INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder that leads to a progressive muscle wasting and paralysis. The pathological phenotypes are featured by severe motor neuron death and glial activation in the lumbar spinal cord. Proposed ALS pathogenic mechanisms include glutamate cytotoxicity, inflammatory pathway, oxidative stress, and protein aggregation. However, the exact mechanisms of ALS pathogenesis are not fully understood yet. Recently, a growing body of evidence provides a novel insight on the importance of glial cells in relation to the motor neuronal damage via the non-cell autonomous pathway. Accordingly, the aim of the current paper is to overview the role of astrocytes and microglia in the pathogenesis of ALS and to better understand the disease mechanism of ALS.

Key words: amyotrophic lateral sclerosis, astrocyte, microglia, motor neuron, non-cell autonomous toxicity
ALS (SALS) tissue and in transgenic (mutant SOD1 (G93A))
ALS animal models have been documented [16, 17]. Abnormal
regulation of glutamate-dependent excitatory signal has also been
identified in ALS suggesting that excessive synaptic glutamate
and oxidative stress trigger motor neuronal damage. Moreover,
alterated calcium homeostasis, mitochondrial dysfunction, protein
aggregation, cytoskeletal disruption, apoptosis, and inflammation
are associated with motor neuronal damage and cell death [5,
18]. Current medical care for both FALS and SALS focuses
on symptom management. Supportive care can help control
symptoms and make ALS more manageable for patients and their
families, but this care does not significantly improve the disease
progression. Even, to date, there are no effective drug therapies
that slow the relentless progression of ALS [19-21]. In this regard,
the better understanding of pathogenic mechanism of ALS may
enhance the possibility for ameliorating the disease onset and
progression. In this review, we focus on how non-neuronal cells
are associated with the pathogenesis of ALS.

WHAT IS NON-CELL AUTONOMOUS TOXICITY?

In the past when scientists had focused on the study of neuronal
function and activity, the events related to neuronal damage and
cell death were only investigated from a narrow viewpoint. This
view was based on the notion that neurons are damaged due to
the dysfunction and deregulation by themselves (so called
cell autonomous pathway), and this damage was not related to the
dysfunction of any other cell types. As time went by, the view and
knowledge of scientists on the mechanisms of neuronal damage
have more evolved and advanced. Importantly, a growing body of
evidence have proven that non-neuronal cells such as astrocytes,
microglia, and oligodendrocytes directly contribute to the motor
neuronal damage and cell death (so called non-cell autonomous
pathway) in ALS including other neurodegenerative diseases.
Indeed, the disease onset and progression is modulated via non-
cell autonomous pathway in transgenic ALS (mutant SOD1
(G93A)) mice [18]. The mutant SOD1 expression within motor
neurons initiates a damage process and drives the disease onset.
In parallel, activation of astrocytes and microglia by mutant
SOD1 markedly exacerbates the disease progression while motor
neuronal mutant SOD1 has little influence on the progression of
ALS. Thus, the paradigm of the non-cell autonomous toxicity has
been determined and proven in several experimental conditions
of ALS [22, 23].

HOW DO AstrocyTES MIND MOTOR NeURONs?

