MicroRNAs as Effectors of Brain Function

Julie Anne Saugstad, PhD

MicroRNAs

MicroRNAs (miRNAs) are a recently discovered family of small, genome-encoded endogenous RNAs that are transcribed but are not translated into proteins. Early studies in C. elegans revealed that an endogenous small RNA (lin-4) regulated translation of lin-14, a protein required for postembryonic development, through an RNA-RNA interaction. Small RNAs were then shown to mediate gene-silencing via a mechanism known as RNA interference (RNAi). Fire and Mello were awarded the Nobel Prize in Physiology or Medicine in 2006 for the discovery of RNAi. The term miRNA was introduced in a series of back-to-back Science articles in 2001. MiRNA genes produce primary miRNA transcripts which contain at least one, but possibly more, 70 nucleotide hairpin loops. These transcripts are transported into the cytoplasm where they are cleaved by the endonuclease Dicer into an imperfect duplex of 20 to 25 nucleotides. One strand of the duplex is degraded and the other mature miRNA binds to Dicer and forms a complex with argonaute proteins to form RNA-induced silencing complexes. Studies from several laboratories have revealed that the predominant role of miRNAs in RNA-induced silencing complexes is to regulate post-transcriptional gene expression by translational repression, mRNA cleavage, and mRNA decay initiated by miRNA-guided rapid deadenylation. However, emerging studies support that possible involvement of miRNAs in transcriptional and translational activation. Tremendous progress has been made in unraveling the complexities of miRNAs as meta-controllers of gene expression and their impact on cell development, survival, and function, yet miRNA research is still in its infancy. Given the enormous potential for miRNA studies to translate into novel therapeutic strategies for the diagnosis and treatment of many diseases, the quest to examine all aspects of miRNA functions is fully warranted.

MiRNAs and Brain Ischemia

MiRNAs serve essential roles in virtually every aspect of brain function, including neurogenesis, neural development, and cellular responses leading to changes in synaptic plasticity. Accordingly, miRNAs are also implicated in neurodegeneration and neurological disorders. Further, miRNAs are implicated in responses to hypoxia and ischemia, and in ischemic tolerance induced by ischemic preconditioning.

Ischemic tolerance is the response to a short duration of ischemia (preconditioning), which protects cells against a subsequent injurious duration of ischemia. Ischemic preconditioning-induced tolerance is known to require new protein synthesis, and the signature of tolerance is a transient repression of gene expression. We proposed that miRNAs might serve as mediators of new protein synthesis required for tolerance, and thus quantified changes in miRNA expression in preconditioned, ischemic, and tolerant mice induced using varying durations of middle cerebral artery occlusion. We isolated total RNA from the contralateral and ipsilateral cortex of each mouse brain using the mirVana Isolation Kit (Ambion, Austin, TX). The RNAs were labeled, hybridized to the mirVana Probe Set V2 (Ambion) microarray slide that included probes for human, mouse, and rat miRNAs, and microarray slides were scanned on a GenePix 4000B (Axon Instruments, Union City, CA). For initial data analysis, an important consideration was to evaluate the consistency of miRNA expression within each group to identify animal-to-animal and diurnal variations in miRNA expression. For each mouse, the change in miRNA expression from 1 animal was compared with the change of the total group, which showed that miRNA expression was consistent within each treatment group and that regulation of miRNAs was not random in individual mice. This type of data analysis is particularly challenging with regard to human studies where genomics, age, and health will likely impact the consistency of miRNA expression levels in control and patient populations. Our subsequent data analysis revealed that miRNA expression levels were regulated in preconditioned, ischemic, and tolerant mice, and that one prominent predicted target of the miRNAs decreased in preconditioned brain was the global transcriptional regulator, methyl CpG binding protein 2, which had no prior recognized role in preconditioning or tolerance. These studies supported our hypothesis that miRNAs were regulated by preconditioning ischemia, and current studies are focused on elucidating the effects of ischemic preconditioning-regulated miRNAs and their role in endogenous neuroprotection.

