Dysregulation of miRNA biogenesis machinery, miRNA/RNA ratio and miRNA normalizing targets in skeletal muscle of ALS mice

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Introduction: Amyotrophic lateral sclerosis (ALS) is a terminal motor neuron disease affecting muscle and neural tissues. A potential molecular cause common to all forms of ALS is an impairment of the RNA processing machinery in the cell. MicroRNAs (miRNAs) are non-coding RNA (ncRNA) molecules regulating numerous cellular processes. MiRNAs are implicated in the ALS pathology and play a role in impaired RNA processing, microglial dysfunction, inflammation, neuronal death and muscle atrophy.

Methods: *Soleus* (SOL), *tibialis anterior* (TA), *gastrocnemius* (GAST) and *extensor digitorum longus* (EDL) as well as spinal cord (cervical, lumbar and thoracic regions) and brain tissue were obtained from the SOD1/G93A mouse model of human ALS at different stages of the disease spanning from pre-symptomatic (ages 32-35 days) to end stage (ages 156-173 days). RNA was extracted from muscle and neural tissue. mRNA and miRNA expression levels were measured by qPCR. RNA levels were normalized against single-strand DNA concentration. MiRNA levels were normalized by loading equivalent miRNA concentrations in the reverse transcription reaction prior to qPCR analysis.

Results: The expression levels of members of the canonical miRNA biogenesis pathway, *Drosha* and *Xpo5*, and the ALS-related genes involved in miRNA biogenesis, *TDP-43*, *FUS* and *Hnrnpa1*, were upregulated in the TA muscle of SOD1/G93A ALS mice when compared to their wild-type littermates. The ratio of the total miRNA pool relative to the total cellular RNA pool was altered in the TA muscle only as the disease developed. These alterations influenced the expression levels of reference ncRNAs and miRNAs.

Conclusion: We propose that a pathological increase in ALS-related genes causes a dysregulation in the total RNA/miRNA ratio in fast-twitch TA muscle from SOD1/G93A ALS mice. This alteration compromised the accuracy of commonly used miRNA normalization strategies. This issue could be overcome by measuring individual miRNA concentrations and using these values for the reverse transcription step prior to qPCR analysis.