UNIVERZITA KARLOVA V PRAZE

Přírodovědecká fakulta

Studijní program: Speciální chemicko-biologické obory

Studijní obor: Molekulární biologie a biochemie organismů

Ondřej Vaňátko

Glial cells and their role in amyotrophic lateral sclerosis

Gliové buňky a jejich role v amyotrofické laterální skleróze

Bakalářská práce

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Praha, 2018

Poděkování

Chtěl bych poděkovat své školitelce Ing. Miroslavě Anděrové, CSc. a její kolegyni Mgr. Janě Turečkové, PhD. za jejich pomoc, ochotu a trpělivost a za jejich cenné rady.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 9. 5. 2018

Podpis:

Abstract

Amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease) is a progressive neurodegenerative disorder. It affects upper and lower motor neurons in the brain motor cortex, the brain stem and the spinal cord, causing their death, which results in denervation of voluntary muscles. Progressive muscle weakness and atrophy throughout the entire body gradually leads to worsening of the ability to move, speak, chew, swallow and eventually breath. Ultimately it results in affected individual's death due to respiratory muscle failure. Although first identified in 1869, no cure for ALS has been yet found. While early studies focused mainly on the research of motor neurons themselves, the attention has shifted towards glial cells in the past two decades. Glial cells are essential for proper neuron functioning and survival and it appears that they play a major role in ALS progression. The goal of this thesis is to review and summarize findings on the role of glial cells in ALS over the last years, focusing on four specific types of glial cells, namely astrocytes, microglia, oligodendrocytes and NG2-glia.

Key words: amyotrophic lateral sclerosis, ALS, motor neuron, glia, astrocyte, microglia, oligodendrocyte, NG2-glia

Abstrakt

Amyotrofická laterální skleróza (ALS, též známá jako Lou Gehrigova choroba) je progresivní neurodegenerativní onemocnění. Postihuje horní i dolní motorické neurony v motorické kůře mozku, v mozkovém kmeni a míše a způsobuje jejich smrt, následkem čehož dochází k denervaci svalů. To v důsledku vede ke slabosti a svalové atrofii celého těla, postupně dochází ke ztrátě schopnosti pohybu, mluvení, žvýkání a polykání, a nakonec i dýchání, což má za následek úmrtí postiženého jedince. Přestože ALS byla popsána již v roce 1869, snahy o nalezení léku jsou zatím bezúspěšné. Zatímco dřívější studie se zaměřovaly přímo na motorické neurony, pozornost vědecké obce se během posledních let přesunula ke gliovým buňkám. Gliové buňky jsou nezbytné pro správné fungování a přežití neuronů a zřejmě hrají důležitou roli při vzniku a progresi ALS. Cílem této práce je shrnout poznatky o roli gliových buněk při ALS za poslední roky, se zaměřením na čtyři konkrétní typy glií, jmenovitě astrocyty, mikroglie, oligodendrocyty a NG2-glie.

Klíčová slova: amyotrofická laterální skleróza, ALS, motorické neurony, gliové buňky, astrocyty, mikroglie, oligodendrocyty, NG2-glie

Shortcut list

ALS	amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
Cas9	CRISPR-associated protein 9
C/EBP-β	CCAAT enhancer binding protein-β
CNS	central nervous system
c-Ret	oncoprotein (GDNF receptor tyrosine kinase)
CRISPR	clustered regularly interspaced short palindromic repeats
C9ORF72	chromosome 9 open reading frame 72
DNA	2-deoxyribonucleic acid
EAAT2/GLT1	excitatory amino acid transporter 2 / glutamate transporter 1
ER	endoplasmic reticulum
FDA	the Food and Drug Administration
GABA	γ-aminobutyric acid
GDNF	glial cell-derived neurotrophic factor
GFAP	glial fibrillary acidic protein
IFN-γ	interferon-γ
IGF-1	insulin-like growth factor 1
IL	interleukin
iNOS	inducible nitric oxide synthase
LIF	leukemia inhibitory factor
LMN	lower motor neuron
MHC	major histocompatibility complex
MND	motor neuron disease
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
(NGF)-p75	pro-nerve growth factor-p75 neurotrophin receptor
NMDA	N-methyl-D-aspartate
OPCs	oligendendrocyte precursor cells
PNS	peripheral nervous system
ROS	reactive oxygen species
SOD1	superoxide dismutase 1
TGF-β	transforming growth factor-β
TNFα	tumor necrosis factor α
UMN	upper motor neuron

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1 Introduction

Amyotrophic lateral sclerosis is an uncommon worldwide disease affecting people of all genders, races, ethnicities and ages. Being a neurodegenerative disorder, ALS causes death of motor neurons, which consequently results in muscle atrophy and ultimately in death. At first, studies focused mostly on motor neurons, as their deterioration is the main factor in the disease progression. But as new possible causes were being proposed, it became clear that ALS is most likely triggered by complex network of mechanisms. Over the past years, attention shifted towards glial cells, as those play an important role in the maintenance of CNS homeostasis and optimal conditions for neuronal functioning and survival of neurons as well as other cells in the nervous tissue. The aim of this work is to summarize basic facts about ALS, the glial cells and their pathophysiology during ALS.

2 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis falls in a small group of disorders called "Motor Neuron Diseases" (MNDs). MNDs are neurodegenerative disorders characterized by selectively affecting upper motor neurons (UMN) or lower motor neurons (LMN), with ALS accounting for 85 % of MNDs, affecting both UMN and LMN. In the United States, ALS is better known under the name "Lou Gehrig's disease" and is sometimes referred to as "motor neuron disease", rather than falling in the same group.

