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Lost in translation: microRNAs mediate pathological cross-talk between motor neurons and astrocytes

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The central nervous system is estimated to comprise over two hundred billion neurons and glial cells. The crosstalk between these cells and the resultant fine-tuning of neurotransmission underpins every process regulated by the brain and spinal cord. We are still trying to unravel the complex network of chemical signals that drives this inter-cellular communication. However, in the past decade non-coding RNAs have emerged as key players in the cross-talk between neurons and neighbouring glial cells (Schratt, 2009). Non-coding RNAs are a class of untranslated RNA species that contribute to the post-transcriptional control of protein synthesis via binding and subsequent modulation (usually inhibition) of mRNA translation. MicroRNAs (miRNAs) are part of this group of molecules and have been increasingly implicated in inter-cellular communication in health and disease.

In this issue of *Brain*, Hoye and colleagues explore the function of miR-218, a motor neuron-secreted miRNA, in the pathophysiology of amyotrophic lateral sclerosis or motor neuron disease (ALS/MND) (Hoye *et al.*, 2018). ALS is a neurodegenerative condition causing injury and cell death primarily of upper and lower motor neurons, resulting in progressive failure of the neuromuscular system, widespread muscle denervation and death typically within 2–5 years of symptom onset. ALS is a heterogeneous and complex disease and it has become clear that it is non-cell autonomous and that glial cells, in particular astrocytes, microglia and oligodendrocytes, contribute to the neurodegenerative process (Hardiman *et al.*, 2017). The mechanisms through which glial activation contributes to motor neuron death and the causal relationship between these two events have not yet been clearly defined.

The study by Hoye *et al.* focuses on the effect of the motor neuron-specific miR-218 on astrocyte function following its release from dying motor neurons. The authors had identified this miRNA molecule in a previous study, where they found that miR-218 was upregulated 10-fold in the CSF of a rat model of ALS at the end-stage of the disease (Hoye *et al.*, 2017). In this issue of *Brain*, the authors use an elegant combination of miRNA tagging and affinity purification to show that *in vivo* miR-218 is expressed predominantly in motor neurons and only at very low levels within healthy or ALS mouse astrocytes, concluding that miR-218 is

not a major player in regulating protein expression in adult astrocytes. However, using *in silico* bioinformatics tools and a luciferase assay, the authors also show that miR-218 regulates the expression of the glutamate re-uptake transporter EAAT2. This protein is crucial for the correct regulation of excitatory glutamatergic neurotransmission. It plays a key role in the neuroprotective function of astrocytes by preventing accumulation of glutamate in the extracellular synaptic cleft. Reduced expression or function of EAAT2 leads to excitotoxicity, a process clearly linked to the pathophysiology of ALS (Rothstein *et al.*, 1995).

By treating mouse astrocytes with medium from lysed NSC34 cells, Hoyer *et al.* show that the miR-218 released into the medium can be taken up by astrocytes, and that this uptake is associated with decreased expression of EAAT2. Extending these *in vitro* experiments, the authors test their hypothesis *in vivo* in the gold-standard G93A SOD1 transgenic mouse model of ALS. Intracerebroventricular (ICV) injection of an antisense oligonucleotide against miR-218 in mutant SOD1 mice at the symptomatic stage, when motor neurons appear to release this miRNA, successfully decreased the level of miR-218 and restored expression of EAAT2. The authors also report that silencing miR-218 had a normalising effect on the expression of connexin 43 and glial acidic fibrillary protein (GFAP), indicating that this microRNA might have a wider role in modulating glial activation. Consistent with the findings described in this paper, Morel *et al.* had previously identified an exosome-secreted miRNA that displayed a similar function to that described here for miR-218, regulating EAAT2 expression in glia (Morel *et al.*, 2013).

Hoyer *et al.* build on previous evidence, collected mainly from mutant SOD1 rodent models, showing that ALS is a multiphasic disease, where onset is determined by pathophysiological changes in motor neurons and progression is characterized by alteration of glial function (Sun *et al.*, 2015). However, *in vitro* models of ALS (Hautbergue *et al.*, 2017), as well as models of inflammation independent of SOD1 (Frakes *et al.*, 2014), show that glial cells can indeed drive motor neuron loss if they harbour intrinsic abnormalities.

Growing evidence is emerging that miRNAs released by astrocytes via exosomes or complexed with proteins, are important regulators of neuron-astrocyte cross-talk as well as synaptic function and stability. Recently, Chaudhuri *et al.* demonstrated how the presence of inflammatory signals modifies the miRNA cargo of astrocyte-secreted extracellular vesicles and how these regulate neurotrophic signalling to neurons (Chaudhuri *et al.*, 2018). The

concept that some of these secreted miRNAs are then circulated in the CSF and can act as long-distance signalling molecules underpinning disease propagation, is supported by several studies that have identified in CSF miRNAs involved in the regulation of neuronal and glial activity (Waller *et al.*, 2017).

From a therapeutic perspective, miRNAs are known to affect multiple mRNA transcripts simultaneously, which has implications for unwanted off-target effects *in vivo*. miRNAs also have varying targets in different cell types and, as Hoye *et al.* highlight in their discussion, full ablation of miR-218 leads to motor neuron degeneration. Thus, attempting to ameliorate astrocyte dysfunction in ALS by lowering the expression of miR-218 globally within the CNS would be likely to have adverse off-target effects on motor neuron health.