A major pathological feature of ALS is the generation and
migration of new cells, specifically astrocytes, within and around
damaged regions of the spinal cord [24]. Astrocytes respond
to cellular stresses by proliferating and adopting a reactive phenotype
characterized by the development of long and thick processes
with an increased content of glial fibrillary acidic protein (GFAP).
Interestingly, a similar increase in GFAP immunoreactivity
was found when cultured primary spinal cord astrocytes were
exposed to oxidative stress, suggesting that such morphological
changes may be triggered by stress signals [24]. It seems likely that
epigentic alterations induced by mutant SOD1 (mtSOD1) and
other pathological stresses are involved in the transformation of
astrocytes to a neurotoxic reactive phenotype. In this scenario,
non-cell autonomous cell death of motor neurons in ALS could
result from either a loss of normal astrocytic support and/or the
secretion of neurotoxic cytokines. Several studies have proven this
idea as following: co-culture of astrocytes expressing mtSOD1
(G93A) or exposure to conditioned medium derived from
astrocytes expressing mtSOD1 (G93A) damages both primary
motor neurons and embryonic stem cell-derived motor neurons
[25, 26]. Previous studies have suggested that cytokines and other
toxic factors released from SOD1(G93A) astrocytes may trigger
motor neuronal damage [27-30]. For example, in vitro studies by
Ferraiuolo et al. (2011) show that SOD1(G93A) astrocytes are toxic
to normal motor neurons by reducing metabolic support from
lactate release and activating pro-nerve growth factor-p75 receptor
signaling pathway [27]. Interestingly, SOD1 (G93A) astrocytes
specifically express NLRP3 (NACHT, LRR and PYD domains-
containing protein 3) inflammasome complexed with the NLR
protein NLRP3, the adaptor ASC and pro-caspase 1, indicating
that astrocytes mediate the neuroinflammation in ALS [28].
Moreover, transforming growth factor-β1 (TGF-β1) is increased
in SOD1(G93A) astrocytes, and astrocyte-specific overexpression
of TGF-β1 in SOD1(G93A) mice accelerates disease progression
in a non-cell-autonomous manner [29]. On the other hand,
the elevation of Bid, a BCL-2 family protein, in SOD1(G93A)
astrocytes suggests that Bid activation may contribute to astrocyte
activation and motor neuronal damage in ALS [30]. In this study,
Bid is necessary for activating nuclear factor-κB in astrocytes to
mediate pro-inflammatory stimuli, which represents that Bid is
not directly toxic to motor neuron but indirectly modulates the
astrocyte-dependent non-cell autonomous toxicity. Together, it has
been successfully proven that astrocytic cytokines and toxin could
determine disease progression and are critical to the pathogenesis
of ALS.
Excitatory amino acid transporter-2 (EAAT2) is known as a typical glial glutamate transporter that uptakes neurotransmitters glutamate and aspartate from the synaptic cleft [31]. It is believed that EAAT2 uptakes more than 90% of glutamate into glia. In normal condition, astrocytes uptake glutamate and turn it into glutamine, and nourish motor neurons by supplying them as energy source. However, when astrocytes become reactive, the expression of EAAT2 gene is decreased and subsequently an excess amount of extracellular synaptic glutamate may lead to excitotoxicity in motor neurons in the spinal cord of ALS. Indeed, as the dysfunction of EAAT2 is implicated in ALS, the level of EAAT2 is reduced in the motor cortex and spinal cord of ALS patients [32]. Moreover, the decrease of EAAT2 activity impairs motor neuron survival in mouse models of ALS [33]. Otherwise, not only does chemical induction of EAAT2 activity improve motor neuron survival in an in vitro model of chronic excitotoxicity but it also extends the survival of transgenic ALS mice [34, 35]. When EAAT2 transgenic mice is crossed with mutant SOD1 (G93A) mice, it shows a significant delay in motor symptom such as grip strength decline but not in the onset of paralysis [36]. Interestingly, Foran et al., (2011) reports that sumoylated carboxy-terminal fragment of EAAT2 (CTE-SUMO1) is accumulated in the nucleus of astrocytes in the spinal cord of SOD1(G93A) mice [37]. The expression of CTE-SUMO1 in spinal cord astrocytes produces extrinsic toxicity by inducing caspase-3 activation and impairs axonal growth of motor neurons in a co-culture system. This study provides an unconventional role of EAAT2 in that EAAT2 participates in motor neuron degeneration through the direct cytotoxic effect of its truncated peptide but not through the activity of glutamate transporter. All together, growing evidence supports that regulation of EAAT2 activity accounts for motor neuronal survival and death in ALS via a non-cell autonomous pathway.

In comparison to the astrocytic phenotype in ALS, different astrocytic behaviors in relation to the excitotoxicity may be derived due to either the different damage region of CNS (brain versus spinal cord) or the different stress stimuli (bolus excitotoxicity versus chronic oxidative stress). For instance, GFAP-positive astrocytes appear extensively around the damage sites 7 days after injection of N-ethyl-D-aspartic acid (NMDA) while EAAT2- and GFAP-positive astrocytes disappear in a kainic acid (KA)-injected cortical region of the brain [38]. This study shows that two excitotoxic injury models exhibit quite different pattern of astrocyte behaviors such as astrogliogenesis versus astrocyte loss that are distinguished from the pathology of ALS. Accordingly, it will be challenging to pursue how the difference of region or stress stimuli converges and affects astrocyte behaviors in future studies.