2.1 Brief history

Although early descriptions of the symptoms date back to 1824, the disease was first described in 1869 by Jean-Martin Charcot, who also gave ALS its name five years later. The word amyotrophic comes from Greek *amyotrophia*, where *a-* means "no", *myo* refers to "muscle" and *trophia* means "nourishment", therefore *amyotrophia* means "no muscle nourishment". "Lateral" (= on a side) refers to the location of neurons in the spinal cord, "sclerosis" means stiffening or hardening of tissue. People became aware of the disease when a famous baseball player, Lou Gehrig, was diagnosed by ALS in 1939 (hence the name "Lou Gehrig's disease"). Since 1970s, lots of studies were conducted. New approaches and technological advancement made the diagnosis of ALS much easier. Since 2014, people participate in an activity called the Ice Bucket Challenge, increasing awareness of the disease while also raising and donating money to organizations like the ALS Association or ALS Society of Canada (*Amyotrophic Lateral Sclerosis Fact Sheet)*.

2.2 Pathophysiology

ALS develops under a complex network of interactions not restricted to motor neurons only, but including mutual interactions and signaling between motor neurons, glia and immune cells. Ultimately, this interaction leads to motor neuron deterioration. With motor neurons dying, denervation of voluntary muscles begins to appear. At first, deterioration of only a small number of neurons can be compensated via re-innervation by other surrounding neurons. But as the disease proceeds, all neurons innervating a single muscle fiber eventually die, losing the ability to transfer signal to muscle cells (Fig 1). As the muscles slowly degenerate and atrophy occurs, affected ALS patient gradually loses the ability to walk, chew, swallow, move and eventually dies by suffocation due to malfunction of respiratory muscles (reviewed by Zarei et al., 2015).

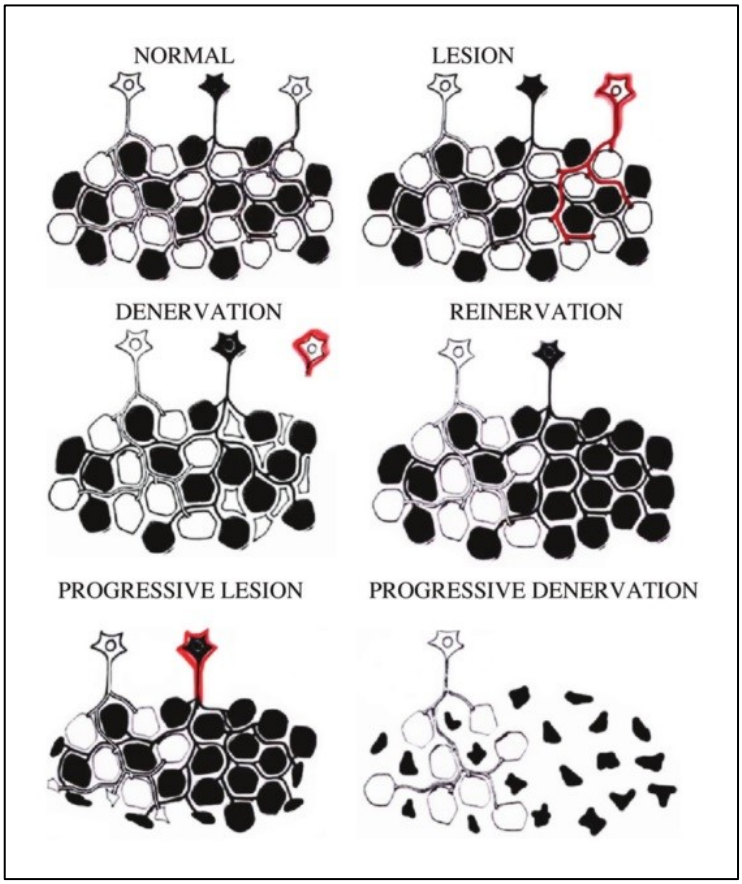

Fig 1. Schematic representation of denervation and reinervation during ALS progression. Loss of motor neuron is compensated by other motor neuron. As the disease progresses, some of the muscle fibers remain denervated, developing an atrophy (Oliveira and Pereira, 2009).

Although the ability to control voluntary muscles is lost, sensory neurons remain intact. ALS patients thus retain their ability to think, feel, taste, smell and hear. An interesting fact is, that despite being voluntary muscles, bowel and bladder function remains mostly unaffected in a lot of cases (Nübling *et al.*, 2014), as well as function of extraocular muscles (McLoon *et al.*, 2014)

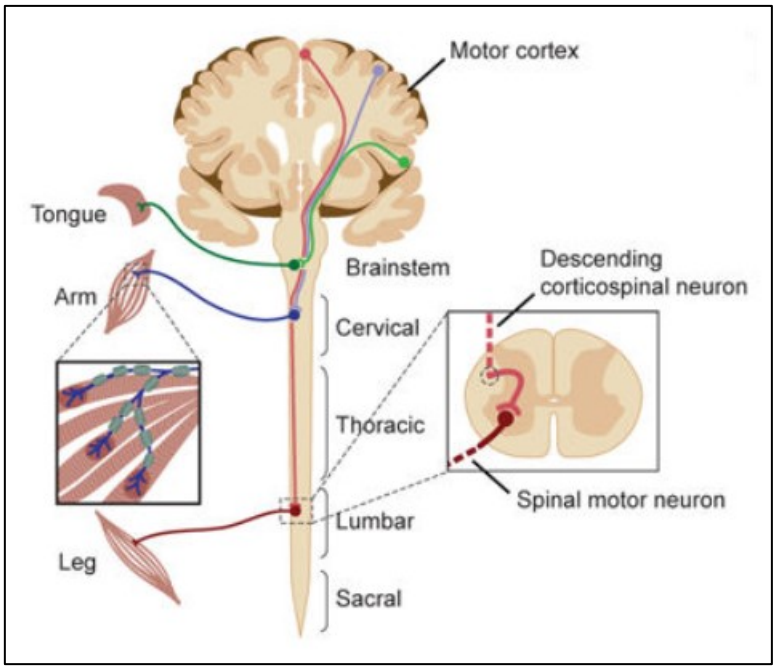

Fig 2. The components of the nervous system affected in ALS. ALS primarily impacts descending corticospinal motor neurons (upper motor neurons) that project from the motor cortex to synapses in the brainstem and spinal cord, and bulbar or spinal motor neurons (lower motor neurons) that project to skeletal muscles (Taylor *et al.*, 2017; edited).