The pathophysiological changes contributing to neuronal injury and perturbation of glial function in ALS are complex and effective therapeutic approaches are likely to require targeting of multiple effectors simultaneously. Previous approaches, aiming to inhibit or activate a single target, have failed to deliver substantial impact on ALS disease progression in the clinic. Astrocyte involvement in ALS is characterised by a complex gain of toxic properties as well as loss of neuroprotective functions, resulting in an aberrant cellular phenotype which is unlikely to be corrected by manipulation of a single target. In this context, engineered miRNA-like molecules with selective cellular targeting and a strong safety profile would represent useful tools in the effort to normalise multiple facets of the cellular pathophysiology underlying ALS. Hoye and colleagues have elegantly dissected through *in vitro* and *in vivo* studies one molecular mechanism contributing to the perturbed cross-talk between neurons and astrocytes in models of ALS (Figure 1). miR-218 has not so far emerged as a prominent candidate in studies of the expression of microRNAs in human biosamples (Waller *et al.*, 2017) and further work is required to ascertain the importance of this pathway in relation to human ALS.

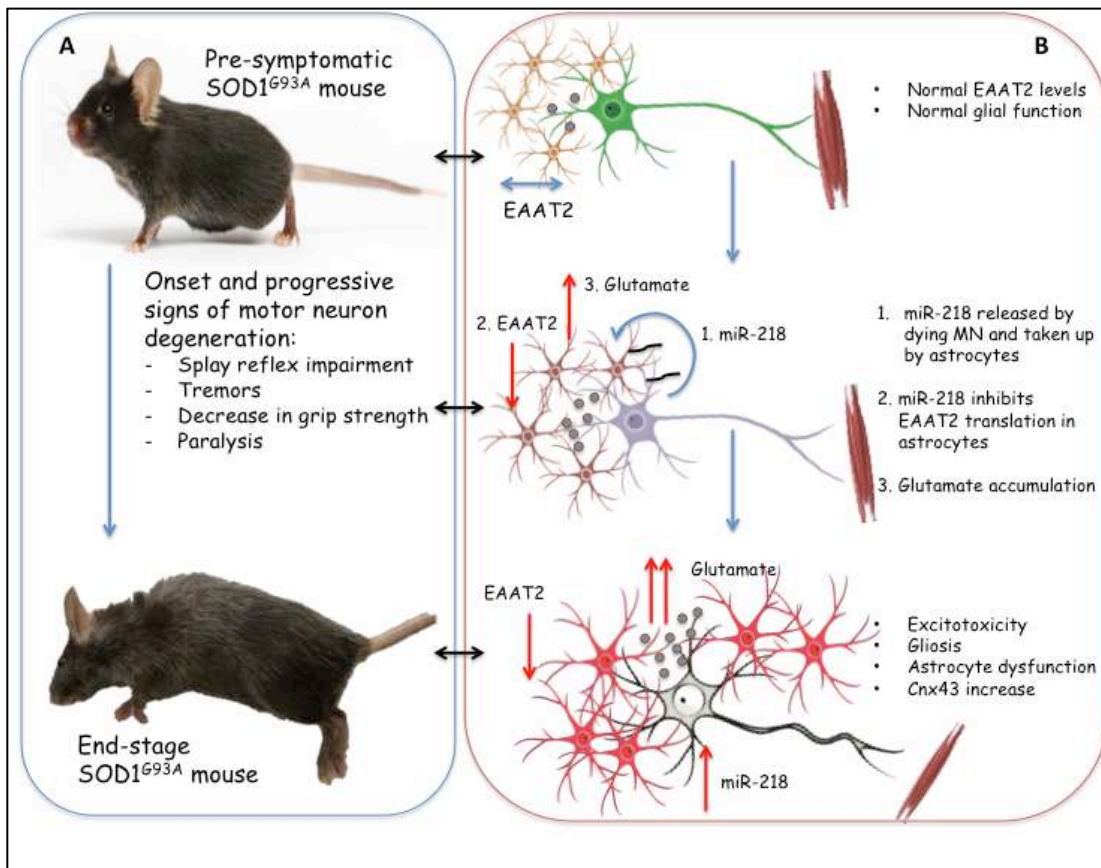


Figure 1. Schematic of the proposed impact of miR-218 in ALS pathology.

A) The SOD1 G93A mouse model of ALS mimics some of the major clinical aspects of the human disease. Prior to adulthood mutant SOD1 mice are pre-symptomatic and do not display clear signs of motor impairment. Between 70–90 days, depending on the genetic background, the mice start displaying signs of denervation and progressive motor neuron (MN) loss, including tremors, splay reflex defects and loss of grip strength. They then quickly progress towards paralysis, when they are euthanized. At the cellular level (B), pre-symptomatic mice do not display major signs of motor neuron loss or gliosis, which only become evident at the symptomatic and end stages of the disease. Hoye *et al.* propose and partly demonstrate that release of miR-218 from dying neurons drives loss of the astrocytic glutamate transporter (EAAT2/Glt1), which contributes to excitotoxicity and exacerbates the inflammatory reaction of astrocytes surrounding the motor neurons. This cascade of events spirals in a toxic loop that impairs astrocyte function and exacerbates motor neuron loss and gliosis.

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Glossary

Non-coding RNA: untranslated RNA species contributing to the post-transcriptional control of protein synthesis via binding and subsequent inhibition of mRNA translation

RILES or RNAi-Inducible Luciferase Expression System: a plasmid-based system engineered to monitor the activity of endogenous RNAi machinery. The miRNA of interest suppresses the expression of a transcriptional repressor and consequently switches ON the expression of a luciferase reporter gene.