CNS Neuroscience & Therapeutics

EDITORIAL COMMENTARY

CNS Neuroscience & Therapeutics

MicroRNA in the Pathophysiology of CNS Injury: Implication in Neuroregenerative Medicine

P. M. Abdul-Muneer
Laboratory of CNS Injury and Repair, Neuroscience Institute, JFK Medical Center, Edison, NJ, USA

Correspondence
P. M. Abdul-Muneer, Laboratory of CNS Injury and Repair, Neuroscience Institute, JFK Medical Center, Edison, NJ 08820, USA.
Tel.: +1-732-321-7000, x62096;
Fax: +1-732-767-2902;
E-mail: pmamuneer@gmail.com
Received 3 May 2016; revision 14 May 2016; accepted 16 May 2016

doi: 10.1111/cns.12579

Central nervous system (CNS) injuries such as traumatic brain injury (TBI) and spinal cord injury (SCI), and cerebral ischemia are major causes of death and disability worldwide, particularly among the young population. Although cause and consequences of these injuries are different, they share several common pathophysiological mechanisms. These injury mechanisms are categorized as primary and secondary. Primary injury is sudden, and as a result of mechanical forces applied to skull and brain. Secondary injury, on the other hand, evolves over time, over a period of days to weeks and even months. Secondary injury of TBI and stroke is characterized by oxidative stress, disruption of blood–brain barrier (BBB), edema formation, inflammation, apoptosis, and delayed neurodegeneration [1–3]. Elucidation of signaling factors controlling the injury-associated pathophysiology has immense importance in both diagnostics and therapy. During and after CNS injury, miRNA plays significant regulatory role in transducing the CNS physiology into pathophysiology and vise versa.

MicroRNAs are short noncoding RNAs that regulate gene expression by antagonizing mRNA translation. miRNA can target and block translation of numerous mRNAs, thereby alter and modulate the expression of myriads of genes involved in a wide spectrum of physiological processes. Moreover, miRNAs have the potential to develop as biomarkers for the real-time diagnostics and prognostics. This article provides an overview of miRNA in mediating the molecular and cellular changes that occur after CNS injury.

miRNAs are abundantly expressed in the mammalian CNS, where they control complex processes associated with neuronal differentiation, maturation, and function [4]. Compared to other signals of CNS pathologies, much less is known about the changes in miRNA expression in CNS injury. However, those known miRNAs attribute significant roles in regulating the pathophysiology of CNS injury. Thus, it is anticipatory to exploit them for the purpose of developing effective therapeutic strategies against CNS injury-associated neurological complications. There are several methods that have been developed to detect miRNAs. Among them, one of the most popular techniques for validating and accurately quantifying miRNAs is quantitative real-time PCR (qPCR). miRNA array is another largely used technique to detect multiple miRNA targets. Other techniques are next-generation sequencing, multiplex miRNA profiling, etc. With this perspective, I provide an overview of miRNAs associated with CNS, their structure, function and biogenesis and roles in CNS development, as well as a brief account on recent miRNA studies in TBI, SCI, and stroke. Finally, an outlook on the feasibilities of miRNA-based therapies as a novel approach to ameliorate the morbidity and mortality associated with CNS injury is provided.

miRNA are small, evolutionary conserved, single-stranded, noncoding RNA molecules composed of 20–24 nucleotides that bind target mRNA and make conformational changes at epigenetic level and regulate the related protein expression [4]. Generally, miRNAs bind to their "target" mRNA through partial sequence complementarity and regulating (generally inhibiting) gene expression at the level of mRNA translation. When miRNA binds to complementary sequence of mRNA, these mRNA molecules are silenced by one or more of the following processes: (1) cleavage of the mRNA strand into two pieces, (2) destabilization of the mRNA through shortening of its poly(A) tail, and (3) less efficient translation of the mRNA into proteins by ribosomes [4].