2.3 Genetics and causes

ALS occurs in two forms – familial and sporadic. Familial form is far less frequent, consisting of 5 to 10 percent of all ALS cases. About half of familial ALS cases are caused by a mutation in one of two specific genes – *SOD1* (Rosen *et al.*, 1993) or *C9ORF72* (Renton *et al.*, 2011). First-degree relatives have about 1 % chance of developing ALS (Wingo *et al.*, 2011). Sporadic form is more often, consisting of 90 to 95 percent of cases. In those cases, ALS occurs randomly with no family history record or clearly associated factors. Random mutations can either directly cause or increase an individual's susceptibility to ALS. Although being the major form of ALS, sporadic form remains less explored than familial form (*Amyotrophic Lateral Sclerosis Fact Sheet*).

So far, several possible mechanisms contributing to ALS have been proposed:

- Glutamate imbalance. One of ALS characteristics is an increased level of glutamate in the brain and the spinal fluid. Under normal circumstances, glutamate works as a neurotransmitter, allowing signal transmission between neurons. In high concentrations, glutamate is neurotoxic, causing over-activation of postsynaptic neurons, which can lead to neuron death via programmed cell death.

- Disorganized immune response. During ALS, some cells undergo a change in their behavior in order to fight ongoing illness, but in doing so they can cause an inadequate immune response resulting in destruction of the body's own neuronal cells. This includes mainly the complex interactions between glia and immune cells.
- Protein misfolding and aggregation. Misfolded proteins can start to accumulate in neuronal cells in prion-like manner and form inclusion bodies. This can happen for various reasons. Due to being misfolded, those proteins cannot function properly on one hand, and can also cause dysfunction of other proteins. (Oliveira and Pereira, 2009).

While these three mechanisms mentioned are expected to contribute to ALS the most, other factors probably play a role in it as well. These include mutation in gene for superoxide dismutase 1, ER and oxidative stress, intermediate filament disorder accompanied by change in axonal transport, mitochondrial dysfunction, glia-mediated neuroinflammation, microglial activation, infiltration of macrophages and T-lymphocytes into the neural tissue (Philips and Robberecht, 2011; Ling *et al*., 2013; Taylor *et al*., 2017). Given the number of contributing factors, ALS is known and regarded as multisystemic disease.

2.4 Epidemiology and life expectancy

ALS affects approximately 0,5-2,5 people out of 100 000, with average incidence being 2 out of 100 000. The incidence varies depending on gender, age, geographic and demographic factors. Men and women are equally likely to develop ALS, although men are slightly more likely to develop its familial form. ALS incidence grows with increasing age, with its rate peaking between the ages of 60 and 75 years. Concerning ethnicity, Caucasians are most likely to develop ALS, covering roughly 90 % of all ALS cases, though this could be due to fact, that most studies have been done in Europe and North America (Chiò *et al.*, 2013). In the 1950s, an extremely high incidence of ALS-like disease was observed on the island of Guam, but it was never properly studied (*Amyotrophic Lateral Sclerosis Fact Sheet*).

Life expectancy depends on the aforementioned factors, as well as social and economic status and access to health care. Half of the affected individuals manage to live longer than 3 years, roughly 25 % manage to live 5 years and only about 10 % live longer than 10 years (Chiò *et al.*, 2009). ALS is fatal in vast majority of cases. Only a handful of people managed to survive ALS or live longer than expected. Notable example was

theoretical physicist Stephen Hawking (1942 – 2018), who was diagnosed at the age of 21, but managed to outlive its prognosis for more than 50 years.

2.5 Treatment

Since no cure for ALS has been yet found, all efforts focus on relieving syndroms and prolonging the life expectancy by providing the best possible supportive care. So far, only two drugs have been approved for medical treatment by the Food and Drug Administration (FDA). One of them is riluzole (sold under the trade names *Rilutek* and *Teglutik*). Riluzole blocks neuronal Na^+ channels, which as a result reduces neurotoxicity regulating the neuron activity. Riluzole has been proven to increase life expectancy by 2 to 3 months (Bensimon *et al.*, 1994). Recently, another drug has been approved by FDA – edaravone (sold under trade names *Radicava* and *Radicut*), which works as an antioxidant. There are also several experimental drugs in different phases of clinical trials, like Ibudilast, NurOwn or Masitinib. Recently, another drug called EH301 was granted a status of orphan drug (*PR Newswire*, 2018).

2.6 Research directions

Most studies are conducted on the genetically modified animal models – rodents, zebrafish and fruit flies – with mutations in *SOD1*, *C9ORF72* or in few other genes (Marchetto *et al.*, 2008; Philips *et al.*, 2013; Frakes *et al.*, 2014; Koppers *et al.*, 2015). Aside from ongoing clinical trials with experimental drugs and development of new ones, there are few areas of focus of research studies:

- identifying mechanisms leading to neuron deterioration and programmed cell death and finding ways to stop this process, with increased focus on the interaction network of glial cells
- finding biomarkers for a faster and easier identification, diagnosis and measuring of the progression of the disease
- understanding the differences and possible similarities between familial and sporadic form of ALS and identifying new genes responsible for or contributing to development of the disease
- determining the role of epigenetics in ALS, identifying environmental factors contributing to development of the disease

There are currently two promising ways to cure ALS in the future. First approach comprises gene therapy, with the advancements in genetic engineering and with the CRISPR/Cas9 technology in particular. CRISPR/Cas9 allows editing of the genome with unmatched precision which makes it a viable tool in treating all different kinds of genetic diseases (Ma *et al.*, 2014; Rodri *et al.*, 2018). Second direction includes studies focusing on stem cells and their possible use in a stem cell therapy. There are experimental clinical trials with stem cells already in progress, but so far, the results were mixed (Glass *et al.*, 2012; Mazzini *et al.*, 2015; Oh *et al.*, 2015; Syková *et al.*, 2017). While stem cell therapy in some cases slows down the progression of the disease, it is clear that much more research needs to be done in terms of understanding the complex interactions, so the stem cells can be introduced to the organism with maximum possible efficiency.