Transient focal ischemia alters miRNA expression in the blood and brain of male rats. However, there are differences...
in responses to focal ischemia in male and female rodent brain; males have greater infarct volumes in response to ischemia than do females.\textsuperscript{18,19} We recently used real-time quantitative polymerase chain reaction profiling to examine miRNA expression in focal ischemia in male and female C57/BL6 mice. These studies revealed that there is a universal, ischemia-induced miRNA profile, which was equally present in both male and female brains, as well as unique miRNA profiles in either male or female brain (J. Saugstad and S. Murphy, unpublished data, 2013). Current studies are focused on validating these miRNA responses, identifying their cellular targets, and determining their functional relevance to ischemia.

**Challenges to miRNA Studies**

We and others have found inconsistencies in miRNA expression levels between different array platforms, highlighting the current technical challenges and limitations of miRNA studies. A recent, rigorous study revealed inherent problems within and between the different assays.\textsuperscript{20} In this study, identical RNA samples assayed on 6 distinct miRNA microarrays obtained from different vendors showed little correlation between the datasets. Only 1 of 6 microarray vendors (Agilent) used probes specifically targeted to the mature miRNA sequence, whereas the others used probes that could detect the mature miRNA sequence but could also detect miRNA sequences in the primary and precursor transcripts. The authors also found inconsistencies between data obtained from the array platforms and NextGen sequencing. This study underscores the complexities and limitations with evolving technologies for miRNA studies and the need to validate changes in miRNA expression using multiple approaches.

The translational goal of miRNA expression studies is to identify specific miRNAs and their targets, which may lead to novel therapeutic strategies for diseases. Thus, it is critical to identify and validate miRNA/mRNA target pairs. The complexity of this task is daunting, as a single miRNA can target hundreds of mRNAs, and 1 mRNA can be targeted by hundreds of miRNAs. Computational algorithms and free energy (\(\Delta G\)) analyses allow for identification of putative miRNA/mRNA targets, but the authenticity of a functional miRNA/mRNA target pair must be validated by additional criteria. As proposed by Kuhn et al,\textsuperscript{21} (1) miRNA/mRNA target interactions must be verified, (2) the miRNA and predicted mRNA target must be coexpressed, (3) a given miRNA must have a predictable effect on target protein expression, and (4) miRNA-mediated regulation of target gene expression should equate to altered biological function.

**Summary**

In the relatively short time since their discovery, the miRNAs have been shown to be essential for neuronal development, survival, function, and plasticity. MiRNAs are regulated in response to ischemia and ischemic preconditioning, and male and female mice show both common and unique responses to ischemia, which may contribute to sexually dimorphic responses to ischemia. These findings warrant further studies to examine the role of ischemia-regulated miRNAs on cell death and/or neuroprotection and to identify new targets for alternative strategies for the treatment or prevention of stroke. Given that miRNAs are encoded within the genome, it is conceivable that mutations in miRNA genes and/or their mRNA target sequences, could disrupt normal post-transcriptional gene regulation and lead to disease phenotypes. This may be particularly true for familial diseases, such as stroke, where protein coding gene mutations have not been identified. MiRNAs are also rapidly emerging as biomarkers for diseases, including brain injury, neurodegeneration, and psychiatric disorders.\textsuperscript{22} Accordingly, there is evidence for altered miRNA expression in peripheral blood isolated from ischemic stroke patients,\textsuperscript{23,24} suggesting the possibility that blood miRNAs can be used as biomarkers for brain injury, including cerebral ischemia. The first decade of miRNA research has greatly impacted our understanding of the mechanisms underlying normal and altered cellular function, despite technical limitations due to the complexity of miRNAs. In the next decade of miRNA studies, efforts to advance and evolve the tools necessary for analysis and validation of miRNAs, should be fully supported, as these tools will be essential in establishing direct correlations between miRNA-mediated post-transcriptional gene expression and disease, a matter of great importance to human health.

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**Disclosures**

None.

**References**


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