miRNA is structurally distinct from mRNA. They are initially transcribed from genomic DNA, largely by RNA polymerase II, in the form of primary miRNA transcripts (pri-miRNA). This primary miRNA molecule displays unique structural property, it folding back on itself to create a double-stranded stem-loop structure held together by hydrogen bonds. These hairpin loop structures are composed of about 70 nucleotides each. The double-stranded RNA structure of the hairpins in a pri-miRNA is recognized by a nuclear protein known as DiGeorge syndrome critical region 8 (DGCR8), named for its association with DiGeorge syndrome. DGCR8 associates with the enzyme Drosha (also known as

ribonuclease III), which cleaves and releases a precursor miRNA (pre-miRNA) from the pri-miRNA [4]. The protein called exportin-5 transports pre-miRNA from nucleus to cytoplasm. In cytoplasm, an enzyme called dicer moves along the double-stranded miRNA and interacts with 5′ and 3′ ends of the hairpin, and cuts away the loop joining the 3′ and 5′ arms into shorter fragments and removes hairpin loop by cleaving RNA about eleven nucleotides from the hairpin base. Then, one strand of each short double-stranded RNA is degraded (passenger strand); the other strand (miRNA) is then associated with a number of proteins collectively known as the RNA-induced silencing complex (RISC), forming miRNA–protein complex. MiRNA–protein complex blocks translation of their mRNA targets as well as they expedite mRNA deadenylation (breakdown of 3′ poly-A tail), which causes the mRNA to be degraded sooner and translate less [4] (Figure 1).

miRNAs are reported as mediators of several molecular, cellular, and biochemical changes that occur after TBI. In rat models of TBI, there are reports on upregulation and downregulation of various miRNAs in different regions of the brain including cortex and hippocampus. Microarray analyses in mouse models of TBI induced by controlled cortical impact (CCI) have revealed significant changes in miRNA expression within the hippocampus. Among the 444 verified mouse miRNAs, expression levels of 35 of them found upregulated, whereas 50 miRNAs found downregulated after TBI [5]. In this model, an in situ hybridization study shows considerable reduction in the expression of miR-107 indicating vulnerability to cell death in ipsilateral hippocampal region. In addition, expression level of miR-21 found upregulated in the TBI-affected dentate gyrus and CA3 regions of hippocampus. Another study, conducted in rodent model of TBI, revealed significant upregulation of eight miRNAs and noticeable downregulation of 13 miRNAs during post-TBI at 24 h after TBI. Interestingly, seven of the 13 upregulated miRNAs later found downregulated at 7th day of post-TBI [6]. In 2010, Liu et al. [7] carried out a time course microarray analysis of miRNA expression and reported that levels of 10 of 156 reported miRNAs found significantly altered from one hour to seven days after injury induced by CCI in rat ipsilateral hippocampal region. Among those 10 differentially expressed miRNAs, miR-144, miR-153, and miR-340-5p confirmed to be elevated at various time points such as 1 h, 1 day, 3 days, 5 days, and 7 days after TBI by quantitative RT-PCR analysis. Further, the authors corelated the elevated expression of these miRNAs with downregulated expression of three target proteins such as calcium/calmodulin-dependent serine protein kinase (CASK), nuclear factor erythroid 2-related factor 2 (NRF2), and alpha-synuclein (SNCA), suggesting critical roles in the pathogenesis of TBI-induced cognitive and memory impairments [7]. In another microarray analysis, Sharma et al. studied 23 miRNAs, which significantly modulated in mice brain injury induced by a free-falling metal rod with a rubber tip of 1 mm diameter. Among these 23 miRNAs, 14 found upregulated and nine downregulated [8]. In 2010, Wang et al. [9], reported a strong correlation between miR-107 and granulin (GRN) a protein involved in wound repair and inflammation, and they speculate that miR-107 regulates GRN expression.

Spinal cord injury is another complicated CNS injury having an estimated annual incidence of 11-60 cases per million people. There have been few studies reported about the miRNA expression changes in SCI. In a model of spinal cord compression in mice, microarray analysis revealed 10 miRNAs expressed differentially after 12 h of injury [10]. In another microarray analysis of a rat contusion model of SCI, variations in the expression level of miRNAs, which are involved in oxidative stress, inflammation, and apoptosis, have been noticed [11]. In this study, authors discerned some miRNAs were upregulated and some were downregulated and a subset of miRNAs were upregulated in initial 4 h and then downregulated at day 1 and day 7 [11]. Likewise, there are several reports on the modulation of miRNAs in animal model of SCI, and these studies provide evidences for miRNA dysregulation during and after SCI. Thus, it is