3 Glial cells

Neurons are considered the most important cells in the nervous system, but they could not function properly without support of the glial cells. Neurons and glia take up roughly the same amount of space in the brain, with ratio of neurons to glia being approximately 1:1, varying in different parts of the brain (von Bartheld *et al.*, 2016). Glia can be divided in two groups – microglia and macroglia. Microglia serve as immune cells for the CNS. All other glia are considered macroglia. Those are - from the most abundant types - astrocytes, oligodendrocytes, NG2-glia and ependymal cells in the CNS, and Schwann cells and satellite cells in the PNS. There are also some other minor types of glia, but those are generally found in specific locations in the body, they are usually subtypes of major glia and fulfill a very specific role (Blackburn *et al.*, 2009).

In the brain, glial cells have various roles and functions. They provide structural, metabolic and nutrition support, participate in sustaining the blood-brain-barrier and regulating the blood flow, maintain ionic and chemical homeostasis, provide insulation for neuronal axons, have immune function, line cavities and produce the cerebrospinal fluid. They also play major roles during the brain development, such as guiding the neuronal growth or reducing the number of already formed synapses. Unlike neurons, glial cells maintain the ability to proliferate, and some of them are engaged in the process of gliogenesis and possibly neurogenesis (reviewed by Jäkel and Dimou, 2017).

Due to their ability to proliferate, glial cells are the primary source of brain tumors (Goodenberger and Jenkins, 2012). Their dysfunction has been connected to variety of neurodegenerative diseases, such as schizophrenia (Windrem *et al.*, 2017) or Alzheimer's disease (Yang *et al.*, 2011).

A detail discussion of the roles of microglia, oligodendrocytes, NG2-glia and astrocytes follows, since these represent the most discussed types of glia, in progression of ALS.

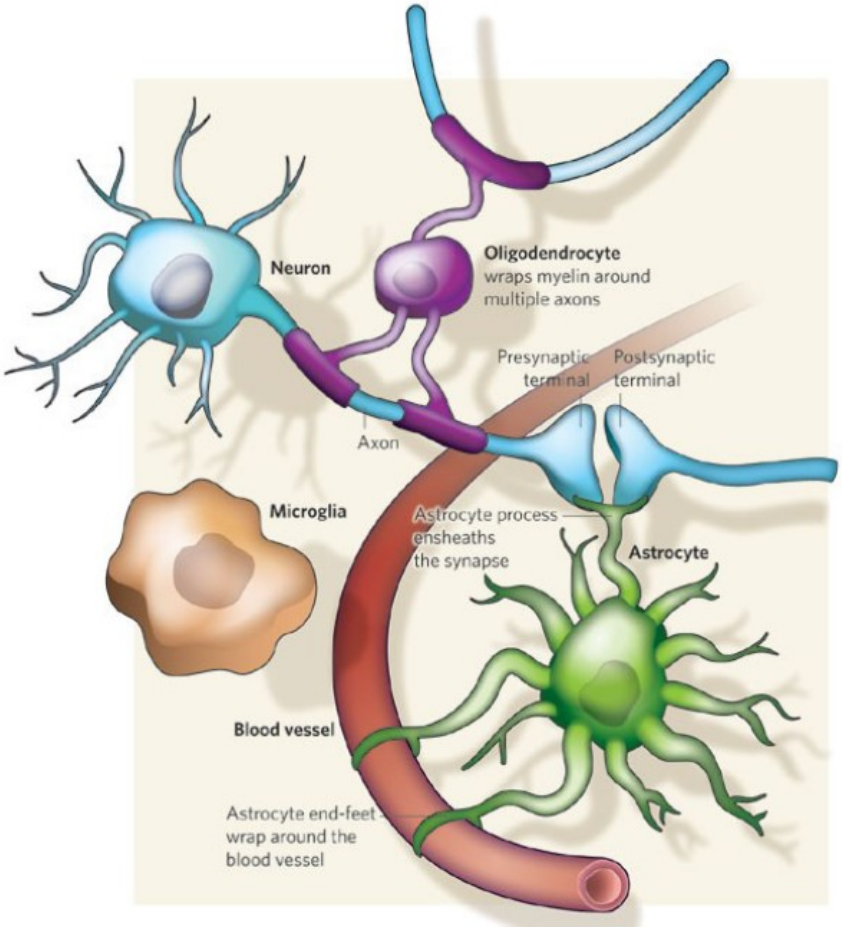

Fig 3. Schematic representation of neuron-glia interactions in the CNS. Microglia are effector cells of the immune system. Oligodendrocytes myelinate neuronal axons to secure signal transmisson. Astrocytes extend processes towards neuronal synapses and blood vessels (Allen and Barres, 2009).

3.1 Microglia

As other cells of the immune system have very limited access to the brain thanks to the blood-brain-barrier, microglia carry out the immune function in the CNS. Unlike other glial cells, microglia are of mesodermal origin, as they are derived from the hematopoietic stem cells. They account for 10 to 15 percent of all glia. Throughout the CNS, they are found in evenly distributed large non-overlapping regions. In their resting state, microglia have a small body with long branched processes. However, they can change their morphology depending on current health state of the organism (reviewed by Kettenmann *et al.*, 2011).

