Published in final edited form as:
Stroke. 2013 June ; 44(6 0 1): S17–S19. doi:10.1161/STROKEAHA.113.000985.

MicroRNAs as Effectors of Brain Function

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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 *Ceanorhadbitis 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[1]. Small RNAs were then shown to mediate gene-silencing via a mechanism known as RNA interference (RNAi)[2]. Andrew Fire and Rob 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[3–5]. MiRNA genes produce primary miRNA transcripts containing at least one ~70 nucleotide hairpin loop. These transcripts are transported into the cytoplasm where they are cleaved by the endonuclease Dicer into an imperfect duplex of 20–25 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 (RISCs)[6, 7]. Studies from several laboratories have revealed that the predominant role of miRNAs in RISCs 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. Thus, while 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, miRNA research is still in its infancy. While technical challenges have hampered miRNA studies, new technologies are evolving. 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 are fully warranted.

MiRNAs and Brain Ischemia

MiRNAs serve essential roles in virtually every aspect of brain function, including neurogenesis[8], neural development[9], and cellular responses leading to changes in synaptic plasticity[10]. Accordingly, miRNAs are also implicated in neurodegeneration and neurological disorders[11]. Further, miRNAs are implicated in responses to hypoxia and ischemia[12], and in ischemic tolerance induced by ischemic preconditioning[13].

Disclosures : none

Ischemic tolerance is the response to a short duration of ischemia (preconditioning), which protects cells against a subsequent injurious duration of ischemia[14]. Ischemic preconditioning-induced tolerance is known to require new protein synthesis[15] and the signature of tolerance is a transient repression of gene expression[16]. 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 (MCAO). We isolated total RNA from the contralateral and ipsilateral cortex of each mouse brain using the mirVana Isolation Kit (Ambion, Austin, TX, USA). The RNAs were labeled, hybridized to the mirVana Probe Set V2 (Ambion) microarray slide which included probes for human, mouse, and rat miRNAs, and microarray slides were scanned on a GenePix 4000B (Axon Instruments, Union City, CA, USA)[13]. For initial data analysis, an important consideration was to evaluate the consistency of miRNA expression within each group to identify animal-to-animal and/or diurnal variations in miRNA expression. For each mouse, the change in miRNA expression from one animal was compared to the change of the total group, and 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 humans 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 decreased miRNAs was the global transcriptional regulator, methyl CpG binding protein 2 (MeCP2), which had no prior recognized role in preconditioning or tolerance[13]. 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[17]. However, there are differences in responses to focal ischemia in male and female rats: male mice have greater infarct volumes in response to ischemia than do female mice[18, 19]. We recently used real-time quantitative PCR (qPCR) 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 (Saugstad J and Murphy S, 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[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, while the others used probes that can detect the mature miRNA sequence but can 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 one mRNA can be targeted by hundreds of miRNAs. Computational algorithms and free energy (ΔG) 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[21]: (1) miRNA/mRNA target interaction must be verified, (2), the miRNA and predicted mRNA target must be co-expressed, (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

Within the last decade, 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 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 which disrupt normal post-transcriptional gene regulation could lead to disease phenotypes. This is 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[22]. Accordingly, there is evidence for altered miRNA expression in peripheral blood isolated from ischemic stroke patients[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 hugely impacted our understanding of the mechanisms underlying normal and altered cellular function, in spite of any technical limitations due to the complexity of miRNAs. In the next decade of miRNA studies, these technological issues will likely continue to be a challenge. Thus, efforts to advance and evolve the tools necessary for analysis and validation of miRNAs, as well as the development of new technologies, 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.

Acknowledgments

I would like to acknowledge my collaborators, Dr. Theresa Lusardi (Legacy Research Institute, Portland, OR) and Dr. Stephanie Murphy (Oregon Health & Science University), for their contributions and continued enthusiasm in regard to our miRNA studies.

Sources of Funding

Funding for these studies was supported by grants from the National Institutes of Health R21NS054220 and R01NS064270 (JAS).

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