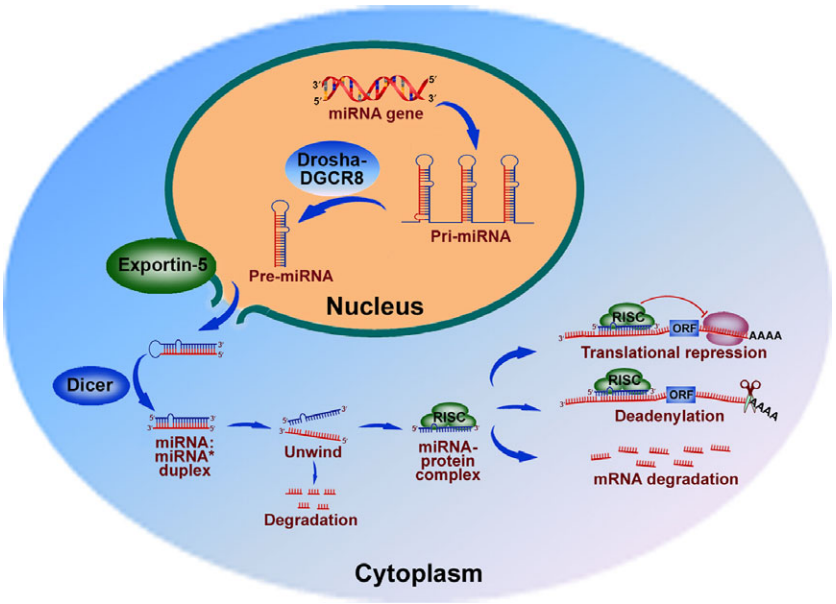

Figure 1 Schematic representation of biogenesis and posttranscriptional suppression of microRNAs: In the nucleus, the nascent primary microRNA (pri-miRNA) transcripts are processed into ~70 nucleotide precursor miRNAs (pre-miRNAs) by Drosha. Pre-miRNAs are transported to the cytoplasm by exportin-5 and the enzyme dicer process pre-miRNA into miRNA: miRNA* duplexes. Then, one strand of short double-stranded RNA is degraded (passenger strand); the other strand (miRNA) is then associated with a number of proteins collectively known as the RNA-induced silencing complex (RISC), forming miRNA–protein complex. RISC acts on its target to downregulate gene expression by different posttranscriptional mechanisms: mRNA cleavage or translational repression or destabilization of the mRNA through shortening of its poly(A) tail.

anticipatory that miRNA analysis can help to develop more effective therapeutic strategies for SCI, as miRNA information has the potential to modulate molecular and cellular changes that occur during and after injury.

Stroke is another leading CNS injury, and one in six people worldwide will have at least one stroke in their lifetime. Stroke leads to cerebral edema, inflammation, and neuronal death that can result in wide tissue damage as well as long-term cognitive and motor deficits. Relating stroke, several miRNA studies have been conducted, and alterations in several miRNAs in the brain at 24 and 48 h were reported in experimental strokes [12]. In a rodent middle cerebral artery occlusion (MCAO) model study, authors have corelated the levels of miRNAs (such as miR-30a-3p and miR-383) to that of aquaporin-4, a common marker for cerebral edema, which were increased with decrease in miRNA level after ischemia [12]. In another miRNA study, it was noticed that miR-132 and miR-664 were downregulated in an animal model of experimental stroke, and this downregulation found correlated with upregulation of matrix metalloproteinase-9 (MMP-9) [13]. It has been established that MMP-9 plays direct role in BBB disruption, hemorrhage, neuroinflammation, and cell death in various neurological diseases [1,3,14]. In 2010, Yin et al. [15] studied the levels of miRNAs that regulate apoptosis following stroke. They reported upregulation of miR-497, which targets the antiapoptotic genes such as Bcl-2 and Bcl-w in infracted area in a mouse model of MCAO. In brief, identification and characterization of newer miRNAs are very critical as they involve in the regulation of stroke related cell signaling mechanisms that could contribute for developing therapeutic strategies against stroke and other pathophysiological conditions.