Microglia fulfill a variety of functions. Under physiological conditions, they constantly survey the local environment within their domains. They act as resident macrophages, phagocyting any foreign material, such as dead cells, DNA fragments or other random debris. Once “eating” enough debris, microglia lose their phagocytic activity and turn

into so called gitter cells. On their surface, microglia express various ligands and receptors for immune cross-talk (Zhao *et al.*, 2013), as well as molecules for communication with other glia. They also express all different kinds of channels. Some of them are specific to microglia only, such as unique K^+ channels very sensitive to changes in extracellular K^+ concentration (Eder, 1998).

Microglia also promote post-inflammatory repair of damaged tissue by secretion of anti-inflammatory cytokines and attracting neurons and astrocytes to the damaged area (reviewed by Jin and Yamashita, 2016). Recently, microglia have been shown to modulate formation of the neuronal network in developing brain by phagocyting excess synapses (Schafer *et al.*, 2012).

3.1.1 Microglia in ALS

A common hallmark of many neurodegenerative diseases, including ALS, is microglial activation. Microglia can be activated by various stimuli, such as changes in neurotransmitter concentrations, pro-inflammatory cytokines like IFN-γ or TNFα, or changes in extracellular concentration of K^+ ions (Dheen a Ling, 2007). Once activated, they undergo morphological and physiological changes in order to restore homeostasis. This includes secretion of pro-inflammatory cytokines (IFN-γ and TNFα, IL-1β or IL-12), chemotactic molecules, neurotrophic factors (IGF-1), anti-inflammatory cytokines (TGF-β), ROS and complement molecules, expression of MHC I/II protein for antigen presentation and rapid proliferation (Iglesias et al., 1997; Lasiene and Yamanaka, 2011).

While there has been some controversy over the role of microglia in ALS, it is clear that microglia are linked to motor neuronal damage via the cell non-autonomous pathway. Apart from microglial activation, several mechanisms have been shown to play a role in ALS. Deletion of nuclear factor kappa B (NF-κB) signaling in microglia leads to impairment of proinflammatory pathways and rescue of motor neurons and extended survival in ALS; however, same deregulation in astrocytes did not prevent microglia-mediated neurodegeneration (Frakes *et al.*, 2014). Ryu *et al.* have shown that ALS-stress induces expression of c-Ret oncoprotein in microglia. Such increase in c-Ret levels result in higher interaction with glial derived neurotrophic factor (GDNF), promoting microglial growth and subsequent deprivation of neurotrophic factors, leading to neuronal damage (Ryu *et al.*, 2011). Increased expression of nitric oxide synthase-2 and cyclooxygenase-2 thanks to increased levels of C/EBP-β was also reported in ALS SOD1 mice (mice with mutation in

gene for superoxide dismutase 1) (Valente *et al.*, 2012). Thanks to the production of chemotactic molecules by microglia, T-lymphocytes cross the blood-brain-barrier and interact with microglia, triggering one of two possible microglia profiles, depending on the stage of ALS: M2 protecting anti-inflammatory profile, or M1 cytotoxic inflammatory profile (Zhao *et al.*, 2004, 2006, 2013).

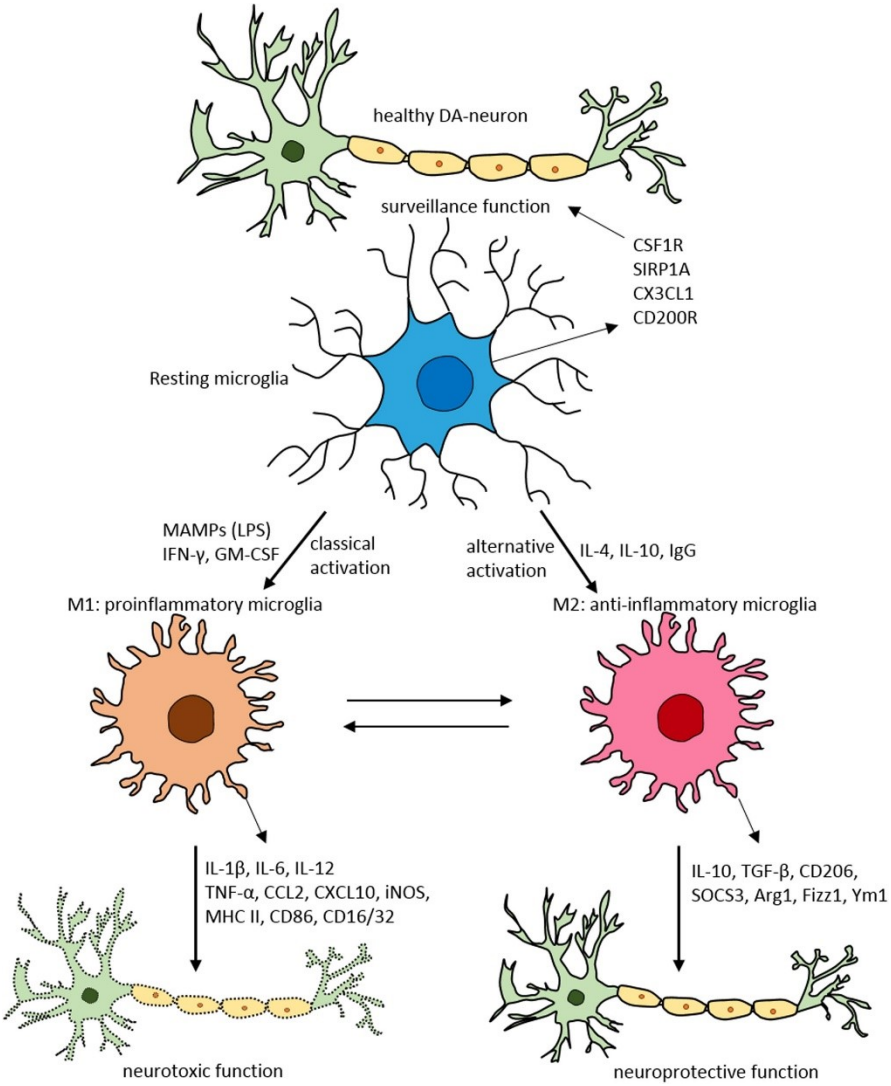

Fig 4. Schematic representation of microglia activation. Upon activation microglia acquire pro-inflammatory M1 or anti-inflammatory M2 phenotype, depending on triggering factors (Subramaniam and Federoff, 2017)