**HOW ARE ASTROCYTES ADAPTED TO ENVIRONMENTAL STRESSES AND WHAT ARE THE SURVIVAL MECHANISMS OF ASTROCYTES UNDER ALS CONDITION?**

Our group has previously addressed this question using primary astrocytes from the spinal cord of wild type (WT) and ALS transgenic [mutant SOD1 (G93A)] mice. Our study shows that astrocyte survival is correlated with the elevation of Ets-2 transcription factor and with Bcl-xL expression [39]. The transcriptional activation of Bcl-xL by Ets-2 compensates oxidative stress by preventing astrocytes from apoptotic or necrotic cell death during the pathogenesis of ALS. Because we observed that motor neurons do not induce Bcl-xL in response to oxidative stress, we suggest that molecular mechanisms of Ets-2-mediated and Bcl-xL-dependent survival pathways may vary among different cell types [39]. Then why are motor neurons of ALS not rescued by the surviving astrocytes? We propose a plausible mechanism that the Ets-2 and Bcl-xL pathway improves astrocyte survival but it occurs too late to prevent earlier motor neuronal damage, or perhaps survived reactive astrocytes release toxic molecules to propagate motor neuron damage (Fig. 1). However, whether this might be expected to occur at an earlier stage, before astrocyte activation is reached its threshold, remains to be further investigated.

![Fig. 1. Astrocytes are associated with non-cell autonomous motor neuronal damage in ALS.](https://doi.org/10.5607/en.2016.25.5.233)
Oxidative stress due to the mutation of SOD1 is highly implicated in the pathogenesis of ALS. Not only does superoxide anion (O$_2^-$) lead to cellular damage including oxidation of DNA and protein and lipid peroxidation but nitric oxide (NO) is also thought to play a key pathogenic role in ALS [40]. Motor neurons are particularly vulnerable to oxidative stress in ALS which is a phenomena attributed to a low level of antioxidant enzymes and a high content of easily oxidized substrates [5, 24, 40]. NO is synthesized by NO synthases (NOSs) from arginine, which is a rate-limiting factor for NO production. We have reported that neuronal NOS (nNOS)-positive motor neurons are depleted while inducible NOS (iNOS)-positive reactive astrocytes are increased in ALS transgenic [mutant SOD1 (G93A)] mice [41]. The expression of iNOS/NOS2 was correlated with the increases of astrocyte activation and NO levels while nNOS/NOS1 expression was decreased in ALS transgenic [mutant SOD1 (G93A)] mice. The high levels of NO interact with superoxide and form highly toxic peroxynitrite. Consistent with findings previously reported by Przedborski and colleagues, increased levels of NO may further exacerbate oxidative stress and trigger motor neuron death [40-42]. As similar to ALS transgenic mice, accumulation of 8-hydroxy-2-deoxyguanosine, a marker of oxidative DNA damage, and elevated levels of peroxinitration damage (production of nitrotyrosine residues by covalent interactions of NO) have also been found in human ALS [43-46]. These data support a prominent role of oxidative stress derived from reactive astrocytes during the pathogenesis of ALS (Fig. 1).

**IS MICROGLIAL ACTIVATION A GOOD SIGN OR A BAD SIGN TO MOTOR NEURONS?**

Despite its controversy, microglia are also known to be linked to motor neuronal damage and the pathogenesis of ALS via the non-cell autonomous pathway [22, 47]. Interestingly, deletion of NF-κB signaling in microglia rescues motor neurons from microglial-mediated death in vitro and extended survival in ALS mice by deregulating proinflammatory microglial activation. In contrast, selective NF-κB inhibition in ALS astrocytes was not sufficient to rescue motor neuron death [48]. In this context, the microglia-mediated damage and toxicity to motor neurons are driven through the diversity of death mechanisms. Using the mice carrying deletable mutant SOD1 transgene by the action of Cre recombinase, Yamanaka and Yamashita have shown that diminishing mutant SOD1 toxicity within microglia significantly slowed the disease progression of ALS. This finding suggests that, in part, microglia contribute to neurodegenerative process of ALS [49].

