Commentary

Research Highlights

To miR or Not to miR: That is the Question in ALS Disease

Williams, A.H.; Valdez, G.; Moresi, V.; Qi, X.; McAnally, J.; Elliott, J.L.; Bassel-Duby, R.; Sanes, J.R.; Olson, E.N. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. *Science,* **2009**, *326*(5959), 1549-1554.

Post-transcriptional regulation represents a powerful means to exert control over gene expression and to enhance plasticity and adaptability at the molecular, cellular and functional levels, even in a complex organ such as the nervous system. Approximately 80% of the human brain genome is transcribed into RNA, yet only about 2% of the genome is transcribed into protein, which emphasizes the capability of various steps of RNA signalling and RNA-based mechanisms to contribute to gene control. In this regard, one of the hottest research topics in biology today is the nuclear processing and cytoplasmic formation of microRNAs (miRNAs) that occur in all eukaryotic cells. miRNAs are small, noncoding portions of ribonucleic acid binding to the 3'-untranslated region of target mRNAs and leading to translational repression or degradation of the target. Since the requirement for target complementarities is only partial in eukaryotic cells, one miRNA can potentially have hundreds of targets, and each mRNA can be regulated by many miRNAs. Because there are almost 16,000 miRNAs in animals, plants, and viruses, and more than 1000 different known human miRNA sequences (each of approximately 20-25 nucleotides), exact prediction of the actual mRNA targets would appear to lie just beyond our grasp.

At least 20-30% of human protein-coding genes are likely controlled by miRNAs. Recent studies have demonstrated that miRNAs are key regulators of diverse biological processes, such as cell proliferation, growth, differentiation and apoptosis. A growing body of evidence indicates that miRNAs are highly expressed in the CNS with several being found specifically in brain, where they play important roles in normal and/or pathological development and functioning. For instance, miRNAs have a role in brain morphogenesis, neurogenesis, neuronal differentiation, dendritic spine generation, synaptic formation and plasticity. In addition, some miRNAs have been implicated in neuropsychiatric disorders, epileptic seizures, traumatic spinal cord and brain injuries, brain cancer and ischemia, Parkinson's, Alzheimer's, and Huntington's diseases, Rett, Fragile X and Tourette's syndromes. Although certain factors such as the genome itself, stress, RNA oxidation, lack of neurotrophic proteins and environmental influences have been identified as possible determinants of changes in miRNAs expression, we are only now beginning to understand the impact of this novel class of gene regulators on the CNS. Identifying the complex roles of miRNAs and their targets thus promises to bring new insights to many aspects of neuronal function and dysfunction, and to be decisive for future research, by fostering potential application of miRNAs as biomarkers, diagnostic instruments and therapeutic tools for many neurodegenerative and neuroinflammatory diseases.

Giving strength to this idea, a study recently published in *Scienc*e by Williams and co-authors describes that microRNA-206 (miR-206), a skeletal muscle-specific miRNA that is dramatically induced in a mouse model of Amyotrophic Lateral Sclerosis (ALS) in coincidence with the onset of neurological symptoms, is a modifier of disease pathogenesis. ALS is characterized by progressive degeneration of all upper and lower motor neurons, denervation of target muscles, atrophy, paralysis, and finally death due to respiratory failure. There is currently no effective treatment for ALS and identification of the specific cellular mediators, especially the bidirectional signalling between motor neurons and skeletal muscles at the neuromuscular synapse, remains a major challenge in the search for novel and successful therapeutics.

In order to distinguish if the increased level of miR-206 in ALS might be an innocuous correlate, a contributor to pathology, or part of an ultimately inadequate compensatory effort, Williams and colleagues generated targeted mutants in which miR-206 expression is abolished in mice expressing G93A-SOD1 (a mutation in the ubiquitous free radical scavenger enzyme Cu,Zn superoxide dismutase, that is the most frequent cause of familial ALS). Loss of miR-206 didn't apparently affect disease onset, but rather accelerated its progression. Exacerbation of disease symptoms in miR-206$^{-/-}$ ALS mice was accompanied by skeletal muscle atrophy, kyphosis, paralysis, and diminished survival by about one month. The notion that motor neuron pathology plays a key role in ALS, whereas miR-206 is present exclusively in muscles, led Williams and co-authors to speculate that the miRNA might affect nerve-muscle interactions. They then sought to prove that reinnervation of denervated muscles by motor axons is delayed in the absence of miR-206, without impairment of axonal regeneration. The authors demonstrated that miR-206$^{-/-}$ / G93A-SOD1 neuromuscular junctions are disorganized, showing deficient colocalization of nerve and post-synaptic sites. Next they asked how miR-206 might promote a partially successful compensatory response to denervation in ALS. Using reporter constructs, the *Science* paper goes on to demonstrate that one role of miR-206 is to repress the translation of histone deacetylase 4 (HDAC4) mRNA, the strongest computationally predicted target of miR-206, also implicated in the control of neuromuscular gene expression. Furthermore, the skeletal muscle phenotype of HDAC4 null mice was opposite that of miR-206$^{-/-}$ mice, in terms of reinnervation following injury. As predicted for a miRNA and its substrate, this result suggests that miR-206 and HDAC4 might have contrasting effects on retrograde signals required for reinnnervation, and that miR-206 might function to counteract the negative influence of HDAC4. In search of muscle-derived synaptic organizing factors that are affected in a contrary way by miR-206 and HDAC4, the authors next proved that fibroblast growth factor binding protein 1 (a secreted factor interacting with fibroblast growth factor family members to potentiate their bioactivity) is significantly down-regulated following denervation in miR-206-/- mice, but up-regulated in HDAC4 skeletal muscle knockout mice. The consequent implication is that the salutary actions of miR-206 are mediated by muscle-derived factors that sustain nerve-muscle

interactions in response to motor neuron injury, and that miR-206 slows ALS progression by sensing motor neuron injury and promoting compensatory regeneration of neuromuscular synapses.

In a broader perspective, these observations with miR-206 open a new scenario on the composite mechanisms underlying ALS, and further our understanding of miRNA-related neurodegenerative and neuroinflammatory states in general. Initial support for a functional relationship of miRNA with RNA-binding proteins such as FUS/TLS and TARDBP/TDP43, known to be involved in miRNA processing and to be mutated in ALS, moreover suggests promising roles for regulatory RNA in the pathogenesis of ALS, also encouraging further exploitation of miRNA-based strategies in ALS and related diseases. Is miR-206 only the tip of the iceberg? That is the question for the generation of new RNA-directed gene-therapy against ALS.

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