Another mechanism contributing to neurodegeneration is microglial senescence. Studies have shown that over time, microglia undergo morphological changes and gradually lose the ability to replicate, forming a population of senescent and dysfunctional cells, possibly contributing to neurodegeneration thanks to only limited neuroprotection (Streit, 2006; Koellhoffer *et al.*, 2017).

In conclusion, microglia are essential for maintaining the state of CNS and their activation is beneficial as a response to acute and reversible stress. On the other hand, prolonged activation by chronic stress (such as in ALS) causes neurotoxicity, and therefore needs to be regulated.

3.2 Oligodendrocytes

They originate from their precursor cells, oligodendrocyte precursor cells (OPCs, also called NG2-glia), and they are the last cells to be formed in the CNS. During development, abundance of oligodendrocytes is produced, but only those, which receive necessary factors from surrounding neurons, survive, while other undergo apoptosis (Baumann and Pham-Dinh, 2001). There are two types of oligodendrocytes – myelinating and non-myelinating (satellite). Myelinating oligodendrocytes have a small body with up to tens of processes, extending to and myelinating neuronal axons. The function of satellite oligodendrocytes is not yet very well explored but is possibly connected to regulating homeostasis (Barres and Raff, 1999). Analog of myelinating oligodendrocytes in the PNS are Schwann cells.

The main role of oligodendrocytes is insulation of neuronal axons. Several processes extend from their body, enveloping a neuronal axon. Thanks to the way myelination is carried out, oligodendrocytes allow saltatory conduction, providing huge increase in the speed of signal transmission. They are also known to produce factors such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and insulin-like growth factor-1 (IGF-1), which are important for neuron growth and survival (reviewed by Nave, 2010).

3.2.1 Oligodendrocytes in ALS

In both ALS patients and mice models, degenerative changes in oligodendrocytes are present. As the disease progresses, these changes become more apparent, eventually leading to their death. Though this loss is compensated by increased proliferation and differentiation of OPCs, and the overall number of oligodendrocytes does not change, newly arised oligodendrocytes do not maturate properly and there is a clear loss of function (Philips *et al.*, 2013). There is a reduction in expression of many oligodendrocyte markers, such as monocarboxylate transporter 1 (MCT1) (Lee *et al.*, 2013). MCT1 is a lactate transporter; lack of MCT1 results in impaired trophic support for neurons and can possibly affect myelination, as both processes are regulated by lactate (Rinholm *et al.*, 2011; Lee *et al.*, 2013). Oligodendrocytes degeneration has been shown to precede motor neuron death in SOD1 mice models. Demyelinated neurons lose the ability to effectively transmit signal and slowly degenerate because of the lack of trophic factors (Kang *et al.*, 2013; Philips *et al.*, 2013).¨

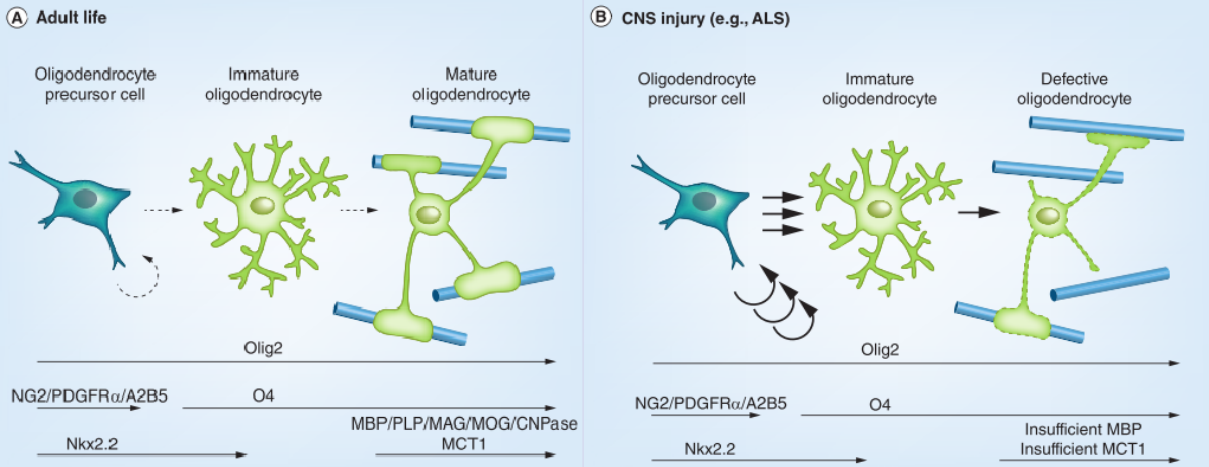

Fig 5. Oligodendrocyte during adult life and in ALS injury. A) Oligendrocytes are in a stable myelinating state and there is a pool of mitotically active NG2 cells is maintained, but their proliferation and differentiation is regulated. B) In ALS, there is an increased proliferation and differentiation of NG2 cells, which, however, function abnormally, as their myelinating supportive function is impaired (Nonneman *et al.*, 2014, edited).