In conclusion, miRNAs, the novel class of posttranscriptional gene regulators, have been increasingly recognized as therapeutic targets and as biomarkers for various CNS disorders. So far, much less is elucidated about their role in the pathophysiology associated with CNS injuries. However, the available studies have demonstrated that miRNAs as major mediators of molecular, cellular, and biochemical changes occur during and after CNS injuries. Compared to any other biomolecules, miRNAs have greater potential to develop as viable biomarker to monitor the different stages of the pathophysiology of CNS injuries. Thus, there is immense scope for developing miRNAs as targets for developing therapeutic strategies against CNS injury-mediated ailments. Thus, an in-depth study elucidating various miRNAs, critically involved in modulating the pathophysiology of brain injury, has paramount importance.

Conflict of interest

The author declare no conflict of interest.

References

1. Abdul-Muneer PM, Schuetz H, Wang F, Skotak M, Jones J, Gorantla S, et al. Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. *Free Radic Biol Med* 2013;**60**:282–291.
2. Abdul-Muneer PM, Chandra N, Haorah J. Interactions of oxidative stress and neurovascular inflammation in the pathogenesis of traumatic brain injury. *Mol Neurobiol* 2015;**51**:966–979.
3. Abdul-Muneer PM, Pfister BJ, Haorah J, Chandra N. Role of matrix metalloproteinases in the pathogenesis of traumatic brain injury. *Mol Neurobiol* 2015; doi: 10.1007/s12035-015-9520-8.
4. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;**116**:281–297.
5. Redell JB, Liu Y, Dash PK. Traumatic brain injury alters expression of hippocampal microRNAs: potential regulators of multiple pathophysiological processes. *J Neurosci Res* 2009;**87**:1435–1448.
6. Hu Z, Yu D, Almeida-Suhett C, Tu K, Marini AM, Eiden L, et al. Expression of miRNAs and their cooperative regulation of the pathophysiology in traumatic brain injury. *PLoS One* 2012;**7**:e39357.
7. Liu L, Sun T, Liu Z, Chen X, Zhao L, Qu G, et al. Traumatic brain injury dysregulates microRNAs to modulate cell signaling in rat hippocampus. *PLoS One* 2014;**9**:e103948.
8. Sharma A, Chandran R, Barry ES, Bhomia M, Hutchison MA, Balakathiresan NS, et al. Identification of serum microRNA signatures for diagnosis of mild traumatic brain injury in a closed head injury model. *PLoS One* 2014;**9**:e112019.
9. Wang WX, Wilfred BR, Madathil SK, Tang G, Hu Y, Dimayuga J, et al. miR-107 regulates granulin/progranulin with implications for traumatic brain injury and neurodegenerative disease. *Am J Pathol* 2010;**177**:334–345.
10. Nakanishi K, Nakasa T, Tanaka N, Ishikawa M, Yamada K, Yamasaki K, et al. Responses of microRNAs 124a and 223 following spinal cord injury in mice. *Spinal Cord* 2010;**48**:192–196.
11. Liu NK, Wang XF, Lu QB, Xu XM. Altered microRNA expression following traumatic spinal cord injury. *Exp Neurol* 2009;**219**:424–429.
12. Jeyaseelan K, Lim KY, Armugam A. MicroRNA expression in the blood and brain of rats subjected to transient focal ischemia by middle cerebral artery occlusion. *Stroke* 2008;**39**:959–966.
13. Copin JC, Goodyear MC, Gidday JM, Shah AR, Gascon E, Dayer A, et al. Role of matrix metalloproteinases in apoptosis after transient focal cerebral ischemia in rats and mice. *Eur J Neurosci* 2005;**22**:1597–1608.
14. Abdul Muneer PM, Alikunju S, Szlachetka AM, Haorah J. The mechanisms of cerebral vascular dysfunction and neuroinflammation by MMP-mediated degradation of VEGFR-2 in alcohol ingestion. *Arterioscler Thromb Vasc Biol* 2012;**32**:1167–77.
15. Yin KJ, Deng Z, Huang H, Hamblin M, Xie C, Zhang J, et al. miR-497 regulates neuronal death in mouse brain after transient focal cerebral ischemia. *Neurobiol Dis* 2010;**38**:17–26.