and approved the final version to be submitted.

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