A study from 2016 shows that *in vitro*, oligodendrocytes contribute to motor neuron death in ALS via SOD1-dependent mechanism, mediated by both soluble factors and cell-to-cell contact. In this study, SOD1 knockdown in OPCs resulted in reversing the pathogenic oligendrocyte phenotype in both familial and sporadic ALS cases, except for those with the mutation in *C9ORF72* gene (Ferraiuolo *et al.*, 2016).

Though oligodendrocytes are relatively understudied, they seem to play a role in ALS onset and progression, but ALS does not represent a primary oligodendrocyte disease.

3.3 NG2-glia

NG2-glia, also called synantocytes, polydendrocytes, or oligodendrocyte precursor cells (OPCs), are the fourth most abundant type of glia in the CNS. On the surface, they express NG2 chondroitin sulfate proteoglycan, hence their name. NG2-glia represent the largest population of resident precursor cells (about 4-8 %). They can be found both in grey and white matter and they are also present in neurogenic niches (Aguirre *et al.*, 2004). In different parts of the brain, NG2-glia have distinct morphological and physiological properties (Bakiri *et al.*, 2009).

NG2-glia represent an immature neural cell population that can differentiate into different mature neural cell types under different conditions. I*n vitro*, they have been demonstrated to give rise to new oligodendrocytes, astrocytes and in some cases, neurons. *In vivo*, NG2-glia differentiate primarily into oligodendrocytes (and into astrocytes in some cases), but their neurogenic potential is still highly discussed, and studies have produced mixed and controversial results so far (reviewed by Valny *et al.*, 2017).

Apart from their progenitor function, not much is known about NG2-glia. It is well established that NG2-glia are a part of neuron-glial synapses, receiving both excitatory and inhibitory signals from neurons, though function of these synapses is still unclear (Gallo *et al.*, 2008; Sakry *et al.*, 2011).

3.3.1 NG2-glia in ALS

Not a lot of research has been done in terms of role of NG2-glia in ALS. NG2-glia quickly replace dead oligodendrocytes thanks to increased proliferation and differentiation, but the new oligodendrocytes are dysfunctional both in terms of myelination and trophic support (Kang *et al.*, 2013; Philips *et al.*, 2013). Interestingly, some studies suggest that NG2-glia response to injury/disease is triggered by demyelination, rather than death of neural cells (Franklin, 2002; Steiner *et al.*, 2006; Sirko *et al.*, 2013).

3.4 Astrocytes

Astrocytes, apart from having many other functions, serve as the neuronal scaffold, fulfilling an important structural role. Their name comes from Greek *astron*, meaning “star”, since they are star-shaped. In the CNS, they can be found in two forms. Fibrous astrocytes have long, unbranched processes, and can be found mostly in the white matter. Protoplasmic astrocytes are more prevalent, with shorter and more branched processes, and are found in the

gray matter. Common astrocyte feature is the expression of GFAP (gliar fibrillary acidic protein); this is useful for astrocyte identification via immunohistochemistry (Takamiya *et al.*, 1988; Cahoy *et al.*, 2008). Via its processes, one astrocyte can be attached up to thousands of neurons. Astrocytes are connected with each other through gap junctions, forming a functional syncytium, which is important in astrocyte signaling. Astrocytes can be non-electrically excited, generating intracellular waves of Ca^{2+} rapidly spreading through the syncytium, causing release of glutamate, ATP and GABA (Bennett *et al.*, 2003).

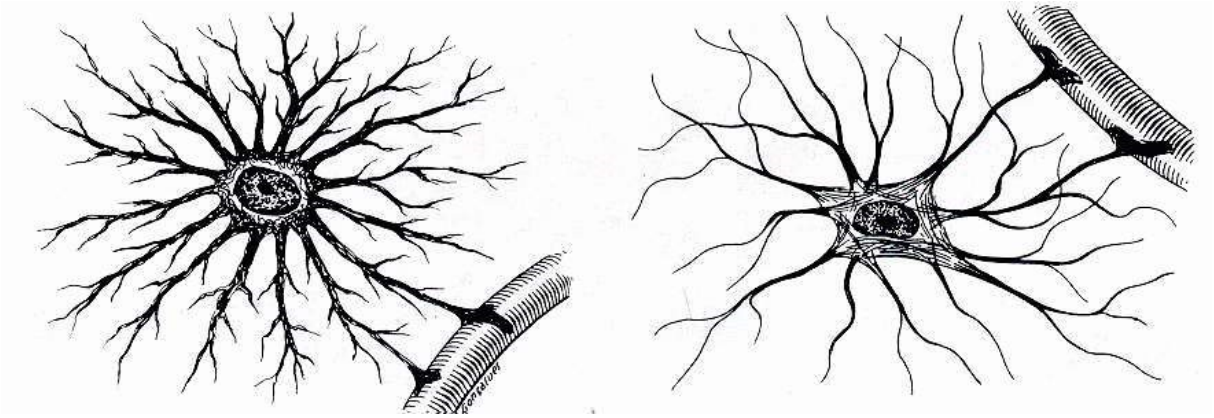

Fig 6. Morphological diference between protoplasmic (left) and fibrous astrocytes (right). Source: Histology II, Lab. of Fish and Shellfish Pathology

In the CNS, astrocytes have many roles. They help sustain the extracellular homeostasis of neurotransmitters, metabolites and ions. They are responsible for developing and maintaining the blood-brain-barrier, and they also contribute to regulation of the blood flow (Abbott *et al.*, 2006; Koehler *et al.*, 2009). Astrocytes provide a lot of different kinds of support for neurons. During development, they are important for neuronal growth and migration, as well as formation of new synapses (Nishida and Okabe, 2007; Stevens *et al.*, 2007). Astrocytes express different transporters, regulating concentration of glutamate, GABA, ATP or K^+ ions in the synaptic cleft, preventing neuronal excitotoxicity (Barbour *et al.*, 1988; Erecińska *et al.*, 1996; Yang *et al.*, 2005). They control the metabolic exchange between neurons and the bloodstream and provide nutritional support for the neurons, serving as a glycogen reserve, as well as providing neuron with sources of energy, such as lactate (Bouzier-sore *et al.*, 2002). Furthermore, astrocytes can possibly promote myelinating activity of oligodendrocytes by releasing leukemia inhibitory factor (LIF) (Ishibashi *et al.*, 2006), and they play a role in formation of glial scar after an injury (Stichel and Müller, 1998; Faulkner, 2004).

