most potent triggers for neurotoxicity, and in reports analogous to the paper by Fritschi et al., amyloid-β oligomers seems to be present in human Alzheimer’s disease CSF at concentrations many orders of magnitude lower than in brain tissue lysates, if at all (Xia et al., 2009; Esparza et al., 2013; Savage et al., 2014).

In conclusion, with the efforts of Fritschi et al., as well as several other groups in recent years, the streetlight is now beginning to swing around to illuminate the human brain directly.

Hopefully this is where we will find the keys to developing effective therapeutics for Alzheimer’s disease.

David L. Brody and Michael L. Gross
Washington University, USA

Correspondence to: David L. Brody
E-mail: brodyd@neuro.wustl.edu
doi:10.1093/brain/awu261

References

A serum microRNA signature for amyotrophic lateral sclerosis reveals convergent RNA processing defects and identifies presymptomatic mutation carriers

This scientific commentary refers to ‘Serum microRNAs in patients with genetic amyotrophic lateral sclerosis and pre-manifest mutation carriers’ by Freischmidt et al. (doi: 10.1093/brain/awu249).

Defective RNA processing has occupied centre stage in the pathogenesis of amyotrophic lateral sclerosis (ALS) since the identification of TARDBP (also known as TDP-43) inclusions in 95% of cases and pathogenic mutations in RNA processing genes such as TARDBP, FUS and MATR3 (Sreedharan et al., 2008; Vance et al., 2009; Johnson et al., 2014). FUS and TARDBP are known to regulate mRNA transcription, splicing, stability and transport (Tollervey et al., 2011; Rogelj et al., 2012) but they are also part of the large Drosha complex that regulates microRNA (miRNA) biogenesis (Gregory et al., 2004). Dysregulation of miRNA expression has been shown in many cancers and more recently in Alzheimer’s disease and is predicted to play a mechanistic role and/or be an indirect biomarker of disease.

In this issue of Brain, Freischmidt et al. (2014) report that levels of a specific subset of miRNAs are reduced in the serum of patients with familial and sporadic ALS, and that these reductions are even detectable in presymptomatic carriers of pathogenic ALS mutations (Freischmidt et al., 2014). Some caution is required as patient numbers are small and some samples required pooling for analysis, but if these results can be replicated in larger cohorts then this will become a landmark study. Robust serum miRNA biomarkers would aid early diagnosis and therefore early treatment, and identify at-risk asymptomatic mutation carriers with
the prospect of presymptomatic treatment. ALS shows great genetic heterogeneity, but the miRNA signature seems to be common to all genotypes and to sporadic cases. This implies that miRNA dysregulation reflects dysfunction of a common disease pathway, perhaps more as a consequence of disease rather than being directly causal. A key question with respect to any biomarker is whether it reflects a genetic trait or the disease state. The observation that levels of specific miRNAs are significantly lower in those with clinical signs of ALS compared with asymptomatic mutation carriers suggests that both may be the case. Again, larger cohorts and longitudinal studies are now required to determine whether the changes in serum miRNA levels reflect disease activity.

In their search for differentially abundant serum miRNAs, Freischmidt et al. used Affymetrix™ miRNA 3.0 arrays with probes for 1773 miRNAs in nine cases with familial ALS and 10 control subjects, and identified 30 miRNAs that were significantly downregulated in the patients. Levels of the four most significantly downregulated miRNAs (miR4745, miR3665, miR1915, and miR4530) showed similar changes when measured by quantitative RT-PCR in a second cohort of 13 cases with familial ALS and 13 control subjects, and in a cohort of 14 cases with sporadic ALS compared with 14 control subjects. Despite significant genetic heterogeneity, there was a surprising uniformity among the downregulated miRNAs: when Freischmidt et al. screened 18 presymptomatic mutation carriers and eight control subjects using miRNA microarrays, they found that 22 of 24 significantly downregulated miRNAs overlapped with the discovery set of 30 miRNAs. Lastly, the authors identified a common sequence motif, GDCGG (with D being G, A or U), in the majority of the downregulated miRNAs. Enrichment of this sequence motif suggests an upstream RNA binding protein that regulates this subset of miRNAs. One candidate is the CGGBP1 RNA binding protein, which has a preference for CGG repeats (Naumann et al., 2001), but there may be many others. These results are a remarkable step forward in terms of biomarker discovery, but urgently require replication and validation in cohorts with ALS and frontotemporal dementia, where there is striking overlap in terms of TARDBP and FUS pathology, and causal genes.

It is interesting to speculate as to why miRNA exists in the serum, and what implications these changes in miRNA profiles might have for diagnosis and therapy. Although the role of miRNA in serum has not been clearly established, two possible sources have been proposed: cytoplasmic miRNA that is packed into exosomes and actively secreted or miRNA that is released by cells undergoing apoptosis or necrosis (Valadi et al., 2007). Serum exosomes may circulate throughout the body and, via targeted or non-targeted fusion with cells, deliver packages of miRNAs, which have the potential to change protein expression inside that cell (Creemers et al., 2012).

The common sequence motif may indicate dysregulation of a master miRNA binding protein and may explain why this particular subset of miRNAs is downregulated. The identification of this RNA binding protein could provide a more robust upstream diagnostic and predictive biomarker, or a therapeutic target if changes in miRNA are shown to be mechanistic in initiating or propagating neurodegeneration. Freischmidt et al. note that downregulation of these miRNAs may have functional effects as one of them, miR1915, regulates the expression of Bcl2. Given that miRNAs can target hundreds of mRNAs, it will be important to correlate changes in miRNAs with changes in the abundance of other mRNAs and the proteins that they encode.

Acknowledging that this is a pilot study that requires replication and validation, the discovery of a miRNA signature in serum would have major implications for ALS. If these biomarkers are shown to be disease-specific, their discovery would aid early diagnosis and presymptomatic testing. If their levels are shown to mirror disease activity, they would have predictive value and could be used to measure the efficacy of any therapeutic intervention. This would be transformative in accelerating the pace of drug discovery for ALS and potentially other neurodegenerative disorders.

Youn-Bok Lee,1 Boris Rogelj2 and Christopher E. Shaw3
1King's College, London, UK 2Jozef Stefan Institute, Ljubljana, Slovenia
Correspondence to: Christopher E. Shaw
E-mail: christopher.shaw@kcl.ac.uk
doi:10.1093/brain/awu262

References