On the other hand, in order to examine whether proliferating microglia leads to motor neuron degeneration in ALS mice, Gowing et al. (2008) generated double transgenic mice with CD11b-TK(mut-30) and mutant SOD1(G93A) in which a 50% reactive microglia is specifically reduced in the lumbar spinal cord [50]. Unexpectedly, reduction of reactive microglia had no effect on the degeneration of motor neuron. This study implies that proliferating microglia-expressing mutant SOD1 (G93A) does not play a pivotal role in triggering neuronal damage in an animal model of ALS. This study raises a question regarding whether different stages of microglia are involved in different modes of action for protecting versus being involved in the damaging of motor neurons through yet unidentified mechanisms. We suggest that future studies are necessary to uncover the precise action mechanism behind the obscure role of microglia in ALS.

**WHY IS IT NOT CONSISTENT TO OBSERVE THE ROLE OF MICROGLIA IN THE NEURODEGENERATIVE PROCESS OF ALS?**

Is microglia activation beneficial or disadvantageous to motor neurons? Microglia function is necessary for surveilancing the condition of motor neurons and for restoring tissue injury in response to acute and reversible stress: microglia are beneficial before the threshold limit reached. However, constitutive activation of microglia by a chronic and irreversible stress such as ALS stress may transform them as a non-cell autonomous player to be toxic to motor neurons: microglia are disadvantageous after they become fully activated.

We have previously found that the expression of c-Ret is altered in motor neurons of the lumbar spinal cord in ALS transgenic [mutant SOD1 (G93A)] mice and ALS [mutant SOD1 (G85R)] and (G93A)] motor neuronal cell lines [51]. c-Ret oncoprotein is a protein kinase receptor and responds to glial cell line-derived neurotrophic factor (GDNF). c-Ret-mediated signal transduction is important to maintain cellular activity and survival function. Notably, the levels of non-phosphorylated and phosphorylated c-Ret were markedly elevated in active microglia of the lumbar spinal cord of ALS mice in an age-dependent manner. Our findings suggest that ALS stress-induced expression of c-Ret in microglia may trigger non-cell autonomous toxic signals and exacerbate damage responses in motor neurons by disturbing the GDNF signaling pathway in motor neurons [51]. Our previous study does not provide a direct evidence that microglia contribute to non-cell autonomous motor neuronal damage in ALS. However, based on our findings, we suggest an indirect contribution of microglia to motor neuronal damage. For instance, the increased level of c-Ret in microglia elevates interaction with GDNF. As a result, the c-Ret and GDNF interaction promotes the survival of
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Microglia whereas the subsequent deprivation of NFs by activated microglia may lead to motor neuronal damage (Fig. 2).

CONCLUSIONS

A vicious cycle of ALS stresses transforms astrocytes and microglia from Dr. Jekyll to Mr. Hyde

In the pathogenesis of ALS, non-motor neuronal cells such as astrocytes and microglia undergo a series of molecular and cellular changes in that these cells become unprofitable to motor neurons, leading to irreversible neurodegeneration. The mechanism of non-cell autonomous motor neuron death is closely associated with the pathophysiological change in ALS that is apparently distinguished from cell autonomous pathway.

Neuroinflammation is now identified as a key contributor to motor neuron damage in ALS [52-54]. Reactive astrocytes and microglia are triggers of neuroinflammation that accelerate disease progression [55, 56] which is further exacerbated by ongoing neuronal injury [53]. Inflammatory cytokines released by astrocytes and microglia may facilitate glutamate excitotoxicity thereby linking neuroinflammation and excitotoxic death [18, 57, 58].

Taken together, previous findings suggest that the molecular and cellular adaptation between astrocytes, microglia, and motor neurons may be differently modulated by epigenetic components upon ALS stresses. In this paradigm, due to chronic oxidative stress or other irreversible mechanisms, a critical threshold limit is reached and that reactive astrocytes and microglia trigger the pathological processes that subsequently lead to a non-cell autonomous death of motor neurons in ALS. This idea suggests that future therapeutic strategy for the treatment of ALS should be aimed at specific interception of pro-oxidant and pro-death signals in a cell-type specific manner [59-62].

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