Recent studies implicate a few new, previously unknown functions. Astrocytes seem to be able to modify the electric signal via secretion of their own transmitters called gliotransmitters (Santello and Volterra, 2009). They are also believed to play a role in regulation of stem cells differentiation (Jiao *et al.*, 2008) and neuronal axon regeneration (Anderson *et al.*, 2017) and to possibly be involved in long term potentiation (Han *et al.*, 2013).

3.4.1 Astrocytes in ALS

As a response to injury or disease in the CNS, astrogliosis occur. Astrocytes change their morphology and physiology, depending on localization and severity of the illness. That includes change in their molecular expression patterns, proliferation, hypertrophy and formation of a glial scar. In ALS, astrogliosis is most likely triggered by mutant SOD1 expression (Pekny and Pekna, 2016).

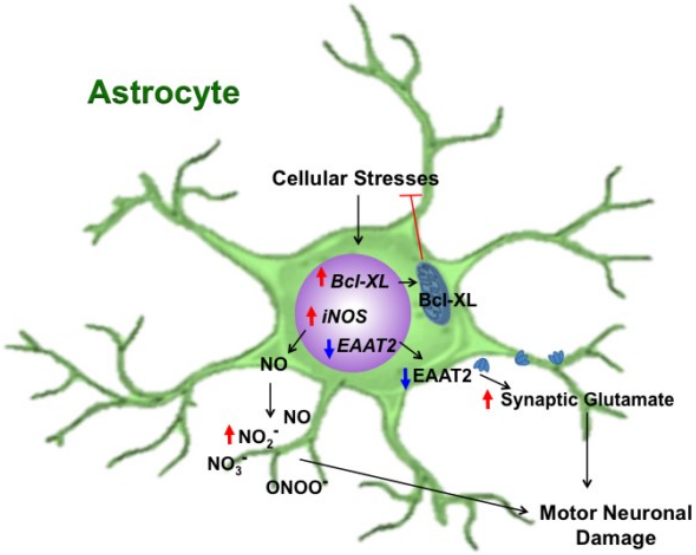

Fig 7. Change in molecular expression of some factors in activated astrocytes. Increased iNOS and decreased EAAT2/GLT1 expression lead to increased NO release and decreased glutamate uptake in the synaptic cleft. Elevation of glutamate and NO levels induce motor neuron damage via non-cell autonomous pathways (Lee *et al.*, 2016).

Astrocytes can induce motor neuron degeneration by direct cytotoxicity, loss of homeostatic/supportive function or combination of both. So far, plenty of mechanisms have been proposed to play a role in ALS.

One mechanism thought to play a key role in ALS is dysfunction of glutamate transporter EAAT2/GLT1 thanks to limited expression, resulting in ineffective glutamate uptake from the synaptic cleft, causing neuronal excitotoxicity (Yang *et al.*, 2010). Moreover, astrocytes lower the expression of motor neuron AMPA receptors GluR2 subunit, leading to increased Ca^{2+} permeability (Van Damme *et al.*, 2007). They can also release high levels of D-serine, which acts as co-activator of NMDA receptors, exacerbating glutamate neurotoxicity (Sasabe *et al.*, 2012). Higher levels of cyclooxygenase 2 have been observed, leading to increased prostaglandin E2 synthesis, which induces release of glutamate from astrocytes (Seifert *et al.*, 2006). Other important mechanism is mitochondrial dysfunction, leading to increased levels of ROS, which has been linked to neurodegeneration in SOD1 mice (Cassina *et al.*, 2008). Increased levels of iNOS and NADPH oxidase have been observed in human SOD1 astrocytes (Marchetto *et al.*, 2008).

Astrocytes also contribute to motor neuron degeneration by impaired production of neurotrophic factors and reduced metabolic support (Van Damme *et al.*, 2007). In addition, astrocytes produce soluble molecules with selective toxicity to spinal cord motor neurons. Another proven mechanism includes activation of (NGF)-p75 signaling pathway involved in direct motor neuron toxicity (Ferraiuolo *et al.*, 2011).

4 Conclusion

ALS is a serious progressive neurodegenerative disease affecting motor neurons. Though first described almost 150 years ago, its exact causes have not been yet identified and current options for its treatment are very limited. Together with understanding new important homeostatic and protective functions, the role of glial cells in the progression of ALS has been described. It was discovered that ALS develops under a complex network of interactions not restricted to motor neurons only, but including mutual interactions and signaling between motor neurons, glia and immune cells.

In general, there is a disruption in subtle balance in the communication between neurons, astrocytes and microglia. While this disturbance is compensated in the early stages of the disease, prolonged exposure to disease factors causes overactivation and dysfunction of both astrocytes and microglia. This consequently causes death of oligodendrocytes. Though oligodendrocytes are being replaced by replicating and differentiating NG2 cells, new oligodendrocytes have impaired both myelinating and supportive function. Demyelinated neurons slowly degenerate and denervation of voluntary muscles appear. Affected ALS patient eventually dies due to respiratory muscle failure.

While there has been a lot of publications about roles of astrocytes and microglia in ALS, not much about contribution of other cells types to the disease is known. Clarification of mechanisms of interaction between different cell types in ALS pathology can eventually lead to finding new therapeutic targets, such as support of remyelination or neurogenesis.

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